Ocular Hazard from Picosecond Pulses of Nd:YAG Laser Radiation

Abstract. *Seven rhesus monkeys (14 eyes) were exposed to 1064-nanometer radiation in single pulses of 25 to 35 picoseconds from a mode-locked Nd:YAG laser. Threshold injury resulted from single pulses with a mean energy of 13 ± 3 microjoules. Electron microscopy of the retina revealed that damage was highly localized in the photoreceptor and pigmented epithelial cells at the outer retina. Membrane disruption, distorted outer segments, and abnormal melanin granules resembling fetal premelanosomes were observed.*

Recent developments in the production of intense ultrashort pulses of radiation from mode-locked lasers have promoted great advances in optical chronography, in the study of basic processes in atomic and molecular physics, and even in research on controlled nuclear fusion (*1*). One example under investigation at Los Alamos is the mode-locking of Nd:YAG (Nd^{3+}-doped yttrium aluminum garnet) lasers to produce picosecond pulses which can be focused optically on a pellet of fusionable material (*2*). However, the ocular hazard from picosecond pulses has received little attention. Our primary objective was to define threshold damage to the retina in terms of the energy of a single pulse entering the eye. Another major objective was to investigate by electron microscopy the nature and possibly the basic mechanisms of effects on the mammalian retina of exposure to picosecond single pulses of 1064-nm radiation.

Pulse trains of 25 to 35 psec separated by 10 nsec between pulses were provided by a mode-locked Nd:YAG oscillator operating in the TEM$_{00q}$ transverse mode. A Pockels cell placed between crossed calcite polarizers provided a means of selecting single pulses from the train. Each pulse was amplified, diffracted through a 2.8-mm aperture, and directed into the eye of the monkey. The Gaussian profile of the beam was approximately 3.8 mm in diameter at the $1/e$ points as measured at the aperture. Beam divergence was less than 1 milliradian, producing an image diameter on the retina estimated to be about 25 μm.

Energy entering the eye was monitored by means of a beam splitter–photodiode combination, the output of which was displayed and photographed on a fast oscilloscope. The output amplitude of the photodiode was correlated with the reading of a sensitive cone radiometer (*3*). The cone radiometer was checked against an Eppley cell, which had been calibrated with a standard of irradiance from the National Bureau of Standards. A calibration between photodiode and cone radiometer was performed before and after each animal exposure.

Seven rhesus monkeys (14 eyes), ranging in age from 18 to 24 months, were selected as the experimental animals with ocular characteristics most likely to provide a useful correlation with man (*4*). Each animal was anesthetized, its pupils were dilated, and specula were inserted in both eyes to keep the lids open. The animal was immobilized for exposure on a stereotaxic platform which secured the head while providing coaxial adjustment of the eye to the laser beam. Fundus photographs were taken before and after exposure and at specific later times, according to a systematic schedule of killing the animals which provided retinal tissue for histology and
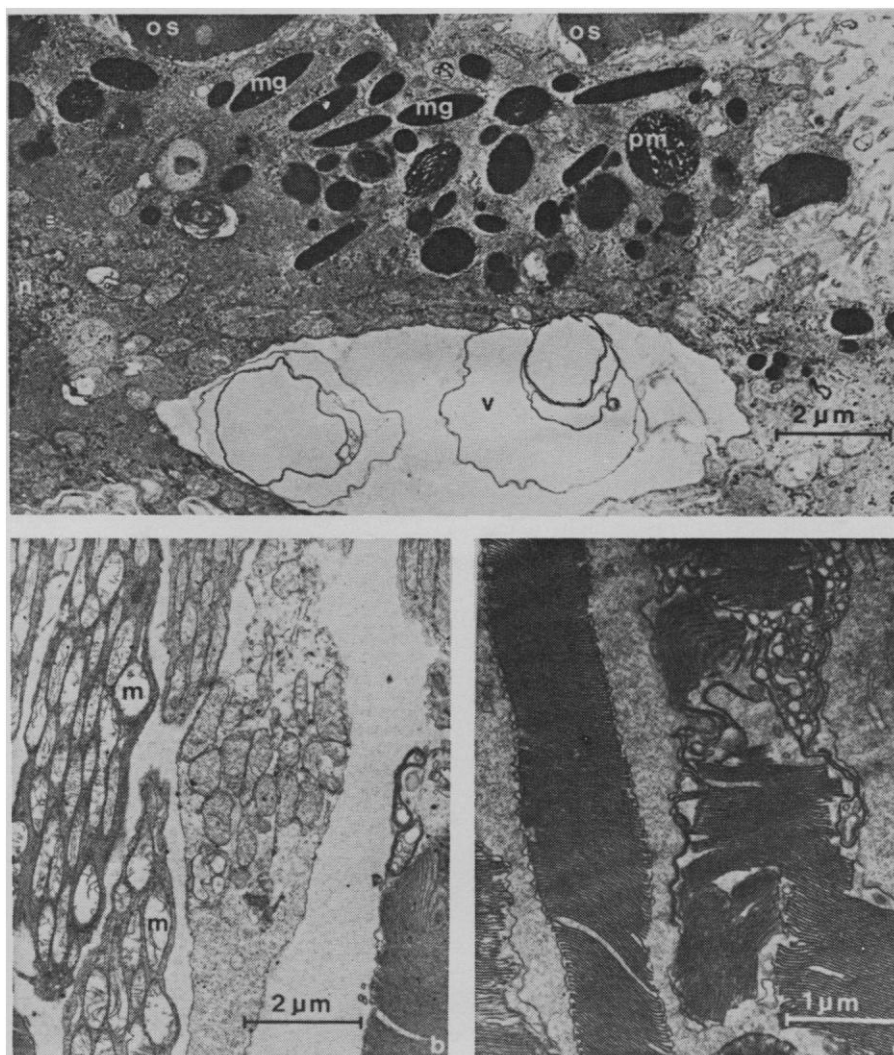

Fig. 1. (a) Pigment epithelium 3 days after exposure to a single pulse of laser radiation with energy 56 μj. Five of the melanin granules resemble fetal premelansomes (*pm*); the other melanin granules (*mg*) appear normal, but disoriented. A light halo can be seen in the cytoplasm surrounding most of these granules. The large vacuole (*v*) marks an area of separation of the pigment epithelium from Bruch's membrane. The nucleus (*n*) is convoluted. Photoreceptor outer segments (*os*) can be seen at the top of the pigment epithelium. A high concentration of ribosomes is visible. (b) Photoreceptor inner segments 1 hour after exposure to 59 μj. The swollen mitochondria (*m*) at the left are adjacent to relatively normal mitochondria. This is an example of damage localization. (c) Photoreceptor outer segments 105 days after exposure to 13 μj, the energy for threshold damage. Again, note the localization of damage between adjacent cells.

electron microscopy at intervals of 1 hour, 24 hours, 48 hours, 72 hours, 20 days, and 105 days. One animal is still alive for future investigation.

Threshold lesions were determined as follows: each eye received 18 exposures; 6 in the nasal paramacula, 6 in the macula, and 6 in the temporal paramacula. The appearance and size of a lesion within 5 minutes after exposure was the criterion for reducing or increasing the optical density of neutral filters in the beam. Lesions were classified as none, threshold, mild, moderate, and severe. With the exception of the first animal irradiated, the grade of lesion never exceeded moderate. The characterization of a threshold lesion depended primarily on size and time of appearance. For a detailed description of the characterization of threshold lesions, see (4). Usually, but not always, a threshold lesion appeared 24 hours after exposure, and was estimated to have a diameter ranging from 25 to 50 μm.

From a total of 243 exposures, 20 were selected as representative of a threshold lesion. The mean energy entering the eye was 13 μj with a standard deviation of $\pm$ 3 μj. Threshold energies ranged from 8 to 17 μj. Based on an estimated spot diameter of 25 μm and a transmittance through the ocular media of 0.76 [figure 2 in (4)], the radiant exposure at the retina was 2.0 joule cm^{-2} and the power density 67 Gw cm^{-2}. The first monkey to be irradiated received inadvertently one exposure of 282 μj at the cornea. This produced a massive hemorrhage into the vitreous. Another exposure of 150 μj produced a subretinal hemorrhage. All subsequent exposures were confined to energies below 100 μj.

The specimens were prepared for electron microscopy by the method of Kuwabara and Gorn (5). In lesions ranging from threshold to moderate, the inner retina beyond the outer plexiform layer was undamaged. Pathological effects were confined to the pigmented epithelium and the photoreceptor cells. Moreover, the damage seemed to be highly localized; and it was not unusual to find a normal outer segment with badly distorted segments immediately adjacent. The same phenomenon was observed for inner segments and for adjacent pigment epithelial cells. Another feature was the abnormal appearance of the melanin granules, which were disoriented, were surrounded by a thin band of diffuse cytoplasm, and often presented a stri-

ated appearance similar to that seen in melanosomes during fetal development. This effect was previously noted in an albino rat which had been exposed to white light for several days (5). Membrane disruption in the outer segments, mitochondria, lysosomes, nuclei, and endoplasmic reticulum was a common finding. These effects were observed at 1 hour and 24 hours after exposure. Enhanced metabolic activity, evidenced by abnormal concentrations of ribosomes, was present at 24 hours and extends out to 20 days. Even at 102 days in a threshold lesion some photoreceptor cells exhibited swollen mitochondria in the ellipsoid, pathological nuclei, and distortion in the outer segments; also, some melanin granules still presented a disrupted or striated appearance. Some of these findings are illustrated in Fig. 1, a, b, and c.

These effects are different from those noted in conventional retinal burns, such as burns produced by a clinical xenon photocoagulator (6), which suggests that physical mechanisms other than thermal injury may be significant in retinal damage at high-power densities and short exposure times. In addition to thermal denaturation, nonlinear effects have been suggested as possible mechanisms leading to retinal damage. Cleary and Hamrick (7), for example, have demonstrated that Q-switched pulses produce sonic transients in the mammalian retina.

We are of the opinion that the melanin granules in the pigmented epithelium represent the major sites of photon absorption and subsequent loci of the physical effects producing highly localized retinal damage. The random distribution of melanin granules adjacent to outer segments and organelles in the pigmented epithelium and subretinal space could account for this highly localized damage if the range of effects extended only a few micrometers beyond the sites of absorption. This seems highly likely whether we attribute the damage to local heating effects or to sonic transients or some other nonlinear phenomenon. Membrane disruption in the outer segments would produce ionic and osmotic imbalances, which could interfere drastically with the electrophysiological and metabolic relations between the inner and outer segments and lead to pathological manifestations throughout the photoreceptor cell such as swollen mitochondria, ruptured disks, and abnormal effects in the nucleus and the synaptic pedicle. Localized membrane

disruption could also account in part for the release of hydrolytic enzymes from the lysosomes into the cytoplasm and the general disorganization of the endoplasmic reticulum.

It is interesting to compare the thresholds for damage to the retina with Q-switched pulses and picosecond pulses. According to Vassiliadis et al. (8), the energy required at the cornea to produce threshold damage in the retina of the rhesus monkey for 30-nsec Q-switched pulses of Nd : YAG radiation is 280 μj. Ebbers and Dunsky (9) report a threshold of 164 μj for 10-nsec pulses. We measured the threshold energy with Q-switched pulses by using the same exposure techniques reported here, and obtained 68 $\pm$ 12 μj for 15-nsec pulses. This is to be compared with 13 $\pm$ 3 μj for 30-psec pulses. Thus, according to our data, the energy required for threshold damage with a 15-nsec Q-switched pulse is five times that required with a 30-psec pulse. If the data of Vassiliadis or Ebbers and Dunsky are used this factor is even larger.

WILLIAM T. HAM, JR.
HAROLD A. MUELLER
ARNOLD I. GOLDMAN
Department of Biophysics, Virginia
Commonwealth University, Richmond
BRIAN E. NEWNAM
L. M. HOLLAND
L Division and H Division,
Los Alamos Scientific Laboratory,
Los Alamos, New Mexico 87544
T. KUWABARA
National Eye Institute,
National Institutes of Health,
Bethesda, Maryland 20014

References and Notes

1. D. J. Bradley and G. H. C. New, *Proc. IEEE (Inst. Electr. Electron. Eng.)* **62**, 313 (1974).
2. K. Boyer, *Astronaut. Aeronaut.* **11** (No. 1), 28 (1973); J. L. Emmett, J. Nuckolls, L. Wood, *Sci. Am.* **230**, 24 (June 1974).
3. R. C. Williams and H. A. Mueller, *Appl. Opt.* **5**, 135 (1966).
4. W. T. Ham, Jr., H. A. Mueller, R. C. Williams, W. J. Geeraets, *ibid.* **12**, 2122 (1973).
5. T. Kuwabara and R. A. Gorn, *Arch. Ophthalmol.* **79**, 69 (1968).
6. I. H. L. Wallow and M. O. M. Tso, *Am. J. Ophthalmol.* **75**, 610 (1973).
7. S. F. Cleary and P. E. Hamrick, *J. Acoust. Soc. Am.* **46**, 1037 (1969).
8. A. Vassiliadis, H. C. Zweng, N. A. Peppers, R. R. Peabody, R. C. Honey, *Arch. Environ. Health* **20**, 161 (1970).
9. R. W. Ebbers and I. L. Dunsky, *Aerosp. Med.* **44**, 317 (March 1973).
10. Supported by H and L Divisions, Los Alamos Scientific Laboratory, and by contract DADA-17-72-C-2177 with the U.S. Army Medical Research and Development Command. In conducting the research described in this report, the investigators adhered to the "Guide for laboratory animal facilities and care" of the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences–National Research Council.

22 April 1974