Ruby laser effects on the monkey eye

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Laser pulses of moderate energy but of large retinal subtense produce significantly different retinal lesions than a clinical type of exposure of high energy density and small retinal subtense. A retinal photoagulation that leads to a satisfactory retinal adhesion, whether produced by white light or laser, is characterized by sharp boundaries, coagulation necrosis of the entire retinal exposure area, and migration of pigment into the area of insult. A low energy density retinal burn of large retinal area is frequently not visible ophthalmoscopically. Histologically, the lesion is not demarcated by sharp boundaries, little coagulation necrosis is seen, and eventually the entire retina becomes involved. Pulsed ruby laser radiation was presented in Maxwellian view to the intact monkey eye. The pulse duration was about 2.0 msec. and the flash energy was varied between 1 and 250 joules. Gross damage to the globe was characterized by corneal pitting, lenticular disruption, bubbles and hemorrhage in the vitreous, and loss of light reflex. Energy levels above 100 joules produced a marked degree of periorbital edema. Histological observations revealed extensive primary damage in the pigment epithelium and choroid and secondary retinal detachment and degeneration peripheral to the area exposed. Progressive retinal detachment and dedifferentiation secondary to the laser lesion are found to occur for a considerable time post exposure.

Pulsed ruby laser radiation can be an important tool for the visual psychophysicologist. The wavelength of the energy is such that it will be maximally absorbed by only one of the four known photopigments, and the high energy values available will allow flash bleaching that could produce a temporary protanopia without disturbing side effects typically produced with high intensity light adaptation. It is theoretically possible to produce an irreversible protanopia. However, the destructive effects of ruby laser radiation on ocular structures are well known, and the effects of large area retinal irradiation must be elaborated before ruby laser can be used in human psychophysical studies.

Typically, a laser exposure is performed by directing unfocused radiation into the pupil of the eye. The laser beam, being coherent, is focused by the lens of the eye into a spot on the retina that is approximately 100 to 150 μ in diameter.1 In clinical photoagulation, the use of optics external to the eye has allowed the laser beam to be presented as a cone of light which can illuminate an area up to 2 mm. in diameter. The use of a short focal length positive lens which brings the beam to a focus in the center of the lens allows illumination of large retinal areas with the disadvantage of creating a "hot spot" inside the lens where the energy is focused. The major advantage of this type of presentation, a Maxwellian view, is that the lens of the eye is effectively bypassed, and the area illuminated is a function of the diameter of the image and the focal length of the lens.

A Maxwellian presentation was used in the present study: (1) to approximate the

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psychophysical viewing conditions used in this laboratory for the production of a transient protanomaly, and (2) to elaborate the retinal effects of laser pulses of large angular subtense.

Method

The left eyes of seven monkeys of the species *Macaca cynomolgus* and *Cercocebus torquatus atys* were exposed to laser pulses of approximately 2 msec. duration with energy levels from 5 to 250 joules. The right eyes of three of the *Macaca cynomolgus* were also exposed.

A pulsed laser with a seven inch ruby rod approximately ¾ inch in diameter was used. Liquid nitrogen cooling was used. Energy values were computed from a calibration curve of exit energy from a short focal length simple objective lens. A portion of the beam was split off into a small copper cone calorimeter and the exit energy of the lens focused in the mouth of a large copper cone calorimeter. Calibration curves of energy from the lens as a function of the small calorimeter reading were constructed. The small calorimeter was used to monitor each flash of the laser for calculation of the energy at the eye.

The animals were lightly anesthetized, the eyelids retracted, and the pupils dilated with a 1 per cent solution of cyclopentolate hydrochloride. The animal was placed in the path of the laser flash at a position which focused the flash in the center of the animal's lens, presenting a Maxwellian view that subtended a retinal area of about 78.5 sq. mm. or 24 per cent of the total retinal area.

The exposed eye was enucleated at different time intervals post exposure with the animal under deep, preterminal anesthesia. The intact globe was dissected free of all extraneous tissue, and immersed in acid alcohol for ten minutes. The anterior portion was dissected away, the vitreous sponged out and both portions of the eye returned to the fixative for five minutes. Following fixation the globe was dehydrated, embedded in paraplast, and sectioned at 8 μ. The sections were stained with hematoxylin and eosin, or gallocyanin, or Mallory's "azan" aniline blue.

Fig. 1. Fundus photographs of normal and laser exposed mangabey eye and retinal sections from exposed eye. Normal fundus, *Cercocebus torquatus atys* (A); laser exposed fundus 9 mo. post exposure (B); chorioretinal scar, 9 mo. post exposure (×100; reduced ⅓) (C); neural retinal, 9 mo. post exposure (×100; reduced ⅓) (D).
A series of four Macaca cynomolgus received single pulse laser exposures in both eyes. The retinal subtense of these exposures in Maxwellian view was 43.2 degrees which yielded a retinal area exposure of 1.13 sq. cm. The energy per flash ranged from 1.2 to 7 joules delivered in 1.5 msec., and the energy densities ranged from 1.0 to 6.2 joules per square centimeter. The animals were put to death painlessly six days post exposure.

**Results**

**Gross observations.** At all energy levels superficial corneal pitting and clouding could be seen immediately after exposure. Energy levels of less than 10 joules permitted limited visualization of the retina immediately following exposure. The central area of the retinal burn often manifested acute hemorrhage. Peripheral blood vessels were blackened, and the central artery and vein had loss of sheen and darkening. The retina peripheral to the exposed area was generally gray in color and no light reflex could be elicited. Gassing and hemorrhage into the vitreous humor was frequently observed. Exposures in the range of 100 joules and above resulted in a marked degree of periorbital edema.

Three animals in the first series were put to death painlessly 9, 12, and 15 months post exposure. In all three animals (exposures 8, 9, and 10 in Table II) the retina was completely degenerated in the exposed eye at time of killing. The mangabey, exposure 8, displayed a normal cornea and lens. However, the retina contained no normal neural elements, and degeneration of associated tracts could be traced throughout the visual system. The degeneration of the retina is demonstrated in Fig. 1.

Two macaques exposed to high energy single pulses (exposures 9 and 10) displayed complete opacity of the cornea and softening of the globe. A small mass of glial elements about 2 mm. in diameter was found in the periphery of the eye of exposure 9. In section the mass was found to be an encapsulated clump of pigment. Exposure number 10, sacrificed at 15 months, was found to be aphakic. A finding of major interest in this animal was a gross lesion, in the nonexposed eye, running from the disk to about 2 mm. temporal to the fovea. Although care was taken to cover the nonexposed eye during exposure, enough energy was apparently able to penetrate the tape and the upper lid to produce a large lesion. In both animals total degeneration of the optic nerve, optic tract, and lateral geniculate body could be traced from the destroyed eye.

**Histological observations.** The major primary damage produced by a millisecond range laser pulse is found in the pigment epithelium and choroid. Energy levels on the order of 30 joules cause immediate detachment of almost the entire retina with portions of the retina being blown back into the vitreal cavity, and depigmentation of the underlying choroid. Fig. 2 presents two views of an enucleated globe exposed to a 29 joule pulse. In the lower figure, the bulb is transilluminated from the front. Pulse energies considerably below this level result in destruction of the pigment epithelium and choroid in the area of exposure, infiltration of free blood cells into the area destroyed, and a progressive retinal detachment that may or may not be self-limiting.

For purposes of quantification, the histopathology of the eye was lumped into eight gross categories. Table I presents the exposure series and Table II, the types of damage found following exposure. Fig. 3 is a representative sample of normal retina and some of the eight damage categories. The gross categories are not exhaustive in any sense and were chosen only as indicators in an attempt to elaborate the insult and indicate the time course of other changes. The exposure numbers of the individual sections are indicated in the lower left corner of the sections and correspond to the exposure given in Table I. The magnifications are noted in the lower right corner of each section. The figure was made of 8 by 10 inch enlargements of 4 by 5 inch photographic plates. The true magnification of each section must take into account the photographic reduction in size for publication.
Fig. 2. Dissected globe, surface illuminated (upper) and transilluminated from the front (lower), after exposure to a 20 joule 1 msec. laser flash. Maxwellian presentation.
Table I. Exposure series

<table>
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<th>Exposure No.</th>
<th>Energy (joules)</th>
<th>Energy density (cal./sq. cm.)</th>
<th>Post exposure (days)</th>
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Table II. Damage found in monkey eyes after exposure to laser rays

<table>
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<tr>
<th>Exposure No.</th>
<th>Retinal detachment</th>
<th>Pigment loss (P. E.)</th>
<th>Choroid damage</th>
<th>Microlesions or scattered pigment granules</th>
<th>Degeneration of inner and outer segments Fibrous elements</th>
<th>Pyknotic nuclei Free blood cells Loss of architecture</th>
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Sections A and B in Fig. 3 are control sections from an unexposed Cercocebus torquatus eye. Section A is stained with gallocyanin\(^2\) and section B with Mallory-Heidenhain "azan" aniline blue.\(^3\) Section C is a gallocyanin stained section of Macaca retina showing retinal detachment in the region of Bruch's membrane with some free blood cells in the area. The area shown is peripheral to the laser lesion and shows some choroidal damage although the pigment epithelium is relatively intact. Section D is a higher power view of a portion of section C showing pyknotic receptor cell nuclei in the outer nuclear layer and the distorted outer plexiform layer. Section E is a gallocyanin stained section showing retinal detachment through the fovea and complete absence of the pigment epithelium. Degeneration of the receptor outer segment has occurred and the ellipsoid portion of the inner segment is club or mushroom shaped. Macrophages and fibroblasts are found in the choriocapillaris. Clumps of pigment granules are seen in the region normally occupied by the outer segments and pigment epithelium. Section F is a higher power view of the area between Bruch's membrane and the outer limiting membrane seen in section E. Section G is a gallocyanin stained section from exposure 4. This section shows clumping of pigment probably from the pigment epithelium, and isolated spindle-shaped melanin granules from the pigment epithelial cells are seen in and around the receptor inner and outer segments. Some degeneration of the receptor segments can be detected. Club-shaped ellipsoids can be found with no outer seg-
Fig. 3. Composite showing normal retina (A and B) and the damage categories listed in Table II. Sections are perpendicular to the retinal surface, and the light path is from the bottom. See text for description.

ment. Section $H$ is a Mallory triple stained section from exposure 7. In this section the receptor segments scleral to the outer limiting membrane are completely degenerated. The number of receptor nuclei is reduced but some viable cells are seen. The vitread processes of the nerve cells are prominent. Several tracks of granule lesions are evident. Section $I$ is a gallocyanin stained section from exposure 7 showing complete disruption of the retinal architecture and scattered pigment granules. The remaining viable nerve cells are probably a part of the inner nuclear layer. Section $J$ is a Mallory triple stained section of peripheral retina from exposure 7. Ghost inner seg-
ments are seen scleral to the outer limiting membrane, the retinal architecture has completely deteriorated, and isolated melanin granules are found scattered throughout the section. Section 6 is a hematoxylin and eosin stained section from exposure showing retinal detachment, pigment clumps, receptor outer segment degeneration, club-shaped ellipsoids in the inner segment, and early degeneration of the retinal architecture in the inner nuclear layer.

Discussion

Total retinal detachment and blindness following a single laser pulse was a rather unexpected outcome in the first series of exposures. Ham and associates had found that the retinal dose required to produce a chorioretinal lesion decreased as the area radiated increased with image diameters ranging from 0.18 to 1.1 mm. However, Jacobson, Cooper, and Najac extended the range of image diameters to 1, 2, and 4 mm. and found the threshold dose required to produce a burn increased as a function of image diameter. Both studies used white light as the source and found the burn threshold to be about 1.85 cal. per square centimeter for a 1 mm. image diameter. Jacobson, Cooper, and Najac found that the retinal dose required to produce a burn with a 4 mm. image diameter exceeded 2 cal. per square centimeter.

The minimum energy density required to produce a burn in the rabbit retina with a pulsed ruby laser has been determined by Ham and associates. The threshold energy density, 0.85 joules per square centimeter delivered in 200 μsec, is relatively independent of image diameter within the range of 0.5 to 1.0 mm. No ruby laser lesions of large image diameter have been reported. However, if the relationship of image diameter to energy density for a burn reported by Ham and associates and Jacobson and associates holds for pulsed ruby laser, we should find that the threshold energy density required for a 1 mm. image diameter is about 3/4 of the energy density required for a burn with a 4 mm. image diameter and an image diameter larger than 4 mm. would require a greater energy density.

The devastating effects of the first series of exposures indicated that energy densities in calories per square centimeter (1 cal. = 4.18 joules) were considerably too high. However, they indicated that total detachment occurred in the first six days post exposure and laser-induced trauma should be easily discernible at this time.

A second series of exposures was carried out with four Macaca cynomolgus. The animals were exposed to one laser pulse in each eye and painlessly put to death six days later. Pre- and postexposure ophthalmoscopic examinations were carried out for each exposure and again at time of killing. The energy densities in this series of exposures ranged from 1.0-6.2 joules per square centimeter. The calculated image diameter was 11.25 mm., assuming a posterior modal distance of 15 mm. The image diameter is calculated from the arc segment of a 15 mm. diameter circle, intercepted by an angle of 43.2 degrees originating on the circumference of the circle.

Results

Gross observations. Energy densities below 2.6 joules per square centimeter delivered in 1.5 msec, did not produce an observable lesion. Cobblestoning or small irregularities of the fundus were seen six days post exposure in three eyes. There was usually some graying of the fundus immediately and by six days post exposure the retinal architecture appeared to be totally detached with a loss of definition of the disk and surface vessels.

Histological examination. Degenerative changes were always found in laser exposed retinas. The characteristic appearance of degenerating photoreceptors has been described by Krückmann. Briefly, following insult to the photoreceptors the
outer segment breaks up into platelets, there is dissolution of the inner segment, the nucleus passes scleral through the outer limiting membrane, and the resulting cleft is filled in by glial elements. This course of events according to Krückmann is schematized in the upper left plate of Fig. 4. The upper right plate in Fig. 4 demonstrates the appearance of a laser exposed retina six days after exposure. The left center plate is a high power view of several degenerating receptors. The loss of definition of the outer limiting membrane is readily apparent in these plates.

The lesion boundary is very difficult to detect if secondary degeneration of the retina is allowed to progress as long as six days. However, careful examination of the pigment epithelium reveals a sharp boundary between normal and abnormal cells in the pigment epithelium. The cells in the exposure area are poorly stained, swollen, and multinucleated. The neighboring pigment epithelium shows a regular arrangement of low cuboidal single nucleated cells. Laser insulted pigment epithelium is shown in the right center plate of Fig. 4, and the abrupt transition from abnormal to normal cells is shown in the lower plate. The entire field of the lower plate is less than 130 μ.

Discussion

It is impossible to calculate the energy actually arriving at the retina without knowledge of the transmission characteristics of the ocular media because transmission through the eye varies as a function of wavelength. The energy package in electromagnetic radiation is the quantum, and the total amount of radiant energy is the sum of all the individual energies of the individual quanta. The quantum energy is determined by the wavelength of the radiation with all quanta of the same wavelength having the same energy. Since the quantum energy is directly proportional to the frequency of the radiation, short wavelength radiation contains higher energy quanta than long wavelength radiation. In the visible range of the spectrum, a laser pulse of X quanta at the wavelength 4,000 Å would contain twice as much energy as a laser pulse of X quanta at the wavelength 8,000 Å. However, when calculating the amount of energy incident on the fundus of the eye from these pulses we would find the relationship reversed by the absorption spectrum of ocular media. Geeraets and associates determined the per cent of radiant energy absorbed in the human ocular media over the range of 3,500 to 15,000 Å, and their data indicate that nearly 100 per cent of the energy at 4,000 Å is absorbed before it reaches the fundus. Less than 5 per cent of the energy at 8,000 Å is absorbed by the ocular media and, assuming 0 per cent refractive loss, 95 per cent of the energy impinges on the retina and choroid. The results reported in this paper are unique to the ruby wavelength and cannot be generalized to lasers producing light of other wavelengths.

The relationship of the pigment epithelium to the retina and choroid is unique both in structure and function and any damage to the pigment epithelium is deleterious to visual function. Ham and associates have determined that the "threshold dose" of radiation, whether delivered from a non Q switched laser or pulsed intense white light, causes an abrupt rise in temperature in the pigment epithelium. The temperature required for a "threshold" burn is estimated to be close to 100° C. Suprathreshold doses drive the temperature above the vapor point of water and create disruption of the retina from escaping steam bubbles. Vapor bubbles in the vitreous have been observed following laser coagulations. Temperatures well below the vapor point of water are probably lethal to the cells in which the higher temperatures occur and, thus, destruction of a restricted area of pigment epithelium probably results with exposure intensities well below the defined threshold.

The changes in retinal morphology seen in the present study at different times post exposure indicate the following course of events: absorption of laser energy in the
Fig. 4. Composite showing schematized course of photoreceptor degeneration after Krückmann; degenerating photoreceptors in laser exposed retina (upper right, center left); laser exposed pigment epithelium (center right); and the abrupt transition from abnormal to normal photoreceptors.
pigment epithelium causes a localized temperature elevation in the region of the pigment epithelium receptor outer segment junction with no discernible damage to more vitread retinal structures. Subsequently, the attachment of the receptor outer segments with the pigment epithelial cells degenerates and the receptor ellipsoid begins to enlarge and become club-shaped. The area of the outer segment pigment epithelium junction is infiltrated by an eosinophilic fluid ground substance that mechanically breaks the receptor outer segment pigment epithelium junction in the area surrounding the lesion. The fluid infiltration and mechanical detachment continue to increase in area until the entire retina is dissected away from its relationship with the pigment epithelium and the retina slowly dedifferentiates from a highly organized sensory structure to a neural-glial epithelium.

Ikeda and Foulds\textsuperscript{1-2} have caused separation of the neural retina from the pigment epithelium by creating a small retinal hole by suction and injecting hyaluronidase into the posterior vitreous. This treatment is followed by a slow total detachment of the retina through the area of the junction of receptor outer limbs and the pigment epithelium cells. A total detachment resulted in some degeneration of the outer segments within 48 hours. With long-standing detachment, i.e., 32 weeks, the neural elements of the retina were replaced with glial elements. Within the 32 week period from treatment to replacement of glial elements with neural tissue, the degenerative changes found closely parallel our findings following laser exposure. The findings of Ikeda and Foulds\textsuperscript{1-2} and the degenerative changes seen following vitaminosis A\textsuperscript{13} suggest that interference with the structure or function of the pigment epithelium or with the intimate association of the receptor outer segments and the pigment epithelium cells result in degeneration of the retina.

We would like to thank Dr. Hisako Ikeda for sending us some slides of her material for study.

REFERENCES