Thresholds for retinal vitreal and contained hemorrhages were determined for 1064 nm laser light at 30-nsec and 4-nsec pulsewidths. Rhesus monkeys received graded exposures from a neodymium-yag laser onto either the macular or extramacular region of the retina. Contained hemorrhages appeared as concentric ring structures with white punctate centers. The vitreal hemorrhage was characterized by the presence of choroidal blood in the vitreal chamber at the exposure site. The 30-nsec contained hemorrhage threshold (ED50) was 1.7 mJ on the macula and 2.1 mJ for an extramacular exposure. The 30-nsec vitreal hemorrhage macular threshold was 2.3 mJ, and the extramacular threshold was 6.6 mJ. The threshold for the 4-nsec pulsewidths to produce a hemorrhage (vitreal or contained) on the retina (macula or extramacular) was 340 μJ. Invest Ophthalmol Vis Sci 27:1176–1179, 1986

Laser safety standards are a result of many studies aimed at setting a maximum permissible exposure (MPE) for the various wavelengths and pulsewidths obtainable with operational laser systems.¹ The American National Standards Institute (ANSI) MPE is based on threshold data for the minimum visible lesion (MVL). Little has been done, however, investigating the effects of suprathreshold ocular exposures. Two such effects are the vitreal and contained hemorrhage in which choroidal blood either passes through the ruptured retina into the vitreal chamber or is contained beneath the retina. Both have been observed and reported for visible laser radiations. However, the hemorrhagic lesion endpoints have not, in general, been adequately defined with respect to parameters such as threshold energies, wavelength and pulsewidth of the source, or pathology of the damaged tissue; nor have permanent and transient effects on vision been investigated. These parameters are important in defining risks, evaluating the transient or permanent nature of the injury, and in considering medical treatments with lasers.

Using a neodymium-yag (Nd:YAG) laser producing 30-nsec and 4-nsec pulses at a wavelength of 1064 nm, we report thresholds for vitreal and contained hemorrhages at macular and extramacular positions, and discuss the experimental indications of mechanism.

Materials and Methods. Apparatus: The Nd:YAG flashlamp pumped laser used in this study was built by the Los Alamos National Laboratory. An Isomet Model 420 intracavity Q-switch was used to obtain 30-nsec pulsewidths. To generate 4-nsec pulses, the Q-switched 30-nsec laser pulses were directed into an intracavity Pockels cell driven by a laser triggered spark gap with a 4-nsec charge line.

To accurately position the beam on the retina, an inverted Zeiss fundus camera and animal translation stage were used. A helium-neon alignment beam, collinear with the Nd:YAG laser beam, was used to position the fundus camera line-of-site with the 1064 nm beam path. Spatial filters and lenses produced a collimated beam with a 1/e² diameter of 3 mm at the corneal plane. The beam divergence was less than 1 mrad. Additional details of the experimental setup and beam diagnostics have been published elsewhere.²

Animal models: The experimental subjects were 18 rhesus monkeys (Macaca mulatta) weighing between 2 and 6 kg with both sexes equally represented. Each animal had normal corneas, lenses, and retinae, with ±0.5 diopter or better refraction and no astigmatism.

Prior to exposure, subjects were preanesthetized with 10.0 mg/kg ketamine (IM), and anesthetized with a single injection of 18.0 mg/kg sodium pentobarbital into the saphenous vein. Eyes were dilated with 1% tropicamide applied topically. The eyelids were retracted with an ophthalmic speculum and the cornea irrigated with lactated Ringer’s solution to prevent dessication. The subject was then placed on a stage (having 5° of freedom) in front of the fundus camera so the retina could be viewed and positioned such that any retinal area could be accurately exposed. Each eye received one macular and one or more extramacular exposures, none of which were made over any observable venation. The exposed retina was viewed immediately after exposure to observe lesion development.
Fig. 1. Funduscopic photograph of rhesus monkey retina which has been exposed to a neodymium-yag laser, demonstrating three lesion types: minimum visible lesion (vertical column of four reflective circular areas at center), vitreal hemorrhage (far left and far right), and contained hemorrhage (upper center). The right-hand vitreal hemorrhage is centered on the macula.

Eyes used for histopathology were perfused in vivo with Karnovsky’s fixative. They were enucleated post-mortem approximately 2 hr after exposure. Eyes were post-fixed in Osmium tetroxide, dehydrated in a graded ethanol solution and embedded in LDX-112. The specific techniques used for eye preparation have been described previously. Thin sections were cut with an ultramicrotome and stained with Toluidine Blue.

The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act of 1970 and the “Guide for the Care and Use of Laboratory Animals,” prepared by the Institute of Laboratory Animal Resources-National Research Council. This investigation also conformed to the ARVO Resolution on the Use of Animals in Research.

Lesion definition and data analysis: Visible lesions were placed in one of three categories: minimal visible lesion (MVL) without hemorrhage, contained hemorrhage (CHL), or vitreal hemorrhage (VHL). The MVL appeared as a small spot on the target site within 1 hr after exposure. CHLs were large structures, approximating the shape and size of the macula. They exhibited concentric ring structures with a white punctate center, and varied somewhat in ring diameter and color. A VHL was differentiated from the other lesion types by the leakage of blood into the vitreal chamber. Examples of each lesion type may be seen in Figure 1.

The 4 nsec hemorrhage threshold was taken from both macular and extramacular exposures and combined VHL and CHL data. Thirty nsec thresholds were
Table 1. Vitreal and contained hemorrhage thresholds (ED$_{50}$) for 30-nsec Nd:Yag (1064 nm) laser radiation

<table>
<thead>
<tr>
<th>Type hemorrhage</th>
<th>Retinal position</th>
<th>ED$_{50}$ *</th>
<th>Confidence limits (95%)</th>
<th>Lowest energy w/hemorrhage*</th>
<th>Highest energy w/o hemorrhage*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.04–2.2†</td>
<td>1.6</td>
<td>3.3</td>
</tr>
<tr>
<td>Contained</td>
<td>Macula</td>
<td>1.7</td>
<td>0.6–2.7</td>
<td>1.6</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>Extra-macular</td>
<td>2.1</td>
<td>1.4–2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitreal</td>
<td>Macula</td>
<td>2.3</td>
<td>0.6–3.9</td>
<td>2.1</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Extra-macular</td>
<td>6.6</td>
<td>1.6–27.3†</td>
<td>1.8</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>4.2</td>
<td>3.1–280</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Total intraocular energy in millijoules.
† 90% confidence limits. 95% Fiducial limits not valid.
‡ ED$_{95}$ and ED$_{90}$. No valid fiducial limits could be calculated.

resolved into VHL and CHL data for both macular and extramacular exposures.

The effective doses for 50% probability of damage (ED$_{50}$) with confidence limits were calculated by probit analysis.$^5$ Insufficient lesion data at high energy levels prevented fiducial limit calculation for the extramacular VHL ED$_{50}$s. Therefore, the ED$_{05}$, ED$_{50}$, and ED$_{95}$ are reported for this data. Limited lesion data also lowered the macular CHL ED$_{50}$ fiducial limits to 90%.

**Results.** The ED$_{50}$s and their confidence limits for the 30-nsec pulsewidth are summarized in Table 1. Listed also is the lowest energy observed to produce a hemorrhage and the highest energy that did not produce a hemorrhage.

Figure 2 demonstrates a CHL produced by an exposure of 2.7 mJ and a VHL from an exposure of 2.2 mJ. Both exposures were of 30 nsec duration. Damage to the retina due to a CHL was characterized by retinal detachment. The retinal pigment epithelium (RPE) and Bruch’s membrane had both been penetrated with blood filling the space between RPE and neural retina. The outer nuclear layer (ONL) had pyknotic cells and the outer plexiform layer (OPL) was swollen with serous exudate (Fig. 2A). The most prominent features associated with VHL damage were a portal through the neural retina and choroidal hemorrhage which through that portal entered the vitreous chamber (Fig. 2B).

The retinal hemorrhage ED$_{50}$ for the 4-nsec pulsewidth was calculated by probit analysis to be 340 μJ with confidence limits at the 95% level of 260 μJ and 440 μJ. The highest energy exposure producing no hemorrhage was 330 μJ. The lowest energy exposure to produce a hemorrhage was 270 μJ.

**Discussion.** The 30 nsec ED$_{50}$s were consistent with previously reported data$^6$ in that the macular thresholds are lower than the extramacular thresholds for both contained and vitreal hemorrhages. The macula’s lower threshold may be related to the decreased thickness of the retina and internal limiting membrane (ILM) thickness in this area.$^7$ From observations on the formation of numerous VHLs, it was noted that development frequently proceeds through an intermediate CHL stage. The CHL increased in diameter until it ruptured, forming a VHL. The elapsed time from exposure until the time of rupture never exceeded 10 sec. On two occasions the foci of rupture occurred some 100 μm from the exposure site. This shift between exposure and rupture sites could be due to local variations in thickness and shear strength of the retina and ILM.$^7$

In Figure 2A, a typical CHL, the laser radiation was absorbed principally by the RPE with damage pene-
trating into the choroid. Choroidal blood has passed through the damaged Bruch's membrane and the RPE and caused the neural retina to separate from the RPE and be pushed upward. Serous exudate in the OPL is thought to be plasma filtering through the outer nuclear layer. ONL cell damage is thought to result from RPE damage and not directly from interaction with the 1064 nm laser light.8 With the VHL, Figure 2B, the higher energy density of the exposure has caused greater damage to the choroidal vaclature than in the CHL. The increased free blood pressure coupled with anteriorly directed shock waves from the RPE have created a portal for blood passage from the choroid to the vitreous compartment. Neuronal retinal detachment and OPL enlargement are also present.

Within the nanosecond pulsewidth range, both thermal and mechanical damage mechanisms may be operative with the dominant mechanism for lesion formation shifting from thermal to mechanical as the peak power increases. With suprathreshold exposure energies such as those required to produce hemorrhages, the effects are generally cataclysmic and can be attributed to both mechanisms.9,10 The hemorrhage threshold determined using a 4 nsec pulsewidth (340 μJ) was found to be a factor of six below the contained hemorrhage threshold for 30 nsec pulses (1.9 mJ), suggesting that at the shorter pulsewidth the mechanical damage mechanism was more dominant.

**Key words:** hemorrhage, neodymium-yag, laser, retina, monkey

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**References**


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Full-Field Electroretinograms in Miniature Poodles With Progressive Rod-Cone Degeneration

Michael A. Sandberg, Basil S. Pawlyk, and Elior L. Berson

Full-field electroretinograms (ERGs) were recorded between the ages of 4 and 34 months from 8 miniature French poodles with inherited progressive rod-cone degeneration (PRCD) and from 11 normal miniature poodles. Three stages of retinal function were observed in the affected dogs, although the ages of transition from one stage to the next were variable among dogs. Rod and cone ERGs were normal in amplitude in the first stage, rod ERGs were reduced in amplitude and cone ERGs were normal in the second, and rod ERGs were extremely reduced in amplitude or nondetectable and cone ERGs were reduced in amplitude in the third. On average, these poodles with PRCD lost 7.2% of remaining rod amplitude per month and 2% of remaining cone amplitude per month. Affected poodles had normal rod and cone b-wave implicit times over all three stages, in contrast to humans with the early stages of progressive forms of retinitis pigmentosa who have delayed rod and/or cone b-wave implicit times. Invest Ophthalmol Vis Sci 27:1179–1184, 1986.

The miniature poodle with autosomal recessive progressive rod-cone degeneration (PRCD) initially shows symptoms of night blindness, followed by impaired day vision, eventually resulting in blindness. A similar sequence of events is a well-recognized feature of the typical forms of human retinitis pigmentosa.2 Electroretinograms (ERGs) in response to Ganzfeld (full-field) stimulation in the early stages of human retinitis pigmentosa have been characterized not only by reduc-