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BIOLOGICAL EFFECTS OF LASER RADIATION

Final Scientific Report - Volume II
(Review of Our Studies on Biological Effects of Laser Radiation—1965 to 1971)

Samuel Fine

Submitted October 17, 1978
(1 July 1963 to 30 September 1971)

Supported By
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DA-49-193-MD2436

Department of Biophysics and Biomedical Engineering
Northeastern University
Boston, Massachusetts 02115

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A106 235	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) BIOLOGICAL EFFECTS OF LASER RADIATION. Volume II. (Review of Our Studies on Biological Effects of Laser Radiation—1965-1971),		5. TYPE OF REPORT & PERIOD COVERED Final 1 July 1963 - 30 September 1971
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Samuel/Fine		8. CONTRACT OR GRANT NUMBER(s) DA-49-193-MD2436
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Biophysics and Biomedical Engineering Northeastern University, Boston, MA 02115		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102A/3S161102BS05
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick Frederick, MD 21701		12. REPORT DATE October 17, 1978
		13. NUMBER OF PAGES 207 pages
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for Public Release; Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Laser biology; free radical formation; tissue culture; gross and histological changes, argon laser irradiation; hazards and backscattered viable cells; carcinogenic potential; ultraviolet cataract formation; mechanisms; models; protective devices; psychological studies.

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

Many of our studies on biological effects of laser radiation from 1965 to 1971 are reviewed. These include: continuation of free radical studies; differential effects on cell growth patterns in tissue culture; effects on biochemical preparations; attempts to alter the immunological capability/virulence ratio of influenza virus; gross and microscopic descriptions of lesions, their natural history, and alterations in calcium and magnesium; argon laser irradiation studies; occurrence and significance of backscattering of viable tumor cells;

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carcinogenic potential of the radiation; cataract production at ultraviolet wavelengths; uses and hazards in dentistry; degradation of the energy within a closed filled cavity; charged particle production; usefulness of sonic and ultrasonic signature characterization; studies on focal hepatic injury and repair; reversible depigmentation investigations and relationship to melanin granule as a target site; development of pulse-duration dependent models; analysis and development of threshold injury models; CO_2 laser development and biological and biophysical studies; in-vivo and in-vitro tests of effectiveness of laser protective material; use of closed circuit TV; categorization of non-radiational hazards; contributions to provisions for safe use of lasers; and psychological studies.

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Note on Content

This volume contains a review of our studies on biological effects of laser radiation from 1965 to 1971. It does not contain a review of those studies from 1963 to 1965, which are contained in volume 1, the studies on CO_2 laser irradiation of the rabbit eye, which are contained in volume 3, nor studies on frequency doubling in biological tissues, which are contained in volume 4 of this final scientific report.

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

Abstract

In this volume are documented some of our contributions relating to biological effects of laser radiation which were supported in whole or in part by this contract. These include:

1) continuation of the studies on free radical production which we were the first to show occurred on laser irradiation of biological tissue;
2) differences in laser-irradiated cell growth patterns from those in non-irradiated controls in tissue culture;
3) continuation of our studies on laser-induced changes in biochemical preparations with a view towards production of medically useful compounds which we initiated;
4) attempts to affect the immunological capability/virulence ratio of influenza virus in order to explore facilitation of vaccine production;
5) extensive gross and histologic long-term studies on laser-induced lesions in normal adult animals;
6) studies on laser-induced will-circumscribed focal hepatic injury and repair in order to investigate the natural history of such injury, the alterations in calcium and magnesium, and to establish whether the laser could be used as a tool for this area of research;
7) probably the first biological studies on argon laser irradiation, where we observed problems associated with incision of larger vessels and reactive bleeding necessary in applying the argon laser to ophthalmology and associated heating problems with use of argon and other continuous lasers in attempting to eradicate tumors;
8) the first studies to show that pulsed laser irradiation could result in backscattering of viable cells from an irradiated tumor site and

consequently potentially cause dissemination of tumor on use of high peak power irradiation for therapeutic purposes;

9) the first large-scale and perhaps still only animal studies on the carcinogenic potential of high peak power irradiation;

10) tne first studies to show definitely that cataracts can be reproducibly produced at ultraviolet wavelengths; proposals for initiation of ultraviolet studies at 337 nm; effects of ultraviolet laser radiation at 325 nm on the skin;

11) dental studies with a view to exploring the potential and hazards associated with use of lasers in dentistry;

12) the first studies to show and develop an understanding of the mode and significance of degradation of high peak power radiation within a closed, filled biological cavity such as the cranium (or cell on a microscopic basic), including the production of pressures, sonic, ultrasonic and hypersonic frequencies;

13) initiation of those studies directed towards characterization of charged particles obtained from a biological target;

14) the first attempts at characterization of biologically significant molecules by determination of the sonic and ultrasonic signature on laser irradiation;

15) integration of gross, microscopic, enzyme and temperature studies together with model studies in determining aspects relating to focal hepatic injury and repair;

16) continuation and expansion of our studies which first showed reversible depigmentation in normally black-haired mice, the similarity of the thresholds thus obtained on normal mode and Q-switched irradiation to those for the eye, and the significance of the melanin granule as

a target site;

17) probably the first (melanin granule) model to explain the observation that a marked decrease in energy is required for threshold injury with decrease in laser pulse duration, and the development of additional melanin granule models for injury on pulsed laser irradiation;

18) the early development of models relating to thresholds for injury associated with continuous visible (laser) radiation in order to attempt to determine a safe transpupillary power on a worst case thermal basis, and extension of this model to other than minimal target (retinal) irradiation diameters;

19) the first CO_2 laser biological and biophysical studies, including experimental data for both threshold biological injury and tissue ablation and fitting of this data by thermal and heat flow models;

20) development, construction and dissemination of data regarding the design of inexpensive CO_2 lasers early after their initial discovery;

21) development of a new method for detecting and measuring the frequency of surface vibrations using the speckle pattern from a He-Ne laser;

22) the first use of in vivo biological targets to test the effectiveness of laser protective material; in these studies, the Q-switched to normal mode relative effectiveness obtained for threshold injury was shown to be much greater at suprathreshold injury levels (indeed, the initial Q-switched versus normal mode studies, which had been reported under this contract showed marked qualitative differences in effectiveness);

23) studies on hazards and protective devices associated with 10.6 micron irradiation;

24) problems associated with closed circuit TV for observation and with

single signal systems for safety;

25) probably the first attempt at categorization of non-radiational non-electrical toxic and explosive hazards associated with ancillary materials and laser media;

26) the early development and outline of procedures and techniques for safe operation of lasers;

27) major contribution to the first significant laser hazard survey--the Massachusetts Laser Survey;

28) provision of information to numerous agencies, groups and organizations in areas relating to laser interaction, hazards and safety, programs for hazard control and use of lasers as, for example, those relating to lasers used during NASA's first manned moon landing;

29) psychophysical studies including the first human psychophysical studies to determine heat sensation thresholds for CO_2 laser radiation as a function of time, intensity and area.

In addition, the following are outlined and detailed in the accompanying volumes of this final report:

30) many of our biological studies to 1965 in which are included details concerning our findings. Most of those discussed below are related to our initiation of the following areas of bio-laser research.

    a) free radical production;
    b) _in vivo_ spectroscopy specific element documentation;
    c) changes in specific macromolecular preparations which differed from those produced by heat, per se;
    d) production of localized lesions in mammalian fetuses through

the intact uterus;

e) lesions deep to the surface on irradiation of the abdomen of mice, and their etiology;

f) regression of non-irradiated tumors in some animals in which one of the tumors of multiple-tummor implant animals was irradiated--this may have been due to immunological changes, and was not unique to the laser radiation modality;

g) the effect of interfaces on energy entrapment;

h) analysis of errors associated with use of thermocouple;

i) Q-switched versus normal mode effects.

31) effects of CO_2 irradiation on the rabbit eye, including determination of thresholds for injury, measurement of intraocular (anterior chamber) pressures together with lens changes on suprathreshold irradiation, analysis of macromolecular changes and identification of a process for attempting to maintain constancy of temperature on increased heat flow into eye (See Vol. 3);

32) production of frequency doubling in specific eye and skin related biological tissues on ruby laser irradiation at 694 nm (See Vol. 4).

This contract, furthermore, provided the initial impetus which then permitted us to direct our attention to areas relating to the rupture force and tensile strength of aortic tissues and effects of various modalities of sterilization and maintenance on these tissues, and towards cardiovascular research related areas. For this reason, some credit was given to the contract in our publications relating to the above two areas.

This note does not give specific credit to the various members of the research group associated with each project discussed above. These are given in the publication references included in the sections of the volumes of this final report.

FREE RADICALS AND ELECTRON SPIN RESONANCE STUDIES

Some aspects relating to free radicals in biology are discussed in Reference 2. The initial electron spin resonance studies carried out under this contract are discussed in volume 1 of this report. Further electron spin resonance studies of melanin containing tissues after pulsed ruby laser irradiation were carried out by Stratton, Pathak and Fine (1). Results were compared with those obtained following irradiation at 77^{o}K or 300^{o}K with ultraviolet or visible radiation using a 1600 watt Xenon lamp and suitable filters.

Human skin and hair and mouse skin exhibited E.S.R. signals due to unpaired electrons, without irradiation if the samples were pigmented. These signals which are attributed to melanin had a "g" value of 2.0035 and consisted of a single almost symmetrical line of width 7-8 gauss at 77^{o}K. This intrinsic melanin signal was not destroyed by temperature changes over the range 77^{o} to 380^{o}K but was reversibly affected by the moisture content of the samples. White skin and hair with very little melanin content gave little or no signal.

Laser irradiation of pigmented human skin at 77^{o}K produced an enhancement of the melanin signal. There was no change in "g" value, line width or shape relative to the intrinsic signal. It seemed likely that the radiation had increased the number of detectable unpaired electrons in similar environments to those already present in the unirradiated samples. However, such observed additional free radicals appeared to have short lifetimes at room temperatures, as illustrated by the small signal obtained by irradiating at 300^{o}K and then cooling the samples to 77^{o}K within a few seconds after the laser pulse.

We attributed the laser induced signal following pulsed ruby laser irradiation of mouse skin, to increase in the free radical content of the intrinsic melanin,

(1) Stratton, K; Pathak, M.A. and Fine, S. "E.S.R. Studies of Melanin Containing Tissue After Laser Irradiation", N.E.R.E.M. Record, I.E.E.E., 1965.

(2) Pryor, W.A. "Free Radicals in Biology" Volume 1, Academic Press, 1976

in this case chiefly present in the residual hair of the C57BL/6 skin. The skin itself is almost unpigmented, since there are no epidermal melanocytes, except for the tail. The E.S.R. signals from well plucked skin were negligible and unchanged by irradiation, whereas those from skin with the pigmented hair merely clipped were enhanced by laser irradiation as previously observed. The response of human hair to laser irradiation was similar to that of skin, and again radiation induced signals were only detected at low temperature. Warming an irradiated sample of skin or hair to 300^{o}K for a few minutes and then recooling destroyed the radiation induced portion of the ESR signal, but did not affect the stable intrinsic melanin signal. We did not detect any differences between the effects on the ESR signals of continuous visible (600-700mu) and laser radiation.

U.V. and ionizing radiation produce different ESR spectra in both white and pigmented skin and hair. $Co^{60}\gamma$ radiation does not specifically enhance the intrinsic melanin free radical component but produces a complex spectrum, partly attributable to proteins, in both skin and hair. U.V. radiation enhances the melanin signal in pigmented samples but also produces other radicals with greater line width, in both melanised and unmelanised skin.

In conclusion, pulsed ruby laser irradiation enhanced free radical signals due to melanin present in skin and hair. It did not produce similar signals in white hair or fair skin. The ESR signals were analogous to those produced by comparable doses of continuous visible (600-700 mu) radiation and differed from those obtained after U.V. or ionizing radiation. In spite of the visible damage to the tissues and the luminous vapors emitted, laser radiation did not induce any E.S.R. spectrum except that characteristic of melanin. On the other hand, both U.V. and ionizing radiation induced other free radical signals, in both melanised and non-melanised tissues.

TISSUE AND CELL CULTURES

Further tissue culture studies were reported under this contract by Hardy, Hardy, Fine and Sokal (1). They studied the effects of ruby laser irradiation on mouse fibroblast culture.

Mouse fibroblasts (L929) were suspended in Eagle's medium (2.5 x 10^6 cells in 1 ml.) Four tubes were submitted to 4 exposures of pulsed laser radiation at 3 min. intervals (10 joules/cm^2 at 6943Å). The irradiated cells and non-irradiated controls were transferred to fresh culture medium and were incubated at 35°C for 4 days. Cell counts wer performed daily. Non-irradiated controls underwent lag phase for one day and exponential growth for 2 days leveling off on the fourth day. The irradiated culture remained in lag phase for 2 days but then underwent exponential growth at a markedly higher rate than the controls, yielding 5 times as many cells on the fourth day as compared to the controls. The experiment was repeated 4 times with essentially the same results. It appears that while laser radiation prolonged the lag phase, it also stimulated mitotic activity.

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(1) Hardy, L.B., Hardy, F.S., Fine, S. and Sokal, J. "Effects of Ruby Laser Radiation on Mouse Fibroblast Culture", Federation Proceedings, 26:688, April, 1967.

STUDIES ON INTERACTION WITH MACROMOLECULAR BIOCHEMICAL PREPARATIONS AND WITH VIRUSES

Many of the macromolecular studies supported by this contract are discussed in Volume 1 of this report. In vitro effects of pulsed neodymium laser radiation (1060 nm) on human gamma globulin were reported by Cohen and Fine (1). The results indicated that pulsed laser radiation of IgG at this wavelength produced effects different from those of thermal denaturation upon precipitin reactions with rheumatoid factor.

Similarly laser irradiation at 694.3 nm of 1 percent saline solutions of human (HGG) and rabbit (RGG) gamma globulin produced different effects than that of a thermal nature per se upon the precipitin reactions with rheumatoid factor (2). The studies on heat treatment of globulin solutions at 63^{o}C for 15 minutes caused aggregation and measurably more precipitation with rheumatoid factor than untreated globulins, whereas the laser irradiation reduced or destroyed the capacity for the globulins to precipitate rheumatoid factor. Precipitin reactions between rabbit anti-HGG and laser irradiated HGG demonstrated a shift of position of precipitin curve peaks, as well as reduction of total amount of precipitation. Ultracentrifugal analyses did not reveal additional components with laser irradiation of HGG, as were observed with heat treatment of globulins. Immunochemical agar gel diffusion and immunoelectrophoretic analyses revealed slight differences of laser irradiated from native globulins.

It was concluded that the interactions of high intensity electromagnetic (laser) radiation with globulins differed from those of heat per se, as demonstrated by differences in the serologic activity, certain physicochemcial changes and other pertinent parameters of the respective reactions.

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(1) Cohen, E. and Fine, S. "In Vitro Effects of Laser Irradiation on Human Gamma Globulin," Federation Proceedings, 27:1, 473, March-April, 1968.

(2) Cohen, E., Klein, E. and Fine, S. "Effects of Laser Irradiation on Some Serologic Properties of Human Gamma Globulin," Life Sciences, Vol. 7, Pt 2, Pg. 569-581, 1968.

It appeared that the serological effects differed from those obtained by other laboratories employing X and gamma irradiation of specific protein solutions. Attention was also directed towards comparison with effects of ultrasound, particularly since the production of ultrasonic waves by laser radiation had been observed, particularly when a dissolved gas is present in the medium (3). Further possible mechanisms and potential uses for laser modified macromolecules are discussed in the paper (2).

Studies on the effects of pulsed laser radiation at 6,943Å and 10,600Å on infectivity of influenza virus were conducted by Klein and Fine _et al_. (4). The studies carried out indicated that virulence might be changed by pulsed laser radiation in both directions. Under the appropriate experimental conditions it could be increased; in the presence of dyes which specifically complex with the nucleic acid component, a decrease in virulence might be brought about. The activity was determined by infectivity titration in embryonated hen eggs. The effects were considered as possibly due to deaggregation of virus particles or to changes within the structure of the virus itself.

The authors believed the laser related studies to be important in the following respects. Changes in virulence may be of interest in regard to theoretical as well as practical aspects or virology. The public health aspect of qualitative or quantitative changes in virulence due to pulsed laser radiation may be of significance, since laser devices are used both in the open atmosphere and in enclosed spaces, where infective particles may be present. Furthermore, an understanding of the laser related mechanisms by which virulence is decreased could provide a basis for attenuation of a pathogenic strain, thus facilitating the production of vaccines. Reference was made to laser-induced acoustic breakage of

Tobacco mosaic virus for Q-switched ruby laser irradiation by Hamrick and Cleary (5).

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(3) Fine, S., Klein, E., Fine, B. S., Litwin, M., Nowak, W. B., Hansen, W. P., Caron, J. and Forman, J. "Mechanisms and Control of Laser Hazards and Management of Accidents," Proceedings of the Second Conference on Laser Technology, (Illinois Institute of Technology), April, 1965.

(4) Fine, S. and Klein, E. "Lasers in Biology and Medicine," Laser Focus, July 1969.

(5) Hamrick, P. E. and Cleary, S. F. "Laser-Induced Acoustic Breakage of Tobacco Mosaic Virus," Nature, 220:909-910, Nov. 30, 1968.

STUDIES ON NORMAL ANIMALS

Many of our studies on the effects of pulsed laser irradiation on normal animals are discussed in volume one of this report in conjunction with comparative studies by other investigators. Other studies oriented towards the eye, and towards frequency doubling are discussed in accompanying volumes or sections of this report. The section below details some additional studies on normal animals. The purposes of these studies were to determine the effect of laser radiation on normal in vivo tissue; to study the mechanisms associated with injury and repair per set as well as those due to laser radiation; and to determine the potential for the use of the lasers in injury and repair studies.

Studies were carried out on the effects of laser radiation on the skin and body wall of the mouse. The pathology of those two regions was discussed (1). This report concerns the pathologic effects of laser radiation on the skin and body wall of the mouse and discusses how these effects differ from thermal and other injuries.

Studies described here were carried out with single pulses obtained from ruby crystal lasers at 6943 Å at energy levels of 3 to 100 joules per pulse and a pulse duration of the order of 1 millisecond, and at higher energy levels, and with neodymium glass units at 10,600 Å at energy outputs ranging from 300 to 900 joules per pulse. The radiation was unfocussed or focussed to a spot size of 1 to 2 mm at the skin surface by a simple lens system. Anesthetized Swiss (white) and C_{57} (black) mice of various ages and both sexes were used, the hair clipped and the area depilated chemically prior to irradiation. Over 300 mice were used in these studies.

Animals were sacrificed immediately following exposure and at intervals thereafter ranging from 1 to 26 days. The animals were necropsied, except for the 66 irradiated mice and non-irradiated controls which were kept for lifetime follow-up.

Since data on thermal injury in mice was not readily available in the literature, 28 lesions were induced by mono- or bipolar electrocoagulation for comparision with the effects of laser radiation. Other lesions were induced by a steel rod which had been kept in boiling water prior to application or by pipetting of boiling water onto the surface of the skin. An additional group of 132 mice was exposed to a mercury lamp.

Skin lesions were visually examined and photographed periodically. Skin and internal lesions were recorded at necropsy. Material for histologic examination was fixed immediately in 10 per cent buffered formalin, embedded in paraffin and stained with Fontana stain for melanin and by the reticulin-trichrome and Mayers hematoxylin stains. Frozen sections were cut in some instances and stained with oil red O. Serial sections were not made routinely, but multiple levels were sectioned in the absence of a gross lesion.

Following unfocussed irradiation of one hundred and ninety mice, immediately following irradiation a slight discoloration was seen over an area up to 10 mm in diameter, corresponding to the laser rod diameter, in almost all mice that had been exposed to 70 to 100 joules. Below 70 joules, lesions were seen in a decreasing proportion of mice. No immediate lesions were recognized on gross observation below 20 joules. From 300 to 900 joules(10600 A), the lesions measured about 15 mm in diameter, corresponding to the larger diameter of the rod, and consisted of a paler outer zone surrounding a reddish-brown area with central destruction of the epidermis.

The lesions were similar at different anatomic sites except that rupture of the skin of the forehead with exposure of the skull occurred in 3 animals receiving approximately 860 joules at 10,600Å to this area.

After reflection of the skin, a few petechiae were seen occasionally on the deep surface following irradiation at 20 joules. These were present more frequently as the energy was increased to 70 joules. Above 70 joules, diffuse hemorrhage occurred. Above 300 joules, hemorrhage was absent from the central 3 to 4 mm of the lesion on the inner surface of the reflected skin. Beyond this area, there was a circular region of necrosis 5 mm in width, separated from normal tissue by a hemorrhagic ring 1 mm in width.

Diffuse hemorrhages were occasionally seen in the abdominal and chest wall muscles at energy levels of less than 70 joules and were almost always present above this energy level. The hemorrhages involved areas up to 20 mm in diameter. Even the more severe hemorrhages did not result in rupture of the muscle sheath. At energy levels above 300 joules the lesion of the muscular layer coincided with corresponding zones on the surface of the skin. The degree of hemorrhage was not constant at a given energy level. Lesions in the underlying viscera, especially the liver, were sometimes produced at 20 joules and were almost always present at 30 joules and above in contrast to that observed visually at the skin surface. Injury to the intestine and the brain were usually responsible for death occurring within the first few days after irradiation.

One day after irradiation, the skin lesions appeared as a patchy, superficial, reddish-brown, dry area measuring up to 10 mm in diameter. Two days later, a firmly adherent crust, approximately 1 mm thick, covered the entire surface in the majority of cases after 70 to 100 joules.

On the fifth day after irradiation, the scab tended to separate. Below this level, the lesions were less severe. Above 70 joules, the skin lesion was adherent to a larger area of whitish discoloration and loss of transparency in the superficial muscle sheath over and close to the site of the hemorrhage.

Following the focussed irradiation of ninety-two mice, a greyish-white umbilicated skin lesion, 1 to 2 mm in diameter, was present immediately after exposure to energy levels ranging from 10 to 100 joules. The severity of the resulting lesion was proportional to the energy delivered. Exposure to energies above 300 joules resulted in umbilicated lesions, 3 to 4 mm in diameter, surrounded by a pale circular zone. A reddish discoloration was present within the central crater. Lesions from 300 to 900 joules were indistinguishable by gross observation. The effects on the deeper tissue at energy levels up to 100 joules were similar in appearance and size to those observed after unfocussed irradiation. Above 300 joules the effects on the deeper tissues extended over a smaller area than in the lesion following the corresponding amount of unfocussed radiation and appeared to be better tolerated by the animal.

The healing process was similar to that following unfocussed irradiation. The crust in the central crater at all energy levels separated and the umbilicated lesion flattened within 4 to 5 days.

Following recovery, the majority of mice showed no delayed visible abnormalities. The lesions healed within 2 to 3 weeks after irradiation, depending on the size and severity of the lesion, and left no macroscopic scar. In several black mice scattered throughout the groups, temporary failure of melanogenesis during the recovery period was observed, but the subsequent hair generation showed no abnormal pigmentation. In 1 mouse a chronic ulcer was present 1 year following irradiation. Other than depigmentation no consistent differences were found between the reaction to the laser irradiation in black and white mice.

Twenty-two of the sixty-six mice,which were kept for lifetime follow-up,died at intervals ranging from 3 week to 16 months following irradiation.

Microscopic studies were reported in the references on the skin and underlying tissue immediate lesion, following both focussed and unfocussed radiation. The significance of the melanin granule, and the effect of the radiation on it were discussed. Microscopic studies of the healing lesion were carried out; polymorphonuclear infiltration, necrosis of small dermal blood vessels, inflammatory exudate, development of nuclear pyknosis and karolysis, presence of macrophages, nerve degeneration, ulcer and crust formation represent some of the findings. The effect of pigment on the depth of the lesion was noted. The fact that many of the pathologic changes found following laser irradiation have also been observed in various types of burns was discussed. The vascular perforations and scattering of melanin granules appeared, however, to be exceptions.

It was thought that the damage to deeper tissue following laser irradiation was proportionately more severe than in thermal burns with comparable degrees of surface injury. This was not a species difference since lesions in mice subjected to thermal burns or to electrocautery during the present experiments closely resembled those in the larger animals used in other reported studies. It was not known whether the differences from thermal injuries previously described were due merely to the uniqueness of the distribution of heat produced by laser radiation ,due to radiation "penetration," or whether some other factors such as those associated with phase transformations, charged particle production or wavelength alterations were of significance. The relationship to the work by Moritz, on the pig, and by Sheline, Alpen, Kuhl and Ahokas on the rat was discussed in the reference; the importance of the properties of the laser radiation was considered--the short pulse duration, high power density, wavelength--as well as the properties of the tissue including interfaces, importance of phase transformations, entrapment of energy, vascular rupture, transformation of radiation energy to ultrasonic and other types of energy. Some attention was therefore given to the mechanisms involved in the interaction.

In further studies, the gross and microscopic pathology of the internal viscera following laser radiation was reported (2). Visceral lesions were produced in mice by normal mode pulsed ruby and neodymium irradiation at energy density levels ranging from 4 to 400 joules/cm^2 and by continuous wave CO_2 laser irradiation at power levels ranging up to 24 watts. The gross and microscopic changes following laser irradiation resembled basically lesions produced by other heat producing agents, but differed in selectivity for pigments and production of pressure effects. The variations in the lesions of different organs appeared to be due primarily to differences in pigment content, tissue structure and response to injury. The rapid temperature rise during pulsed laser irradiation led to vaporization of tissue fluids causing large intercellular spaces with cellular distortion. The pressure waves produced changes which appeared to resemble blast injuries. Lesions produced by CO_2 lasers showed primarily thermal changes. The mechanisms of the interaction between radiation and tissue component were considered in regard to the parameters of the laser output. Some possible similarities to blast injury on pulsed irradiation of the thorax (or full bladder) were noted. The differences between lesions produced by pulsed irradiation and those produced by electrocautery, hot surfaces or ultrasonic irradiation are discussed. Reference is made to the biophysical studies, including temperature measurements, made by Fine et al.; and the relationship between the temperature obtained and the histological (microscopic) findings.

The significance of backscatter of viable cells, perforation of blood vessels and differences in the effects of pulsed high peak power systems in the visible or near infrared to those produced with CO_2 lasers was considered.

In an abstract report on the effect of laser radiation on the skin and underlying tissue of mice during controlled hair growth, the selective action of pulsed laser radiation at 6943 Å and 10600 Å on pigmented structures in the skin of (black) C-57 mice was compared with the effects of other physical agents on pigment formation (3). Laser irradiation during the active phase of hair growth (7 - 9th day) when melanin production is most marked in the hair bulb, resulted in interruption of melanogenesis for one hair generation. There were no permanent histological changes and hair growth, per se, was not affected. This temporary depigmentation differed from pigment loss produced by continuous laser radiation sources (CO_2 at 10600 Å) and other types of physical trauma (heat, diffuse light, ionizing radiation), which appear to be permanent and associated with destruction of the melanocytes. The effect of X-radiation appeared to differ further from laser irradiation in that maximum depigmentation occurs during the resting phase. Laser irradiation carried out on each day of the hair cycle indicated progressive increase in energy threshold levels for induction of depigmentation, as the resting phase was approached. Conversely, injury to the underlying tissues by pulsed laser radiation was most severe during the resting phase and became less marked as the amount of melanin increased with the activity of this cycle. The protective action of melanin was demonstrated against radiation at higher intensities than previously available. Temporary depigmentation for the duration of the hair cycle suggests the loss of melanogentic components which are reconstituted only at the beginning of a new hair cycle. The inhibition of melanogenesis without inhibition of cell division indicated other than thermal effects of laser radiation.

Other studies were carried out on the surgically exposed canine liver *in vivo* (4). These studies were carried out using sterile technique at 6943 A to 60 and 500 joules to determine the feasibility of using higher power density radiation for treatment of specific pathological conditions in human liver. Unfocused beams and radiation focused on the liver surface and deep to the surface were employed. Lesions produced by unfocused beams were well-circumscribed, whitish, and friable. Tissue destruction and crater formation to varying depths were observed following focused irradiation. Bleeding was minimal when capsular rupture occurred. When the capsule was intact, a small halo of blood was contained beneath it. Open liver biopsies were done at intervals and examined histologically. Gross examination revealed increasing consistency and progressive decrease in size of the lesion. Biopsies taken immediately after irradiation revealed sharp borders of demarcation, cytoplasmic and nuclear changes. Seven days post irradiation lobular architecture remained intact although hepatic cell necrosis was severe. Alteration in microscopic characteristics of serial biopsies occured over the next several months. Fibroplasia of repair was more severe at higher energy levels. Alterations in blood chemistry and liver function were obtained.

In further studies carried out on the effect of pulsed laser radiation on the liver of the neonatal rat *in vivo*, Edlow et al (5) observed a dark red swollen lesion which became flatter and paler until, after seventeen days, a previously recognizable sesion might no longer be grossly visible.

Studies on focal hepatic injury and repair, using the laser as a tool, were continued. MacKeen et al (6) reported on this with specific emphasis on the calcium and magnesium concentration in such lesions. Single, well circumscribed 8 mm hepatic lesions were produced in male rats through abdominal muscle following skin reflection, with a 65 joule, 1 msec ruby laser pulse. At intervals, the animals were exsanguinated, perfused with saline, and the lesion excised. Lesion, adjacent tissue, uninvolved liver, non-irradiated perfused and non-perfused controls were sampled. Ca and Mg levels were determined by atomic absorption spectroscopy. Perfused control liver tissue Ca was 58 ppm (ug/gm dry weight [d.w.]) and magnesium, 430 ppm. The calcium concentration in unperfused normal liver averaged 140 ppm. The lesion calcium 5 minutes post irradiation (p.i.) was 122 ppm. This increased to 840 ppm at 24 hours and to greater than 3,000 ppm dry weight in 14 days. The total lesion calcium increased for the first week, then fell coincident with a decrease in lesion size. In lesions, the magnesium concentration was 330 ppm immediately post irradiation. The level fell slightly, returning to the original level at 8 days. Lesion total Mg rose at 8 days post irradiation then decreased with time. No significant calcium or magnesium alterations occurred in tissue adjacent to the lesion or in uninvolved liver. Our studies showed that calcium may accumulate early following focal liver necrosis and increase during the period of progressive cellular autolysis, without similar marked change in magnesium.

The above--gross and microscopic studies on laser irradiated mice, together with comparative electrocautery and thermal injury investigations; studies on internal viscera; effect on melanogenesis; studies on injury and repair following irradiation of the surgically exposed canine liver; effect on the liver of the neonatal rat; and studies on calcium and magnesium concentrations and amounts in focal hepatic injury and repair--represents some of the studies on normal animals carried out with pulsed ruby and neodymium laser radiation. An outline of some of the CO_2 studies and ultraviolet studies is considered in other sections of this report.

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TUMOR-RELATED LASER RADIATION STUDIES AND POTENTIAL FOR CARCINOGENESIS

In addition to studies discussed in Volume 1 and the other accompanying sections of the report, a number of tumor-related studies were carried out. These included (i) investigations on experimental malanomas in mice using an argon laser, (ii) determination of the viability of tumor cells back-scattered from a tumor, irradiated with pulsed irradiation, and (iii) exploration of the potential carcinogenic effects of pulsed laser radiation. This latter group could have been included in studies on normal animals, but is discussed in this section.

In studies using the argon laser carried out in collaboration with Roy Paananen at the Raytheon Company (1), it was observed that some of the pressure associated effects, previously observed on pulsed high power irradiation of tissue (and tumors) were not present. Furthermore, relatively well-controlled, well-demarcated incisions of small vessels could be made with the argon laser. Whereas there was minimal bleeding on incision of small vessels, incision of larger vessels resulted in bleeding. This was, in part, related to the fact that the vessel wall was destroyed, without "coagulation" of blood; consequently the blood could readily extravasate through this incision. The argon laser differed from the CO_2 in that the beam was visible. Although major eye-related studies were not carried out, it was evident from the above studies (probably the first argon studies to be carried out) that this relatively well-controlled beam in the visible region of the spectrum would be of potential value for ophthalmological use. Our conclusions in regard to this ophthalmic use of argon lasers has subsequently been born out.

Those problems, which we noted and which we presented at meetings in regard to the potential for destroying a vessel wall without associated "coagulation" of blood, and those relating to delayed reactive bleeding have been observed in further use of the argon unit in ophthalmology. We observed bleeding both at the time of irradiation and delayed bleeding. We also noted that although a tumor could be reduced, and perhaps its growth delayed using the argon laser, there were certain problems associated with its use. The tissue had to be exposed to the beam, (the absorption coefficient of tissue is high at visible wavelengths), and the transformation of the irradiation to heat occurs with, of course, attendant problems, such as thermally associated pain. The basic questions, as to whether the argon laser was superior to contact heat for tumor destruction (was there a wavelength effect?) or whether it was superior to the cautery knife for cutting and cauterizing, had not been resolved at the time of our studies. The major obvious advantage of the argon laser for tumor heating was that it was a non-contact system and, in this regard, might prove preferable. Although its control and wavelength were obviously suitable for ophthalmologic studies, these specific avenues were not pursued by our group due to limited funding.

Another problem to which we directed our attention was whether pulsed high power irradiation of tumors with lasers might result in spread of viable tumor cells from that site. If so, then we could consider this result as a potential hazard or problem associated with use of pulsed laser irradiation as a therapeutic modality for tumor destruction in man. In April, 1966, the principal investigator reported at the American College of Physicians on behalf of his co-investigators and himself for the first time, that viable tumor tissue was indeed spread from the site of irradiation. Although this was not included in the original abstract to the College (2), it was considered of sufficient importance to report, and was discussed in the Journal-American by Abramson (6).

In the presentation to the College, it was shown that "following pulsed laser irradiation of exposed tumors, the backscattered material from the tumor, reimplanted into animals resulted in growth of tumors." It was concluded that "extreme care must be utilized in consideration of high peak power laser radiation for the treatment of localized malignancies since the possibility exists that some of the scattered material may contain viable tumor cells and may be driven deep into the tissue or metastasize by blood and lymphatics." We later showed that the characteristics of the tumor associated with reimplantation might differ from that of the original tumor. These results, of significance for irradiation of tumors in man, were confirmed by others and extended (4, 5).

A third area of importance in regard to laser irradiation is whether laser irradiation of normal tissue results in tumor production (at this site), and if so, to what extent. Certainly, we are aware that there is increased tumor production in severely burned man; we were interested in the carcinogenic potential on laser irradiation of tissue, in order to improve management capability of accidental laser injury. In order to attempt to answer this problem, a large series of mice were irradiated and followed (3). These studies reported by Bock et al., provided information heretofore not available. These were the first studies of any magnitude which attempted to answer this specific important problem.

In these studies by Bok et al., a total of 1579 ICR Swiss and C57/BL mice were exposed to pulsed ruby laser radiation at three levels of intensity (10, 20 and 40 joules) and at various stages of the hair growth cycle. Skin tumor production was not observed in these animals. Additional groups of mice were treated once with ruby laser radiation, (10-50 joules) followed three weeks by repeated treatment with croton oil. Tumor production was not seen in the 280 C57/BL mice so treated; however, a total of 14 tumors were observed in the 280 ICR Swiss mice treated similarly. The appearance of tumors was unrelated to the stage of hair growth cycle and only slightly related to the intensity of the radiation. A repeat study using 300 Swiss female mice exposed to ruby and neodymium laser radiation (150 joules/pulse), followed by croton oil, produced only a single papilloma in 65 weeks.

It was concluded that under the specific conditions of these experiments, laser radiation at these wavelengths did not exhibit complete carcinogenic activity or tumor-initiating activity; however, other potential effects of laser radiation on carcinogenesis must be investigated before it can be concluded that laser radiation at these wavelengths does not affect tumor production.

In summary, then, three different sets of studies were first carried out under this contract; the argon studies, the studies on the potential for spread of tumor cells on pulsed irradiation, and the carcinogenic potential of laser irradiation. Our results in these three areas are of obvious significance for the use of these lasers in man.

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CLINICAL STUDIES

Since there was no evident major advantage for urgent use of pulsed lasers in the 1960's as a therapeutic modality in man without preceeding animal studies, studies in man were not,in general, carried out under this contract. It was felt that the studies in man should be preceded by studies in animals and associated in vitro investigations, in order to determine the benefits, the risks, and the benefit/risk ratio. It was felt that this was especially warranted in regard to dermatological studies; in regard to (non-hazard) clinically related ophthalmologic studies, it was felt these could be carried out by skilled groups with extensive animal and in vitro related laser experience, and with highly controlled, sophisticated reliable laser equipment.

However, we did carry out one group of psychophysical studies using the CO_2 laser; these will be reported in another section.

The contract did permit the investigators to remain current in regard to clinical studies. These were discussed in their reviews listed below (1 - 7), as well as in other sections of this report. Knowledge of ongoing clinical studies also permitted the investigators to provide pertinent information regarding safety and accident prognosis and evaluation to individuals, and to industrial and governmental organizations, some of whom were engaged in Department of Defense related research and development. Furthermore, our other non-clinical investigations permitted us to provide information of value for clinical application.

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ULTRAVIOLET STUDIES - EYES AND SKIN

As early as 1965, interaction of laser radiation at ultraviolet wavelengths with biological systems were initiated under this contract. The studies were carried out with the pulsed nitrogen laser in collaboration with H.G. Heard of Energy Systems and with radiation at 2650 Å quadrupled from neodymium at 10,600 Å in collaboration with W. Haynie of Eastman Kodak Company. In both cases the energy and energy density achieved per pulse were very low; consequently, there were no major changes observed. The authors thought that with equipment available at the time of the review (1968-69), possibly studies yielding positive results could be obtained.

A review of ultraviolet lasers, biological studies and applications was presented at the Conference on Biological Effects of Ultraviolet Radiation, the proceedings of which were published by Pergamon Press in 1969 (1). In this review, reference was made to the possibility of frequency doubling to ultraviolet wavelengths in skin and eye as previously suggested by Fine and Zaret, respectively, and to production of second harmonic generation in crystalline amino acids in vitro on ruby laser irradiation by Rieckhoff and Petocolas.

Other potential areas of investigation discussed in this report included disinfection and sterilization at a distance, occurrence of two-photon processes and frequency doubling in biological tissue, the use of holography and optical spatial filtering techniques at ultraviolet wavelengths to obtain information concerning cells and cellular processes, adaptation of the ultraviolet laser to microbeam studies, and the use of ultraviolet lasers for in vivo and in vitro diagnostic determinations, including those involving fluorescence. Reference was also made to potential hazards of ultraviolet radiation at high intensities as available from lasers. These hazards include those of standard low-intensity ultraviolet sources in addition to other injurious changes which may be induced as the intensity is increased. The injurious effects may thus exceed in rate of onset, and complexity, the acute burns and the delayed reactions, such as mutagenesis or carcinogenesis and photosensitization encountered with standard ultraviolet sources. Hazards of ultraviolet laser radiation were therefore considered to be of particular significance in regard to the eyes and the mucocutaneous tissues.

With the advent of the helium cadmium ultraviolet laser, operating continuously at 325 nm., studies were carried out by the investigators on the eye and skin, at the 1 to 10 milliwatt level (2,3).

There were several reasons for undertaking these studies. One was interest in determination of hazards at this wavelength. A second related reason was to determine whether cataracts were produced at this wavelength and the mechanism of such cataract production. A third reason was to determine whether the laser could be used as a tool for cataract production in experimental animals in order to study cataracts.

Although one might have expected cataracts to have been readily produced on exposure to ultraviolet radiation in vivo, this had not been the case previous to our report. As stated in the paper by Kinsey (5), "quantitative determinations of the absorption of ultraviolet radiations by different structures of the eye are of importance, since various pathological conditions such as cataract, retinal damage and functional visual disturbances have been variously ascribed to these radiations."

There is growing evidence that long term exposure to ultraviolet light causes undesirable changes in the lens. A certain amount of evidence -- but none of it conclusive (Duke-Elder (7)) -- can "be brought forward to associate senile cataract" (the usual type of cataract) "with those long ultraviolet rays in sunlight which can penetrate to, and are absorbed by the lens." (Underlining mine). This includes comparison of cataract occurence frequency and age of occurence.

a.) Under various climatic conditions

b.) With various types of employment, such as outdoor workers versus indoor workers

c.) The common observation that senile cataracts usually begin in the lower quadrant of the lens where the incident light falls most directly.

Ultraviolet light produces changes in the lens. Pirie, (6) Lerman (8) and Zigman (9) reported that the yellow or brown color found in aged lenses might be the product of exposure over the years to sunlight. Their studies were basically biochemical in nature and were not carried out on *in vivo* eyes. Indeed, although as discussed below (2), we had reported the production of cataracts on exposure to 325 nm. ultraviolet radiation, the statement was still made in 1972 in an editorial in Annals of Opthalmology that, "in past experiments, lenses were exposed to intense ultraviolet light for 300 hours or more without causing cataracts."

Indeed, in their extensive studies on the effect of ultraviolet on the eye, Pitts and Tredici (10) primarily discussed injury to the cornea as observed by gross and slit lamp examination. Their studies appear to have been carried out between 200 and 300 nm. From their data, it appeared that the action spectrum for rabbit and primate were similar. A minimum threshold for injury (ergs/cm^2) occurred at about 270 nm. In addition, although the normal histology and to some extent, normal electron microscopy of the cornea is discussed in their paper, information on alterations at this level are not discussed. In their paper, they do reference the momentual work of Verhoff and Bell (11) and the significant paper by Cogan and Kinsey (12). In their further studies, Pitts *et al.* (10) used corneal light scattering measurements as a method for determining the threshold for ultraviolet injury. Their studies were carried out in humans. However, they did not report any information relating to lens injury, nor any long term follow-up.

Therefore, at the time at which we carried out our studies, it was not certain, based on the ophthalmic literature, that cataract formation could be directly related to ultraviolet radiation of the eye. Our studies, (2,3) as discussed below, showed conclusively that cataract formation (in the rabbit *in vivo*) indeed was directly related to irradiation of the eye with a He-Cd. laser at 325 nm; that ultraviolet radiation at this wavelength will indeed consistently and reproducibly produce cataracts; that this may be one of the hazards associated with ultraviolet radiation at 325 nm. and at other wavelengths) and that the HeCd laser (or other ultraviolet lasers as at 337 nm.-- see below), can be used as a tool for production of cataracts.

In summary, in our studies (2 3) weanling rabbit eyes were exposed *in vivo* to the beam of the HeCd ultraviolet laser. Anterior subcapsular and cortical cataracts were consistently and rapidly produced at the power levels employed. The changes in the intervening cornea were grossly reversible, while those in the lens were not. Localized cortical cataracts produced by an ultraviolet laser persisted for over 2 years. Maturation of the cataract was not observed to that time. The available evidence supports the belief that the damage was produced photochemically within the absorbing cells.

Our methods and results were as follows. During irradiation, the animals were anesthetized, pupils dilated, one eye used as a control, and corneal surface temperatures measured prior to, intermittently during and immediately following the irradiation using a thermocouple. The cataract produced by the UV laser appeared similar to those few produced previously by incoherent UV light (11,7). The change began in the subcapsular region, directly in the line of exposure, as an apparent acute necrosis of epithelial and some adjacent cortical cells. This produced the early subcapsular opacity. The opacity apparently spread slightly along the cells receiving initial injury, but remained limited in extent. New lens fibers insinuated themselves, separating the main necrotic mass from the anterior capsule,and the localized cataract became displaced from the anterior surface. No widespread cataractous changes (i.e., mature cataracts) were observed with the exposures and time intervals used, although the cataracts produced persisted up to 2 years. The finding of rather prompt development of cataractous changes from such injury is consistent with the relatively nonspecific sequence of events that may occur in the lens with other forms of acute, localized trauma that cause localized epithelial and/or cortical cell necrosis.

The rapid appearance of the cataractous change was considered as due to a direct injurious (i.e., abiotic or photochemical) effect on the lens epithelium and superficial cortex by the UV light, which was here capable of reaching it and being absorbed by the superficial cells. Some of the absorbed UV might be changed into heat locally within the absorbing cells, some into fluorescence (which was very striking during the process or irradiation), and some could be absorbed and produce a photochemical reaction within the cells. Although intralenticular temperatures were not measured in this study, corneal surface temperatures were determined. Corneal lesions were produced following irradiation, but the measured increases in temperature were slight and too small to have caused thermal protein denaturation. The small increases in temperature of the unirradiated corneal periphery could have resulted from increased flow of limbal blood. These measurements substantiated our conclusions that the cataractous changes were not totally thermal.

These studies were significant in two regards. First, we showed that long term exposure to ultraviolet radiation at this (and other) wavelengths can result in cataract formation, immediate or delayed; and these cataracts might be photochemical rather than photothermal in nature, although a combination of the two could not be ruled out. In addition, we showed that the He Cd laser could be used as a tool for cataract production in order to study cataracts.

We also concluded that the possibility, once raised, that retinal damage may occur in the aphakic eye appeared to be more significant with advent of UV light generated by a laser source.

In regard to protection, we stated that unlike protective devices for laser wavelengths in the visible and near-infrared spectra, the means for ocular protection at this UV wavelength appeared simple. Ocular protective material to attenuate the beam should be carefully assessed, however. Only certain spectacles and plastic laboratory glasses provide marked attenuation of the beam at ultraviolet wavelengths.

Although our studies were carried out with relatively low CW He Cd lasers, the development of high powered systems would make the accidental production of cataracts (as well as corneal injury) more probable.

Our continuation of ophthalmologically related ultraviolet studies had to be terminated because of lack of funds. However, if funding had been continued, we would have carried out studies at 337 nm. using pulsed high p.r.f. lasers, as well as those at 325 nm.

Our basis for wishing to carry out studies at 337 nm. was as follows:

We thought that studies at 337 nm. should enable us to provide information of value for setting safety standards - for the mode of operation of the commercially available lasers at this wavelength. Another reason for studying the effects of radiation on the eye at this wavelength (337 nm.) was based on the spectral transmission properties of the eye and the various components of the eye in the ultraviolet. There were several particularly significant publications relating to this area -- one by Kinsey (5) and others by Bachem, (14) Cogan and Kinsey (12) and Boettner and Walter (15). Basically, this data can be summarized as follows: Below 290 nm., all the ultraviolet energy is absorbed by the cornea. Between 300 nm. and 400 nm., the cornea absorbs correspondingly less radiation, until at 400 nm., it transmits 94% of the radiation impinging on its surface. At 330 nm. the cornea transmits about 81%, at 350 nm., 86% of the radiation. Well over 90% of the radiation which impinges on the lens at these two wavelengths is absorbed by the lens. As the wavelengths increase from 350 to 400 nm., a corresponding smaller percentage of the radiation is absorbed by the lens. It is within the range of 330 t0 350 nm., therefore, that the lens appears to have the highest fractional absorption of the radiation impinging on the eye. (It should be noted that most of this data was obtained from rabbits and some allowance must be made for extrapolation to the human eye).

Data from other studies (in which the principal investigator was involved), led him to believe that the effects of 337 nm. are probably primarily photochemical, not photothermal. He expected 337 nm. to be cataractogenic, and the cataracts to be photochemical in nature. (Although it is possible to attempt to distinguish between photochemical and photothermal injury, based on electron micrograph studies following irradiation at 325 nm. and 10.6 μ, when both photothermal and photochemical effects occur simultaneously, determination of etiology of the injury is difficult.)

In summary then, the purpose of studies at 337 nm. would have been:

1.) To provide information regarding hazards and thresholds to cornea and lens at this wavelength for the mode of laser operation available (pulsed mode).

2.)To determine the cataractogenic potential at this wavelength.

3.) To determine injury to other tissues of the eye, in addition to the lens, at this wavelength (particularly to the cornea).

4.) To determine whether effects observed at this wavelength are photochemical or photothermal at irradiation levels not far removed from threshold.

5.) To determine whether lasers at this wavelength provide a tool for the study of cataractogenesis and their prevention and treatment (other than cataract surgery).

6.) In combination with our studies at 325 nm., to determine which wavelengths might provide the optimum tool for the studies in purpose 5, above.

We believe that studies, as detailed in our unfunded but reviewed proposals, including those submitted to N.S.F. in 1974 (16), have been carried out at this wavelength and our expectations confirmed, by studies subsequent to our proposal.

In addition to the ophthalmologically related studies, investigations were carried out on the effect of 325 nm. laser radiation on the guinea pig skin (4). This investigation undertook to study the possible protecting role of pigmentation against ultraviolet laser radiation. Ears of 15 guinea pigs ranging from "albino" to black were irradiated with a (15 mW), 325 nm. 1.5 nm. diam beam from a Spectra-Physics He-Cd Laser for 16-30 minutes. Biopsy specimens were taken immediately, 24-72 hrs., and 1 month after radiation. NaBr split, dopa, and EM preparations were examined.

In lightly pigmented skin immediately after radiation there was a vacuolization of epidermal cells and development of small edematous lesions in the papillary dermis. Small dermal areas, presumably occupied by peripheral nerves, were also injured, and the myelin sheath of large nerve fibers also showed damage. After 24 hours, extensive dermal edema developed, and often an infrabasal separation of the epidermis occurred with necrosis of the overlying epithelium. At the periphery of the lesion there was a varying degree of epidermal damage, including cellular vacuolization near the nucleus, and destruction of nuclei. NaBr split preparations showed that the 24-hour lesion was about 1 mm in diameter, both in the epidermis and in the dermis, which corresponded to the beam diameter. In NaBr preparations this lesion was characterized by separation of epidermal cells and the replacement of the collagen bundles in the dermis by a homogenous, eosinophil material.

By contrast, in heavily pigmented skin there was greater injury to the regions of cells which was occupied by melanosomes, and the injury to the dermis was considerably less than in lighter colored skin.

Since there was an elevation in temperature of less than $2^{o}C$, the effects are considered photochemical and not photothermal in nature. A macrosocopically observable hyperpigmentation followed the laser radiation 2 to 3 days post irradiation.

In summary, then, this and the associated contracts were 1.) the first to show that cataracts could be reproducibly and readily produced in vivo at ultraviolet wavelengths; 2.) the first to discuss the reasons why 337 nm. irradiation result in photochemical cataractogenesis, and 3) probably the first to carry out studies on skin using the ultraviolet laser at 325 nm.

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DENTAL STUDIES

Previous dental studies are discussed in Volume One of this report. Further details and studies carried out under this contract on the effect of both ruby (694 nm) and CO_2 (10.6 µ) radiation on dental tissue *in vitro* (1,2) are discussed below.

A. Ruby Laser Irradiation Studies

At 694 nm., extracted human teeth with sound enamel were exposed to laser radiation at exit beam energy levels of 12 and 25 joules. Focused and defocused beams were used. A lens system provided spot size approximately 0.1 and 0.3 mm. in diameter at the target site, resulting in calculated energy densities of 10^4 joules per square centimeter to energy densities in excess of 10^5 joules per square centimeter per pulse at the surface of interaction site. The duration of irradiation was of the order of 1 millisecond per pulse. The ruby rod was cooled by liquid nitrogen during the experiment. The teeth were photographed, plastic embedded,and sectioned at 100 microns.The x-ray diffraction pattern, microhardness, and solubility of irradiated enamel were studied. In an attempt to estimate the upper limit of temperature in the pulp chamber during irradiation, a thermocouple was positioned in the pulp chamber; its exposed part was shielded to protect it from direct irradiation.

Craters were produced, somewhat conical in shape, on irradiation. As expected, the greater the energy, the larger the crater. The area surrounding the crater appeared chalky white with glasslike fusion of the surface enamel. In addition, there appeared to be an alteration in the enamel prisms extending from the crater apex toward the dentino-enamel junction.

The radiation was more intensely absorbed by blackened surfaces then by normal enamel. The authors believed that this property could possibly be used to selectively treat those surfaces which typically show the discoloration usually accompanying the caries process.

The microscopic examination of ground sections in white light, polarized light, and microradiographically showed alteration of enamel structure in similar areas. This zone of altered enamel was seen adjacent to the crater, on the surface, and extending from the apex of the crater to the dentino-enamel junction. It is possible that the observed shattered appearance of the enamel may have been the result of the laser radiation being reflected, scattered, and absorbed by the enamel crystals. Other factors,considered by the authors,include the generation of sonic and ultrasonic vibrations accompanying the interaction of laser radiation with dental tissues, and high pressure and temperature gradients which could also be of significance. The changes observed in birefringence of the enamel were considered indicative of changes in the structure of the enamel as the result of laser irradiation. The precise nature of these changes in enamel structures was not fully understood at the time of the report.

The x-ray diffraction patterns and the microhardness tests, however, did not indicate changes inthe hydroxyapatite nature of the enamel which had been irradiated. If there had been conversion of hydroxyapatite to some other compound, it is possible that changes would have been detected in microhardness and/or in the x-ray diffraction pattern. However, as noted, the determination of microhardness on a convex surface that had been polished to a flat smooth finish is technically difficult. We felt that the findings relative to the microhardness of laser treated and untreated enamel required further investigation.

The high energy densities from focused laser beams produced rapid and intense heating of enamel at the point of impact, sufficient for vaporization, as evidenced by crater production. The authors were aware (and had reported) that attempts to measure temperatures at the point of "impact" could result in large errors because of the direct action of laser radiation on temperature-monitoring devices. Possibly the measured temperature rise in the pulp chamber (80-90°C) was consequently due in part to the direct absorption of laser radiation by the thermocouple placed in the pulp chamber.

The findings of this investigation did not include a study of the bound water or the organic matter normally found in enamel. The loss of these components could not have been detected by the methods of analysis used. However, considering the energy densities occurring at the point of impact in the enamel and the intense heating which resulted, both water and organic matter must have been lost from the enamel. This might have occurred even at some distance from the region of visible crater formation.

In regard to clinically associated hazards, the authors concluded that laser radiation could be damaging to both soft and hard oral tissues. Serious problems associated with the application of laser radiation to the treatment of oral disease would necessarily include adequate protection of the eyes of both the patient and the operating team. Other factors to be considered would include prevention of abscess formation both locally and systemically from scattered particles produced at the site of irradiation.

In summary, the authors concluded that on pulsed (694 nm.) irradiation of teeth *in vitro*, under experimental conditions of this study:

1) No change was found in radiodensity of enamel adjacent to the craters.
2) There was no change in the hydroxyapatite configuration of enamel isolated from the surface enamel surrounding the craters.
3) There was no change in microhardness of laser irradiated enamel.
4) There was alteration in the enamel rod structure in the area of laser impact which was observed with the light microscope. There was also change in birefringence of this enamel as seen in polarized light. This suggested a disruption of the pattern of enamel rods.
5) The coating of the enamel surface with dyes modified the interaction of laser radiation with enamel.

B. CO_2 Irradiation Studies

The purpose of the CO_2 studies (2) was to determine the feasibility of fusing enamel (in extracted human teeth) by using high-power-density continuous-wave (CW) radiation at 10.6 μ as made available by CO_2 lasers. The gross and microscopic findings of the interaction of CW radiation on calcified tissues were compared with short-pulse duration ruby irradiation. Studies were also carried out on the gross and microscopic findings of the effect of CW CO_2 irradiation on components of the tooth. A nominal 20 watt laser was used.

Following irradiation, the enamel in the region of exposure appeared fused, chalky white and opaque. It was hard and brittle and fractured easily.

There was no evidence of closure of the fissures, and the margins of the white, opaque material could be penetrated with an explorer, Both the region of irradiation and the surrounding region of the crown were hot to touch, as expected. Results of attempts to fuse synthetic hydroxyapatite to enamel fissures were generally unsuccessful. Occasionally there appeared to be fusion of the synthetic hydroxyapatite to the enamel.

Other changes were observed deep to the site of irradiation. Some of these were possibly due to conductive heat. The x-ray diffraction pattern of the postirradiation enamel showed lines consistent with the presence of a small amount of alpha calcium orthophosphate as well as those associated with hydroxyapatite.

The studies carried out indicated that there was further need to study the thermal properties of the heterogenous structure of teeth. This was needed to better understand the interaction of CO_2 radiation with these structures and to develop a better understanding of the effect of specific therapeutic modalities which have an associated thermal component, whether deliberate or accidental. Such studies might assist in attempts to improve dental therapy and provide improved methods for prevention of dental caries and for prophylaxis.

DENTISTRY

1. Lobene, R and Fine, S. "Interaction of Laser Radiation with Oral Hard Tissues," Journal of Prosthetic Dentistry, 16:3,589-97,May-June, 1966.

2. Lobene, R., and Raj Bhussry, B., and Fine, S. "The Interaction of Carbon Dioxide Laser Radiation with Enamel and Dentin," Journal of Dental Research, Vol. 47, No. 2, 311-317, March-April, 1968.

MECHANISMS OF INTERACTION OF LASER RADIATION WITH BIOLOGICAL SYSTEMS, BIOPHYSICAL STUDIES

A. Outline of Mechanisms and Associated Studies Reported by 1965

Some of the mechanisms considered to be associated with the interaction of (laser) high peak power radiation with biological systems to 1965, including those determined under this contract, are discussed at length in an accompanying volume of this report. By 1965, Fine et al (1,2) had separated the factors responsible for hazards into the following categories:

A. The laser radiation and its interaction with the biological system.

B. The pumping source, especially flash tubes.

C. The high voltage and currents required for the operation of the laser system

D. The laboratory or field environment in which the system is used.

The mechanisms and hazards associated with the beam interaction (category A) were considered as dependent on the properties of the radiation and the biological system (1,2).

In 1965, Fine et al, (1,2) stated that the parameters of the radiation which should be considered include energy and energy density, power and power density, wavelength and, possibly, coherency and polarization; the characteristics of the biological systems included both the reflectivity, turbidity, electromagnetic and acoustic absorption coefficients, specific heat, thermal conductivity, presence of interfaces and closed cavities, heterogeneity of the tissue and elasticity. Biological characteristics included the biochemical activity, local and general response to injury, the capacity for repair or compensation and the relative sensitivity of the tissue for genetic changes or somatic aberrations (including malignant transformations). Laser radiation of tissue cultures had been shown to result in chromosomal defects which were subsequently transmitted to later generations of cell cultures. (Rounds, personal communication, 1965).

For Category A, the mechanisms which must be considered in the interaction were further subdivided into seven (7) categories. (1965)

1. Degradation of energy with the production of temperatures sufficient to cause thermal changes per se in the biological system.

2. Degradation of energy within a closed filled cavity (such as within the cranium) accompanied by phase transformations resulting in the production and transmission of perssure and possibly shock waves.

3. The production of sonic, ultrasonic, and hypersonic frequencies.

4. Effects due to alteration of wavelength associated with excitation of molecules and scattering of the primary radiation, as well as frequency multiplication.

5. Induction of photochemical reactions.

6. The formation of free radicals and both charged and uncharged light and heavy particles.

7. The possible importance of high electric gradients particularly at high peak densities.

In this and the associated references (1,2) the following statements were made by Fine et al regarding the above seven mechanisms. These were contained in our contract supported 1965 reference, and substantiated the significance of the above processes in laser-biosystem interactions. "Although degradation of energy with production of temperatures sufficient to cause protein denaturation or thermal burns may be the most immediately obvious gross effect, it is not necessarily the most significant in regard to the short or long term effects. Insofar as the skin is concerned, healing may be accompanied by keloid formation. The possibility of malignant transformation must not be neglected-- a higher incidence of squamous cell carcinoma in the region of old healed scars due to burns is well documented (12, 13) Other sequelae of burns include infections, chronic ulceration and scarring with deformities, resulting in limitation of function.

"Insofar as the eye is concerned, burns of the retina and choroidal layers at 6943 Å and at 10,600 Å can occur at low energy and power levels (14). Burns of these tissues by incoherent radiation have been well-documented -- lesions having occurred on looking at the sun during an eclipse (15), or even at a photographic arc lamp. As laser frequencies increase towards the ultra-violet, damage to the cornea can occur at wavelengths shorter than 2950 Å, to which the cornea is relatively opaque. At wavelengths in the infrared, cataracts can be produced, by absorption of the energy by the iris pigment epithelium, with subsequent damage to the underlying lens epithelium and superficial cortical lens fibers by the heat produced. Such cataracts, experimentally produced (16) by focused light on the iris, were limited to the zone of contact between lens and pigment epithelium of the iris (i.e., pupillary zone of iris), and did not occur beneath the peripheral (non-contacting) iris. Delayed posterior subcapsular lens changes occured 60 to 90 days after exposure.

"Whether unfocused radiation (i.e. not directly focused on the iris), from either a coherent or incoherent light source, will produce a cataractous change is not yet clear. True thermal cataracts (i.e. the so-called glassblower's cataract) are rare today (17), and are occasionally confused clinically with a more common flocculent deposit on the lens capsule (pseudoexfoliation of the lens capsule), entirely unrelated to infrared exposure. On the other hand, wavelengths in the longer ultra-violet region (2950 Å to 3050 Å) are not known to give rise to cataract formation. These radiations do produce latent (approximately 8 hours) painful, superficial, corneo-conjunctical burns (kerato-conjunctivitis), which, generally, are easily cleared up with simple treatment. This type of exposure is, perhaps, of greater importance near the continuous operation of gas lasers, where the production of ultra-violet may be very high, and/or the exposure may be long.

"Shorter wavelengths of electromagnetic radiation (i.e. x and gamma radiations) not only produce a superficial burn, but they do give rise to cataractous changes, usually after a considerable (measured in months) latent period. Retinal damage is by far, the most serious of the ocular complications, for such damage is irrepairable. Retinal damage from focused (i.e. inherent dioptrics of the eye) coherent or incoherent light, at minimal or threshold levels, is limited mainly to the pigment epithelium and photoreceptor layers, and is discussed in detail elsewhere (14, 18). Moderate to severe levels of irradiation at the retinal surface cause greater destruction of both retinal and choroidal layers, with frequent hemorrhages into the vitreous body, with their attendant complications of scarring and even retinal detachment.

"Degradation of energy within a closed filled cavity, such as the eye or skull, differs from that occurring on a free surface. Interaction of radiation at a sufficiently high energy density with the media may result in phase transformations to a vapour or gaseous phase. Since the total volume of the cavity is fixed, high pressures will occur at the site of interaction. The pressures will be transmitted with relatively little attenuation to regions distant from the site of interaction, if a quasistatic pressure rise is assumed. This can result in tissue destruction due to direct effects and due to temporary interference or disruption of the vascular supply at some distance from the site of impact. Consequently, severe and fatal injuries can be produced, although the local effect of the initial direct lesions would not be vital to the functioning or survival of the organism.

"Studies directed at elucidation of this mechanism of injury has been reported by Fine and Klein (19,20) and Earle et al. (21). Following unfocussed irradiation at 6943 Å, 1 millisecond pulse duration, at energy levels in excess of 40 joules directed at the forehead of mice, death followed within less than 30 seconds in 10 out of 23 animals (19). Intracranial hemorrhages were present in the meningeal spaces in the ventricles and conducting system, and within the substance at the base of the brain at regions distant from the site of primary interaction, rather than the more generalized distribution of lesions observed on gross and microscopic examination following radiation directed at the closed, intact cavity.

"Further studies were reported under this contract in 1965 by Fine et al utilizing pressure transducers to determine the relevance of the previous observations."With the pressure transducer inserted within the closed cranial cavity, a much higher pressure response was obtained than when the pressure transducer was exposed to the same radiation with only skin and a section of skull bone interposed. (Fig. 2). Consequently, the effects of irradiation of regions with relatively rigid, closed, filled cavities, on both a macroscopic and microscopic level, differed from those involving regions without rigid boundaries.

"Other regions in which injury may be partially dependent on the presence of quasistatic pressure states, on a macroscopic and microscopic level include the thorax and abdomen. Similar considerations apply to radiation directed at the skin overlying certain anatomical sites, such as the joints, the vertebral column, enclosing the spinal cord and nerves, the anterior, later and posterior aspect of the neck (with its subjacent blood vessels, nerves, glands and trachea) the meatus of the ear, the nares, and other areas where major blood vessels, nerve trunks, or important structures are superficially located. (Fig. 7). Damage to these vulnerable areas lying beneath the skin surface may produce severe disability or even death. (22).

"The presence of sonic frequencies associated with the interaction during laser irradiation of in vivo and in vitro systems has been reported.(23). In further studies, both sonic and ultrasonic incoherent pressure vibrations have been detected on non-Q-switched ruby laser irradiation (in the 20 joule range) and on Q-switched irradiation (in the 1 joule range) of heads and chests of mice. On irradiation of the anterior surfaces of the chest and head, these frequencies were observed, when pressure transducers were coupled to the posterior surfaces of mice (Figs. 3, 4).

"Bubble formation and cavitation may be associated with such vibrations, particularly if a disolved gas is present in the media. Bubble formation can result in the production of free radicals (24). In the case of cavitation, collapse of the cavity in a liquid may result in very high pressures and possible shock waves in the fluid adjacent to the cavity wall. Incoherent sonic and ultrasonic pressure waves that originate along the path of the beam will result in significant mechanical energy transport to regions distant from the primary source of laser interaction. However, the intensity decreases with distance.

"These modes of energy transfer may be of significance, insofar as immediate tissue damage and long-term effects are concerned. The importance of such mechanisms will be dependent upon the efficiency of the energy conversion from electromagnetic to mechanical modes and the energy and power density in the beam.

"Chiao, Townes, and Stoicheff (25) have shown that irradiation of certain crystals by a 50 megawatt, 30 nsec., ruby laser results in the production of intense (1 kilowatt), coherent, hypersonic waves (10^{10} c.p.s.) via stimulated Brillouin scattering. Studies carried out by Garmire and Townes (26) have shown that, in a similar way, intense, coherent, hypersonic waves can be generated in liquids such as water. Giuliano (27) has investigated damage in dielectric solids caused by hypersonic waves (13 g.c.), which were created via stimulated phonon processes both within the crystal and within the liquids surrounding the crystal. Gigacycle waves are rapidly attenuated, thus giving rise to possible damage only in regions near the laser beam itself.

"As the beam is scattered, coherent hypersonic waves will be generated along the beam path. These frequencies may result not only in cell death, but in cell alterations, possibly of long term significance, particularly in the skin and ocular tissues.

"The presence of wavelengths other than those of the primary radiation may be of significance, insofar as long term effects are concerned. Irradiation at high energy levels and power densities produced incoherent re-radiation from the excited atoms, some of which occurs at wavelengths shorter than those of the incident wavelength. Should this occur deep to the surface, energy quanta are produced at sites not normally exposed to these wavelengths. This may result in tissue or cellular alterations other than those normally produced. Should these wavelengths lie in the ultra-violet region, absorption by nucleic acids and proteins may occur with subsequent long term effects.

"Frequency multiplication has been produced in crystals such as quartz and KDP on exposure to laser irradiation at 6943 Å (28,29). Frequency doubling has been observed in amino acid crystals (30). It is possible that frequency doubling may also occur in tissues, particularly in regions where crystalline structures such as melanin granules are present. Should this occur in the skin or eye and in hydroxyapatite crystals in bone, coherent quanta of energy will be produced at wavelengths which are normally attenuated by intervening tissue layers. In this way, secondary effects, early or delayed, may be produced which differ from those obtained at low radiation levels.

"Photochemical reactions may also occur at the wavelength of the primary radiation or at wavelengths produced as secondary phenomena. Because of the relatively high intensity of the radiation, reactions may occur, which would not occur at a significant extent at the same wavelengths at lower energy and power levels. Laser radiation has been shown to induce functional and/or structural changes in proteins, including gamma globulins, enzymes, and other macromolecules of biological origin in vitro and in vivo, which may be due in part to photochemical reactions. Such changes have been produced both in the presence or absence of photosensitizing agents acting as energy transfer agents. (31).

"Photosensensitizing agents may be normal endogenous formation (i.e. melanin, hemoglobin and other chromophores), pathologic endogens formations (eg. ochronosis in Alkaptonuria), or may be wholly exogenous (dyes, drugs, vitamins, industrial chemicals). Photobiological reactions may result in (primary) photo-irritation, or in photosensitization by stimulating immunological incompatibilities with protracted allergic manifestations.

"Free radicals have been considered as factors in the biological effects of ionizing radiation, genetic changes and malignant transformation. Electron spin resonance measurements following irradiation of black mouse skin and fibrinolysin preparations indicated with high probability that free radicals are produced in these biological materials on laser irradiation. Irradiated skin of white mice and collagenase gave no signal (32).

The effects of laser radiation on the incidence of malignant changes in intact mammals is currently under investigation.

"The presence of charged particles within the plume ejected from an abdomen of a black mouse has been shown (33). High speed photographs (8,000 - 18,000 frames per second) were taken of the motion of plumes moving through an inhomogenous magnetic field on laser radiation in the thirty joule range focused on the abdomen of black mice. Spiral plume macroscopic motion and confinement of the luminous part of the plume to a relatively small (1 cm^3) volume was observed (Fig. 5) in agreement with the motion of charged particles moving in an inhomogeneous magnetic field (field strengths 0-300 gauss, field gradients of the order of 200 gauss/cm.). Comparison with plumes ejected from the abdomen of black mice on laser irradiation by focused beam in the thirty joule range in the presence of zero applied magnetic field showed rapid dissipation of the plume over a large volume with no spiral trajectories (Fig. 6). Some varities in trajectory was observed during these studies.

"Observations of the decay of plume luminosity also indicate the existence of charged particles within the plume. The plume is self-luminous, since its visibility persists beyond the period of target luminosity and laser pulse duration by at least an order of magnitude. Order of magnitude calculations show the radiational cooling of particles less than 10 microns in diameter to be extremely rapid. Although these particles may be charged, they cannot explain the long persistence of plume luminosity. In general, no other particulate matter of larger diameter was visible with sufficient density in the high speed photographs to be totally responsible for persistence of the visible plume. If the plume contains a high density of ions and electrons, recombination of these constituents is slow enough to explain the persistence of plume luminosity. Other mechanisms, such as bremstrahlung, may contribute to plume luminosity.

"The presence of charged plume particles was substantiated by probe studies. In studies carried out in this laboratory, copper probes initially held at ground potential were positioned near the path of the plume. If the ions and electrons in the plume have unequal directed velocities as the plume expands, a voltage would be induced in the probes. (Direct laser irradiation of the probes produced no detectable probe voltage change. However, during experiments on charged plume particles, precautions were taken to avoid direct irradiation of the probes.) Probe voltage changes were detected when copper probes were placed near the path of the plume ejected from various physical and biological targets on irradiation in the 20 joule range. By positioning two probes a measured distance apart along the approximate plume path, a voltage difference or current flow was detected between the two probes as a function of time. This data verified the presence of charged plume particles and allowed an estimate to be made of the average velocity at which the plume expands. Charged particle velocities as high as 10^4 cm/sec. were detected.

"Other studies were carried out using streak photography. The plume particle velocities measured with this technique were in agreement with the velocities obtained by probe measurements at some distance from the animal. However, streak photography indicated that the observable initial plume particle velocities near the animal surface were at least five times greater than those velocities measured by probes placed at some distance from the irradiated animal surface. An analytic model of the plume will exceed the velocity of directed plume motion by at least an order of magnitude (greater than 10^6 cm/sec.). Because the electron mass is extremely small, these velocities represent very low electron energies.

"There are several possible mechanisms for ion production within the plume. Ionization can occur on ejection. Ionization can occur just after the plume itself is ejected-- heating being due to continued absorption of laser radiation following ejection from the target. Another possible mechanism for creating charged particles is through inelastic collisions between free electrons and uncharged atoms present in the plume. Elucidation of the actual mechanisms for ionization is currently under investigation.

"From the preceeding discussion, the plume ejected from biological material during the following laser irradiation, can best be described as a physical plasma.

"The high electromagnetic power densities that are available from lasers, especially Q-switched lasers, indicates that direct field ionization and secondary ionization due to rapid electron acceleration may occur within biological material. A one megawatt per mm^2 beam has an associated electric field of the order of 10^7 volts/meter. Focusing of the usual laser beam to a 1 mm. spot produces strong transverse and longitudinal fields. Bond rupture and direct field ionization may be expected at field strengths of 10^7 volts/meters. Should these effects occur in a certain region, one can expect increased conductivity within that region. However, separation of the effects produced by a high electric field from those due to other energy transformations is difficult.

"It is impossible to extrapolate with accuracy the relative importance of the mechanisms of energy transformation at low levels of energy and power on irradiation of small animals, like the mouse, to the potential effects in man at higher energy and power levels. The relative biological significance of the mechanisms discussed is dependent on the degree to which they occur at the various energy and power levels, and their relative effectiveness in producing a specific biological response. The data obtained on severe injuries in "scaled down" systems can, however, serve as guidelines for precautionary measures until their possible implications for larger mammals or man have been critically analyzed."

Summary and Comments

In discussing the above, which was reported under this contract by 1965, it is evident that marked effort had been directed in this contract by 1965 to mechanisms of interaction. Although the principal investigator and his associates are sometimes credited with the studies relating to the effects of pulsed laser radiation on the forehead of mice, there seems to be a lack of knowledge on the part of authors of publications in the field that this contract was responsible for attempting to determine and understand the mechanism -- that pressure elevations were observed, that sonic and ultrasonic frequencies were measured, that histology was carried out and this reported by 1965. In carrying out these studies both reflective and blackened pressure transducers were used, and thermocouples were inserted coincident with the pressure transducer to estimate the upper bound of temperature at that site. (The thermocouple was not directly exposed to the radiation). This was done in order to differentiate between transmitted pressure, sonic and ultrasonic waves, and effects due to thermal stressing of the transducer. Our studies indicated the effects were indeed due to pressure changes measured by the transducer, and not due to thermal stressing of the transducer. Attempts were made in these studies to calibrate the transducer; calibration of transients was difficult and therefore waveforms are given on a relative milliwatt scale, rather than on an absolute pressure scale. In our studies, care was taken to establish that the waveforms observed were not due to pulsed operation of the laser system, *per se*.

Indeed, in conjunction with our studies on mouse forehead irradiation, measurements were made on cardiac and respiratory charges, as measured by e.c.g. telemetry and impedence pneumography, in order to attempt to assist in determining the mechanisms of transformation of energy within a closed filled cavity. Although it appeared to us that cessation of respiration preceded cardiac arrest, post irradiation, this was not sufficiently reproducible to permit a definitive statement to be made.

In addition to the above, attempts were made to modify the interaction using dyes, and were reported in the 1964 Boston Laser Conference proceedings. Black and green dyes applied to the forehead of mice appeared to alter (increase) the intracranial injury, on ruby laser irradiation. The hope in some of the dye studies was to modify interaction of tumors.

In conjunction with the above sonic and ultrasonic studies to which we devoted considerable time and effort, attempts were made to characterize a compound (in solution) by its "sound wave pattern." Studies on sonic analysis associated with the interaction during laser irradiation of in vivo and in vitro systems were carried out. These investigations were performed by positioning of a microphone at a fixed distance from a site of interaction. Differences in frequency and amplitude were noted when different light absorbing substances were present. Although differences could be obtained, and were determined to arise from the solution (or irradiation site) by shifting the microphone position, the sound waveforms obtained did not appear to be sufficiently specific to be able to use the technique (at that time and with the sensitivity available) for differential characterization of compounds by the above signature studies. It is possible that further investigation of this area is warranted.

In regard to the fourth mechanism, frequency multiplication, studies were carried out under this contract on in vivo frequency doubling with a Q switched ruby laser, and are reported in an accompanying volume of this report.

The fifth mechanism, induction of photochemical reactions has, of course, been observed by many investigators. Our studies on transformations in plasmin (or fibrinolysin) lipase, and blood group substances (1965) may in part be dependent on photochemical reactions. These are discussed in an accompanying volume. Similarly, the cataracts observed on the HeCd laser irradiation were considered in whole or part to be photochemical rather than photothermal per se.

Some considerable time and effort was directed in this contract by 1965-66 towards studies on bubble formation and cavitation on irradiation. Bubbles could be readily produced in solution, and these appeared, in some cases, to be cavitation bubbles. Whether any of the tissue distension observed on a microscopic scale in our studies is related to "bubble formation and cavitation," is uncertain. However, the occurence of these "formations" could be significant in regard to mechanisms for injury.

The potential for malignant transformation studies mentioned above are discussed in an accompanying section of this report.

Studies on free radicals-mechanism 6 - were continued and are discussed in accompanying sections. However, in no case were studies carried out on free radical formation occuring at the time of irradiation (i.e. during irradiation). Studies of this type may still be warranted; certainly the qualitative and quantitative aspects of short-lived free radical formation during irradiation cannot be determined from the studies reported in this contract. Obviously, much work has been carried out in the physical sciences on the latter part of mechanism 6.

In regard to mechanism 7, attempts were made to possibly separate direct irradiation effects from high field gradient effects by orienting the animal surface parallel to the beam; using a high peak power Q-switched system (in the mid 1960's), and focusing it just anterior to the animal surface.

No evident alterations were observed. Whether a high electric field gradient was present at the site of focusing and therefore at the surface of the target (which possesses some conductive characteristics) would remain to be determined.

The above studies were reported in the literature, at widely attended conferences, and in personal discussions with groups carrying on bio-laser work. It is expected that this and the associated contracts will be given credit and priority for our work and findings discussed above, and for our attempts to elucidate mechanisms underlying laser effects, in vitro and in vivo, as reported by us by 1965.

The remainder of references 1 and 2 are concerned with hazards relating to B, C, and D, and to mechanisms for control of laser hazards and management of accidents, to laboratory design, protection standards and dosimetry, and will not be considered further here.

B. Focal Hepatic Injury and Repair

A second set of studies carried out in an attempt to better understand the interaction of pulsed ruby laser radiation with biological tissue is discussed by Fine et al in "Focal Hepatic Injury and Repair Produced by Laser Radiation" published in the American Journal of Pathology (3). The purposes of these studies were (1) to obtain further information concerning the interaction, and to correlate physical and biological finding in regard to injury, (2) to study focal hepatic injury and repair, and (3) to determine whether the laser could be used for production of sterile focal hepatic lesions in order to study focal hepatic injury and repair. In this study radiation from a 100 joule (nominal) ruby unit was directed at the liver through intact abdominal muscle after reflection of the skin. In vivo temperature measurements were made in the liver of rats by using a solenoid actuated thermocouple injection system with a 20 msec delay time, to prevent direct thermocouple irradiation. Methylene blue was applied to the thermocouple needle in order to follow the tract for the determination of the location at which temperature measurements was made; a cone calorimeter was used for energy measurements; thermofax paper and photography were used for studying the beam energy distribution; the animals were followed post-irradiation; and biopsies taken periodically for light microscopy and histochemical enzyme studies.

Grossly, the well-demarcated lesions following irradiation were initially pink-to-grey with a red margin. They became white, smaller and regressed by 32 days. Microscopic changes were noted and discussed; among these the occurence of giant cells and calcium deposition in the lesions and alteration in dehyd ~genase activity were of interest. The measured temperature post irradiation was extrapolated back to the time of irradiation. It was found that oxidation enzyme activity was absent from that part of the lesion close to the surface, where the temperature was over 100°C. At 3mm. from the surface, the temperature achieved was 48-64° C; in this region, which appeared histologically normal, the enzyme activity was not decreased. In the zone intermediate between the above two, the enzymes activities were decreased but not absent. Consequently, there appeared to be a relationship between the temperature elevation (and temperature-time-history) and the biological alterations observed. In addition, microscopic observations of tissue disruption with occurrence of intracellular spaces histologically in the superficial part of the lesion was associated with calculated temperature elevations in excess of 100°C. These observations lent credence to the concept that temperature elevations considerably in excess of 100°C on laser irradiation may result in steam formation on a microscopic basis in the irradiated tissue, with associated pressure, distension and compression of surrounding tissue. At temperatures above 300°C, carbonization and ablation of tissue is expected; such temperatures were not measured or such observations made. Consequently, our understanding of the mechanism of interaction was extended by noting that at lower temperature levels, enzyme and tissue changes occur,and are related to temperature elevation (temperature-time-history) and that at temperature levels above 100°C, pressure-related changes may occur as noted in the "mouse head shot" studies, in agreement with our previous interpretations.

The development of the lesions into a fibrocalcific module containing multinucleated giant cells which occurred was in agreement with the development of liver lesions produced by heat or cold. However, the dissociation of liver cells from their trabecular arrangement in the superficial layers, the rupture of blood vessels, and the distension of tissue spaces, and sharp margination differed from those produced by the latter forms of injury; and was ascribed to the effect of short pulse duration irradiation of sufficient energy density to produce perhaps other than solely thermal effects per se. The production and evolution of the lesion was of interest, and showed that the pulsed(ruby) laser, even as then available, could be used as a tool for the production of sterile, focal lesions in the liver in order to study the problem of focal hepatic injury and repair.

C. Analysis of Target Geometry - Effect of Physical Characteristics of Biological Target Site.

Another biophysical area to which attention was directed was the importance of the physical characteristics of the biological target site in modifying the interaction of the beam with the biological system. These physical properties included the geometry of the target, whether time varying or time invariant, its refractive index (or indices in the case of a heterogenous system) and its thermal properties. As discussed in part 1 of this report, one of the studies which brought this problem to the fore was the observation of transient outward hemispherical distension of the abdominal skin of the mouse, on ruby laser irradiation (Fig. 6.)

Based on this and associated observations, together with questions which arose concerning the cause of the distension, the characteristics of the elevation which occured and the basis for these pressures (or pressure differences), and the effect of the time varying distension on energy disposition distribution, an analysis was carried out by our group and reported under this contract (4); this was done in an attempt to develop an understanding of the significance of some of the factors, and to attempt to explain our observations.

Attention was directed to the general parameters affecting the variation of energy deposition within the biological systems, when unfocused and focused radiation is directed at such a system (as for example, the abdomen of a mouse). The "focal depth" and "focus" within a biological heterogenous medium such as the skin was considered to be a function of target geometry, refractive index, and degree of collimation of the incident beam. Factors considered of importance in homogenous, multi-layered, non-time-varying refractive material included the thickness and the refractive indices of the layers. In many cases, such as that of epidermis, the layers are too thin to produce appreciable refractive displacement.

For reflective focusing, the fractional change in refractive index between adjacent layers is most significant. The high water content of biological material might result in similar refractive indices of adjacent layers on a macroscopic basis. Consequently the most important macroscopic refractive and reflective focusing effects were considered in general to occur either at the exterior surfaces bounded by air, or at those interior surfaces that enclose vacuoles, cavities, or steam pockets. To a first approximation, the remaining tissue might be characterized by a single, macroscopic refractive index for monochromatic light. However, since biological tissue is inhomogenous on a cellular scale, microscopic variations in refractive index may introduce local heating effects and radiation scatter. (Studies attempting to determine methods for measuring the heterogeneity of the refractive index in tissue were carried out under this contract). Effects due to scattering were neglected in this report (4), but considered in progress reports.

More complicated target geometries, such as observed in studies on time-varying skin distension (Fig. 6) during laser irradiation could occur. This distention rate, the high curvature, the thickness of skin layers, and the water content of the encapsulated steam affect refractive focusing. It was considered that the tissue posterior to the steam pocket could develop time-varying negative curvature, which could defocus the transmitted radiation. However, this curvature of the tissue surrounding the steam pocket could focus the reflected radiation within the steam pocket.

Special cases were considered. For the case of the converging cone of light, on irradiating a flat, refractive diathermanous surface at normal incidence, the focal point of the beam in tissue would differ from that observed in air; with a refractive index greater than air, the focal point will be at a greater depth (using simple ray tracing techniques, and neglecting absorbance and scattering). If a spherical surface is irradiated, the energy distribution will usually differ from that expected in air, due to refractive differences. For example, a parallel beam will be focused, (for usual refractive indices); a converging beam will have its location of focus altered depending on the radius of curvature of the system and the convergence and convergence point of the beam (in that condition where the beam is orthogonal to the surface, its degree of convergence will not be affected).

In this paper, (4) the effects of the differing beam and target geometries and target characteristics on the initial energy distribution within the target were examined. For example, consider a simple case -- a beam focused on a target with constant absorptive and refractive properties. Neglect scattering and other factors. There are two opposing factors which come into play. As the beam crossection decreases towards the expected region of focus within the target, the power and energy density in the region of the beam (e.g., along its axis) increases. However, the beam attenuation due to energy deposition in the tissue would tend to result in decreased beam intensity. Using a dimensionless axial versus depth analysis, it was shown that under some conditions the initial temperature would continue to increase with depth, whereas for other beam parameters, a minimum energy deposition and associated temperature could be reached either at the surface or at some distance between the surface and the focal depth. Indeed, on a three dimensional basis, the temperature distribution might be complex, as for example, of a saddle point type. Consideration was given in the report to the use of the analysis used for determining thermal parameters of the tissue and to scattering.

Examples were given in this report of biological target geometries to which this analysis would be applicable. These included a large number of possible target geometries and the resulting consequent initial temperature distributions within experimental media. For example, when radiation is directed at the forehead of mice,the curvature, refractive index, and reflectivity of the media may result in focusing an incident, collimated beam. When the skull has appreciable thickness, the relative refractive index of bone must be considered. An example of an approximately hemispherical, refracting target was the sub-cutaneous or intradermal tumor module. It was stated that in order to evaluate the radiation density as a parameter which may result in regression of a specific volume of tumor tissue, focusing effects due to curvature and refractive index of the nodule and surrounding tissues must be considered (as well as, of course, scattering).

Consequently, as indicated above, the analysis had several purposes. One of these was to show how some the the physical factors which should be considered in an attempt to determine the energy distribution in a biological system would affect such energy distribution, and secondly to provide examples where such situations occurred in practice. It should be noted that although the examples given were on a macroscopic level, irradiation on a microscopic level including single cell or intracellular irradiation for selective injury production or probing requires similar analysis.

D. Reversible Depigmentation: Threshold, Mechanism, Significance

The next area I wish to discuss is reversible depigmentation in rodent skin and associated threshold values for this process. This bio-effect and some of our thoughts relating to this bioeffect are discussed in a number of our publications including reference 5. By depigmentation, we mean the regrowth of white hair at the site of (pulsed ruby laser) irradiation of normally black haired mice. (The pigment in these mice is in the hair). By reversible, we mean that the hair in subsequent cycles post irradiation was again black (contained melanin).

The reasons for discussing these studies under "mechanisms" are several. First, the observation of reversible depigmentation was in itself interesting. Although depigmentation had been shown to occur following X-irradiation, such depigmentation was permanent and associated with the loss of melanocytes; the depigmentation in the case of laser irradiation evidently persisted only for one hair cycle generation. Secondly, the reversible depigmentation could be produced at energy density levels of about 0.5 joules/cm^2 to 40 joules cm^2, on normal mode pulsed ruby laser radiation (in contrast to Q-switched irradiation where the threshold for depigmentation appeared to be lower). This threshold for such reversible depigmentation "injury" was therefore about 1 joule/cm^2 on normal mode ruby irradiation. This was similiar to the estimated energy density required at the retina (at that time) for irreversible retinal injury on normal ruby laser irradiation (0.7 joules/cm^2 at the retina). We produced depigmentation on Q-switched irradiation; in this Q-switched mode the energy density for depigmentation appeared to be less than for normal mode irradiation. This was in agreement with studies on irreversible retinal injury (0.07 joules/cm^2 Q-switched vs 0.7 joules/cm^2 normal mode, at the retina) on pulsed laser irradiation.

We therefore had two factors in these studies which concerned us; fist, the cause of depigmentation and its reversibility, and second, the decrease in energy density required with decrease in pulsed laser irradiation for threshold injury. That this decrease in energy density with increase in power density was not considered to be necessarily true only for threshold injury, but that the type (and mechanism) of injury might indeed change as the power density was increased (shortened pulse duration) was evident from the first studies to be reported on comparison of normal mode and Q-switched ruby irradiation. In conjunction with Dr. Maiman and his group at Korad, we carried out studies on the effects of Q-switched ruby irradiation on mice; comparative normal mode studies were carried out. These, the first comparative biological studies on the effects of power and power density (at short pulse duration, Q-switched versus normal mode) to be carried out and published (Fine, Maiman, Klein, and Scott, Life Sciences, March 1964), showed that damage to the skin and underlying structures at high peak power (Q-switched) occured at a lower energy density level than on normal mode irradiation. Furthermore, the effects appeared to be qualitatively different. (I remember, fourteen years later that the Q-switched lesions at Korad appeared more severe, and the "sound" of the interaction of the beam with the tissues was different, it was more "acute.")

There were two factors which we considered significant and common in injury to the skin (or reversible depigmentation) and to the retina. First, they are both melanin granule containing tissues. Secondly, for both tissues, the threshold for injury decreased as the pulse duration was shortened. These problems concerned us since 1964. Our attention was consequently directed towards the melanin granule, its roles as an energy absorbing and transfer agent, and as an entity which might assist in answering the problem relating to the decrease in energy density with pulse duration required for threshold injury. To quote from the NEREM 1965 paper, "The factors producing inhibition of melanogenesis, limited to the duration one cycle had not been previously described and are not understood. If melanogenesis is maximal during the 7th-9th day, at least some melanin pigment (or precursors) would be located in close proximity to the cellular constituents (e.g. melanosomes) responsible for its synthesis. The energy is probably absorbed by the melanin. Energy, absorbed by the pigment; may then be transferred to the enzymes and other components involved in melanogenesis, resulting in their deactivation. This effect may be due to temperature rise within the pigment, with transfer of energy to the adjacent region. Other mechanisms of energy transfer including a consideration of the rate at which the energy is delivered are under investigation. Apparently at the period of maximum sensitivity all or most of the melanogenetic system is producing melanin and therefore more sensitive to irradiation. It appears that melanogenetic elements are not formed for the remainder of the cycle. It is probably that the irradiation itself does not initiate a new cycle." (Above underlining not in original paper).

In summary, then, " Alterations have been produced in the skin of mice at energy density levels of 0.5 joules/cm^2. This is of the same order of magnitude as the threshold levels for irreversible damage to the rabbit retina. Further study at lower energy density, as well as at other wavelengths are being carried out. The studies may assist in obtaining meaningful information applicable to threshold determination and concerning the melanogenetic system." As evident from the above, we considered the melanin granule important in understanding the mechanism for threshold injury.

E. Melanin Granule Models

Initial calculations and further thought were given to the above problem. Based on the above, attention of our group was directed towards melanin and the melanin granule. This led to a single mechanism melanin granule model, which was first reported in considerable detail by Fine at the April 1966 Quantum Electronics Conference in Phoenix. It does not appear as such in the abstract (6), since the abstract was submitted previous to full development of the model. Confirmation of presentation of this model at the QEC Conference is found in the 1965-66 Medical R and D Contract Progress Report and in the progress report by S. Fine on Research Grant R01-RH-00361, Health, Education and Welfare (1965-1967). In the 1965-66 HEW Progress Report, an outline of the model is given in considerable detail. This report was, of course, available to the public and to reviewers.

The June 1965 - September 1966 H.E.W. Progress Report contains the following information concerning this "Single Mechanism" model, reported on at the Q.E.C. in 1965.

"Analytic studies have been carried out in conjunction with the experimental studies to develop a model which would account for the marked decrease in energy threshold for injury with decrease in pulse duration. Attempts have been made by several authors to explain laser induced changes in pigmented tissue using models for macroscopic heating. Ham _et al_ have employed macroscopic models for reinal tissue injury where the pulse duration is greater than 200 microseconds. However, the macroscopic thermal models have not been successful in describing the lowering of the threshold by an order of magnitude for Q-switched pulses, where the pulse duration is 100 nanoseconds or less. It has been suggested that tissue injury at these short pulse durations may be due to 'other than thermal effects.' Analytic studies have been carried out in the author's laboratory to show that the order of magnitude difference between Q-switched and non-Q-switched laser injury levels may be the result of heating an array of melanin granules, which are possible prime target sites for energy absorption of the laser radiation both in the skin and in the eye."

In the discussion below, the optical problems of granule heating have been neglected in favor of obtaining information regarding the "thermal time constants" associated with heat flow from an array of microscopic spheres.

"The melanin granules are assumed to be spherical in an infinite medium. The granules are assumed to be opaque and non-scattering with an absorption cross-section, $\sigma = \pi a^2$, where a is the granule radius. The medium in which the granule is embedded is assumed to be essentially optically transparent to the incident radiation. Despite the assumption of opacity, heat is assumed to be uniformly generated within the granule model. In the initial model, the granule spacing is assumed to be sufficiently great so that heat flow from one granule is not perturbed by its nearest neighbors. The temperature rise at the boundary of the granule can then be obtained as a function of time.

"The relationship between the incident energy density and pulse duration necessary to raise the temperature at the boundary of the granule to a predetermined critical temperature, such as the temperature necessary to cause denaturation, has been obtained. In general, because of heat flow from the granule, it is evident that the shorter the pulse duration the less the total energy required to reach a predetermined critical temperature. This would relate to the experimentally observed decrease in total energy required to produce a threshold lesion as the pulse duration is decreased from milliseconds to nanoseconds.

"Because of the size and the properties of the granule and the thermal properties of the adjacent tissue, considerable heat flow occurs during the pulse. This radial heat flow is not significant for pulse durations shorter than 100 nanoseconds, if uniform energy absorption is considered throughout a thin, flat, partially opaque slab as in models used by other authors. The assumption of discrete absorption sites of the size of the melanin granule permits a thermal model to be obtained which predicts a decrease in the total energy required for threshold injury as pulse duration is decreased."

This single mechanism model is further discussed in reference 7 (Hansen and Fine, Applied Optics, January, 1968). Figure 3 in this reference shows that as the pulse duration is shortened, the energy required to reach a specific peri-granular temperature decreases, for a specific granule size. In addition, it shows that as the granule size (radius) increases, a greater incident energy density is required to reach the perigranular temperature for injury.

The above model, presented at a major international meeting in 1966, and documented in progress reports of that year, was the first of our models to successfully explain the decrease in (laser) energy density required for threshold injury to the retina (and for reversible depigmentation) with decrease in pulse duration, (with increase in laser power density).

A second melanin granule model developed under this contract to explain the decrease in energy density required for threshold injury with decrease in pulse duration was published in Applied Optics in January, 1968 by Hansen and Fine (7). As in the previous model, the melanin granules in the retinal pigment epithelium are considered as the primary absorption site for injury. The second model differs from the single mechanism model in the following manner. The single mechanism model assumes that a specific temperature must be reached in the perigranular region to produce injury, and the injury is thermal. The two-mechanism model assumes that at long pulse durations (4 microseconds to one-tenth second in the case considered), injury is essentially thermal; the temperature for threshold retinal injury being obtained from extrapolation of a drawn curve based on data from W.T. Ham, Jr. The data in this curve show that as the pulse duration is shortened, the temperature required for injury increases. Our extrapolation of this curve (data) results in a temperature of 100°C being required for injury at a pulse duration of 4 microseconds. The second part of this two-mechanism model requires that for pulse duration shorter than 4 microseconds, injury is related to steam production, and occurs at 100°C. There are consequently, two parts to this model: 1.) at long pulse durations, injury is essentially thermal and 2.) at short pulse duration, injury occurs at 100°C with steam production,

Both the single and two mechanism models agreed with the experimental observations at that time which indicated that the energy density for threshold injury decreases as the pulse duration is shortened. The two-mechanism model may have had the following advantages. First, for short pulse durations, disruptive effects including melanin granule scattering were reported to occur on threshold injury; the requirement of steam formation and possibly pressure related effects might therefore be in agreement with this biological observation. Secondly, as the pulse duration is increased (beyond 4 microseconds), the latent heat of vaporization is no longer required for production of injury -- the thermal component of the injury process becomes active. Consequently, a minimum energy density could be required for threshold injury -- in our example at 4 microseconds pulse duration. As the pulse duration is lengthened beyond this limit, the energy density required for threshold injury is of course again increased. Of course, at very long pulse durations, the model for heat generation within an array of melanin granules in tissue reduces to the model for uniform heat generation within the irradiated tissue density. It should be obvious that this two-mechanism model predicts a minimal energy for threshold using a specific pulse duration; or in a specific pulse duration range which, of course, might have been other than at 4 microseconds. This 4 microsecond specific pulse duration for minimal threshold energy density occured because of the parameters used, and because a straignt line extrapolation of data from Ham, et al. was used with data spread neglected, etc. Whether indeed a minimum energy density for threshold injury with variation in pulse duration was found experimentally was left to further work by other investigators.

In the publication (7), attention was directed to the fact that other than the two mechanisms discussed may be responsible for injury and that the temperature - time - history is important. A number of mechanisms which might contribute to the occurrence (and observation) of threshold injury, however threshold injury is defined, are discussed in the preceding parts of this section of the report and in accompanying volumes.

We think our models were significant in focusing attention on specific mechanisms, target sites and methods of analysis. We considered these aspects to be applicable to microirradiation including partial cell irradiation, as well as to the types of studies outlined (7).

In summary, then, a single and two mechanism model was developed to explain the decrease in energy density required for threshold injury with decrease in pulse duration. The single mechanism model was presented in 1966, based on earlier reversible depigmentation work in 1965. The progress reports to both the Medical Research and Development Command (1965-66) and to the National Institutes of Health (1965-66) contain information regarding our single mechanism model. Consequently, information regarding our model development was available to others in the field preceding by far our publication date of January, 1968.

F. Models Relating to Mechanisms of Interaction Associated with Continuous Laser Radiation in the Visible (at 632.8 nm.)

At the time (1967) at which we reported on a model for estimating the threshold for injury on continuous He-Ne irradiation at 632.8 nm (8, 9) (and for other wavelengths in the visible), a number of studies had been carried out (34,35,36).

We as well as others had considered that for the normal eye the primary ocular target at 6328A was the chorioretinal region. This was based on the high percent transmission of the ocular media anterior to the retina at this wavelength. Although the focusing properties of the eye did not in itself determine the primary ocular target since visible radiation can be focused in the vitreous, the absorption coefficient of the vitreous is less than that of the chorio-retinal region. The primary target site and consequently the gross injury threshold region at a specific input power was therefore considered to occur in the retinal choroidal region.

Experimental studies at 6328A had been reported by Kohtiao, et al. (34,35). They produced lesions in retinas of chinchilla rabbits on 2.5 second exposure to a 25 milliwatt multimode He-Ne laser. The retinal image size was 0.25 mm; the retinal power density was therefore of the order of 40 watts/cm^2, assuming most of the incident radiation reached the retina. A temperature rise of 7.8°C was recorded in the lesion, with a rapid decrement in temperature elevation laterally. Increasing the exposure time to as long as 30 minutes did not apparently increase the intensity of the lesion; data on exposure shorter than 2.5 seconds at this power level was not given. No lesions were observed following irradiation at a power level of 2 milliwatts for 1 hour and at a power level of 5 milliwatts. In other experimental studies, a retinal temperature elevation of 12^{o}C to 20^{o}C was produced for a 1.2mm diameter threshold lesion on exposure to intense visible light (36).

The above experimental data did not include injury thresholds for the very small retinal image diameters which could be encountered with highly collimated lasers, and which would represent a "worse case" situation. Direct temperature measurements for very small retinal image diameters were considered difficult. For example, if a thermocouple were used, its thermal characteristics, physical size and inaccuracy in placement could result in considerable error in temperature measurement. Therefore, to assess the hazards from highly collimated lasers, we felt that one might calculate the maximum retinal temperature elevation utilizing suitable models for heat conduction in retinal tissue.

A calculation of the retinal temperature elevation under "worse case" viewing conditions was carried out (8,9), and the results compared with experimental data. The "worst case" viewing conditions included the following factors: minimal diameter retinal image diameter (10 microns (37)); uniform distribution of laser power density across the retinal image; steady-state heat flow conditions reached. In order to develop an adequate threshold injury model, both the absorption of radiation in the various ocular media and the allowable temperature elevation at the site of injury had to be known. Measurements performed by several groups indicated that at 6328A, light suffers only a small reflection loss at the anterior surface of the cornea, and then passes virtually unattenuated through the cornea, aqueous, lens, and vitreous. However, based on <u>in vitro</u> data, from 15% to 40% of the incident radiation at this wavelength was considered to be absorbed in a thin layer of retinal pigment epithelium, the region at which maximal absorption of energy per unit volume occurs at this wavelength(38,39). Sufficient energy absorption in the pigment epithelium can elevate the temperature in this region to a level where an ophthalmoscopically visible retinal lesion is produced. In order to quantitatively assess laser hazards,a safe human retinal temperature elevation might be assumed. Based on the experimental temperature values given above and an estimate of the "body temperature elevation encountered in fever" we assumed that a human retinal temperature elevation of 2^{o}C could be considered safe over a protracted time.

We then developed a model based on the above constraints. The model was as follows: A cylindrical volume of retinal pigment epithelium in which laser radiation is absorbed and in which heat is uniformly generated is considered as the target site, the site of heat absorption. The maximum transient temperature elevation, U_{τ} is considered to occur at the center of the irradiated volume of pigment epithelium, at the end of the radiant pulse of time duration τ. This temperature rise can be obtained from equations in Carslaw and Jaeger (40), and are given in our publication.

The maximum steady-state temperature rise is obtained by solving the steady state heat flow equation, and is given in the publications. Results are discussed below.

Based on the fraction of retinal power absorbed by the pigment epithelium, its estimated thickness from experimental data, an estimated volumetric heat capacity of the pigment epithelium to that of water, a retinal image diameter of 10 microns, and a diffusivity equal to that of water, it was calculated that about 50 microwatts into the eye would result in a temperature rise of 2^{o}C (for 100% absorption in the pigment epithelium and neglecting blood flow). For 40% absorption, the total power entering the eye at 632.8 nm was calculated to be about 100 microwatts. We therefore felt that on a worst case basis, allowing a temperature rise of about 2°C, the maximum safe power at 632.8 nm (and for other visible lasers) should be about 100 microwatts entering the eye for continuous irradiation. This value of 100 microwatts should be compared with protection standards set for intrabeam viewing by the eye at 632.8 nm and for argon lasers.

It should be noted in regard to the above that on direct viewing of the sun 10 mw enters the pupil; this is distinctly hazardous. A 0.1 microwatt He-Ne laser beam, when viewed directly, is extremely bright. Consequently, the above two factors, as well as the studies done on thresholds including the excellent published work of Paul Lappin, set some guideposts on how well the model fits the data. Furthermore, temperature rise obtained with the model was compared with that observed in the above cited references. One could obtain reasonably good agreement by assuming a fractional absorption (less than 100%) in the pigment epithelium.

The above model is a thermal type model; the effects relating to threshold injury (or "non-injury" at 2°C rise) are considered as due to thermal processes. If photochemical mechanisms for injury are shown to be significant in man in the visible, they may of course result in "safe values" lower than the above incident transpupillary irradiation values.

An error in the equation, noted by Professor Frank Barnes and Chia-lun Hu, was communicated to me. The addendum was published as a letter to the editor in Applied Optics. Barnes and Hu were not given credit for this in the letter. I wish to take this opportunity to thank them and to correct this oversight.

G. Extension of Our Studies on Application of Thermal Models to Retinal Threshold Injury for Other than 10 Micron Target Diameters

The work discussed in the above section related to development of "worst case" thermal models for establishing "safe" values for visible, continuous radiation such as produced with a He-Ne laser. The above thermal models were based on absorbance of a specific fraction of the radiation within a 10 micron cylinder of the pigment epithelium, and on the assumption that a temperature rise of $2^{o}C$ can be sustained for a considerable period of time.

Further model studies were reported by Hansen and Fine at the Laser Industry Association meeting in October, 1968, and are published in the Proceedings of the meeting (10). (Since then, the LIA initials have been changed and now refer to the Laser Institute of America. The principal investigator and several other members of the board at that time were responsible for considering such a change.)

Basically, the conclusions reached in this proceedings report were as follows:

1.) For pulse durations in excess of about 10^{-5} seconds, the melanin granule boundary temperature will be influenced by its nearest neighbors. This influence tends to smooth out the temperature distribution within the pigment epithelium. For pulse durations exceeding the above by an order of magnitude, the granule models, discussed in E above, tend towards the cylindrical model considered in F above. Here light is uniformly absorbed, and heat uniformly generated within the cylindrical target volume within the pigment epithelium.

2.) For the 10 micron retinal image diameter, 90% of steady state conditions are estimated to be reached in about 3 milliseconds (90% steady state temperature). This is considerably less than the 100 milliseconds time estimated to be required for a reflex blink reaction.

3.) As the diameter of the beam at the pigment epithelium target site is increased, the time required to reach 90% steady state temperature is increased. For example, for a 250 micron diameter retinal irradiance, the time is 600 milliseconds, for an 800 micron diameter beam at the target site retinal irradiance, the 90% steady state temperature time is 6 seconds. Consequently, avoidance reactions become significant for larger size beam diameters at the pigment epithelium, in that they prevent the irradiated region from reaching steady state temperature. The larger the beam diameter, the more significant is this avoidance reaction time effect (e.g. blink reflex), particularly if the injury is dependent on peak temperature elevation. (As discussed below, we think the temperature-time-history, is, of course, of importance as are other factors.)

4.) For the cylindrical model discussed, the peak temperature will always occur at the center of the heated volume of the pigment epithelium. The larger the diameter the slower the rate of rise of this central temperature to the peak temperature, as discussed above. After the heating pulse ceases and cooling begins (for a fixed pulse duration irradiation) as the image diameter increases, the central temperature falls more slowly and consequently, the central temperature may be higher, on the <u>average</u>, for a larger image diameter, than for a smaller one. It may therefore exceed a "critical" temperature for a larger period of time. Although the larger diameter irradiance region may therefore "catch up temperature wise," to that of the smaller diameter irradiance, there is no way of being certain that it will exceed the spatial-temperature threshold for injury, without much more information concerning the processes necessary for "injury." This analysis also illustrates some of the difficulties in experimentally determining injury thresholds for 10 micron irradiation by extrapolation from 800 micron diameter irradiation studies.

5.) If the threshold for injury were a function only of temperature elevation, then for the model considered, the region of injury would be less than the region irradiated.

6.) Light scattering, particularly for small image sizes (10 microns) should increase the irradiation threshold; this was in agreement with the studies of King and Geeraets, in which an increase in retinal energy density was required to produce injury, with decrease in image size.

7.) For a given image size, the transpupillary power for threshold injury increases with increase in threshold injury temperature. As shown in a graph in the publication, for a 10 micron spot size, the following are approximate values, based on our heat flow model. For a 2^{o}C elevation about 100 microwatts, for a 10^{o}C about 1 milliwatt, for a 20^{o}C about 2 milliwatts, for 30^{o}C about 3 milliwatts, for a 50^{o}C rise about 5 milliwatts. The other information in the graph shows that for a fixed peak temperature rise, the incident power must be increased with decrease in pulse duration, as expected. Also, as expected, the delivered energy density required for threshold injury increases with increase in pulse duration. This is based on our thermal model assumptions.

The above represents some of the conclusions in the LIA publication. Since this extension of the worst case analyses paper has not been published elsewhere, it is included as an addendum.

H. Biophysical Studies at 10.6 Microns with the CO_2 Laser

As well as carrying out studies and developing models for pulsed systems and for continuously operating systems in the visible such as with the He Ne laser at 632.8 nm., attention was directed at the mechanism of interaction of 10.6 micron irradiation, as made available with the CO_2 laser, with biological systems, in particular, with the eye and skin.

We were the first to carry out and report on the biological effects of CO_2 laser radiation (41, 11). We then extended and consolidated these studies. Considerable effort was directed towards development of simple inexpensive CO_2 lasers by our group, which we subsequently used for studies over several years. George R. Peacock was the individual most responsible for development of the units.

An early version of these units was reported in the American Journal of Physics (42). A modified unit was built in our laboratory; complete details including a parts list and alignment procedures were described and reported to the Surgeon General's Office responsible for this contract. Details were made available to other groups carrying out biological studies. Information regarding the details of our units are discussed in the section on instrumentation and development. Many of our CO_2 studies are outlined in an accompanying volume (Mackeen and Fine) of this report; some are discussed in the historical review, where some of our CO_2 work is reviewed, as well as that of others. Some is discussed in greater detail in the body of the report by Mackeen and Fine.

The purpose of this section is to outline one group of biophysical studies oriented towards mechanisms (11), reported in 1966. In this publication, the following five results were reported: 1) Measurement of the absorption coefficient of rat skin and diaphragm tissues between 9 and 11 microns showed that the absorption coefficient of the tissue at 10.6 microns was high -- about 130 to 270 cm^{-1} (Hardy's data is shown in conjunction with our data). 2) Thresholds for injury were obtained for exposure of abdomens of shaved and depilated C57 mice to 10 micron irradiation, for power densities between 2 and 5 watts cm^2 and exposure times between 20 milliseconds and 5 seconds. (A visible criterion for injury threshold was used). The experimental data were shown to be well fitted by a range of straight line log-log plots (the logarithm of the incident power density and exposure time for threshold injury was, of course, an inverse relationship -- the greater the power density, the shorter the exposure time). 3) The experimental threshold data (power density vs. exposure time) was well fitted by a one-dimensional heat flow equation for constant temperature rise for threshold injury (where the absorption coefficient at 10.6 microns is considered infinite), i.e., the tissue is considered opaque. The temperature rise predicted by this equation at the center of the lesion (to match the data) was $22^{o} \pm 15\%$, based on this simple heat flow equation

$$[\{\text{power density}\}\ (\text{exposure time})^{\frac{1}{2}}\ \text{equals a constant}]$$

given in the paper, and in which the tissue was considered opaque. The actual temperature rise measured using a thermocouple injector was $22^{o}C \pm 20\%$ in good agreement with calculated values. Our experimental threshold data were also well fitted by theoretical isotherm curves for $22^{o}C$ rise, using a diathermanous (non-opaque) medium with alphas of 150 and 270 cm^{-1}.

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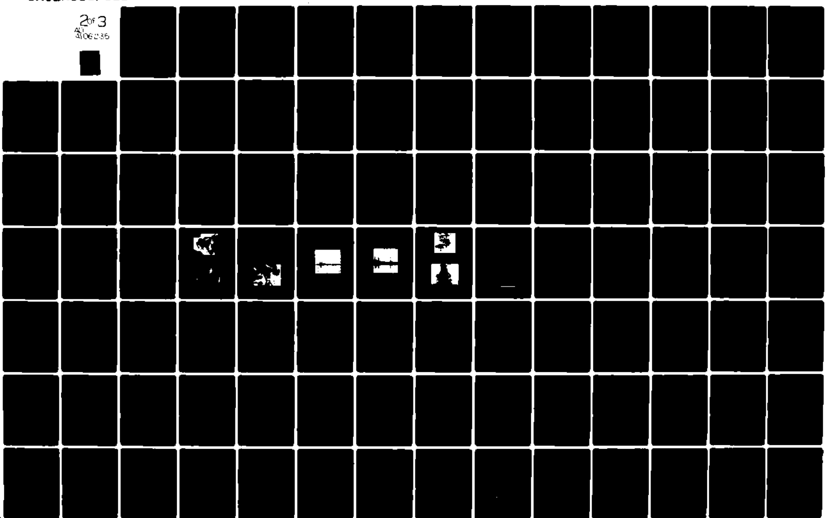

(Probably a slightly lower temperature for injury would have provided a better fit to the data observed, for an alpha of 150 cm^{-1}). Over the range of time studied, it appeared, therefore, that a gross threshold lesion within one hour (our criterion for threshold of injury; the "burn threshold") was therefore produced at a constant temperature rise of about $22^{o}C$. In summary, then, our data and modelling indicated that we should be able to estimate the value of CO_2 laser power density and exposure time which would result in approximately 100 percent probability of producing a mild "immediate" skin lesion in skin of mice, over the range of 0.5 to 5 seconds, and perhaps extrapolate from those numbers to other power density and time exposure ranges.(4) The power density-time data could be well fitted by a curve of the form (power density) $(\text{exposure time})^{+\frac{1}{2}}$ = constant for threshold injury. Therefore, the energy density-time date for threshold injury could obviously be well fitted by a curve of the form: (energy density) $(\text{exposure time})^{-\frac{1}{2}}$ = constant, or equivalently, The energy density required for threshold injury varies directly as the square root of the exposure time. In our studies, the equation which well fitted the data was energy density = 3.6x $(\text{exposure time})^{+\frac{1}{2}}$ (based on the heat flow equation and the temperature used). For exposure times much larger than those used in this study, one would expect the equation to not hold. One would expect a constant threshold power to be required, and the basic equation would then be that the required energy density varies directly with the time, and not its square root. It is interesting that if one uses the square root equation, and 100 milliwatts as the threshold, the time of exposure required to satisfy the equation

(Power) $(\text{time})^{1/2}$ = 3.6 is twenty two minutes, which is quite long (but of course not "infinite time"). The differences in types of applicable equations arise, in part, from our assumptions, such as no radial heat flow (radial heat flow neglected) during the irradiation, used in the analysis in this paper. (5) In conjunction with the above studies, experiments were reported on tissue ablation (hole drilling) in rabbit, dog and monkey tissue on irradiation at 10 microns. A graph of depth of penetration versus exposure time, at an incident power density of 100 watts/cm^2 is presented, for ablative *in vivo* penetration of rabbit skin. Curves drawn on the graph, based on a model developed by Landau for calculating material losses in melting and ablating solids, fall below the experimental points, but do agree with the data points within a factor of two. It was felt that the experimentally observed greater depth of penetration beyond that calculated from Landau's model, (for a given exposure time) might be due to factors which include changes in thermal constants of tissue with temperature, and the temperature at which ablation occurs.

The above studies were considered to provide information concerning CO_2 lasers interaction with tissue --that indeed the experimental data was well fitted by an opaque and diathermanous model with a high absorption coefficient; that the experimentally determined temperature elevations agreed with that of the model; that models for tissue penetration appeared to be capable of development; and that the information obtained and models developed were pertinent to hazards, not only in this infrared range, but to thermally induced injury in the visible, and to thermal injury (not photochemical) in the ultraviolet. Furthermore, the information developed in this paper is of value in assessing and developing

CO_2 (or other infrared) lasers as tools in surgery for cutting and coagulation. This data was published by 1966.

SUMMARY OF MECHANISMS

The studies discussed in this section of the report are as follows: biological, biophysical,model and analytic studies relating to degradation of energy with production of temperatures sufficient to cause thermal changes, degradation of energy within a closed filled cavity with accompanying phase changes, associated pressure, sonic and ultrasonic frequencies, effects due to alteration of wavelength, the possibility of frequency multiplication, the induction and importance of photochemical reactions, the pressure and importance of free radicals, charged and uncharged particles, the studies on focal hepatic injury and repair, the analysis of target geometries, the investigations of reversible depigmentation, the mechanism for its production and its significance including that relating to model development for eye injury, the development of the melanin granule models and those relating to continuous laser radiation inthe visible, and the biophysical studies with the CO_2 laser.

Although the above represent only some of our work relating to mechanisms of interaction of laser radiation with biological systems, it is evident that our studies in this regard, supported by this contract, have made a significant contribution to this field. A number of our studies in this area were presented in part or in whole previous to publication. This report documents the priority of our work in specific areas as discussed above which was carried out under this and associated contracts.

MECHANISMS OF INTERACTION OF LASER RADIATION WITH BIOLOGICAL SYSTEMS

BIOPHYSICAL STUDIES

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INSTRUMENTATION AND DEVELOPMENT

This contract has contributed to instrumentation and development in several areas. Three of these will be discussed briefly. They are 1) development of CO_2 lasers for our use and that of others, 2) development of a method for detecting and measuring the frequency of surface vibrations using a He-Ne laser, and 3) thermocouple injection system for measurements of tissue temperature on laser irradiation. Two other areas - television systems fo monitoring the interaction and dual visual signal systems are discussed under hazards and protection.

1. Development of CO_2 Lasers

Inexpensive CO_2 lasers had been constructed previous to our report. We described (1) a method for increasing the power output by using a simple technique for lengthening the active gas tube and by introducing a cooling system, and a novel combination Brewster-Angle window holder which also served as an electrode.

Twenty watts of output power was obtained at 6%-7% over-all efficiency from a 3-meter tube with a 1-cm bore diameter, using the above techniques. The laser gas tube was constructed from commercial, water-jacketed Cenco (West improved with T-S joints), male-female condenser columns 65 cm in length with a clear bore of 1 cm diam. Occasionally, a drip tip had to be sawed off to assure a 1-cm bore. Four of these tubes were fitted together using vacuum grease at the ground-glass joints. An aluminum center electrode was inserted in the middle of the gas tube. The over-all laser-tube length could be quickly altered by additional condenser columns as space or power requirements dictated.

A closed-loop water-cooling system was constructed by interconnecting the condenser column water jacket with Tygon tubing, using a large, plastic rubbish barrel as a water reservoir.

Combination aluminum electrodes and Brewster-angle window holders were turned down on a lathe and fitted with vacuum grease to the female ends of the condenser column gas tube. Sodium chloride Brewster-angle windows were secured to the aluminum with low-vapor-pressure epoxy such as Torr-Seal. The useful lifetime of these windows was in excess of a few months when kept in an atmosphere of less than 45% relative humidity. This was accomplished by heating the air near each NaCl window with a 100-W light bulb. Plastic bags were employed to cover the windows when the laser was not in use. When the NaCl flats required re-polishing, they were, without damage, slid off the aluminum by heating with a torch and applying slight hand pressure.

Large radius mirrors were fabricated from glass flats using amateur telescope-makers' equipment. Gold was vacuum deposited onto the surface of these mirrors after a 1-2 mm diam hole had been drilled in the center of the output mirror. A larger beam diameter with higher total power at reduced coherence was obtained using a sodium chloride flat as the output mirror. The 4% first surface reflection from the sodium flat was sufficient to yield a laser output of 20 W from this narrow-bore gas tube.

With this system we continued biological studies. This CO_2 laser was successfully Q-switched using an external rotating prism, as reported in the 1966 SGO progress report.

A second improved CO_2 laser (3) was then developed to extend our biophysical studies.

It consisted of three sections: a mirror holder containing a spherical gold mirror, the water-cooled laser gas tube containing Brewster angle windows to which NaCl flats were attached and electrodes, and a second mirror holder containing a dielectric mirror. Shutters, which could be pulsed were inserted in the cavity to provide variable beam pulse durations. Further details concerning the unit are provided in the accompanying volume by Mackeen and Fine. More complete details are given in the complete Northeastern University report on the unit.

Mr. G.R. Peacock was responsible for the major thrust in development of these units. In addition to the individuals listed in the publications (W. Peter Hansen and Larry Feigen) Joel Cohen spent time in development of the intra-cavity shutter, so that a variable, well determined pulse duration, could be obtained and Donald Mackeen carried out modifications and tuning of the system, during its use.

2. A Method for Detecting and Measuring Frequency of Surface Vibrations Using a Helium-Neon Laser

Previous to our studies (2), the speckled appearance of a uniform diffuse surface under illumination by a CW helium-neon laser has been discussed and mathematical explanations for the effect reported. Rigden and Gordon showed that the speckling was caused by a specific arrangement of scatterers on the surface, and that for each arrangement the pattern was constant. It followed then, that if the surface were to vary in position with time, the speckle pattern would also vary accordingly. When any given position of the surface relative to the observer was repeated, the speckle pattern should also be repeated.

We, therefore, postulated that if the laser beam were chopped at the same rate as the frequency of some mode of vibration of the surface in question, a stationary speckle pattern corresponding to that vibration mode would be observed. This pattern would either appear to flicker on and off, or, at higher frequencies, would appear continuous and stationary due to the image retention properties of the eye. If the speckle pattern appeared to vary in time, then the beam chopping rate would differ slightly from the rate of vibration of the surface. If more than one mode of vibration were present in the surface, then a stationary pattern superimposed on a moving or washed-out background should be observed. The purpose of our report was to determine the feasibility of detecting and measuring the frequency of vibration of a surface, based on the above. This procedure was considered to be similar to that which employs a stroboscope to stop action.

The method for determining the validity of the above was to impinge a chopped helium-neon beam on a piece of sponge rubber coupled to an audio oscillator driven loudspeaker. Visual observation of the speckle pattern on the sponge rubber target was carried out. With the loudspeaker stationary (with the audio generator off), a clear speckle pattern was observed both with the chopper off and with the chopper rotating at any arbitrary speed. When vibration was induced, the speckle pattern was observed to disappear. The frequency of the audio generator was then increased until a stationary pattern was again observed. It was found that for a stationary pattern, the frequency of the chopper and the audio generator agreed to within 2Hz at frequencies from 40 to 125 Hz. Lower frequencies were detectable, but there was perceptible flicker. Initial experiments were carried out to measure the frequency of movement (vibration) of the chest wall of rats due to respiration and heartbeat, but were not successful, possibly because of low frequency of the vibrations and lack of sensitivity of our system. Possibly more sensitive systems could today provide information of value in this regard, or in other biomedical areas.

3. Thermocouple Injection System

As discussed by Nowak et al., (4), thermocouples exposed to direct or scattered laser radiation may give readings equivalent to temperature values which are much greater than that of the actual tissue temperature. Although this may appear self-evident, data in the literature at the time of publication indicated that a number of investigators were reporting tissue temperature changes which were considerably in error, presumably for the above reasons. To overcome this problem for in vivo temperature measurements, a solenoid actuated thermocouple injector system with a delay time of 20 msec. was developed to allow temperature measurements to be made without absorption of pulsed laser radiation in the thermocouple wires and junction. Temperature readings were therefore obtained 20 msec. after the laser pulse (5).

These types of injectors have been developed by others. However,our use of them in the laser related situation was reported and the information was made available.

INSTRUMENTATION AND DEVELOPMENT

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LASER HAZARD AND PROTECTION STUDIES

Because of this contract, we have contributed to the above area in several ways. These include:

1. Experimental study of the interaction of laser radiation at several available wavelengths and power levels, in order to evaluate hazards.

2. Development of models to aid in understanding the mechanism, type, and degree of hazard at various wavelengths or wavelength ranges, and power levels.

3. Determination of the biophysical factors relating to the studies in (1) above.

4. Experimental studies on and analysis of the use and limitations of protective devices and systems for decreasing hazards.

5. Development, presentation and publication of programs, procedures and techniques for safe operation of lasers; specific hazard analysis and protection.

6. Major participation in development and carrying out of the Massachusetts Laser Survey - the first such major survey of laser hazards in a state.

7. Provision of information to other Department of Defense contractors regarding implementation of procedures for minimizing hazards in specific situations.

8. Provision of information to other non-Department of Defense governmental agencies and industry in regard to laser related hazards and their minimization.

9. Development of laser safety legislation within the state of Massachusetts.

10. Membership on ANSI Committees in regard to laser safety, service on other laser safety committees.

Area 1: Experimental studies on the interaction of laser radiation with biological systems.

These are discussed in the other sections and volumes of the report, and will not be further discussed here. The sections include experimental studies relating to pulsed and/or continuous visible, CO_2 and ultraviolet radiation carried out by our group.

Area 2: Model Studies

These are discussed primarily in the section on mechanisms of interaction in the several parts of the report, and in those areas in which model studies are correlated with the experimental studies. They will not be discussed further in this section.

Area 3: Biophysical Factors Relating to Experimental Studies

These are discussed together with the experimental studies, or in separate accompanying sections of this report; and will not be referred to further in this section.

Area 4: Experimental Studies on and Analysis of the Use and Limitation of Protective Devices and Systems for Decreasing Hazards

a. Safety Improvement for Unattended Lasers (1). This very simple report suggested the use of a cap or flap to cover the exit port of a laser such as a He-Ne laser when it is not in use. The importance of a gravity drop system was mentioned; it is interesting that in a commercial system reviewed much later, the safety mechanism attempted to use a powered system as the safety element and gravity for the operational stimulus, the opposite of our suggestion. (It was the opposite of that which should have been done). The small report also discussed consideration of attenuation filters, wedge systems and diffusing screens. For infrared or ultraviolet radiation, caps incorporating the above and a material which converts the radiation to a visible image are suggested. It should be noted that at the time of this report, simple methods for beam containment (caps, flaps, etc.) were not used.

b. Preliminary Report of Biologic Testing of Laser Protective Material (2). Previous to these studies (2), experimental studies had been carried out to determine the ocular tissue injury threshold on exposure to specific laser wavelengths. The characteristics of protective material, in particular, its optical density at the laser wavelength, required to reduce an incoming suprathreshold beam to a safe level at the eye had been based on the above data, together with calculations or in some cases, physical measurements on the material. Biological testing of these materials to determine whether they indeed do protect the eye, as calculated, even at high power density levels, had not been carried out previous to our study. It could not be stated with certainty whether the calculated values would accurately predict the degree of safety actually present, or whether some unexpected, unpredicted injury might not occur to various ocular tissues.

We therefore, carried out what we believe to be the first studies on the degree of protection offered the eye on pulsed ruby laser radiation, in which the eye itself served as the indicator; our studies were carried out to determine the degree of protection actually afforded by a number of such materials interposed between ruby laser radiation and rabbit eyes in vivo. An attempt was then made to correlate the optical density of the protective material at the ruby wavelength with the severity of ocular tissue alterations, and with the degree of protection expected.

Both a normal mode and a Q-switched ruby laser were used as radiation sources to irradiate the rabbit eyes with and without interposition of sample protective materials. These protective plastic shields were secured one-half to one inch in front of the eye. The samples evaluated were selected plastic materials used in the Glendale Optical Company Laser-Gard spectacles, thin plastic films manufactured by the American Cyanamid Company, and glass used in American Optical Company spectacles for protection against ruby laser radiation. The optical density of all the samples at 694.3 nm, were supplied by the manufacturer. Ophthalmoscopic examinations were performed before, immediately after irradiation, and at intervals during the following week. Some animals were killed two weeks post irradiation, the eyes examined, fixed and examined histopathologically.

There were several findings of interest. In agreement with the first paper published on a comparison of Q-switched and normal mode irradiation (Fine, Maiman, Scott, Klein), it was found that there was indeed a qualitative difference in the interaction, and in the hazard. For example, the Q-switched retinal ruby laser threshold had been reported by a number of groups to be about one-tenth that of the normal mode threshold energy. From our studies here of the unprotected eye, we found that 2.7 joules Q-switched ruby suprathreshold irradiation of the eye caused much more severe injury than 20 joules suprathreshold normal mode irradiation; even 1 joule Q-switched irradiation of the eye caused a much more severe lesion. Indeed, interposition of a filter of 2.8 O.D. between the 2.7 joule Q-switched beam and the rabbit eye was necessary to produce a lesion roughly equivalent to the 20 joule normal mode lesion. Consequently, in this case, the energy ratio for "equivalent suprathreshold irradiation injury" was over 1000:1 rather than 10:1; Q-switched suprathreshold irradiations can therefore be considered much more hazardous than the 10:1 threshold value ratio.

A second finding was that, in general, the filters appeared to protect as expected from calculations. The degree of protection offered by the plastic materials increased as the optical density increased, both for the normal and for the Q-switched modes of irradiation. In general, there was no detectable injury to tissues of the eye other than to the retina following interposition of the filters. In some instances, what appeared to be alterations in the corneal epithelium after normal-mode irradiation cleared within 24 hours. These corneal surface irregularities would occasionally limit our examination of the retina immediately following irradiation. With clearing, the fundus could be examined within 24 hours.

A third finding was that in those irradiated eyes in which even a clinically extremely mild or questionable lesion was observed, histological examination, when carried out, revealed changes. It was concluded, therefore, that if a retinal lesion could not be found by ophthalmoscopy combined with careful and serial sectioning, the exposure must be near or subthreshold.

We also carried out multiple irradiations rather than single exposures, to determine protection against injury. We contended that the use of multiple irradiations rather than single exposures to an eye with and without interposition of filters might provide a good criterion for establishing thresholds for injury as well as determining the degree of protection offered by laser progective glasses (2). Another finding was that our values for normal mode and Q-switched ruby thresholds were in general agreement with those reported by Geeraets, et al.

Our reported studies were preliminary studies. We believed more detailed studies would be necessary to evaluate the exact capabilities of representative protective materials. We stated that the use of eyes in vivo might be of considerable significance if these "protective" materials exhibit nonlinear protection, particularly at very high power densities, such as those occurring with picosecond pulses.

I would expect that such studies involving picosecond pulses have been carried out since this question was raised by us in 1971. We ourselves did not pursue these picosecond studies, due to lack of funding.

c. Hazards and Protective Devices Associated with 10.6 micron radiation (3). An analysis was carried out on some of the materials found in CO_2 protective devices. These were reported to some extent in the above reference, and in a more complete report, printed at that time, which is appended. Briefly, required properties of a beam protective device are outlined. These include low transmission at the wavelength under consideration; low reflectance; no intense visible light generation which could prove to be a hazard; pre-failure warning; no production of dangerous or unpleasant substances or fumes; sufficiently long protection time; indication of lack of protectiveness; comfort; minimal impedance of communication and vision; no fogging in use; protection at all angles of incidence; cessation of material reaction problems when irradiation stopped. Other physical properties are mentioned.

Attention was directed to the reflectivity of metal rims or hinges in normal glasses, their limited protection and fogging characteristics, problems and advantages of materials such as cellulose acetate and glass; and of welders goggles systems. We carried out studies and reported on non-uniform heating of glass. Effects included the production of thermal stresses; the production of stress fractures; bright light production at the site of irradiation and its capability for producing eye injury; and the significance of scratches and imperfections in fracture production. The effects on glass of CO_2 radiation were compared with those on cellulose acetate. With cellulose acetate there was slow ablation; no visible light production; and production of an acrid odor as warning. On irradiation of quartz from an American Optical unit for CO_2 protection, it was found that the unit would not fracture under about 10^3 watts/cm^2 irradiation, even when the posterior surface was cooled with freon, while the anterior surface was irradiated. Polarized light studies showed the production of stress patterns in the quartz similar to those in glass; the residual stress under polarized light studies was, however, much smaller in quartz than in the glass. Re-irradiation of the quartz in this stress region did not result in fracture; light emission was also less; reflection appeared to be definitely less.

In very high power studies, attention was given not only to protection (indirect) against inadvertent CO_2 irradiation, but against emission at other wavelengths - visible and ultraviolet - from the site of irradiation. Although attempts were made to measure "ionizing radiation" (x-rays and above) during some of these and other irradiations at various power and wavelengths, we did not obtain any indication of the presence of such short wavelength irradiation. (Studies attempting to determine whether x-ray or snorter wavelengths were produced on laser (non-CO_2) irradiation were initiated by us in 1964.)

In connection with the above hazard-protection studies, attempts were made to cut the caps off sealed quartz vials, which contained radioactive material, at a distance, in order to determine whether the laser beam could be used as a tool for such tasks at a distance. Our power levels were too low; possibly today this has become a simple procedure.

d. Use of Closed Circuit Television Systems for Observation (4). This is discussed in an accompanying volume of this report. As indicated in the publication, there are problems associated with the use of such systems. For example, sufficient radiation at the incident wavelength reflected from the site or produced at the site at other wavelengths may injure the camera. The reflected radiation, of course, can itself be hazardous to the eye.

e. Dual Visual Signal System to Enhance Safety (5,6). Often, a single visual signal, such as a light bulb, is used to indicate activation or energization of the system. The problem with this is obvious - when the bulb is burned out (or the visual or auditory alarm system non-operational), one does not know whether the laser system is not operational or whether the visual signal has failed (e.g., bulb burned out). Since the beginning of our studies we have advocated, designed, developed, used and proposed a dual set of flashing visual signals, switching from green to red, to enhance safety during operation of the laser equipment. With this dual system, one visual signal light must be on and the other off at all times. If both are off, it tends to mean the indicator device is non-operational, for whatever reason (lack of power to device, burned out bulbs, etc.). The reasons for having the system flash or pulse is two-fold; it attracts attention to the signal system and it tends to alert those who are color blind.

As stated above, we have used this system, found it very satisfactory, and brought it to the attention of the contract monitors and the laser community. We expect that it has been incorporated in some systems and standards.

Area 5: Development, Presentation and Publication of Programs, Procedures and Techniques for Safe Operation of Lasers; Specific Hazard Analysis and Protection.

a. Safety note: Toxic and explosive hazards associated with lasers (7). This was the first and possibly still only report attempting to categorize non-radiational, non-electrical, toxic and explosive hazards associated with ancillary materials used in laser studies as well as with the active laser media. These could be encountered either during manufacture or its use. Despite the potential significance of this paper in organization of non-radiational and non-electrical hazards, and the fact that it was published in 1968 in Laser Focus, a journal which is well known in the laser area, and the fact that one of the authors has sat on standards committees, there has been no attempt to reference this contract supported paper in any of these standards; it is therefore included as an addendum to this section.

Basically, elements and compounds which were used either in the manufacture of lasers or in their operation at the time of the report were separated into six groups: relatively innocuous; inflammatory or explosive; ocular or respiratory irritant; anoxial agent; protein poison; material injurious to the hematopoietic system or liver. The materials are discussed concisely and briefly. As concluded in the paper, its purpose was to alert and not alarm. For improved safety, areas to which attention could be directed include: employee education, safety engineering assessment, available handbooks of industrial toxicology and laboratory safety, methods for first aid treatment, and color and number coding of chemicals as suggested by NFPE.

b. Implementation of Procedures and Techniques for Safe Operation of Lasers (5,6,7,8). In these references are listed some of our thoughts in regard to development of programs, procedures and techniques for safe operation of lasers. It is difficult to separate analysis of hazards from aspects relating to development of a safety program.

In reference 5, the hazards were broken down into the following four categories.

1. The laser radiation and its interaction with the biological system.
2. The pumping source, especially flash tubes.
3. The high voltage and currents required for the operation of the laser system.
4. The laboratory or field environment in which the system is used.

For example, as well as the beam characteristics, in category 4, the problems differ, depending on whether the system is contained within a closed environment, such as a room, whether the beam is directed through the atmosphere and if so, whether on a vertical or horizontal axis. The population at risk, qualitatively and quantitatively differ in the above three cases; thought must consequently be given to this aspect. Even as early as 1965 (5) we were able to categorize a number of mechanisms which must be considered in the interaction of the radiation with biological systems. By 1966 (6) we were able to propose a laser safety program - a tentative program for the control of laser hazards and management of accidents. An outline of the program is given below. Since this was probably one of the first programs to be outlined in such detail, a more complete description is given in an addendum. It should be noted that the term Laser Hazard Control Officer is used rather than Laser Safety Officer. Although the latter term is perhaps preferable from a public relations point of view, it is probable that laser hazard control more accurately describes the functions of the individual, particularly for a system operating in an outdoor environment.

The general program outlined consisted of the following:

Control of Laser Hazards

- General Administrative Procedures
  - Personnel
    - Laser Hazard Control Officer
    - Deputy Laser Hazard Control Officer
    - Laboratory Laser Hazard Control Officer
  - Medical Supervision
  - Records
- Laboratory Design and Operation
- Protection Standards and Dosimetry

Management of Laser Accidents

These areas were then discussed in greater detail in the publication (6) and are discussed in somewhat less detail in reference 8. In regard to areas relating to laboratory design and development, the advantages of the dual signal system and the advantages and problems associated with the use of television and similar systems are discussed above. Other areas discussed include problems associated with non-visible beams (infrared and ultraviolet), problems of semi-conductor off axis radiation, consideration of automatically resettable interlocks (with built in manual override system) as possibly the optimum in some cases, room design, layout and ventilation.

Area 6: Massachusetts Laser Survey (9,10)

This contract was concerned with laser safety surveys for several reasons. First, a number of D.O.D. and other governmental contractors in Massachusetts were engaged in laser research development and application. Secondly, developments in the field of laser hazards, laser safety and laser legislation based on the above were of interest to the Medical Research and Development Command. Direct contract funds were not specifically used in support of this survey; the availability of our group with experience in areas relating to laser hazards and laser safety, because of our work under this contract, made participation in and contribution to the survey possible.

Our contribution to this survey included the following:

a) Presentation of a two day lecture course at Northeastern University for individuals associated with the federal and state government which included the following:

Theory of solid-state, gas and semiconductor lasers;

Engineering asepcts of lasers;

Definition of parameters and methods of measuring of these parameters;

Basic physics and anatomy of the eye;

Photometric and radiometric units;

Ophthalmologic aspects of laser hazards, including threshold data and site of injury;

Biological effects and non-ophthalmologic hazards, including mechanism of interaction;

Chemical hazards;

Hazards associated with atmospheric scattering; and

Laser eye protection.

b) Participation in development of the forms used in the survey.

c) Participation in survey planning and in the site visits.

d) Development of information from the survey.

It should be noted that funds from this contract were not used in support of this survey; however it would not have been possible for members of our group to provide the above course, which required planning and development, nor to participate in the site visits and in development of information, if our work had not been supported under our contract over a period of time by the Department of Defense.

The course presentation and our participation were carried out at essentially no charge to the federal and state government organization involved in the survey.

Areas 7 and 8: Provision of Information Regarding Laser Safety to Various Groups and Organizations Including Department of Defense Contractors

The following represents some of the groups to whom information has been given in regard to mechanisms of laser interaction, hazards and safety, programs for hazard control, and use of lasers.

1. The Massachusetts Institute of Technology
2. The Mitre Corporation
3. The University of Maryland (including information relating to studies which were carried out on first moon landing)
4. Eastman Kodak
5. Xerox Corporation
6. Westinghouse Air Brake
7. Western Electric Company
8. Radio Corporation of America
9. The Raytheon Company
10. Avco Everett Research Laboratories

11. The National Aeronautics and Space Administration
12. The Department of Transportation
13. Arthur D. Little Company
14. Comsat
15. Micronetics
16. Maser Optics
17. General Lasers
18. Lumonics
19. Spectra Physics
20. Northeastern Radiological Research Laboratory
21. Department of Public Health, State of Massachusetts
22. The Division of Radiological Health, U.S.P.H.S.
23. The Division of Occupational Health, U.S.P.H.S.
24. The Martin Company
25. Department of Defense

It would not have been possible to provide information to the above groups relating to laser studies, if our work had not been supported by this contract.

Area 9: Development of Laser Safety Legislation Within the State of Massachusetts

The principal investigator has served as an unpaid consultant to the Department of Public Health, over ten years. He has been involved in assessment of laser hazards in academic, industrial and commercial environments for the department; in determination of methods for minimization of hazards and improvement in safety in laser related equipment and studies for the department and in the development of legislation. The Department of Defense contract permitted development of expertise necessary to carry out these assigned tasks.

Area 10: ANSI Committees

The principal investigator has served on several committees and chaired one committee in the initial laser related document. The contract assisted in the maintenance of the capability by the principal investigator to contribute to the above.

Summary:

This contract has enabled contributions to laser hazard and protection studies to be made in the following areas. These include:

1. Experimental study of the interaction of laser radiation at several available wavelengths and power levels, in order to evaluate the hazards.

2. Development of models to aid in understanding the mechanism, type, and degree of hazard at various wavelengths or wavelength ranges, and power levels.

3. Determination of the biophysical factors relating to the studies in (1) above.

4. Experimental studies on and analysis of the use and limitations of protective devices and systems for decreasing hazards.

5. Development presentation and publication of programs, procedures and techniques for safe operation of lasers; specific hazard analysis and protection.

6. Major participation in development and carrying out of the Massachusetts Laser Survey - the first such major survey of laser hazards in a state.

7. Provision of information to other Department of Defense contractors regarding implementation of procedures for minimizing hazards in specific situations.

8. Provision of information to other non-Department of Defense governmental agencies and industry in regard to laser-related hazards and their minimization.

9. Development of laser safety legislation within the state of Massachusetts.

10. Membership on ANSI Committees in regard to laser safety; service on other laser safety committees.

These areas are detailed above.

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PSYCHOPHYSICAL STUDIES

Many of the early reported studies relating to the interaction of laser radiation with biological systems were oriented towards suprathreshold studies or towards determination of thresholds for production of injury, particularly to the eye. Mechanism oriented studies and the use of the laser as a tool in such areas as ophthalmology and microsurgery represented other areas of endeavor. Some psychophysical and flash blindness studies were carried out, including those by Sperling.

We have discussed a number of these areas in the other sections of this report, and will not comment on them further here. This section of the report discusses two areas. The first is concerned with initiation of studies to determine the visual threshold for laser radiation in the near infrared, the mechanism for visual detection of such radiation at and above threshold, and the effect of such characteristics as coherence on spectral sensitivity. The second area is concerned with determination of thresholds for heat sensation on CO_2 irradiation of human skin *in vivo*.

John Campbell was responsible for designing, developing and constructing the equipment for the first of these studies, and carried out the studies to the point discussed in this section. Further details regarding specific aspects are discussed in progress reports and in reports submitted to the Surgeon General on this area of endeavor. John Campbell also assumed responsibility for carrying out the second of these studies - the CO_2 studies, and for analysis of the data. His work in our laboratory terminated when he went on active duty at Ft. Knox, where he continued to work in psychophysical laser studies. This is, in part, the reason that studies were carried out only to the point discussed, and not pursued further in our laboratory.

1. Studies Directed Towards Determination of Visual Threshold for Near-Infrared Laser Radiation; Mechanism for Detection, Qualities and Characteristics.

There are several groups of reports which interested us, in regard to directing our attention towards vision-related psychophysical aspects of near-infrared laser radiation. Representative of one group of reports are those by Wald and his collaborators (1,2), in which the sensitivity of the human eye for both photopic and scotopic vision is shown to extend beyond 1000 nm., into the near-infrared. The second group of reports are represented by those published by Brindley and by Lewis (3,4). In these publications, the following was reported. When one eye of each of a number of observers was irradiated with band-limited infrared radiation, from 711 nm. to 887 nm,, respectively, and the color of that band compared with the color of radiation shorter than 700 nm. directed at the other eye, the following was observed. Light of very long wavelength (beyond 690 nm.) appears more orange to the normal eye than light of wavelength about 690 nm. For each very long (near infrared) wavelength study (711 to 887 nm.) a unique shorter wavelength in the visible could be found which visually matched it perfectly. As the near infrared irradiation wavelength was increased from 711 nm. to 887 nm., the matching wavelengths appeared to be correspondingly decreased from 688 nm. to 641 nm. A theory for this extension to the near infrared for vision is given by Lewis. Extension of Lewis's theoretical curve to about 1100-1200 nm. indicates that the corresponding visible wavelength would fall in the range 550 to 600 nm.

A somewhat different set of observations reported by Vasilenko, Chebotaev and Troitskii (5) was representative of a third set of results of interest to us. They used a Ne-H_2 gas laser operating at specific wavelengths - 984.6 nm., 1114 and 1117 nm., 1152 nm. and 1179 nm. Observers looked at the radiation for ten flashes and compared the color with the spectrum from an incandescent lamp seen through a monochromater. The comparison matching wavelengths, 560 nm. on irradiation at 1114 and 1117 nm., 576 nm. on irradiation at 1152 nm., and 584 nm. on irradiation at 1179 nm. compare closely with the second harmonic of the laser wavelength. The authors concluded that the results indicate the wavelength observed was the second harmonic of the laser wavelength.

It should be noted that the two sets of studies discussed above differ. In the former (3,4) the longer the near infrared wavelength, the shorter the matching visible wavelength; in the latter (5), the longer the irradiating infrared wavelength, the longer the matching visible wavelength. The former were carried out at low irradiation power levels, the latter possibly at higher power levels (possibly hundreds of milliwatts per pulse). Possibly the mechanisms in the two cases might be different; the observations by Vasilenko et al. might depend on specific non-linear properties of the eye, which become accentuated as the power level is increased.

We undertook to answer three questions: 1) can infrared laser radiation be seen - what is its "effective spectrum"? 2) is the infrared to visible transformation effectiveness, for laser irradiation qualitatively and quantitatively, the same as or different from that for low peak power radiation (e.g., is coherence important) and 3) what are the mechanisms involved in answer to question 1 and 2, particularly if differences are noted (e.g., if power is important and frequency doubling does occur).

We attempted to begin to obtain answers to the above questions in two ways. First, by carrying out psychophysical studies initiated in 1966, as discussed in this section and second, by carrying out studies in frequency doubling in biological tissue in vitro, discussed in an accompanying section of the report. (As discussed in that section, our interests extended into areas beyond that relating to answering the above questions). *In neither of* the above did we carry out studies in the infrared; in both we used visible radiation to initiate our studies. The reasons for this are as follows: We could not extend the psychophysical animal studies into the infrared, since John Campbell went on active duty to Ft. Knox. In regard to the frequency-doubling studies, our contract was terminated before we could carry out these in vitro studies at other than ruby wavelengths. Infrared studies were, however, planned.

Furthermore, in neither of the above studies, did we use human subjects. In the 1965-67 period, we were ignorant of approximately how far visual thresholds, if they existed, would lie below threshold injury, particularly in the infrared region. Indeed, in the infrared region, the threshold for ocular injury could well be below that for vision (1-4). Specifically, the levels used by Vasilenko et al. to obtain their data did not appear to us to be at a level which we could consider as safe. Although we considered the possible use of subjects whose eyes had to be enucleated for specific reasons, such as presence of intra-ocular tumor, even in this situation we would have hesitated to carry out human psychophysical studies involving human subjects because of possible problems such as tumor spread. Furthermore, such specific subjects were not readily available to us.

Our initial psychophysical studies were therefore oriented towards determining the visual threshold for laser radiation in the near infrared of an animal - the hooded rat. Our first studies in this area were directed towards determining the sensitivity of the hooded rat to visible radiation wavelengths; the studies were not carried out beyond this point in the laboratory, because of John Campbell's transfer.

The following is from the introduction to one of our reports by John Campbell regarding our projected studies.

"The present experiment was designed with two missions in mind. Its tactical mission was to specify the visual sensitivity of a convenient laboratory animal, the rat, to laser radiations in the near infrared under conditions of dark adaptation and light adaptation. Its strategic mission was twofold: to provide data for creating tentative, safe limits for use as guides in selecting energy levels to be employed in a similar experiment using humans and to reveal technical problems inherent to this type of research. The paradigm adopted utilized a learning procedure (a modification of D'Amato's learning procedure) in conjunction with a psychophysical procedure (the method of stimuli). Since most information on laser-induced irreversible lesions is founded upon the study of pigmented mammalian eye, internal consistency dictated the use of a rat having pigmented eyes. Consequently, the hooded rat was employed.

"What was known about the visual sensitivity of the hooded rat to electromagnetic radiation? Pertinent information came from recent psychophysical analysis by Muntz (1967) and an electroretinographic analysis by Dodt and Echte (1961). Both experiments demonstrated that the hooded rat is visually sensitive to wavelengths extending from at least 430 to at least 700 millimicron (mu) and that the eye of the hooded rat, like the human eye, possesses two spectral sensitivity functions (luminosity functions) - a scotopic curve representing the eye's spectral (wavelength) sensitivity when dark adapted, and a photopic curve representing the eye's spectral sensitivity when light adapted. In the case of the hooded rat, unlike the case with the human, the band of wavelengths separating the wavelength to which the eye is most sensitive under scotopic conditions (i.e., about 510 mu) and the wavelength to which the eye is most sensitive under photopic conditions (i.e., about 535 mu) is rather narrow, meaning a relatively small change in spectral sensitivities concommitant with changes in retinal adaptation (i.e., the "Purkinje shift") of about 25 mu."

Our studies were consequently initiatied and carried out using the hooded rat as the subject, with stimuli in the visible.

The test apparatus which was built for these studies consisted of the following: an enclosure into which the rat was placed as well as a visual display, the front of which fit flush with the inner front wall of the enclosure. As regards the enclosure, the copper tubes comprising its floor, the sheet aluminum forming its inner walls, and the steel level mounted in front of the visual display could be electrified by passing current generated by a shocking device to them via a grid scrambler (Grason-Stadler, E1064GSP). The shocking device constructed was a quasi-constant current source which generated an alternating current that acted as a shock. The device produced current pulses.

The front of the visual display consisted of a 4" x 4" piece of transparent plexiglass which served as a viewing port. Six inches behind the viewing port was a 4" x 4" piece of white translucent plastic which acted as a screen. A one-inch diameter aluminum disc coated with magnesium oxide (a "uniform diffusing surface") was centered on the front of this screen and functioned as a target. The remainder of the screen acted as a field. Radiation constituting the discriminative stimulus (S^D) passed via two front surface mirrors through a motor-driven sectored disc, a series of diverging lenses, a set of neutral density (N.D.) filters (Kodak) and then onto the target. The S^D was switched on during trials and off between trials by moving a sheet of metal, attached to the arm of a solenoid, out of, and into the path of radiation. The source of this radiation was either a projection lamp or a laser: when using a projection lamp to produce the S^D, an S^D of specific wavelength composition could be achieved by interposing particular combinations of color filters (Corning) between the projection lamp and the target. When using lasers to produce the S^D, the wavelength of the S^D was governed solely by the laser employed. Radiation constituting the field was supplied by a microscope illuminator, through N.D. filters, and onto the rear of the screen. This radiation was essentially achromatic.

The intensity of the S^D was expressed in terms of luminance (in foot-lamberts or ft.L), when the source of this radiation was the projection lamp and in terms of radiance, when a laser was to be used.

The level system was designed so that it actuated programming and recording equipment only if the rat depressed the level 1/16" with a force equalling or exceeding 140 grams. These specifications allowed the rat to engage in "bar-holding" behavior, a frequently encountered problem in avoidance learning studies, without such behavior serving as an effective response. Thus, only a forceful, ballistic-type level depression would actuate programming and recording circuits.

Other equipment employed and integrated into the system included: a programmer; a four-channel non-cumulative type of paper recorder, of which three channels were employed for recording presentations of the S^D and shock as well as the occurrence of level depressions; a noise source for masking potential auditory cues produced by the programmer, and a sound-level meter (General Radio, type 1551-A) used for measuring the intensity (in dB SPL re: 0.0002 microbar) of this noise within the enclosure containing the rat.

The subjects were three male hooded rats (Long-Evans strain) about 160 days old at the beginning of the experiment.

The paradigm adopted was subdivided into a learning procedure and a psychophysical procedure. The learning procedure in turn was subdivided into an escape training or shaping phase and an avoidance training phase. During escape training, rats learned to depress the lever whenever shock was administered in order to terminate the shock. Such a response is termed an escape response (ER), and the development of an ER is a prerequisite to the development of an avoidance response (AR). During avoidance training, rats learned to depress the same lever whenever the S^D, (illumination of target) which was made to regularly precede each administration of shock by several seconds, was presented. Such a response is termed an AR, and its occurrence vs. nonoccurrence (i.e., occurrence of an ER) was the basis for deciding whether or not the rat had detected the S^D on a given trial (i.e., presentation of the S^D). Once rats had learned to make AR's fairly consistently to presentations of the S^D, the psychophysical procedure was begun and the collection of data relevant to specifying the rat's visual sensitivity to several wavelengths under conditions of dark and light adaptation initiated.

After three weeks of being handled (tamed), rats were trained to terminate a periodically administered shock by depressing the lever. This was accomplished by the experimenter's terminating the shock whenever the rat made successive approximations (movement toward front of enclosure, movement toward the lever, standing in front of lever, placement of forepaws atop lever, etc.), to depressing the lever over a number of shock administrations.

Upon the attainment of farily rapid ER's (i.e., less than one second), avoidance training was begun. The procedure initially adopted was similar to one pioneered by D'Amato _et al_., (6-9), using albino rats: D'Amato,and Fazzaro (1966), D'Amato, Keller and Biederman (1965), D'Amato, Keller and DiCara (1964), Biederman, D'Amato, and Keller (1964). Accordingly, on any given trial the S (9.2 ft.L achromatic light) was presented about four seconds before the administration of a shock having an on-time of 0.2 second and an intensity just sufficient to elicit rapid ER's, this intensity being 0.1, 0.2, or 0.3 ma depending on the particular rat being run. The ITI's employed were the same as those used during escape training and the intensity of the field was reduced. Optimal conditions for the acquisition of good avoidance (about 85% AR's) seemed to be an S^D-shock interval of about twenty seconds in conjunction with an S^D which flashed at a rate of 120 $\pm$ 20 flashes per minute. These values were employed during the remainder of the experiment. The specific combination of shock on-time and intensity yielding optimal avoidance was determined for each rat before commencing the psychophysical procedure and was maintained throughout the psychophysical analysis.

Once the rats had reached asymptotic levels of avoidance responding (about 85% AR's) with an intense (9.2 ft.L), nearly achromatic S^D and after running checks to insure that avoidance was mainly attributable to the experimentally defined S^D and not to a correlated stimulus (e.g., auditory),the psychophysical procedure was begun. For each combination of S^D and retinal adaptation state the following procedure was followed. First, a number of preliminary trials were administered in order to estimate the domain of S^D intensities across which different frequencies of avoidance ("I see") versus escape ("I don't see") responses would occur, this domain being termed the threshold region. This information served as a guide in selecting five S^D intensities, one being no S^{D^T}, within the threshold region that were relatively unique as regards the frequencies of AR's versus ER's associated with them. Then, the rats were given about 900 trials, approximately 300 trials being given on each of three days. On any given day the first 100 trials employed an intense (9.2 ft. L), nearly achromatic S^D and served to bring the rats avoidance up to asymptote.

Of the remaining 200 trials, 40 were given at each of the five S^D intensities. It was found necessary to devote the first twenty trials per S^D intensity to adapting the rat to a change in S^D intensity. Consequently, only the last twenty trials per S^D intensity furnished psychophysical data. On each day all trials associated with one S^D intensity were administered before giving trials at some other S^D intensity, a mode of trial administration mentioned by Woodworth and Schlosberg (1954). A different sequence of S^D intensity administration was followed on each of the three days.

The above procedure was repeated for each combination of S^D and retinal adaptation state. Since the rat's visual sensitivity to six S^D's were to be obtained under each of two adaptation states (dark adapted - field illumination of 0.00 ft.L.; light adapted = field illumination of about 1.60 ft.L), a total of twelve sensitivities were to be determined. The six S^D's which were to be utilized are listed *below:*

1. green (530-570 mu), produced by passing the beam of the projection through three Corning color filters (#3484, #4303, and #5120).
2. yellow (560-610 mu), produced by passing the beam of the projection lamp through two Corning color filters (#3480 and #4303).
3. red (610-630 mu), produced by passing the beam of the projection lamp through two Corning color filters.
4. red (694 mu), produced by a CW helium-neon laser.
5. red (840 mu), produced by a CW laser.
6. infrared, produced by a CW helium-neon laser.

Despite the very marked effort in designing, in developing, and in constructing the equipment and in carrying out the initial studies, data which could be analyzed was obtained only from one rat, because of time limitations (i.e., John Campbell's transfer to Ft. Knox). Percent avoidance responses (AR's) were obtained as a function of the stimulus intensity, S^D, for S^D= green (530-570 nm), obtained under conditions of dark adaptation (field intensity = 0.00 ft.L) and light adaptation (field intensity - 0.24 ft.L), and for an S^D = green (530-570 nm), obtained under conditions of dark adaptation. All data were obtained using a shock on-time of 1.0 second and a shock intensity of 0.4 ma, a comgination of shock parameters found to yield optimal avoidance with this rat.

These percent avoidance responses (AR's) were plotted versus the stimulus intensity. The average maximum percent correct avoidance response occurred at the highest stimulus intensity - 86% at 9.2 ft. lamberts. The average minimal percent correct avoidance responses occurring with no stimulus was determined to be 23%. A criterion or sensitivity equal to the base plus one-half maximum-minus-base-difference (23% plus 1/2 (86%-23%)) was used as the criterion for sensitivity. This was 55% average AR's. The stimulus intensities under the specific conditions associated with this criterion were determined from the plots of AR's versus intensity. This was considered as "threshold" light illumination under these conditions. The reciprocal of the value gives an estimate of the sensitivity of the visual system of the animal to the illumination under these conditions.

Three graphs, in which the AR's to green (53-570 nm) under light adapted field conditions, to green under dark adapted field conditions and to yellow (560-610 nm) under light adapted field conditions were obtained.

For the above 55% AR's as a criterion, the intensity for green, under dark field adaptation conditions, was 0.0027 ft. lamberts; for green under light adaptation conditions was 0.0794 ft. lamberts and for yellow, under dark adaptation conditions 0.001 ft. lamberts.

As expected, the eye was more sensitive under dark adaptation conditions than under light adaptation conditions, and it appeared to be more sensitive in the yellow than in the green. However, the threshold values obtained are much higher than expected; and much higher than threshold human values. To what extent this is due to using too high a percentage of correct avoidance responses as the criterion, to what extent it represents insufficient animal training, and to what extent it represents a less than optimal method for determining thresholds and relative thresholds has not been determined. Certainly, for the non-laser visible region it would probably be unnecessary to use other than human subjects; however, as mentioned above, for the laser visible region, one could not have been certain at that time (1966) that human subjects could be used with absolute safety. In the infrared region this was even more questionable, even for "threshold" studies. For suprathreshold (visual response) studies the above was very questionable. Although a different animal species such as monkeys, might have been preferable, we did not have the funds to initiate such studies. Furthermore, the studies which were undertaken were helpful in that they assisted in orienting us towards psychophysical studies, if desirable, and they provided training in psychophysical studies in a laser group for John Campbell who was then further associated with psychophysical studies at Ft. Knox. The studies at Ft. Knox were, however, not a direct extension of the studies undertaken here. The results obtained in the above study were consequently very limited despite the very considerable effort in developing, designing and constructing the equipment. If John Campbell had continued these studies under this contract of at Ft. Knox, further information would have been obtained in this area.

2. Psychophysical Studies at 10.6 microns - Heat Sensation Thresholds for CO_2 Laser Radiation (10)

The fact that 10.6 micron radiation from a CO_2 laser is detected as heat was readily evident by exposing a finger to the radiation. That at higher levels a burn type of reaction results was also quite evident; indeed such an accidental linear burn across the back of the hand resulted in our laboratory from accidental exposure. We followed this accidental burn over a period of time; it appeared to heal in the usual fashion. In previous biophysical studies (Fine, Hansen and Peacock, et al., NEREM, 1966), we had reported that the exposure time required to produce visible threshold injury of rodent skin on CO_2 exposure varied inversely as a function of power density (see accompanying section on mechanisms). That is, for a given crossectional area (A), an inverse relationship existed between the irradiation time (T) and irradiance (H) needed to generate a lesion.

This inverse relationship for threshold lesion production was also reported by Brownell in irradiation of pig skin *in vivo*. Thresholds for two criteria of lesions in depilated porcine skin display this T-H reciprocity at least between the following values of T and H for an A = 2.9 cm^2: (T = 21.6 sec, H = 0.69 W/cm^2) and (T = 0.37 sec) (H = 0.74 W/cm^2) for mild erythema and (T = 39.6 sec; H = 0.74 W/cm^2) and (T = 1.1 sec; H = 7.6 W/cm^2) for spotty white burn (Brownell, A.S., et al., USAMRL Rept. No. 769, 1968).

The purpose of our studies reported below was to determine the reaction times on CO_2 irradiation, using heat threshold as a criterion, and to determine whether a reciprocity existed between reaction time (RT) and irradiance (H), using heat threshold as a criterion.

Two Caucasian males were used in this psychophysical study to specify the range of values of reaction time (RT) and H over which an RT-H reciprocity exists for the case of heat sensations generated by CO_2 laser radiation striking the back of the hand, RT being the time lapse between irradiation onset and raction to heat sensation onset. This was done at 22^o ambient temperature for three values of A. Human heat sensation thresholds displayed an RT-H reciprocity between the following values of RT and H: (RT = 1 sec. H = .320 W/cm^2) and (RT = .75 sec. H = .700 W/cm^2) for A = 3 cm^2; (RT = 15 sec. H = .290 W/cm^2) and (RT = .75 sec. H = .700 W/cm^2) for A = 7 cm^2; and (RT = 30 sec. H = .195 W/cm^2) and (RT =.75 sec. H = .700 W/cm^2) for A = 12.5 cm^2.

We believe these studies to be significant in several regards. First, they showed that an inverse relationship does exist between response time and irradiation intensity, on CO_2 irradiation, using heat sensation as a criterion (for the conditions given).

Secondly, for a large area of irradiance (7 cm^2), a response time of about 30 seconds was required at an irradiance power density of 195 milliwatts. This was in the same range as that determined for threshold injury to the rabbit eye on CO_2 irradiation. (See accompanying CO_2 reports). Thirdly, at power density which is high relative to threshold for detectability (1 $watt/cm^2$ compared to 200 $milliwatts/cm^2$), less than 1 second was required for response time. Fourthly, for irradiances which are only several times higher than long term threshold, considerable time (e.g., 15 seconds at 290 milliwatts) was necessary for the response. Consequently, it is possible that under some relatively lower power irradiance conditions, injury to the eyes may be produced before the sensation of heat results in an avoidance reaction. (Obviously at high power levels such as at 1000 $watts/cm^2$ an avoidance reaction to heat sensation which would prevent injury would not be expected, nor occur).

Fifthly, our studies showed that the CO_2 laser might well serve as a tool in such psychophysical studies.

These studies essentially terminated with John Campbell's transfer to Ft. Knox. It was expected that these and associated studies would have been continued in collaboration with the group at Ft. Knox.

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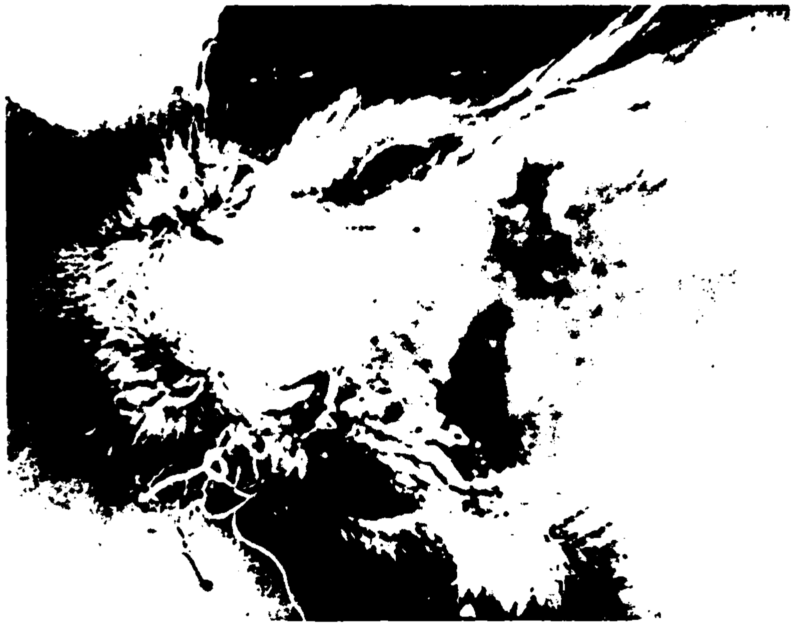


FIGURE 1(a)

Effects of unfocused non-Q-switched 6943 Å, 60 joules (60 joules/cm^2) laser radiation on the shaved skin surface of the forehead of a mouse.

FIGURE 1(b)

Cross sections of brain following unfocused laser irradiation of forehead. Hemorrhages are present within the ventricles, the conducting system and substance of the brain.

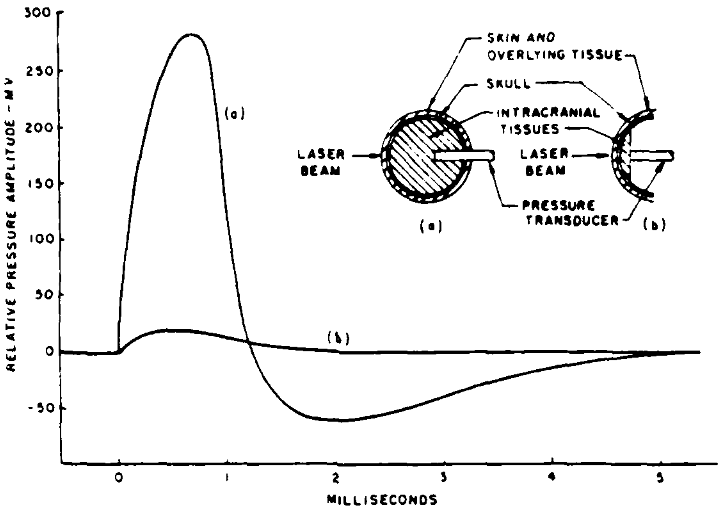
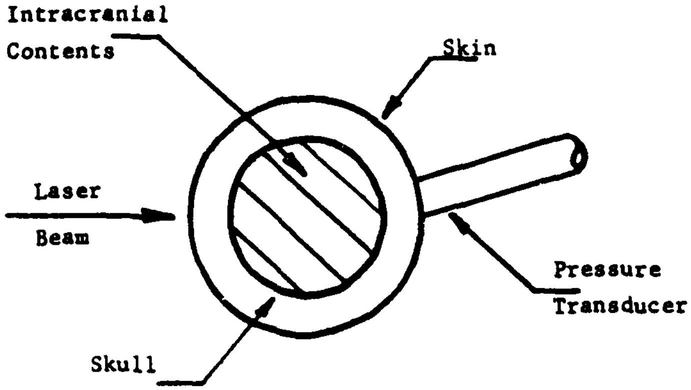


FIGURE 2

Comparison of relative pressure amplitude within the enclosed cranial cavity and pressure amplitude within the cranium with free boundaries. Irradiation at 6943 Å, 25 joules/cm^2, unfocused.

FIGURE 7

Edema and swelling in knee region following non-Q-switched irradiation at 30 joules at 6943 Å.



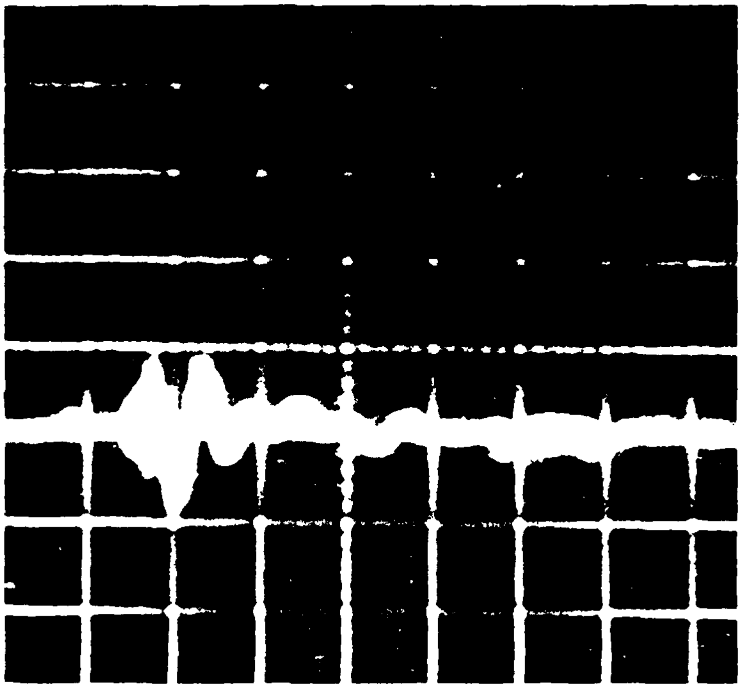
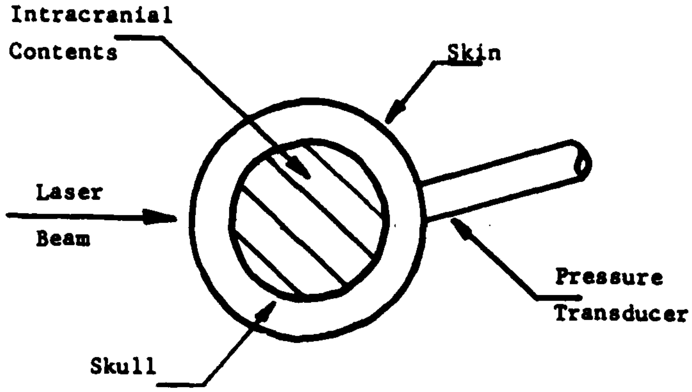


FIGURE 3

Sonic frequencies produced on non-Q-switched ruby laser irradiation of the shaved forehead of mice. Ordinate axis: 0.02 v/cm. (relative pressure amplitude) Abcissa axis: 0.5 msec/cm.



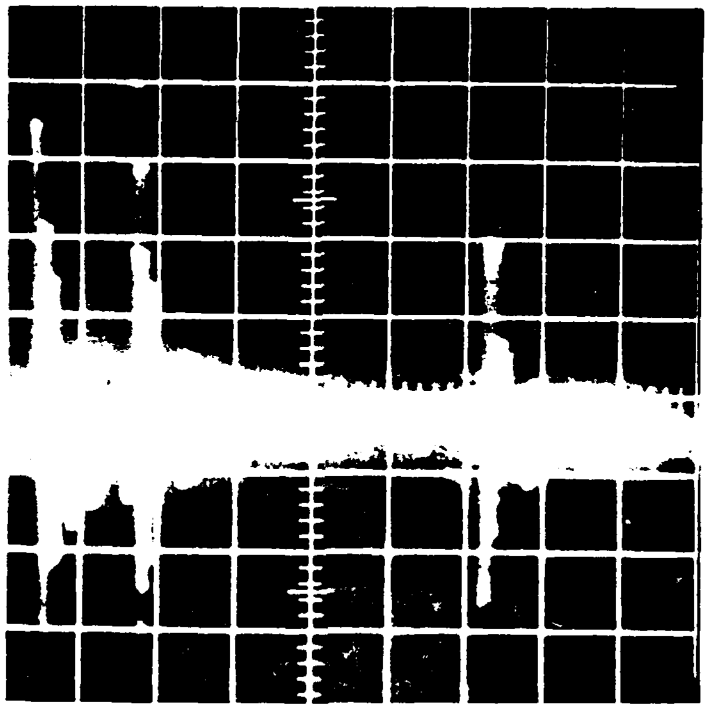


FIGURE 4

Ultrasonic frequencies produced on 1 joule Q-switched ruby laser irradiation of the shaved forehead of mice. Ordinate axis: 0.05 v/cm (relative pressure amplitude) Abcissa axis: 50µ sec/cm. Three spikes produced on Q-switching.

FIGURE 5

Spiral plume trajectory from abdomen of mouse on application of a magnetic field. Irradiation at 6943 Å, 30 joules, non-Q-switched, focused. Single frame of film taken at 8,000 frames per second.

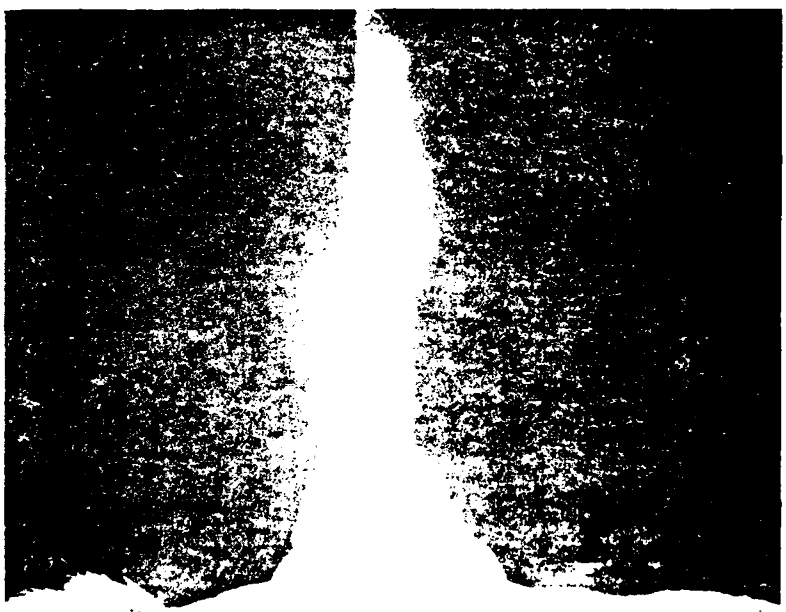


FIGURE 6

Linearly directed plume and outward hemispherical distension of abdominal skin of mouse, irradiation at 60 joules at 6943 Å focused.

ADDENDUM 1

TAKEN FROM

PROCEEDINGS OF THE LASER INDUSTRY ASSOCIATION CONVENTION

PUBLICATIONS COMMITTEE

Samuel Fine

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OCTOBER 24–26, 1968

WASHINGTON, D.C.

Applications of Thermal Models to Retinal Threshold Injury

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I. Introduction

Increased industrial application of visible and near infrared lasers has placed importance upon the quantitative analysis of laser hazards to the eye. For example, in some surveying uses, a He-Ne beam must be visible but not cause ocular injury. This report outlines analytical techniques for estimating the maximum retinal temperature elevation occurring during a step function input of laser power to a retinal image of a given size. These techniques will be used to obtain two results for very small retinal image diameters (less than 40μ) where experimental results have not been obtained. First, it will be shown that under worst-case conditions, exposure to less than approximately 70μW of transpupillary He-Ne laser power is theoretically safe. Second, it will be shown that for a 10μ retinal image diameter, the maximum temperature elevation cannot be limited by a voluntary avoidance reaction on the part of the observer.

II. Continuum Model for the Pigment Epithelium

The primary thermal target site for injury to the eye is defined as the region of highest relative temperature elevation in the eye on irradiation under usual conditions. The location of this region is related to the light absorption characteristics of the eye. Measurements indicate that in the visible region of the spectrum, light passes virtually unattenuated through

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the cornea, aqueous, lens and vitreous (1,2). However, based on in-vitro measurements, the incident light is attenuated by 15 to 40 per-cent in the retinal pigment epithelium (3), the region of greatest absorption of light per unit volume, under usual conditions. The p.e. is a single cell layer, about 10μ thick with small pigment or melanin granules distributed within the cell (4). On a microscopic scale, the melanin granules of the p.e. are the sites in which light is maximally absorbed. As a result of this absorption, the temperature of the pigment granule is raised suffuciently to injure the cytoplasm surrounding the granule. For levels of pulsed laser radiation yielding ophthalmoscopically visible threshold lesions, electron microscopic studies do in fact show cytoplasmic changes in regions surrounding the granules (5).

When the laser pulse duration is short in comparison to the time required for the heating of one granule to appreciably perturb the temperature near another, one can consider the heating of an individual granule imbedded in an infinite medium. Mathematical models based on this assumption and the assumption of spherical granules have been reported (6). These models indicate that a small fraction of the absorbed energy is dissipated from a spherical granule whose diameter is about 1μ during a Q-switched laser pulse (pulse duration approximately 10^{-8} - 10^{-7} sec). However, as the pulse duration is increased, there is significant heat conduction from the granule during the laser pulse. Therefore, for these longer pulses, more energy must be supplied to the granule to raise the granule boundary temperature to a level suffucient for local injury. This result agrees with experimental data insofar as it predicts an approximate order of magnitude increase in the threshold injury energy density as one proceeds from the Q-switched range to the millisecond (non Q-switched) range of pulse durations for ruby lasers.

The following relationship can be used for order of magnitude estimates of the required time, τ, for heat to conduct over a distance, x, in a homogeneous medium of thermal diffusivity, D:

$$\tau = x^2/4D$$

If x is the approximate distance from the border of a melanin granule to the border of its nearest neighbor ($\approx 10^{-4}$ cm) and if D= 10^{-3} cm^2/sec (approximate value for water), then the equation above yields $\tau \approx 10^{-5}$ sec. Therefore, when the laser pulse duration exceeds 10^{-5} sec, the granule boundary temperature will be influenced by nearest neighbor granules. This influence produces a smoothing tendency for the temperature distribution within the irradiated volume of pigment epithelium. This report will be concerned with laser pulse durations exceeding the aforementioned estimates of τ by at least an order of magnitude. Consequently, the granule model will be simplified by treating the pigment epithelium as a continuum where light is absorbed and heat is uniformly generated within a cylindrical target volume (see Fig. 1). This cylindrical volume has a diameter equal to the beam diameter in the region of the retina. The height of the cylinder equals the thickness of the p.e. since, for humans, melanin granules are rather uniformly distributed throughout the p.e. cells (7). For small retinal image diameters and threshold lesions, the heated choroid does not significantly contribute to the heating of the p.e. (8).

With choroidal heating neglected in this model, the maximum temperature rise will always occur at the center of the heated volume of p.e. This temperature elevation can be calculated by solving the time dependent heat conduction equation (9). The solution is:

$$U = \frac{AP_r}{2\ell\rho c} \int_0^{\tau} [\, 1-\exp(-a^2/4Dt)] \cdot \mathrm{erf}(\ell/2\sqrt{Dt})]dt \qquad (1)$$

U = temperature rise (Cent.)

ρc = volumetric heat capacity (cal/cm^3·Cent) ≈ water

D ≈ thermal diffusivity (sec/cm^2) ≈ water

2ℓ = thickness of p.e. (cm)

$2a$ = retinal image diameter (cm)

τ = duration of exposure to step-function input (sec)

P_r = retinal power density (cal/sec·cm^2)

A = fraction of light absorbed in p.e.

t = time from onset of step-function input at t=0 (sec)

Some results of this solution are shown as three transient temperature response curves in figure 2. The first represents the normalized temperature rise for a 10μ image, the second is for a 250μ image and the third is for an 800μ image. The latter two curves cover the approximate range of image diameters that have been used in most of the experimental studies reported in the literature(8, 10-20). These diameters should be contrasted with the much smaller minimum retinal image diameter for humans which has been estimated to have an upper limit of 10μ (21, 22).

The rate of temperature rise for the three curves in Figure 2 can be compared as follows. The time required to reach 90% of the steady state temperature in the 250μ and 800μ cases is 600 msec and 6 sec respectively. These times can be contrasted to the very short time required for a 10 micron image to essentially reach thermal equilibrium - which is 3 msec as shown.

III. Difficulties in extrapolating injury data from large to small retinal image diameters

If threshold retinal injury were related only to the incident retinal power or energy density, then extrapolation of threshold injury levels from large retinal images to a 10μ retinal image could be made simply by using the ratio of the areas of the images. However, the factors that determine injury to a target volume of tissue are the temperature elevation and the length of time over which an elevated temperature is maintained. In other words, injury depends on the time-temperature history of the involved tissue (23).

In the preceding section of this report, it was shown that for a step function of heat generation rate sufficient to raise the peak steady state temperature to a given value, the peak temperature in a 10μ image region rises rapidly to a given equilibrium value whereas the peak temperature of a larger image rises more slowly to the same equilibrium value. This difference in rise times prevents a simple approach to theoretically predicting thermal injury. To see this, assume that a 10μ and an 800μ image are heated to the same maximum temperature by a rectangular heating pulse of given duration. During the heating phase, the time average of the temperature at the center of the "heated cylinder" is lower in the 800μ image than in the 10μ image, possibly causing a slower rate of chemical change leading to injury in the 800μ image. After the heating pulse ceases and the cooling phase begins, the center of the 800μ image cools more slowly than the center of the 10μ image. Thus, during the cooling phase, the central temperature is higher, on the average, in the 800μ image than in the 10μ image. In the cooling phase, chemical change leading to injury may yet occur in the 800μ image but essentially cease in the 10μ image. There is, however, no satisfactory theoretical way to predict whether the 800μ image region will "catch up" and be "injured" in the cooling phase if it has not been "injured" in the heating phase. Similarly, one cannot predict threshold injury in a 10μ image region, under the above conditions, if threshold injury is observed in an 800μ region.

The difficulty of predicting thermal injury is also apparent when one considers possible injury in avoidance reaction time limited exposures. It was shown in the previous section that the peak retinal temperature elevation in a 10μ image region cannot be limited by a voluntary avoidance reaction. However, it is possible that injury can be limited by such a reaction. If thermal injury is a rate process, it is possible that a voluntary avoidance reaction can occur before the chemical processes leading to thermal injury have proceeded far enough to cause threshold injury (particularly if an ophthalmoscopically visible end point is used).

IV. Worst-Case Estimate of Safe Power Levels

Since threshold injury data cannot be extrapolated from large retinal image diameters to a 10μ retinal image, temperatures that are known to be safe for a 10μ image can be considered. These conditions are outlined in this section. A moderate fever temperature (≈ 102 F) can be considered safe for vision over a protracted period of time. While injury may not occur until the temperature rise exceeds 2°C, it is possible to adopt a criterion stating that, "safety cannot be assured for temperature elevations exceeding 2°C." Solving the steady state heat conduction equation for the same geometry as was described for equation 1 yields a relationship between the retinal temperature elevation and the transpupillary laser power as follows (24, 25).

$$U_{ss} = \frac{AP}{4\pi aK}\left[\sqrt{1+u^2} - u + u^{-1}\ell n\left(\sqrt{1+u^2} + u\right)\right] \qquad (2)$$

U_{ss} = steady state temperature rise(deg. Cent.)

A = fraction of light absorbed in p.e.

P = transpupillary power (cal/sec)

2a = retinal image diameter (cm)

2ℓ = thickness of p.e.(cm)

u = ℓ/a

K = thermal conductivity (cal/sec·deg·cm) (assume water)

To obtain a safe value of P for the He-Ne laser, the following worst-case conditions are assumed: no attenuation in the anterior ocular media; A = 100%; 10μ retinal image diameter; heat dissipation not aided by choroidal blood flow; and $U_{ss} = 2^{o}C$. Then, from equation 2, the maximum safe total laser power that can enter the pupillary aperture at 6328A is P = 70μ W. If A is taken to be 40%, P = 180μ W. These results should also hold within an order of magnitude for other lasers in the visible spectrum. Kohtiao et al have reported a measured temperature elevation of 7.8°C for threshold injury in rabbits when exposed to a 25 mW, He-Ne laser for 2.5 seconds. The retinal image diameter was reported to be 250μ . Figure 3 shows various calculated or measured temperature elevations required for injury over a range of pulse durations. The value reported by Kohtiao et al is the lowest measured or calculated value of temperature elevation reported and recorded in figure 3. If this value is adopted as representing the temperature elevation for threshold injury (and there is no satisfactory reason for doing so), then based on equation 1 with A = 100% and a 10μ retinal image diameter, a transpupillary power of about 270μ W would cause threshold injury. For A = 40%, P≈ 700μ W would result in threshold injury. Thus, a 1 milliwatt laser could possibly produce injury.

Other threshold injury temperature elevations can be considered. Figure 4 shows several graphs of the transpupillary power required to raise the central temperature in a 10 micron image diameter through elevations ranging from 2°C to 63°C for pulse durations ranging from 10^{-4} sec to 10^{-1} sec. Threshold area in figure 4 corresponds to the range of temperatures which are associated with injury in figure 3.

V. Summary

In summary, then, for the model presented:

1) For a 10μ retinal image diameter and CW irradiation in the visible, steady state conditions are reached before the human avoidance reaction time of approximately 0.1 sec.
2) There is little reliable time-temperature data available that can be utilized to establish threshold injury temperatures in the retina. Rates of heating and cooling and rate dependent reactions must be considered.

However, a $2^{\circ}C$ temperature elevation can be considered safe for laser hazard estimates.

3) Based on the model presented, a 10μ retinal image diameter and a safe temperature elevation of $2^{\circ}C$, this analysis indicates that on a worst-case basis less than approximately 70μ W of transpupillary power is safe for He-Ne lasers at 6328A. If one assumes that about $8^{\circ}C$ temperature rise will cause threshold injury, then from 270μ W to 700μ W of transpupillary power will be injurious.

4) If the threshold for injury were a function of temperature elevation per se, then the region of threshold injury would be less than the region irradiated.

5) Light scattering, particularly for small image sizes (≈10μ), will result in a higher irradiation threshold for injury than those discussed above. An increase in incident retinal energy density for threshold injury as the image size is decreased has been discussed in an article by King and Geeraets (17).

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Supported by Contracts DA-49-193-MD-2436 and 2437, Surgical Research Branch., Medical Research and Development Command, Office of the Surgeon General, Dept. of the Army: NASA Grant NGR 22-011-007.

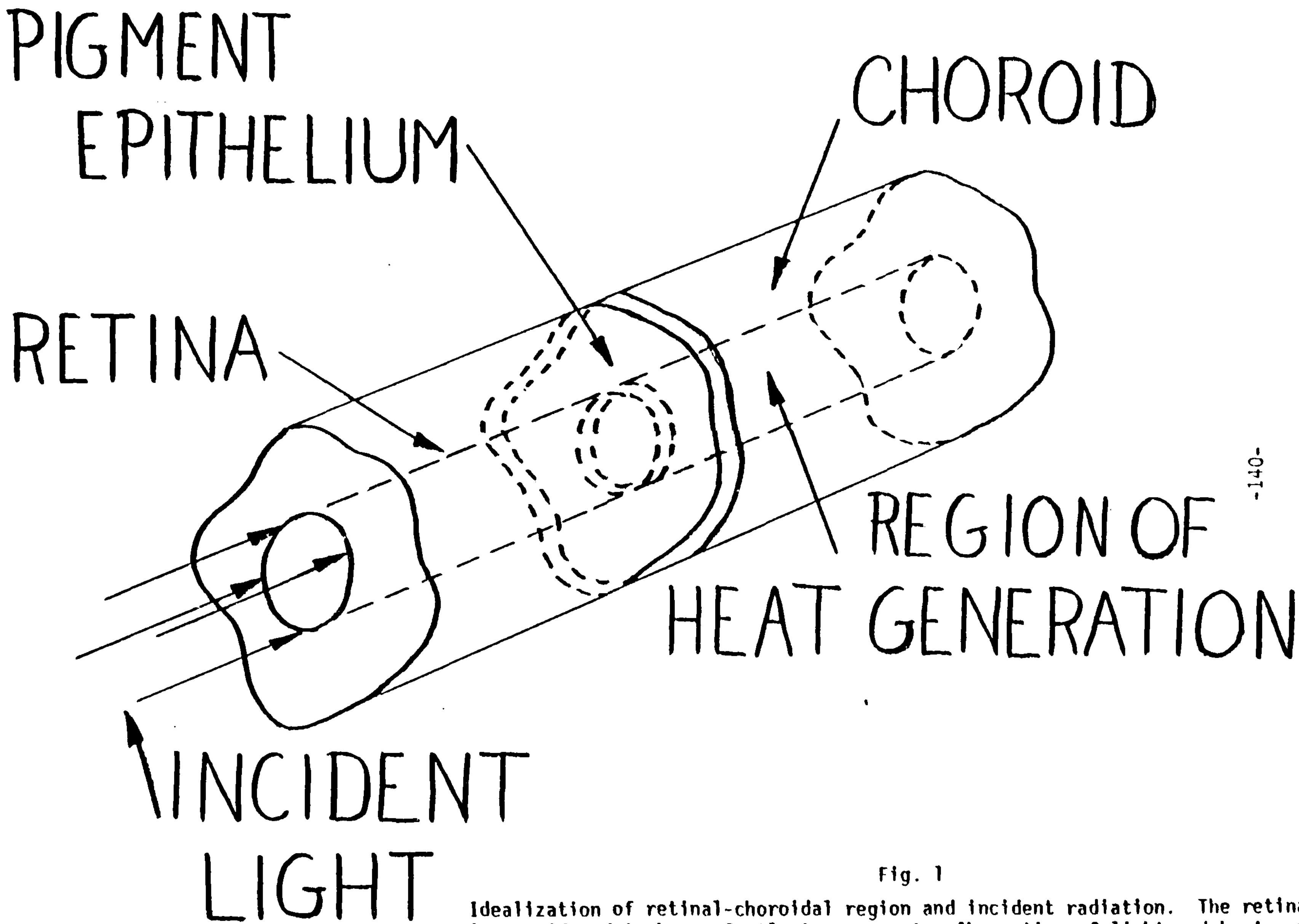

Fig. 1

Idealization of retinal-choroidal region and incident radiation. The retina is considered to be perfectly transparent. Absorption of light and heat generation occur in a cylindrical volume of pigment epithelium and choroid. In this report, choroidal heating is neglected. In the retinal choroidal region. the radiation is assumed to be confined to a cylinder with uniform irradiance at any cross section.

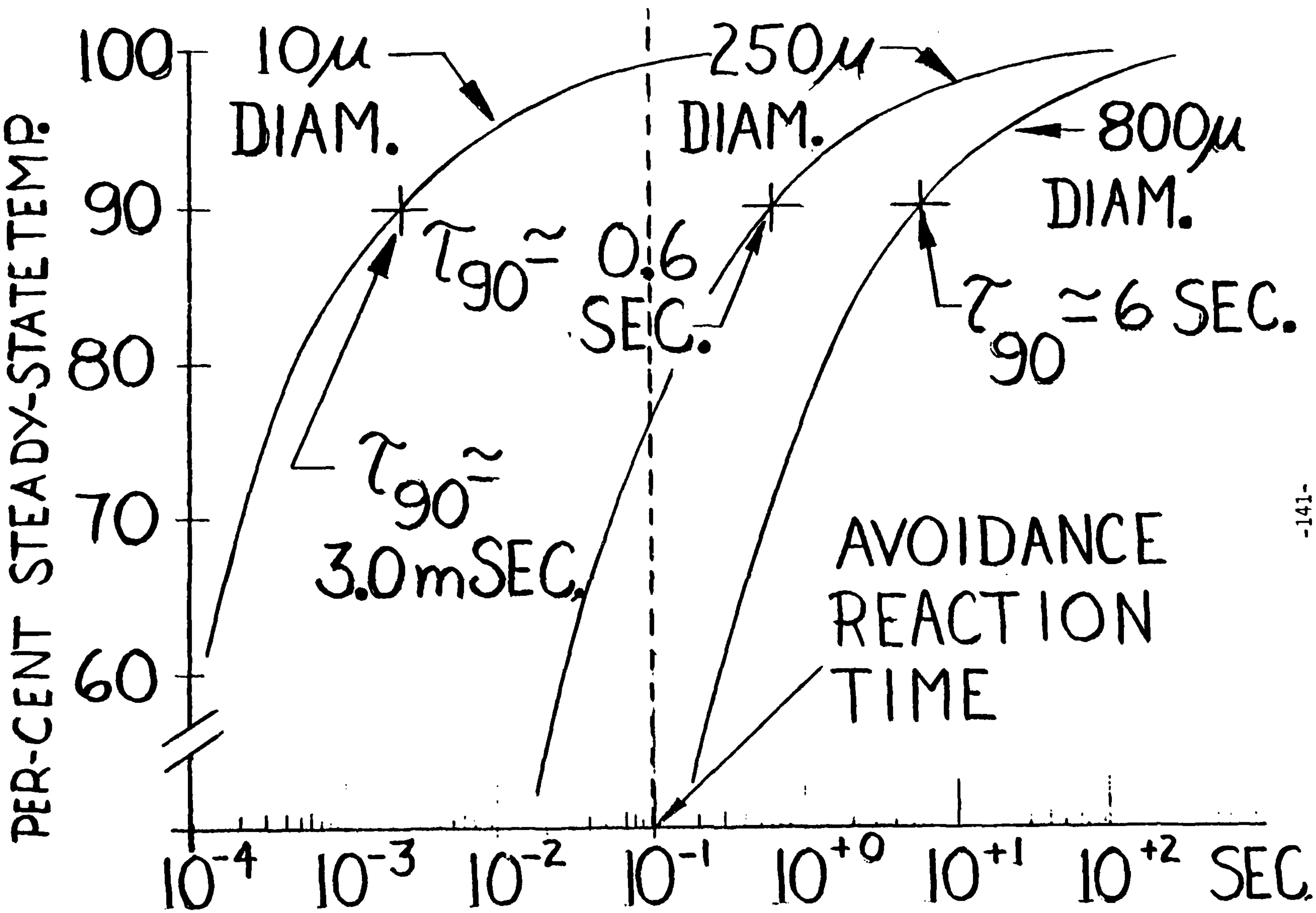

Fig. 2

Normalized temperature response for exposure to a step function of power for various image diameters. The value of 100% represents the asymptotic maximum or steady state value of the temperature rise at the center of the heated volume of pigment epithelium.

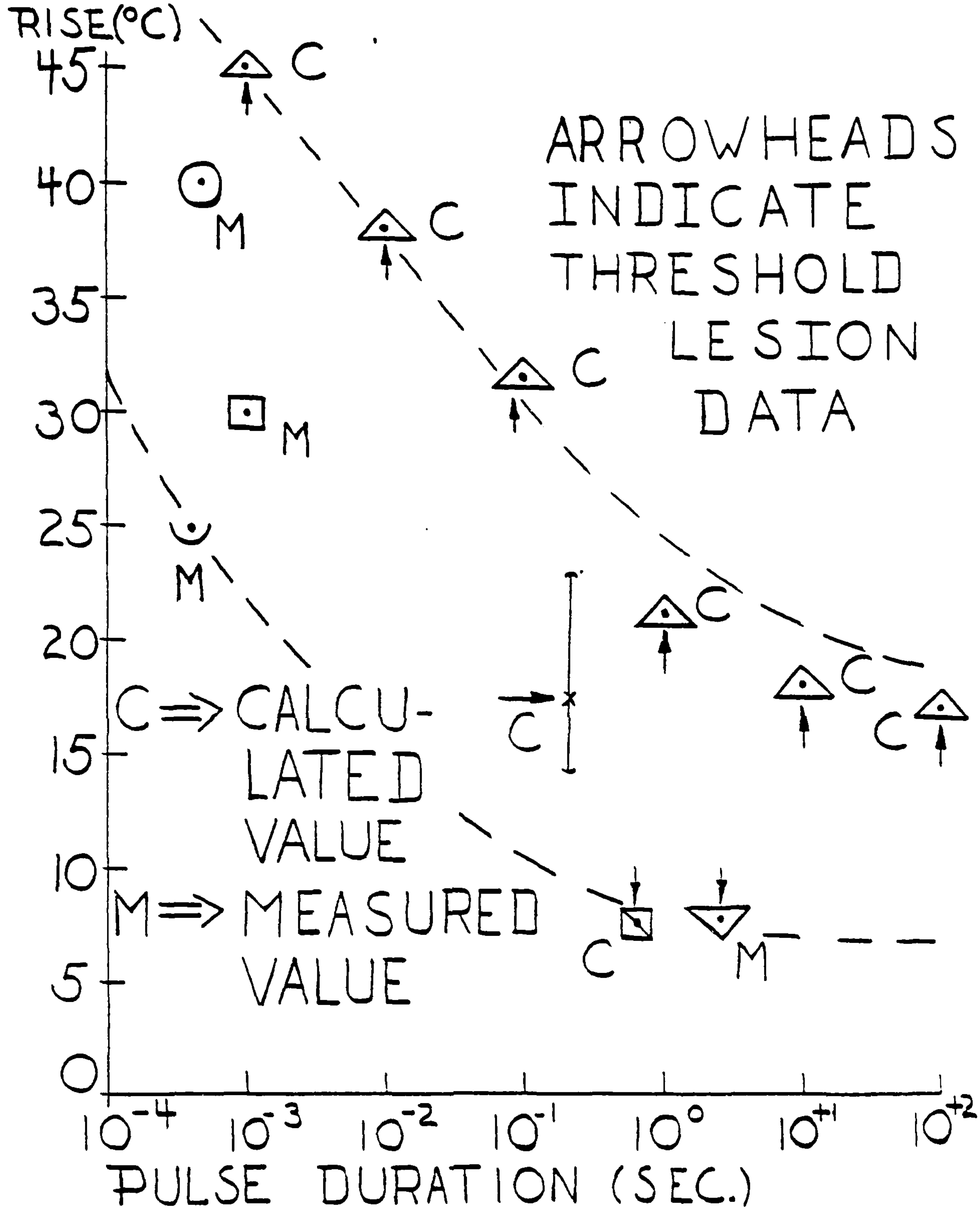

Fig. 3

Calculated (C) and measured (M) temperature elevations from photocoagulation and threshold injury studies that have been reported for various pulse durations. Data was obtained from the following sources: ⊡ Ref. 26; ⊙ Ref. 27; ◬ Ref. 10; ▽ Ref. 15; ◡ Ref. 28; ⧅ Ref. 16.

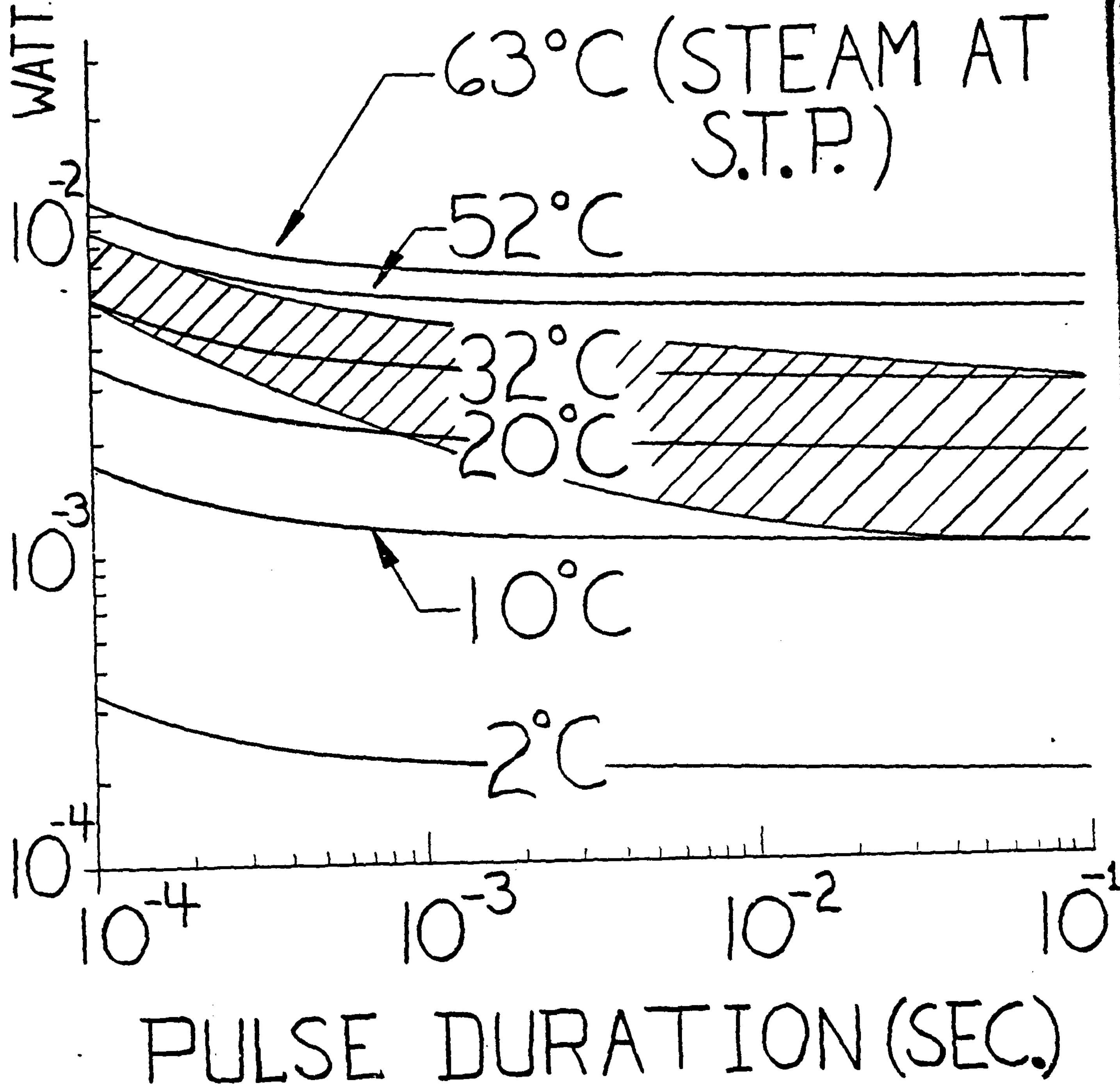

Fig. 4

Transpupillary power required to raise temperature at center of pigment epithelium through elevation shown on various curves

Safety Note: Toxic and Explosive Hazards Associated with Lasers

by DONALD MacKEEN,* SAMUEL FINE* and EDMUND KLEIN**

There is a considerable body of literature concerning laser hazards.[1-3] As a result, those working with lasers are aware of the harmful effects of both laser radiation and hazards associated with electrical circuitry. However, other hazards exist which are associated with ancillary materials used in laser studies as well as with the active laser media. These could be encountered either during manufacture of the equipment or during its use. With the increasing number of gas, liquid and solid-state lasers available,[4] attention should be directed to hazards associated with materials used in laser systems and in irradiation studies, as well as to electrical and radiational hazards. The purpose of this report, therefore, is to provide information regarding the hazards associated with such materials.

In this report,† elements and compounds presently used either in the manufacture of lasers or in conjunction with their operation have been separated into six groups. The scope of each group extends from the relatively innocuous to the extremely hazardous. The majority of these substances might not be considered dangerous when used under proper ventilation or in the usual small quantities. However, it is conceivable that dangerous levels might be reached during manufacture of the laser unit, if the system were to leak, or in the manufacture or burning of materials used in laser studies.

Operating hazards are generally acute (single exposure) whereas manufacturing hazards are acute or chronic, requiring repeated exposures over days or years.

For discussion purposes, the elements or compounds involved have been broken down into six categories, namely:

- Group I – Relatively Innocuous Materials
- Group II – Inflammable or Explosive Substances
- Group III – Irritants to Ocular or Respiratory Tract
- Group IV – Anoxial Agents
- Group V – Protein Poisons
- Group VI – Materials Injurious to Hematopoietic Systems or Livers

Where possible, materials are treated in increasing order of hazardousness. These listings are followed by TLV (threshold limit value).[5,6] Compounds with a systemic effect have a listing of the usual mode of entry into the body (inhalation, ingestion, percutaneous).

Group I—Relatively Innocuous Materials

Argon (Ar), helium (He), xenon (Xe), krypton (Kr), neon (Ne), nitrogen (N), carbon dioxide (CO_2), sulfur hexafluoride (SF_6), FC-25, FC-75, magnesium (as Mg^{++}), aluminum oxide (Al_2O_3), and gallium (Ga).

Argon, helium, xenon, krypton, and neon are generally innocuous—that is, when the concentration in air reaches 50 percent there is air hunger. At a concentration of 75 percent concentration any of these gases is fatal by simple suffocation.[7] Sulfur hexafluoride is also pharmacologically inert when pure[5] and is listed as a simple asphyxiant.[5] Nitrogen exerts a narcotic action when present at pressures above several atmospheres.[6] The TLV for carbon dioxide is 5,000 ppm.[7] The respiratory center is stimulated at 50,000 ppm. At several hundred thousand ppm death results. A fluorinated hydrocarbon, FC-25—used as a high dielectric strength coolant in some lasers—is pharmacologically inert.

Magnesium is generally an innocuous cation, although upon repeated exposure to levels above 15 mg/cubic meter some febrile reactions were noted. Similarly, repeated inhalation of aluminum oxide in concentrations above 50 mppcf (millions of particles per cubic foot) did produce nodular silicosis.[5] Gallium (Ga) has a low order of toxicity.[5]

Group II—Inflammable or Explosive Substances

Acetone, nitrobenzene, toluene, xylene, benzene, deuterated benzene, methyl, ethyl and propyl alcohols; pyridine, cyclohexane, naphthalene dust, deuterium, hydrogen, oxygen, ethyl ether (ether), iodine vapor with ammonia or acetylene, ozone, styrofoam or similar plastic substances when used with liquid nitrogen or liquid helium, liquid N_2—in open containers.

The explosive and inflammable nature of these substances are well documented.[5-8] Benzene and cyclohexane react with oxidizing agents and burn with acrid fumes. When heated, pyridine emits cyanide fumes.[7] Naphthalene dust (and presumably 1 bromo-naphthalene) is moderately dangerous when exposed to flames.[7] Ozone explodes when shocked, on exposure to a flame or to reducing agents.[7] The lower series of alcohols are flammable. When styrofoam or similar plastic foams are used as insulating materials with cryogenic substances such as liquid helium or liquid nitrogen, the oxygen in the entrapped air becomes a liquid. This liquid oxygen in intimate contact with the plastic creates a potential bomb.[7] Hydrogen or deuterium at levels of 4 to 74 percent is highly explosive. Liquid nitrogen in open containers will become contaminated with O_2 from the air—acquiring a bluish color. This mixture is potentially explosive.[3]

Group III—Irritants to Ocular or Respiratory Tract

The rare earths: dysprosium (Dy), erbium (Er), europium (Eu), gadolinium (Gd), holmium (Ho), lanthanum (La), neodymium (Nd), praesodymium (Pr), samarium (Sm), thulium (Tm), terbium (Tb), yttrium (Y), ytterbium (Yb); methyl alcohol, aluminum oxide dust, barium compounds, boron trifluoride, ethyl alcohol, acetone, molybdenum, silica, silicon carbide, styrene monomer, tellurium, acrylates, teflon, toluene, xylene, sulfur, acrylonitriles, zinc oxide, vinylidine chloride, piperdine when heated, tin, TTA plastics, ozone, sulfur oxides, nitrogen oxides, chloroform, formaldehyde, acrylonitriles, pyridine, chromium, antimony, ammonia, beryllium from fire-brick, nickel, phosphine, stibine, selenium, bromine, chlorine, iodine, hydrofluoric acid.

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†Supported by Contracts DA-49-193-MD-2436, 2437, Surgical Research Branch, Med. Res. and Dev. Command, Office of the Surgeon General, Dept. of the Army. NASA Grant NGR 22-011-007.

The rare earths—dysprosium, erbium, europium, gadolinium, holmium, lanthanum, neodymium, praesodymium, samarium, thulium, terbium, yttrium, ytterbium—used in solid-state lasers can cause a high degree of conjunctivitis and can cause opacities following contact with the abraded cornea. When injected into experimental animals these elements produced fatty liver and human exposures to vapors have produced itching and conjunctivitis.[10]

The threshold level value (TLV) for aluminum oxide dust is 50 mppcf. Extensive exposure to this dust has produced nodular silicosis.[7] Inhalation of barium compounds produces dermal and nasal irritation.[5] Boron trifluoride (TLV 1 ppm) in air, can cause pneumonitis. The TLC (threshold limit concentration) for ethyl alcohol is 1,000 ppm. At 1,000 ppm, acetone causes respiratory tract irritation, conjunctivitis and corneal erosion; it is also a central nervous depressant at this level.[5] Molybdenum produces transient respiratory irritation at 5 mg/cubic meter for the soluble compounds and at 15 mg/cubic meter for the insoluble ones. Silica dust produces silicosis at levels exceeding 20 mppcf (amorphous).

Styrene monomer produces conjunctivitis and irritation of the respiratory tract.[5,6] Tellurium TLV is 0.1 mg/cubic meter. When this level is exceeded pulmonary irritation can be produced.[5] The acrylates (thermoplastics) produce irritation to the skin and eyes.[5] Teflon (tetrafluoroethylene) has a TLV of 0.05 ppm in air. When heated to over 400°C, it produces pulmonary edema and "polymer fume fever." Toluene and xylene at levels over 200 ppm produce dermatitis, bronchitis and pneumonitis, conjunctivitis and corneal burns.[5] Sulfur produces similar irritations. Thiopneumoconiosis has also been reported.[5] The TLV for zinc oxide is 5 mg/cubic meter in air. Inhalation of concentrations exceeding this level produce a release of endogenous pyrogens from leucocytes, or the release of a histamine-like substance from the alveolus of the lung or the formation of a metal proteinate causing "metal fume fever," or an irritation of the upper respiratory tract.[5]

Vinylidine chloride, a plastic (TLV 25 ppm in air), can produce irritation on inhalation. When piperidine is heated to decomposition, it yields caustic nitrogen oxide fumes.[7] Tin oxides produce slight cough or dyspnea.[5] TTA plastics should be used under exhaust ventilation. Exposure leads to dermatitis and sensitization.[11] The TLV for ozone is 0.1 ppm.[2] A high concentration causes irritation of the eyes, pneumonia and pulmonary edema. The symptoms may persist for 6 months[5] and can be lethal in a few minutes in concentrations over 1,700 ppm.[12] Ozone is produced by silent electrical discharge and by intense ultraviolet radiation and is a powerful oxidizing agent.[5] Sulfur oxides (TLV 5 ppm) are corrosive, forming sulfuric acid when in intimate contact with moist tissues.[5]

Acrylonitriles cause dermatitis and can cause death from cyanide release.[5] The TLV for acrylonitriles is 20 ppm. Pyridine (TLV 5 ppm) acts as an irritant on inhalation or ingestion causing skin erythema, asthmatic breathing, and paralysis of eye muscles and vocal cords.[5] Chromium and its compounds (TLV 0.1 mg/cubic meter in air) is toxic by inhalation, ingestion or percutaneous absorption.[9] The hexavalent salt is most toxic; it can result in bronchogenic carcinoma. This form of chromium is often encountered in chromic acid cleaning solution. Antimony (TLV 0.5 mg/cubic meter in air) acts as a local irritant; liver damage and pneumoconiosis has been reported.[5] Ammonia (TLV 100 ppm) causes respiratory tract irritation, salivation and bronchial irritation. It is rapidly fatal at 5,000 to 10,000 ppm.[7]

Inhalation of beryllium fumes arising from fire-brick stops used with lasers is dangerous. It can cause pneumonitis, pulmonary fibrosis and granulomatous changes in skin, lungs, liver and spleen.[8] Nickel (TLV 0.5 mg/cubic meter in air) is absorbed by inhalation and acts as an irritant to the respiratory tract.[5] It can act as a carcinogen. Phosphine and stibine are poisonous, volatile compounds of phosphorus and antimony, respectively.

Selenium and compounds (TLV 0.1 mg/cubic meter in air) are toxic by inhalation, ingestion or percutaneous absorption. They are irritants which interfere with enzyme systems. Bromine, chlorine and iodine are irritants to the respiratory tract. This group's action may be looked upon as a reaction with the water of the tissues or from the corresponding acid such as hydrobromic, hydrochloric, and hydroiodic acid. Involved in protein destruction is, therefore, not only the initial heat of reaction, but also the production of a concentrated acid in intimate contact with tissue. Hydrofluoric acid is violently corrosive.[12]

Group IV—Anoxial Agents

Carbon monoxide, cyanides, sulfides, acrylonitriles, silicones heated above 200°C.

The specific anoxial poisons include carbon monoxide (TLV 100 ppm) which causes chemical asphyxiation by producing stable compounds with the hemoglobin of the RBC, thus reducing the oxygen carrying capacity of these cells. The greater affinity of carbon monoxide for the hemoglobin can result in slight oxygen starvation or at higher concentrations nearly instant death. There is considerable controversy concerning chronic effects of carbon monoxide.

Cyanides (TLV 5 mg/cubic meter in air) or hydrocyanic acid (10 ppm in Air) cause nausea and possible convulsions and death by cytotoxic anoxia.[5] The anion reacts within the cell preventing the utilization of oxygen. The compounds can enter the body by inhalation or absorption through the skin, as well as by ingestion.

Hydrogen sulfide or any sulfides in an acid medium (TLV 20 ppm in air) cause initial irritation of the respiratory tract following inhalation.[5] At concentrations of 600 ppm, death ensues in 30 minutes from the systemic effect of anion.[5] In lesser concentration, effects such as weakening of the cardiac muscle and peripheral neuritis are listed.[5] The rotten-egg odor of this gas prevents it from being a more common asphyxiant.

The plastic substances, acrylonitriles, have a TLV of 20 ppm. Their action is that of the cyanide anion.[5] They can be readily absorbed through intact skin.[13] Silicones decompose at temperatures over 200°C, producing carbon monoxide.[5]

Group V—Protein Poisons

Manganese (Mn) and its compounds, indium (In), antimony (Sb) and compounds, barium (Ba) and compounds, nickel compounds, lead (Pb) and compounds, arsenic (As), mercury (Hg), selenium (Se) and compounds, tellurium (Te) and compounds.

Manganese and its compounds (TLV 5 mg/m³) absorbed by inhalation or ingestion can cause permanent damage to the central nervous system, particularly to the extrapyramidal motor system.[5,5]

Indium has shown to be toxic when injected into animals resulting in degenerative changes in heart and liver,[13] but as yet no effect in human beings has been reported.[5]

Antimony and its compounds (TLV 0.5 mg/m^3) are absorbed by ingestion or inhalation.[5] The toxicity by inhalation can produce parenchymatous myocardial degeneration and death from thrombosis.[5]

Barium and compounds (TLV 0.5 mg/m^3 in air), can be absorbed by inhalation or absorption. Soluble barium compounds are irritants and can produce cyanosis, convulsions and paralysis.[5] Nickel compounds have caused some mental aberrations.[14] Soluble nickel compounds[6] have a TLV of 0.5 mg/m^3. Nickel carbonyl is a caustic fuming compound with a TLV of 0.05 ppm. In fatal cases, brain damage and lung hemorrhages were found:[6] absorption is via inhalation or percutaneously. Lead and lead compounds have a TLV of 0.2 mg/m^3 in air. The absorption routes are by inhalation or ingestion. They act by interfering with enzyme systems causing neuromuscular and central nervous system dysfunction.[5] Arsenic (TLV 0.5 mg/m^3) and its volatile form, arsine, (TLV 0.05 mg/m^3) is absorbed by inhalation, ingestion or percutaneously. The metal combines with the sulfhydryl groups of tissues. Arsine is produced by the action of dilute sulfuric or hydrochloric acids coming into contact with arsenic-bearing metals. Changes in nerves, liver and bone marrow may be permanent or fatal.[5]

Mercury metal (TLV 0.1 mg/m^3 in air) at room temperature has a vapor pressure 200 times the maximum allowable concentration.[5] Absorption is by inhalation, ingestion or percutaneously.[5] It produces dermatitis, constriction of visual fields, atrophic lesion of cerebral cortex and cerebellum and liver damage.[5]

Selenium compounds (TLV 0.1 mg/m^3 in air) are absorbed via inhalation, ingestion or percutaneously. Selenium combines with sulfhydryl groups thereby interfering with enzyme systems.[5] An especially dangerous selenium compound is selenium oxychloride, a fuming liquid.

Tellurium (TLV 0.1 mg/m^3 in air) causes red blood cell destruction.[5] Hepatic necrosis has been reported as well as epithelial tubular damage to kidneys, on inhalation or ingestion. Tellurium is also absorbed through the skin.[4]

Group VI—Materials Injurious to Hematopoietic System or Liver

Cyclohexane, toluene, nitrobenzene, xylene, toluidine, hydrofluoric acid.

Cyclohexane (TLV 400 ppm in air) results in weight loss and damage to the hematopoietic system at high chronic levels.[5] The route of absorption is inhalation; this compound is also listed as an irritant and depressant for the CNS.[5]

Toluene (TLV 200 ppm in air) is absorbed by inhalation.[5] It is an irritant, a central nervous system depressant and causes damage to liver. Nitrobenzene (TLV 1 ppm) is fatal to guinea pigs in three hours at 5,000 ppm.[5] Absorption by inhalation, ingestion and percutaneously causes hemolytic anemia and yellow atrophy of liver.[5] It is a depressant for cardiac and smooth muscle as well as for the central nervous system.

Xylene and all isomers—ortho, meta and para—(TLV 200 ppm) are absorbed by inhalation or percutaneously.[5] It is an irritant and depressant to the central nervous system and is a source of possible damage to liver and kidneys. Ortho, meta and para toluidines (TLV 5 ppm) are absorbed by inhalation, ingestion or percutaneously. They are depressant for the CNS and cause formation of methemoglobin.[5] Arsine and stibine are hemolytic substances injurious to liver and kidneys.[1,5]

Hydrofluoric acid (TLV 3 ppm in air) besides being extremely caustic, can cause nephritis and degenerative changes in the liver.[5] It is absorbed by inhalation or ingestion.

Extent of the Problem . . . and Some Recommended Saftey Procedures

The purpose of this paper is to alert, rather than to alarm. It is obvious that the majority of substances listed are only remotely hazardous when encountered in the low levels used at present. Nevertheless, attention should be directed to any potential source of danger.

The frequency of accidents resulting from any of these substances could be lowered by a program of education. A safety engineer could be employed to assess and advise on areas of possible danger. Handbooks of industrial toxicology and laboratory safety[6-9,11,13,15] could be made an integral part of the laser laboratory.

New employees could be given data concerning the nature of any dangerous items with which they might come in contact, plus methods for appropriate first aid treatment. Similarly, the nature of dangerous compounds and appropriate first aid measures could be attached to each new container.[9] Color and number codings could be given chemicals as suggested by the National Fire Protective Association.[12]

In short, if all possible chemical hazards in each laser laboratory area were sought out, assessed, appropriate measures taken and a program of education followed, hazards resulting from these non-electrical and non-radiational sources could be eliminated.

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ANALYSIS OF THE EFFECTS OF 10.6 MICRON RADIATION ON SOME MATERIALS COMMONLY FOUND IN CO_2 LASER PROTECTIVE DEVICES

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Supported by contracts DA-49-193-MD-2436, 2437 and 2680, Surgical Research Branch, U.S. Army Research and Development Command, Office of the Surgeon General, U.S. Department of the Army; and in part by 1-R01-00361-RAD, Center for Radiological Health, United States Public Health Service

ACKNOWLEDGMENT

The author wishes to thank Dr.Ben S.Fine, Armed Forces Institute of Pathology, George Washington University; W.Peter Hansen, Northeastern University; Don MacKeen, Northeastern University, for assistance and information regarding the above studies. The secretarial assistance of Mary Laananen and Evelina Gorman is gratefully acknowledged.

Analysis of the Effects of 10.6 Micron Radiation on Some Materials Commonly Found in CO_2 Laser Protective Devices

Studies have been carried out on the hazards associated with the 10.6 μ radiation emitted by a CO_2 gas laser (1,2). Although radiation at this wavelength can cause severe skin burns and may ignite clothing and hair, injury to the eye is of primary concern, especially injury to the cornea which is the initial site of interaction with the eye (2). Injury to the cornea may heal by the formation of opaque scars, which can impede vision. Injury to the lens, without corneal perforation, has also been observed following 10.6 μ irradiation of the eye (2).

Protective devices which have not been specifically designed for use against radiation at 10.6 μ have been used by many investigators in the laboratory[1]. These devices include celluiose acetate goggles and face masks, and welder's goggles and masks with glass inserts. When the expected hazard is due to low power density radiation, some investigators rely on eyeglasses for protection.

This report contains the results of an examination of the materials contained in the above devices. This examination was performed to determine the degree of protection these materials furnish.

The examination of protective materials would have been considerably simplified if it had been possible to prepare a definitive list of the required properties of an adequate safety device. Unfortunately, a list of this type is difficult to prepare since the device could be employed under unforseeable circumstances, including unusual temperature and humidity conditions. There are some properties, however, which must be included in any such list.

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[1] Since this paper was initiated, a CO_2 laser safety goggle has become available from the American Optical Company. A consideration of this device is given at the end of this report.

These are:

a) The protective material should not transmit intensities of CO_2 laser radiation that are injurious to the underlying region, i.e., the absorption coefficient should be high at 10.6 μ[2].

b) Materials which reflect radiation in sufficient power densities to be a hazard at 10.6 μ should not be exposed to the laser beam[3].

c) Any intense visible light generated from the protective materials which may constitute a hazard should be prevented from reaching the eye.

d) The materials employed should give an indication of being irradiated as a warning to the wearer before protection fails.

e) Materials which emit dangerous or unpleasant substances when irradiated should be avoided except when the emission is used as a warning (see (d) above).

f) The protection time should be sufficiently long[4].

g) The material should give an indication if its protectiveness becomes inadequate under either use or storage.

h) The device should be comfortable, and not awkward.

i) The device should not impede communication.

j) The device should neither impede vision nor fog under normal use.

k) The device should protect at all angles of incidence.

l) Any destructive or hazardous reaction in the device initiated by CO_2 laser irradiation should stop when the irradiation ceases.

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[2] The power densities necessary to cause long or short term threshold ocular injury are under investigation. However, materials of reasonable thickness are presently available which transmit virtually no radiation at 10.6 microns. Some of these materials may initially be employed in safety devices.

[3] e.g. many metals are highly reflective at 10.6 microns, (16).

[4] This time must be much longer than the human reaction time, usually taken as 0.1 sec. (A safety factor of 100 is considered minimal).

Some of the safety devices presently employed have been studied with regard to the above specifications. For example, corrective glasses have metal rims or hinges which reflect the radiation. They do not protect more than the eye area, do not offer protection at all angles of incidence and may fog. Face masks usually protect the face and often the front of the scalp, but sometimes have exposed metal parts, are usually awkward, and impede both communication and peripheral vision.

Cellulose acetate goggles do not protect the hair and lower face, usually impede or distort vision, and may be uncomfortable.

Welder's goggles and masks often have heavily tinted glass inserts which impede vision. The goggles protect only the eye area; the masks are usually awkward, uncomfortable, and impede communication. The masks generally have large opaque areas which reduce the visual field.

Thus, it is apparent that each of the above devices, when used specifically for protection against CO_2 laser radiation, has one or more inherent disadvantages. However, these disadvantages do not preclude the use of these devices until a more specialized safety device is constructed, as long as the prospective hazards and disadvantages are recognized and adequate preparations are made to minimize them.

A complete investigation of a safety device for CO_2 laser radiation must include a consideration of the interaction of the radiation with both the transparent and opaque materials within the device. Most of the materials employed in safety devices (e.g., cellulose acetate, fiberglass, rubber) interact with 10.6 μ radiation in a relatively simple manner by burning or melting. Some of these materials have been investigated and the results are presented below. Glass however does not react in a simple manner. It is well known that glass may fracture if heated non-uniformly, without showing evidence of melting or burning. Considerable attention was therefore directed towards interaction of CO_2 laser radiation with glass. Radiation at 10.6 μ is absorbed essentially at the surface of most types of glass. When irradiated by a CO_2 laser, there are three ways in which the surface of a glass plate may be non-uniformly irradiated:

1) the laser beam may be non-uniform.

2) the beam may be smaller than the exposed glass, or, if the beam is larger, only a portion of the glass may be irradiated.

3) a portion of the glass in the safety device may be covered by other material.

Since the glass portion of most safety devices is supported by opaque material which covers the edges of the glass, non-uniform irradiation of the surface is the rule rather than the exception.

When glass is heated non-uniformly, thermal gradients result. These gradients introduce thermal stresses (8) which may lead to fracture (11,12). This type of fracture in glass is somewhat different from the types usually reported in the literature. Most of the existing literature on the fracture of glass is directed towards one or more of the following areas:

1) The interaction of glass with pulsed lasers which emit radiation at 6943A or 1.06 μ (4,5,14).

2) The reaction of glass to mechanical shock after specified uniform thermal treatment (7,9).

3) The mechanical flaws which contribute to glass fracture (3,6,11,12).

The first and second areas apply only marginally to C.W., CO_2 gas laser irradiation. The reported studies in the third area indicate that experimentation on glass fracture will lead, at best, to general results which are for the most part qualitative. An important conclusion which is drawn from the reports in the third area is that one of the most significant factors which influence the fracture of glass under any type of stress is the presence of microscopic flaws at the surface of the glass. These flaws may be caused either by the way in which the glass has been worked (i.e., polishing, grinding) or, in many instances, by the way the glass is normally handled in the laboratory. It is expected, then, that if the glass is not subject to special care, similar glass samples, tested in similar ways, may behave very differently. In general, we have found this to be the case. However, we have found that there were general recommendations that could be stated if glass was used as a part of a safety device. These recommendations are presented towards the end of this report.

Initial testing was carried out on crown glass microscope slides because they are similar in size and chemical structure, and new slides from the same package probably have similar histories with regard to production and handling. It was expected that these similarities would minimize the spread of the data.

In preliminary studies on microscope slides it was noted that when held by metal clamps, the glass would often fracture along a path from the irradiation site to the clamp, irrespective of near edges. However, when held by wax, samples of glass would usually fracture along a path that went from the irradiation site to the nearest two edges. The latter type of fracture was also noted when the glass was supported by string. Therefore, it was assumed that the metal clamp was grossly affecting the experiment and for that reason wax was used to support all subsequent samples.

In the first set of tests, the power density incident on the glass sample was held fixed at $20 w/cm^2$ while the diameter of the irradiating beam was varied. Table 1 summarizes the results on 1" x 3" x 1/25" microscope slides when the irradiation zone was centered at the center of the surface of the slide.

From the table, it is evident that the glass fractured more quickly as the diameter of the irradiation site increased. It was also observed that the samples always fractured across the 1" dimension, i.e., from the irradiation site to the nearest edge.

When 2" x 3" x 1/25" slides were irradiated, the slides were again observed to fracture more quickly as the irradiation diameter increased. Similar results were obtained on subsequent tests on protective glass, such as filter lens by Jackson products (4 1/2" x 5 1/4" x 1/8") filterweld plate by the American Optical Company (4 1/2" x 2" x 3/32") and (4 1/2" x 5 1/4" x 1/8") and AIRCO lenses 1 15/16" in diameter x 1/8" thick. Noting that the irradiation time necessary to fracture a sample increased as the spot size decreased, the spot diameter was reduced still further. A spot size was found at which the sample did not fracture during ten minutes of irradiation. In addition, smaller spot sizes would not fracture the glass within 10 minutes. The power was then varied and at each power, a spot size was found below which the sample would not fracture for long irradiation intervals. The above experiments were repeated with the irradiation site close to an edge of the samples; all samples that were irradiated in a manner which would previously fracture the glass, were observed to fracture in a shorter time interval. Furthermore, it was observed that at some power densities and spot diameters for which no effect was observed on central irradiation, the sample fractured when irradiated near an edge.

Although in all the above experiments, there were no alterations of the irradiated glass sample which could be seen on direct visual examination until fracture occurred; at higher power densities (of the order of 100 w/cm^2) the glass was observed to alter at the irradiation site. This was first noted as a distortion of objects seen through the sample. At these higher power densities, the glass did not fracture on irradiation, unless the irradiation site was very close to an edge. However, the sample frequently cracked on cooling. In general, it was found that for a given irradiation site and spot diameter, samples which would fracture at power densities insufficient to cause observable change previous to fracture, would either not fracture at higher power densities or would take longer to fracture at higher power densities. However, the glass which did not fracture on irradiation at the higher power densities would frequently crack on cooling.

The observed changes in the samples which were irradiated at the higher power densities indicate that the glass within the altered region had reached a temperature beyond its annealing point. This annealing temperature is dependent primarily on the composition of the glass.

When glass has been heated to beyond the annealing point, all thermal strain within the annealed region is relieved, but permanent residual strain is formed when the glass subsequently cools. For a given ambient temperature, this residual strain increases as the temperature reached beyond the annealing point increases (12). It appears that sufficient permanent strain had been instituted to crack the cooling glass in some cases, but not in others. The samples that reached a lower annealing temperature on irradiation at lower power densities did not usually crack while those irradiated at the higher power densities usually did.

When the annealed samples that had not cracked on cooling were examined, a distortion was usually noted when objects were viewed through the irradiation site.

These samples usually exploded violently after a short period if re-irradiated near the old irradiation site, even at power densities that would not normally fracture the sample. In particular, normal eyeglass lenses were markedly explosive under these conditions.

It is well-known that thermal strain induces stress patterns in glass samples during irradiation. Stress patterns due to residual strain can result post-irradiation if annealing had occurred. The development of these patterns was followed photographically and visually using polarized light. In all samples examined under polarized light, these stress lines were observed to form at the irradiation site and to expand in an outward direction. One of the following four processes was observed:

1) The lines eventually reached a stationary configuration. Direct observation of the sample gave no evidence that irradiation was taking place. The lines disappeared post-irradiation. No evidence of the irradiation could be detected in the sample after it had cooled.

2) The lines eventually reached a stationary configuration. The glass cracked during cooling. Residual lines were still present in the region surrounding the cracks which indicated that some residual stress was still present.

3) The lines reached a stationary configuration. The glass did not fracture on cooling, but the stress pattern did not altogether disappear when the glass cooled. This phenomenon was sometimes accompanied by an observable depression in the anterior surface of the glass after cooling.

4) The glass fractured during irradiation while the stress lines were expanding or after they appeared to become stationary.

The samples which did not lose all the induced stress post-irradiation were more likely to fracture or even to explode during re-irradiation.

Furthermore, it was observed that for those samples in which the stress lines were confined within the edges, the sample did not usually fracture on irradiation, while if the stress lines reached the edge, the sample was more likely to fracture. This observation indicated that the reason the smaller spot diameters and center irradiation experiments discussed earlier usually did not cause fracture, while the larger spots and edge irradiation did, was that in the latter case, the stress lines were more likely to reach the edges of the sample.

However, as mentioned above, some samples were observed to fracture during irradiation even though the stress lines were confined to well within the edges, and the irradiation site appeared to anneal. This phenomenon seems to be somewhat clarified by the observation that was noted earlier in this report, i.e., that the surface condition of the glass may strongly influence the result of irradiation. The samples that fractured under conditions that did not normally cause fracture may have had surface defects. Scratches were shown to result in fracture under irradiation conditions which would not normally cause fracture. Samples which did not fracture were irradiated for ~ 10 min. at an arbitrary power density which caused annealing at the irradiation site. A scratch was then made on the rear surface with a steel stylus without otherwise perturbing the experiment. The samples always fractured shortly after being scratched. Samples were also scratched prior to irradiation. At power densities that caused annealing, these samples invariably fractured.

At sufficiently high power densities, all glass samples were observed to emit a bright white light and a small jet of flame. The flame appeared to be emitted from both sides of thin samples (1/25"), and from the front only of thicker samples (1/8"). These emissions were accompanied by a fine powder or smoke. The light emissions were studied with regard to the possibility that they were intense enough to be hazardous.

Since the maximum power output of the CO_2 laser employed was 20 watts, studies on secondary light emission were carried out using a focused beam. When glass samples were placed at the focal point of an IRTRAN II lens, an intense white light was produced from the irradiation site. This light was painful to observe at a distance of 3 feet from the source, and appeared to result in temporary impairment of visual acuity and persistent after-images (scotomatic glare) in a portion of the visual field. As expected, clear glass appeared equally bright when viewed from front or back; tinted glass transmitted less light as the optical density increased. Only a dull red glow was observed at the back when shade #6 glass[5] was irradiated under the above conditions.

[5]Optical density between 1.93 and 2.36, transmission peak in the green.

There appear to be hazards associated with this source of intense visible radiation. If this source were near the eye, as in a normal protective device, an appreciable fraction of the light would be transmitted through the glass (unless adequately filtered) and enter the pupil. This might result in a hazardous power density at the retina. Kohtiao, et al., (15) have reported findings which indicate that a retinal power density of 54 w/cm^2 incident on the retina due to direct viewing of the sun through a 3mm pupillary aperture is hazardous. Lesions have been observed in retinal studies on people who have given a history of direct viewing of the sun (17).

To determine the power density on the retina caused by the emission from glass, the following procedures were carried out.

The laser beam was focused on the surface of a glass sample to a small spot size. This caused visible light to be emitted. A magnified image of this secondary source was obtained and the size of the source was determined to be 0.2 mm. This was done by inserting a lens and a screen behind the source. The lens formula was then utilized to find the source diameter when the image diameter was measured on the screen.

A 7 mm aperture was placed 2 cm behind the secondary source. This aperture was used to simulate the dark adapted pupillary aperture. The light passing through this aperture was collected by a lens and focused. The power at the focal point was 15 mw. The retinal power density produced by 15 mw entering the eye and focused to 0.2mm is approximately 50 w/cm^2. This power density is similar to that which has been indicated to be hazardous (15).

As previously mentioned, other protective materials have been considered. A 1/4" thick piece of cellulose acetate placed at the focal point of an IRTRAN II lens was penetrated in about 0.8 seconds by a 15 watt CO_2 beam. It smoked and appeared to melt, gave off an acrid odor, but emitted no evident visible light. At lower power densities, correspondingly longer times were required for penetration. For example, at about 10 w/cm^2, penetration occurred after 15 sec, with little or no odor or smoke. At lower power densities, the material melted and was slowly "ablated" from the surface in a manner that might not be observed unless the irradiation site were in direct view. This indicated that at low power densities, irradiation of this type of protective shield might not be noticed until after the protection failed. Fiberglass welders' masks acted in a similar way at low power densities but at high power density a flame appeared at the irradiated site.

RECOMMENDATIONS

If glass is used as part of a safety device, the following guidelines should be observed.

1) the dimensions of the glass should be large in comparison with the dimensions of the beam.

2) the edges of the glass should be covered for several centimeters by opaque material to prevent irradiation close to the edge of the glass.

3) the glass should be checked for stress by polarized light before being used.

4) care should be taken to minimize surface scratches and bruises by rough handling.

5) the glass should be sufficiently tinted (or a tinted back-up plate should be employed) to reduce both scotomatic glare and the possibility of retinal burns due to secondary emissions.

A safety goggle produced by the American Optical Company for use with CO_2 lasers was studied. It consists of 3 sections: (1) a mask consisting of a rubber frame supporting quartz (Vycor) and plastic plates (2) an adjustable plastic headband (3) two spring loaded metal connecting units between the mask and headband to support the mask. The transparent section of the mask is made of quartz plate and a plastic back-up plate with an air space between them.

The quartz was irradiated at all power densities, spot diameters, and locations which had fractured glass plates of similar dimensions. Although the anterior surface of the plate was scarred by high power densities ($\sim 10^3$ w/cm^2) fracture could not be induced. The sample remained intact even when the posterior surface was cooled with liquid freon while the anterior surface was being irradiated.

Observation under polarized light during irradiation yielded stress pattern formations identical to those found in glass under similar circumstances. Post-irradiation observations showed that residual stress was formed in the quartz but the patterns were always much smaller than those noted in glass, indicating that the stress was more localized than that found in glass. Although residual stress lines were present, re-irradiation of the quartz (Vycor) did not result in fracture.

Light emission under high power density irradiation was noticeably less than that observed with glass.

The rubber portion of the mask was then examined with regard to protection against CO_2 laser radiation. The material was a flexible rubber mold, reinforced with a metal strap to hold its shape. This metal is not normally exposed to the beam. The rubber smoked, emitted a strong odor, and was eventually penetrated when irradiated at the following power densities.

1) For a 1.5 cm irradiation diameter ($\sim$9.5 w/cm^2), the rubber was penetrated in 24 seconds.

2) For a 2.5 mm irradiation diameter ($\sim$565 w/cm^2), the rubber was penetrated in 5 seconds.

3) At best focus of the IRTRAN II lens, ($\sim$10,000 w/cm^2), 3 seconds elapsed before penetration.

The characteristics of the American Optical Company safety goggle can now be compared with the requirements for protective devices listed at the beginning of this report.

A) all material in the mask itself has a high absorption coefficient at 10.6 as required.

B) the metal connecting units located at the temples and used to support the mask are exposed to the beam and may be reflective at this wavelength. However, the hazards due to the reflected beam may be reduced by the shape of the reflecting surfaces.[6]

C) there is an untinted piece of plastic behind the quartz which together with the intervening air space acts as a heat insulator. This does not, however, affect the secondary light which is emitted from the quartz under high power density irradiation.

D) the rubber smokes and yields an odor under irradiation, affording a warning of irradiation; the quartz will exhibit a local change in index of refraction, and may glow, under sufficient power density, thus providing a warning. However, there will be no warning provided at low power density.

E) the smoke emitted from the irradiated rubber does not seem to contain hazardous materials.

F) although the quartz insert offers excellent protection for protracted periods of time, consideration should be given to increasing the protection time of the rubber frame.

G) the protection time provided by the Vycor (quartz) was adequate under the conditions of irradiation. However, the rubber charred and blistered and was penetrated under irradiation.

H) the device is quite comfortable, and not awkward.

I) there is no communication impediment.

J) "tunnel vision" only is possible. Redesign of the mask may permit extension of the "visual field".

K) the eye protection afforded by the quartz appears, under preliminary investigation, to be adequate for use with a 20 watt CO_2 laser. The rubber mask can be penetrated in 3 seconds with the highly

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[6] A preliminary investigation of the reflection of 10.6 μ radiation from a flat glass plate and a flat quartz plate was performed. Both samples were irradiated with an ~1.5 cm beam containing 12 watts power at an angle of incidence of 45°. No reflection was observed from the quartz plate using Thermofax paper. No heat was felt by an experimenter when he passed his hand through the air at the expected location of reflected radiation. In contrast, the flat glass plate was observed to reflect enough of the radiation to both darken the Thermofax paper and cause a sensation of heat. It is estimated that 0.2 - 0.5 w/cm^2 was reflected under the conditions described above from the glass plate.

focused beam and may not be adequate under all circumstance. Protection at all angles of incidence is assured by total enclosure of the protected area. The protected area is approximately that covered by a skin diver's facemask, leaving the rest of the head and hair unprotected which, as was previously noted, may represent a hazard.

L) all reaction in the material appears to stop when irradiation ceases. However, there is residual stress in the quartz.

SUMMARY

A consideration of glass, plastic, and quartz has been made with regard to their relative effectiveness as protection against CO_2 laser radiation. It is shown that both plastic and glass can be made ineffective as safety materials either by penetration of the beam, or by fracture upon irradiation with a 20 watt CO_2 laser.

Furthermore, glass can be stressed, with little or no visible indication, in such a way that it will explode violently under otherwise "safe" irradiation. Glass can undergo irradiation without giving visual indications which would otherwise serve as a warning. It may emit hazardous white light due to incandescence when irradiated by high power densities. It may reflect hazardous amounts of radiation.

Vycor, on the other hand, could not be made to fail under irradiation with a 20 watt CO_2 laser. It would not fracture nor be penetrated, nor retain sufficient stress to fracture upon re-irradiation at these power levels. It reflected less radiation than glass during a preliminary study. Although it would become incandescent under high power density irradiation, the emitted light was much less bright than that emitted from glass on similar irradiation.

A rubber mask, made to hold the quartz plate was also tested. It afforded some protection and considerable warning of irradiation by smoking and yielding an acrid odor. However, if is felt that this type mask should have its protection properties improved.

Cellulose acetate and fiberglass of the kind found in chemical goggles and welders' masks, respectively, acted much like the rubber discussed above.

TABLE I.

Occurrence of Fracture on Irradiation of 1" x 3" x 1/25" Microscope Slides at Constant Power Density With Various Irradiation Diameters

Power (watts)	Power Density (watts/cm^2)	Diameter (cm)	No. of Tests	Time Interval During Which Fracture Occurred (sec)	
12	20	0.9	5	7.4 - 18.5	8.1 sec(average)
7.7	20	0.7	10	8 - 15	10.6 "
5.6	20	0.6	10	15 - 29.6	21 "
3.2	20	0.55x0.4	6	27 - 64	47 "

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IMPLEMENTATION OF PROCEDURES AND TECHNIQUES FOR SAFE OPERATION OF LASERS

by

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The material that I am about to present is not my own alone. In its development, I have collaborated with five other people, one of whom is in attendance at this conference. My co-authors are:

1 Dr. Edmund Klein, Roswell Park Memorial Institute;

2 George R. Peacock, Northeastern University;

3 Dr. Ben S. Fine, George Washington University and Armed Forces Institute of Pathology;

4 W. Peter Hansen, Northeastern University;

5 Dr. Martin S. Litwin, Veterans Administration Hospital at West Roxbury, Mass., and Harvard Medical School at Boston.

The development of protective devices and safety measures in the laboratory and in the field are based on two different principles. In the laboratory, personnel can, in principle, be completely isolated from the laser system. In practice, however, one strives to reduce the probability of accidental exposure of personnel to the point where economic feasibility and certain practical problems are the limiting factors. The alternative to not providing a physically-safe environment and not establishing rigid safety rules is the occasion of accidents and their concomitant medico-legal costs. The problem of establishing safe field conditions reduces to one of providing individual protection. This type of protection is typified by safety clothing and goggles.

Consideration of the mechanisms of laser interaction with biological systems has led to a tentative outline of a program for the control of laser hazards and the management of laser accidents[1, 2]. This program is outlined as follows:

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1 S. Fine, et al, "Mechanisms and Control of Laser Hazards and Management of Accidents," Second Conference on Laser Technology (Illinois Institute of Technology), April 1965;

2 R. G. Daniels and B. Goldstein, "Lasers and Masers - Health Hazards and Their Control," Federation Proceedings, Supplement 14, January-February 1965.

Control of Laser Hazards

General Administrative Procedures

Personnel

Laser Hazard Control Officer

Deputy Laser Hazard Control Officer

Laboratory Laser Hazard Control Officer

Medical Supervision

Records

Laboratory Design and Operation

Protection Standards and Dosimetry

Management of Laser Accidents

A. CONTROL OF LASER HAZARDS

1. General Administrative Procedures

a. Personnel

(1) Laser Hazard Control Officer

The laser hazard control officer should be responsible for all factors associated with laser safety within the organization. He should be responsible only to senior management or the chief executive officer of the organization, and carry authority regarding laser safety that can be countermanded only by the chief executive officer.

He should have full records concerning all studies using lasers carried out within the organization, as well as information concerning personnel and the characteristics of the equipment. He should be responsible for monitoring laser equipment or associated experiments, assessing hazards to the extent feasible, and providing safeguards. That he may be able to assess potential hazards prior to their introduction, information regarding proposed experiments should be forwarded to him in writing.

He should provide information regarding the installation of new laser systems, and be present during their initial operation and any subsequent modifications of significance involving experiments in progress.

He should be responsible for providing instructions to all personnel associated with laser studies, and be notified of and responsive to any laser accident that may occur.

The laser hazard control officer may be a medical officer trained in laser hazard control, or an engineer interested in general safety with cognizance of laser hazards.

(2) Deputy Laser Hazard Control Officer

Since the laser hazard control officer may be absent or unavailable at times, a deputy laser hazard control officer should be appointed.

(3) Laboratory Laser Hazard Control Officer

Each laboratory area should have an individual designated as a safety officer to report to the laser hazard control officer on matters concerning laser safety. This safety officer should be responsible for day-to-day safety within the laboratory and the maintenance of adequate records regarding compliance with safety standards. The latter is important since, if no such records are kept regarding experiments, pertinent information related to an accident will not be available.

b. Medical Supervision

The purpose of medical supervision is to deal with the medical and medicolegal aspects of laser hazards. Since the long-term hazards are unknown, a balance must be achieved between possibly applicable procedures and those which can be considered practical.

Medical supervision should include an initial medical examination. The history should emphasize previous radiation exposures and aspects associated with the eyes and skin. The physical examination should give more than usual attention to the eyes and skin, and should be accompanied by laboratory studies. Follow-up studies should be carried out periodically probably at from three-month to one-year intervals and following an actual or suspected accident. The time interval will depend on the equipment used and the studies being carried out.

(1) Radiation Exposure History

A history should be obtained of previous exposures to laser radiation, to high intensity radiation in the radio frequency or microwave regions, and to ultraviolet, x-ray, gamma-ray, and nuclear radiation. Records of previous radiation exposure should be obtained.

(2) General Medical History

Particular attention should be paid to a previous history of injury, infection, inflammation of the eyes, or visual disturbances. Attention to the skin should include information regarding allergy, photosensitivity, previous surgical excisions, and abnormal wound healing.

(3) Physical Examination

The physical examination should include a complete ophthalmologic and dermatologic evaluation, carried out by persons with considerable experience in these areas. For example, the ophthalmologist must have knowledge of other types of radiation injury in addition to knowledge related to the effects of laser radiation.

The eye, of course, should be subjected to the usual tests: visual acuity, refraction, slit lamp examination, and careful ophthalmoloscopic examination under full mydriasis with both white and red free-light. Screening of the critical areas of the macula and fovea by either routine tangent screen examination or campimetry is too tedious and generally not practical. But a rapid method for screening this important central area, and one that may be carried out quickly and simply by a technician or trained aide, involves the use of an Amsler grid. Here, a negative screening adds to the strength of a negative routine type of examination. These special tests may be reserved for the evaluation of lesions detected by other means.

Fundus photography is probably not, in general, useful as a routine screening procedure unless an ophthalmoscopically-detectable lesion or other eye pathology is found. The photographic recording of this lesion may then be of considerable value. Fundus photography may, however, provide a baseline for evaluating the progression of any visible lesion that may develop. Evaluation of a foveal lesion should be done with great care, since eclipse burns frequently produce ophthalmoscopically-detectable lesions in the foveal area with no apparent defect in the central visual acuity.

In the dermatologic examination, particular attention should be directed toward the presence of keratoses and skin malignancies as well as benign dermatoses, particularly those related to photosensitization.

(4) Laboratory Studies

Laboratory studies should include a complete blood count, routine urineanalysis, and blood smear for medico-legal as well as medical purposes.

c. Records

Adequate records should be kept regarding personnel, characteristics of the laser facilities in the organization, operation of laser facilities, and accidents.

2. Laboratory Design and Operation

The attention that should be directed toward laboratory design and operation is dependent on the energy, power levels, and wavelengths used. If high-energy or high-power equipment is used, the operations area should be separated from the charging bank or power supply and from the laser output area. Under these circumstances, interlocks are required to prevent individuals from entering laser firing or capacitor bank areas when banks are being charged or units fired. Information concerning the laser beam interaction can be obtained through the use of closed-circuit television systems or still cameras focused on either the interaction or the meter measurements necessary in the beam area. A sign indicating that the area is a laser laboratory should be posted at the entrance to any such facility.

A dual set of visual signals that switch from green to red can be used to enhance safety during the operation of laser equipment. This enhances the reliability of the safety system, since one visual signal such as a light must be on and the other off at all times. With a single signal, it is unknown whether the laser is "off" or the light has burned out. In some cases, auditory signals may detract from rather than add to safety, particularly since dual auditory systems are impractical.

There should be sufficient isolation between various laser units in the same area to prevent the exposure of personnel to radiation from lasers other than the one with which they are working. Enclosure of the laser head, the irradiation site, and the space between them (when feasible) will assist in the reduction of hazards.

To minimize pupil diameter and consequent light absorption by the eye, the room should be well lit. Painting or coating of the walls with a material that resists fire and absorbs at laser wavelengths will be of value. Full reliance should not be placed on laser glasses during operation, since the interaction of the beam with material may result in backscattered radiation at wavelengths other than those of the incident radiation, for which the attenuation factor of the glasses may be low. This is particularly significant: the effects of long-term cumulative radiation exposure are unknown. Another factor that limits the effectiveness of glasses is the way radiation is scattered in no mathematically-predictable manner but rather, in some instances, predominantly in a specific direction. Glasses that have been exposed directly to unusually-high-intensity radiation should be discarded, since their effectiveness may have been degraded without producing obvious gross defects; for instance, we have observed delayed spallation of goggle filters. Consequently, not looking at the beam or back-scattered radiation is preferable to depending on glasses. A good safeguard is to follow a count-down procedure during the operation of the laser.

Because the beam is invisible at ultraviolet and infrared wavelengths[3], the problem is accentuated. Either the beam should be enclosed or the area so arranged that a person can neither pass through the direct beam, or be exposed to scattered radiation. The solution may be to use shielding. In this event, of course, the area should be monitored before and after shielding to determine the radiation distribution. This problem is particularly acute with continuous, high-power lasers, since constant monitoring of the area by an individual throughout the on-time of the beam is impractical. With semiconductor lasers, the problem of strong off-axis peaks must be recognized. In both these cases, monitoring is required to determine the radiation levels in all regions of the room.

Although definitive information concerning hazards associated with the capacitor discharge system is lacking, sufficient data have been accumulated on electrical

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3 Fine, S., Kline, E., Litwin, M., Peacock, G., Hamar, M. Hansen, W. P., "Biological Effects of High Power Continuous N_2 - CO_2 Laser Radiation at 10.6 Microns", Federation Proceedings, Vol. 25, No. 2., Part I, March-April, 1966.

hazards to indicate the marked dangers inherent in the high-energy system. Even though interlocks and discharge resistors on the capacitor bank are used, it is important to note that time constants are involved and that the discharge system may fail. Manual discharge through a grounding rod should therefore be used as a back-up safety measure prior to contact with the capacitor bank. Such grounding rods, with visible grounding, should be associated with each bank.

In addition to the foregoing, special consideration should be given to the choice of original cables. The cable chosen should be specified as corona-free in addition to having adequate dielectric stength for the laser with which it is to be used. Although actual observation of the cable and continuity tests are very important, the single most-important test is the "hi-pot" or dielectric strength test. These tests can be made with readily-available testing apparatus or the cable manufacturer's testing facility. The dielectric tests will indicate the present strength of the insulation and the corona test the presence of corona. If corona is present, it causes ozone, which is highly corrosive to some dielectrics and may cause ultimate failure of the cable. To be on the safe side, a cable that shows the presence of corona should be replaced.

Since the cables between the power supply and laser head carry high current at high voltage, they should be placed so that accidental contact with them cannot occur. Equipment such as oscilloscopes should be placed so the observer will not have to turn or face toward the beam when the bank is being charged or the laser fired. Meters for measuring high voltages, and oscilloscopes associated with the laser equipment and experiments, should be so arranged and protected as to present minimal hazards to the investigators.

The placement of firing buttons must be sufficiently remote from the charge and hold buttons to preclude the accidental or inadvertent discharge of the laser. Unless automatic recharge of the capacitor bank is required for experimental purposes, charging should be under manual control.

Capacitor discharge may occur through other than the usual discharge circuit, including that due to capacitor breakdown. Hazards associated with this can be minimized by the use of distance and mechanical shielding between the operator and the bank. Discharge of a bank due to capacitor breakdown may be accompanied by a loud sound. This can be minimized by sectionalizing the bank and by sound-damping techniques.

There are three possible problems relative to interlocks:

1 No interlocks on the equipment;

2 Interlocks which do not have a manual override as part of their design;

3 Interlocks which are automatically reset on closure of cabinets or equivalent equipment following disablement for repairs; that is, interlocks with a built-in manual override.

Having no interlocks on the equipment is probably undesirable. Yet, it must be conceded that in this event no reliance will be placed on safety features which could malfunction and leave a bank dangerous at any time.

Interlocks without a manual override must be cheated or jumped to allow for servicing. If the jumper is not removed, an undesirably hazardous condition is introduced. An interlock with a built-in manual override that automatically resets on closure is the type needed in all laser systems. It permits service personnel to troubleshoot the equipment with the door unlocked, or experiments to be carried out where necessary.

Safety regulations associated with laser systems are dependent on the type of the system used and the environment in which it is located. The degree of precaution necessary will depend on a number of factors, including wavelength, continuous or pulsed operation, energy and power available from the laser, and atmospheric conditions. With pulsed, single-shot systems, the unit is essentially safe except when charged (unless a charge has been left on the capacitor bank). The individuals associated with the pulsed laser system must be instructed to charge the capacitor bank, or equivalent system, only when the laser is to be used, and to fire it immediately or discharge it promptly and completely. Under no circumstances must they undertake other tasks while charging and firing of the laser. They must not answer telephones, carry on discussions, or engage in similar tasks at such times.

Since the hazard with pulsed lasers is present only when the bank is being charged and the laser fired, the type of hazard for pulsed, single-shot operation is similar for lasers at all wavelengths in the ultraviolet, visible, and infrared regions.

High PRF and continuous lasers pose common problems, since they may operate for considerable periods of time without sufficient attention being directed toward the prevention of individuals entering the area and the location of individuals within the area. This problem is particularly acute with lasers operating in the ultraviolet and infrared, or invisible, portions of the spectrum. Under these circumstances, the laser area must be considered hazardous at all times – in a manner similar to that of an area containing radioactivity.

As well as the usual room-entry interlock techniques, a second set of barriers similar to those for radioactive or high voltage areas can be used. These barriers may consist of a white tape barrier, or its equivalent, in conjunction with appropriate signs at the same distance. These will act as a second precautionary barrier, thus limiting accidental exposure. A monitoring approach similar to that used for radioactivity can be employed for continuous, or high PRF lasers, particularly for those operating in the invisible portion of the spectrum. This approach can consist of sufficiently-sensitive area detectors appropriately located at the barrier region, and hand-held survey detectors which can be used to monitor and measure the radiation present within an area. The detectors should have the required sensitivity over the wavelength band of interest, and be capable of discriminating against the background radiation present.

For CO_2 lasers, thermofax paper is changed to brown at energy densities of the order of 1 joule/cm^2. Thermocouples will readily detect milliwatts/cm^2, and thermopiles microwatts/cm^2.

Protective face shields should be located outside the entrance to these areas, particularly where continuous or high-PRF units are used in the ultraviolet or infrared region. These protective face shields should be worn by all personnel within the area and should be returned to the shield rack upon leaving.

Consideration can be given to color coding and signs at entrance ways to indicate the class of laser being used. This would keep individuals generally associated with these systems, but not directly involved, aware of the types of units being used.

With high-power, continuous lasers such as the CO_2, fire resistant material is mandatory. Asbestos sheet or cloth has been found suitable for rapidly and flexibly shielding the walls in areas not completely fireproof. The use of several asbestos sheets will permit the top sheet to be replaced after it has burned through, before the underlying sheet is damaged, thus limiting the fire hazard. The presence of flammable material must also be restricted.

Figure D-1 shows a focused CO_2 laser beam in the process of igniting cloth. The ignited cloth continues to burn, thus bringing up the question of whether one should wear a lab coat or its equivalent which can be rapidly unfastened. The advantage in wearing a lab coat is that, should it catch fire, it can be removed more readily than standard street clothing. Extinguishers capable of combating such fires must be available.

Figure D-2 shows an accidental burn on a hand 42 hours post-irradiation with a CO_2 laser. In accidents of this type, severe injury could result from high-voltage equipment in the immediate vicinity on pulling the hand away. Extreme caution must be exercised, in this respect, when setting up equipment.

Windows should be covered to prevent the transmission of laser radiation at visible wavelengths through the window. With ultraviolet and infrared lasers, considerable care must be taken with windows that could be opened inadvertently and thus allow the beams to pass freely to the outside.

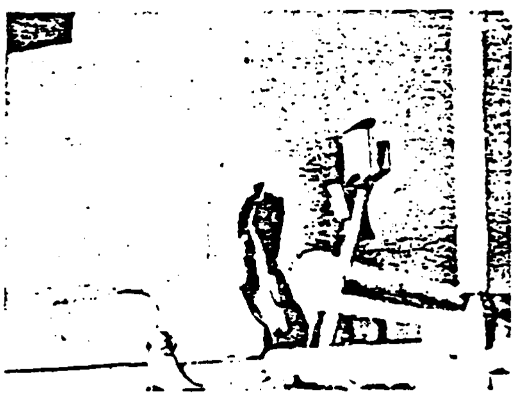

Figure D-1. Ignition of Standard Military Cloth on Irradiation at 10.6 Microns

Figure D-2. Accidental Burn of Hand from CO_2 Laser 42 Hours after Irradiation

Laboratories should be well ventilated, since insufficient ventilation may lead to the accumulation of noxious gases, particularly when these are being used in a continuous-flow gas laser. While this problem is minimized in a sealed system, it is not eliminated; the system may crack and release the enclosed vapors. Examples of these gases are chlorine, bromine, lead, mercury, carbon monoxide, and hydrogen cyanide. The toxicology of the gases and other materials utilized in experimental systems should be investigated, usually by a literature search, previous to their being studied as laser media.

A second reason for adequate ventilation relates to the liquids and gases used to purge and cool lasers. Both liquid nitrogen and liquid helium have been used for cooling lasers, including solid-state and semiconductor units. This may result in the formation of liquid oxygen which can combine with dirt and grease to form an explosive hazard. Styrofoam and similar materials should not be used in liquid nitrogen or liquid helium transfer lines because of the possible explosive hazard associated with the production of liquid oxygen.

In conjunction with the use of cryogenic liquids, it is important that adequate relief valves be present in the system to prevent the buildup of pressure on boil-off. It is also essential that procedures be adopted to prevent an explosive or inflammable gas mixture from being connected to a laser system in lieu of the desired gas mixture. To minimize the hazard of such an error, and of the trace contamination of tanks, it is desirable to operate the laser system several times at low voltage levels following the connection of a new tank to the system.

Good ventilation will also minimize the scattering hazards associated with dust or smoke in the atmosphere.

Other factors include the usual ones of resting responsibility and authority in one individual (laboratory laser hazard control officer) insofar as making certain that the area is secure prior to operation of the unit. He should be responsible for charging the bank and firing the unit. Working in pairs is important. The safety procedures, precautions to be taken, and techniques for mouth-to-mouth resuscitation, along with the names, addresses, and telephone numbers of the physician, laser hazard control officer, and ophthalmologist should be posted.

3. Protection Standards and Dosimetry

Due to the incompleteness of our knowledge concerning biological effects, particularly with regard to long-term effects, it is impossible to set firm standards at this time. A useful guideline for protection insofar as immediate or short-term effects are concerned is the minimal threshold dose of radiation required to produce injury.

Considerable attention has been directed toward determining the threshold doses of pulsed laser radiation for damage to the eye, particularly with respect to injury to the retina-choroidal layers. Biological variability, including heterogeneity, pigmentation, and blood supply, and the extrapolation of studies on animals to man are factors that must be considered. The threshold value obtained will depend on the method used. Such methods include ophthalmoscopic examination and photography, microscopic studies, histochemical and enzymatic techniques, electron microscopic investigation, and the measurement of electrical changes by electroretinography. Studies on the eyes to determine threshold effects have been carried out by Doctors Ham, Geeraets, Fine, and Zaret who are present at this conference. In general, the threshold dose at 6943 Å, non-Q-switched, millisecond exposure, for damage to the retina is in the range of 0.5 to 1 joule per cm^2 incident at the retina. The energy required to produce threshold lesions appears to decrease with decreasing pulse durations.

In our studies, the threshold dose for gross visible damage to the skin of mice appears to be of the same magnitude as that for the eye. The histology may differ due to reflectivity, scatter, and diathermanous properties of the skin of the mice, as well as biological factors. A lower skin threshold dose was obtained at high-peak-power levels with Q-switched systems as compared to thresholds at longer pulse durations.

These data, in conjunction with a safety factor of at least 100, provide guidelines for radiation protection standards at the ruby wavelength.

Order of magnitude calculations indicates that direct viewing of even a 1-milliwatt gas laser at 6328 Å is extremely hazardous. Consequently, considerable care should be taken in the adjustment of even low-power gas lasers.

Standards with respect to high-voltage electrical equipment should comply with acceptable standards of electrical engineering, including those of the ASTM, ASA., and IEEE, as these are referenced in electrical engineering handbooks. The specifications set by the appropriate agencies must be complied with for the systems to be used in the field.

It is necessary that the equipment designed or purchased by a company comply with uniform standards adopted by that company. For example, interlocks associated with all laser equipment should be of one type, grounding rods should be incorporated in the equipment, and grounding techniques for cabinets should be consistent and satisfactory. The laser hazard control officer should be kept informed of all purchases proposed or pending, so that some of these requirements may be incorporated in the specifications.

Dosimetry of two types is desirable: 1) Incorporating a photodetector to allow for the instantaneous measurement of radiation intensity for protective purposes and, 2) recording cumulative exposure for medico-legal as well as medical purposes. These two types of dosimetry are used for personnel exposed to x and gamma radiation. (Dosimetry protection for a single pulse is not feasible because of the short pulse duration.) Further development of adaptive filter glasses may provide some protection. The former type of dosimeter can, however, provide information regarding radiation intensity at a point, for succeeding pulses, or for continuously-operating units. Although a number of problems are associated with the latter type of dosimeter, it offers a means for maintaining exposure below maximum permissible dose levels. Although soft x-rays have been produced at the focal region of a Q-switched laser pulse, the x-ray energy and intensity are low. Most laser systems operate at relatively low voltage (<5 kv) with little associated x-ray hazard. However, the nitrogen laser operating at voltages in excess of 20 kv has a hard tube modulator or equivalent system associated with it. This may result in x-rays. The source, quality, and intensity of the x rays associated with systems of this type should be investigated and precautionary measures instituted. These can be minimized by the usual three factors: shielding, distance, and time. It is important to note that film badge units and other types of x-ray detectors are responsive only over a range of wavelengths. The appropriate detector must be selected to cover the range of interest.

B. MANAGEMENT OF ACCIDENTS

First aid should be restricted to minimal, essential manipulation of the patient as required to arrest hemorrhage, cover the affected region with sterile gauze, and immobilize the affected region, particularly in the event of fracture following electric shock. In the case of respiratory arrest, mouth-to-mouth artificial respiration should be immediately begun and continued until medical attention is obtained. The equipment, techniques, and knowledge necessary to apply controlled countershock in the short period available if ventricular fibrillation occurs and to attempt pacemaking are unavilable in the field. Consideration can be given to the maintenance of cardiac output by external message. All accidents should receive immediate medical attention. An ophthalmologist should be on call at all times. A general physician, also on call at all times, should be available for accidents involving regions other than the eye.

Since specific treatment of the laser-induced injury is not available, medical management of the immediate injury will follow the usual procedures for the treatment of traumatic lesions of comparable degrees of severity.

Retinal damage, as previously mentioned, is irreversible and medical care consists mainly in evaluating the damage and preventing possible complications. Surface burns due to ultraviolet light are generally cared for simply with topical medications, but a severe iritis or irridocyclitis resulting from the impact of strong radiation on the iris may require the use of cycloplegics and perhaps even systematic steroids to control the inflammation.

For lasers operating on either side of the visible spectrum, corneal damage becomes a considerable problem; in the near ultraviolet only superficial burns treated in the usual manner of anesthetics and antibiotics may be sufficient. In the infrared and far infrared, damage may be very marked and produce not only a keratoconjunctivities but severe keratitis leading even to a dense corneal scar. This is often accompanied by an iritis, both of which can be treated with topical and systemic steroids as well as cyclopegic drugs such as atropine and homatropine. The latter form of damage to the cornea, and perhaps iris and lens, may occur with the new CO_2 lasers coming into widespread use (Figures D-3 and D-4). Cataract production due to a rise in local temperature by absorption in the pigment epithelium of the iris in contact with the lens is a distinct possibility when whole white light is used, or perhaps with carbon dioxide lasers. This damage is theoretically and perhaps, backed by some experiments, more serious to the lens where the iris provides no heat shield. The periphery of the lens is more protected by its not being in contact with the pigment epithelium of the iris which provides a heat shield.

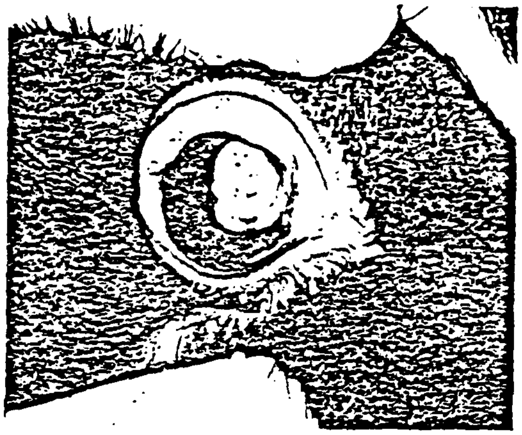

Figure D-3. Irradiation Damage from CO_2 Laser at 5 Watts for 1.0 Second (25 joules/cm^2)

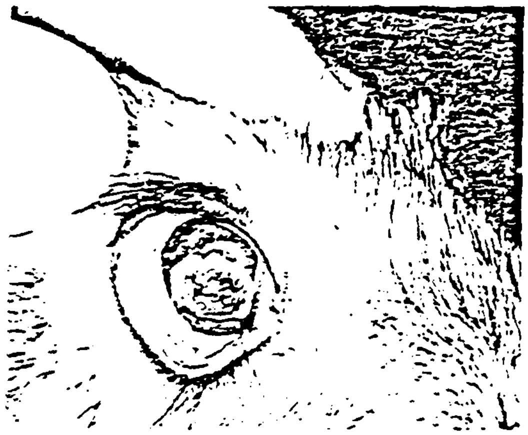

Figure D-4. Irradiation Damage from CO_2 Laser at 9 Watts for 1.0 Second (45 joules/cm^2)

The problem of CO_2 laser damage to the eye is accentuated by the fact that lasers of this type are easy to build and make operational. Since relatively little sophistication is required to construct a CO_2 laser that will operate at power levels from 5 to 20 watts, one can expect a great number of markedly hazardous units to be operational in the near future. Figure D-5 shows a 22-watt CO_2 laser built in our laboratory in three weeks from readily obtainable materials.

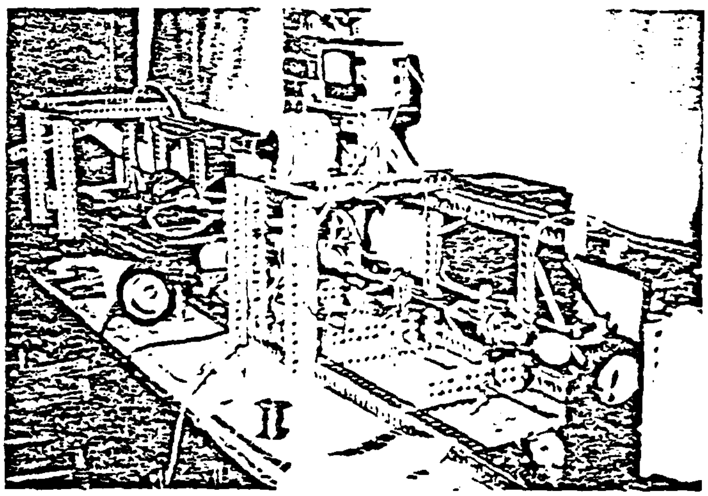

Figure D-5. Twenty-watt CO_2 Laser Designed and Built at Northeastern University

Followup should be carried out with particular attention to delayed anatomical and functional disturbances of the eye and to the possible late manifestations of chronic inflammatory changes in the skin and deeper structures. The delayed effects in the skin and subcutaneous tissues may include scarring, atrophy, indolent ulcers, persisting sinuses, chronic granuloma, and possibly malignant transformations. Treatment of these late sequelae should be in accordance with established dermatological methods of management.

Accidents involving particularly sensitive sites such as joints, cranium, superficially-located blood vessels, and nerves require the immediate attention of appropriate medical specialists. Experimental findings indicate that trauma to these regions cannot be assessed on the basis of the extent of the superficial injury; consequently, the patient should be carefully followed in the event of injury to these regions.

Each accident should be reported to the laser hazard control officer as well as to the attending physician. Abstracts of the medical records should be made available to the lazer hazard control officer.

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BIOLOGICAL EFFECTS OF LASER RADIATION. VOLUME II. REVIEW OF OUR--ETC(U)
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PUBLICATIONS

A. Laser-Related

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B. Non-Laser Related

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C. Laser-Related Presentations as Invited Lecturer, Pertinent to Contract to Which Credit Was Given

1. Conference on Biological Effects of Laser Radiation, Washington, D.C. - Sponsored by U.S. Army Medical Research and Development Command, 1964

2. Conference on Lasers, New York Academy of Sciences, 1964

3. Gordon Research Conference on Biological Effects of Laser Radiation, 1965

4. Conference on the Biological Effects of Lasers, National Institutes of Health, Bethesda, Maryland, October 4-5, 1965

5. Gordon Research Conference on Biological Effects of Laser Radiation, 1966

6. Bell Telephone Laboratories - invited lecturer, 1966

7. The Martin Company - symposium on Biological Effects of Laser Radiation, 1966

8. Boston Medical Physics Society - Lecturer on Biophysical Studies with Laser Radiation, 1966

9. Seminar on Biological Effects of Laser Radiation, University of Texas, Austin, 1966

10. Conference on Development of Lasers in the Biological Sciences, Veterans Administration, Department of Medicine and Surgery, Washington, D.C., August 5, 1966

11. Presentation before the Physicians of the Association of American Railroads, Montreal, June 4, 1967

12. Gordon Research Conference on Biological Effects of Laser Radiation, 1967 (Session Chairman)

13. American College of Obstetrics - District I Meeting - Invited Participant - Biological Studies on Laser Radiation, October 1967

14. Invited Lecturer on Lasers - P.R. Mallory & Co. - Laboratory for Physical Sciences - Biological Effects of Laser Radiation, February, 1968

15. Brookhaven National Laboratory, Upton, New York "Biophysical Effects of Laser Radiation", May, 1968

16. New England Chapter Health Physics Society, "Biological Effects and Hazards of Laser Radiation", May, 1968

17. Case Western Reserve University, Cleveland, Ohio, Summer course on Laser Technology and Applications, presented lecture "Lasers in Biology and Medicine," July, 1968

18. G-APURSI Symposium (International Antenna and Propagation Symposium) "Electromagnetic Waves (Lasers) for Biological and Medical Applications", September, 1968

19. Rutgers University, New Brunswick, New Jersey, Participant in "Evaluation of Laser Hazards Course", October, 1968

20. S. Fine - "Biological Studies Relating to Laser Irradiation, Particulary with Respect to the Eye", Howe Laboratories, Massachusetts Eye and Ear Infirmary, Harvard Medical School, December, 1968

21. S. Fine - "Control of Laser Hazards and Management of Accidents", National Center for Radiological Health, U.S. Department of Health, Education and Welfare, Rockville, Maryland, February, 1969

22. S. Fine - "The Application of Lasers to Biology and Medicine," Conference on Trends and Directions in Biological Sciences of the Thirteen Colleges Curriculum Program Biology Teachers, Clark College, Atlanta, Georgia, March, 1969

23. S. Fine, Participation in Skin Laser Workshop, Second International Laser Safety Conference, Cincinnati, Ohio, March, 1969

24. S. Fine, Lasers--Characteristics, Use, Hazards and Biological Effects, Seminar Series, Environmental Health Engineering and Science, Graduate School of Engineering, Northeastern University, March, 1969

25. S. Fine, Lasers--Characteristics and Uses in Biology and Medicine, Surgical Seminar Series, Boston University School of Medicine, March, 1969

26. S. Fine, Use of Lasers in Biology and Medicine, Laser Applications Course, Washington University, St. Louis, Missouri, May, 1969

27. E. Klein, and S. Fine, *Tissue and Cell Effects of Laser Radiation*--Gordon Research Conference on Lasers in Medicine and Biology, June, 1969

28. S. Fine, "Lasers in Biomedicine," I.E.E.E. Student Branch, Northeastern University, July, 1969

29. S. Fine, "Biological Hazards and Effects of Laser Radiation," in course on Fundamentals of Non-Ionizing Radiation Protection, Northeastern Radiological Health Laboratory, August, 1969

30. S. Fine, Lasers in Industry, Associated Hazards and Protection, National Safety Congress, Chicago, Illinois, October 28, 1970

31. S. Fine, Non-invasive Testing in Medicine, I.E.E.E. group on Engineering in Biology and Medicine, Boston, Massachusetts, November, 1970

32. S. Fine, Uses and Hazards of Laser Radiation in Industry and in Atmospheric Pollution Studies, 24th AMA Clinical Convention, Boston, Massachusetts, November 30, 1970

33. S. Fine, "Bioengineering", Massachusetts Epsilon Chapter, Tau Beta Pi (Northeastern University), September, 1969

34. S. Fine, "Laser Biology", in Laser Fundamentals and Applications course, Polytechnic Institute of Brooklyn Graduate Center, September, 1969

35. S. Fine and E. Klein, "Biological Effects of Laser Radiation," the Theobald Smith Society, New Jersey, October, 1969

36. S. Fine, "Lasers--Biological Effects and Medical Applications," Society of Photo-optical Instrumentation Engineers Meeting, co-sponsored by the University of Rochester Institute of Optics, Rochester, New York, November, 1969

37. S. Fine, Session Chairman, Laser and Ultraviolet Contributed Papers, Fourth Annual Midyear Topical Symposium, Health Physics Society Meeting, Louisville, Kentucky, January 28-30, 1970

38. S. Fine, Lasers in Industry, Associated Hazards and Protection, National Safety Congress, Chicago, Illinois, October 28, 1970

39. S. Fine, Non-invasive Testing in Medicine, I.E.E.E. group on Engineering in Biology and Medicine, Boston, Massachusetts, November, 1970

40. S. Fine, Uses and Hazards of Laser Radiation in Industry and in Atmospheric Pollution Studies, 24th AMA Clinical Convention, Boston, Massachusetts, November 30, 1970

41. S. Fine, "Lasers in Biology and Medicine," in course on Lasers and Optics for Applications, Massachusetts Institute of Technology, Cambridge, Massachusetts, July 30, 1971

42. S. Fine, Guest Lecturer in Graduate Course 2.77, "Biological Effects and Medical Applications on Non-Ionizing Radiation," Fall, 1971, Massachusetts Institute of Technology

43. S. Fine, "Medical Applications, Research and Safety," Boston Section I.E.E.E. 1972 Lecture Series, February, 1972

44. S. Fine, "Lasers in Biology and Medicine," a course on lasers and optics for application, M.I.T., July, 1972

45. S. Fine, "Biological Effects and Medical Applications on Non-Ionizing Radiation," guest lecturer for several sessions in graduate course 2.77, M.I.T., 1972-1973

46. S. Fine, "Biological Effects and Medical Applications of Non-Ionizing Radiation," guest lecturer in graduate course at M.I.T., 1973-9974

47. S. Fine, lectured at Raytheon Research Laboratory on Electrical Hazards and Emergency Management of Accidents, 1974

48. S. Fine, "Biological Effects and Medical Applications of Non-Ionizing Radiation", guest lecturer in summer session course, M.I.T., July, 1975

Most other conferences in which abstracts or papers were published are included in the preceding bibliography.

D. Laser-Related Conference Organization and Planning; Pertinent to Contract

1. Boston Laser Conference, 1963

2. Boston Laser Conference, 1964

3. Institute of Electrical and Electronics Engineers - Member, NEREM Program Committee, 1965

4. Institute of Electrical and Electronics Engineers - Member, NEREM Program Committee, 1966

5. American Association for the Advancement of Science - Session Organizer and Chairman of Session on Biological Effects of Laser Radiation, 1966

6. Laser Industry Association Convention, October 24-26, 1968

7. Course on "Fundamentals of Laser Radiation Protection" given to personnel of U.S. Department of Health, Education and Welfare, 1968

8. Member, program planning committee on seminar series in applications of physical chemical techniques in Biology and Medicine, EMB, IEEE, Boston Section, 1969

9. Laser Industry Association Meeting - Los Angeles, California, October 20-22, 1969

10. Electro-Optical System Design Conference, September 22-24, 1970, New York Coliseum. Planning of sessions, session organization and chairman.

11. Major participant in the organization, planning, and instruction of personnel, and field work related to the first major survey on lasers and laser devices in the United States which was carried out be the State of Massachusetts and Occupational Health and Radiological Health, H.E.W., 1968.

PERSONNEL RECEIVING CONTRACT SUPPORT AND GRADUATE DEGREES OBTAINED

Arnold Aaron	Ph.D. in Engineering
Charles Aaronson	M.S. in Engineering
John Campbell	M.A. in Psychology
John Caron	M.S. in Engineering
Joel Cohen	M.S. in Biology
John Donoghue	M.S. in Engineering
Larry Feigen	M.S. in Physics
James Forman	M.S. in Engineering
Peter Hansen	Ph.D. in Engineering
Karl Hergenrother	Ph.D. in Engineering
Donald MacKeen	Ph.D. in Biology

Note: The above individuals were supported in full or in part for contract related work while carrying out graduate work. In some cases, the support was minimal.

OTHER NON-GRADUATE DEGREE PERSONNEL RECEIVING SUPPORT INCLUDE:

James Blout
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