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US ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY

REPORT NO. 653

RUBY LASER EFFECTS ON OCULAR STRUCTURES

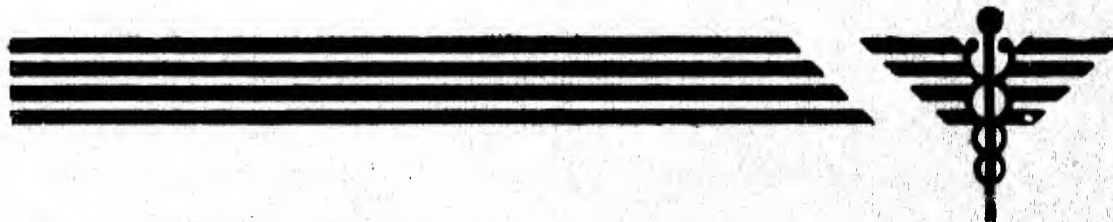
by

Arthur E. Jones, Ph. D.

and

Captain Alan J. McCartney, MC

7 January 1966



UNITED STATES ARMY
MEDICAL RESEARCH AND DEVELOPMENT COMMAND

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In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care as established by the National Society for Medical Research."

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REPORT NO. 653

RUBY LASER EFFECTS ON OCULAR STRUCTURES

by

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Fort Knox, Kentucky

7 January 1966

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Subtask No. 11
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DA Project No. 3A014501A71E

ABSTRACT

RUBY LASER EFFECTS ON OCULAR STRUCTURES

OBJECT

This investigation was carried out to assess the effects of pulsed laser radiation at 6943 Å on the structure of the ocular components. Our interest was primarily concerned with retinal structure.

METHOD

The left eyes of seven monkeys of the species Macaca cynamolgus and Cercocebus torquatus atys were exposed to laser pulses ranging from 5-250 joules with a pulse duration of approximately 1 millisecond. The animals were sacrificed at various times post exposure. With three animals, the left eye was exposed to a 5 joule pulse and the right eye to a pulse of 10, 15 or 20 joules. The animals were sacrificed 1, 6 and 23 days post exposure. All radiation was presented in a Maxwellian view which illuminated about 78.5 square millimeters or about 24% of the total retinal area.

RESULTS

At all energy levels superficial corneal damage could be seen immediately post exposure. By 24 hours superficial corneal damage was no longer evident and no corneal opacities ever developed. At energy levels above 5 joules the lens was totally disrupted and gas bubbles were evident throughout the anterior chamber. The lens damage was probably produced by the Maxwellian presentation. The major interior damage occurred in the choroid. At energy levels above 15 joules, granules from the choroid melanocytes were displaced in all directions causing microlesions of the retinal structures. Extensive retinal damage due to direct absorption of laser radiation was not seen. Retinal destruction appears to be secondary to high temperature thermal effects caused by absorption of the radiation in the choroidal melanocytes and subsequent retinal detachment in the more peripheral areas.

These observations were made following 5 joule pulses. Within 24 hours the pathology was characterized by hemorrhage into the

vitreous and the area of Bruch's membrane, clumping of choroidal pigment, loss of differential staining of retinal neural elements, thrombosis of microvessels, and retinal detachment peripheral to the central burn area. At 6 days, proliferation of connective tissue elements was found in the area of the choroid, an amorphous ground substance was seen in the area of Bruch's membrane and dedifferentiation of visual receptors into a pseudostratified epithelium occurred. The loss of differential staining persisted. A reduction in the glial supporting structure of the retina was evident. Three weeks post exposure disclosed fusion of the retina, choroid and sclera in the central area. Peripheral to this, the retina and pigment epithelium had reverted to simple neural epithelium, most likely as a result of the retinal detachment. In the retinal periphery some differential staining of cells was seen.

Because of the comparable energy densities used in clinical laser photocoagulations, similar pathological changes, including retinal detachment, might be expected to occur in much smaller retinal areas with laser photocoagulations.

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RUBY LASER EFFECTS ON OCULAR STRUCTURES

INTRODUCTION

Pulsed ruby laser radiation can be an important tool for the visual psychophysicist. The wavelength of the energy is such that only one of the four known photopigments will absorb it, 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. However, the potentially destructive effects of ruby laser radiation on ocular structures 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 100-150 μ in diameter. In clinical photocoagulation, the use of optics external to the eye have 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 unfocused image and the focal length of the lens.

A Maxwellian presentation was used in the present study: (1) to approximate the 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 cynamolgus and Cercocebus torquatus atys were exposed to laser pulses of approximately 2 msec duration with energy levels from 5-250 joules. The right eyes of three of the Macaca cynamolgus were also exposed.

A pulsed laser with a seven inch ruby rod approximately 5/8 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% 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 mm^2 or 24% 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 8μ . The sections were stained with hemotoxylin and eosin, or gallocyenin (Lapham *et al*, 1964), or Mallory's "azan" anilin blue (Lillie, 1954).

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 grey 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 of the high energy exposure animals are still surviving and are included as exposures 8, 9, and 10 in Table I. Exposure number 10 has complete opacity and atrophy of the globe. Exposure number 9 does not show atrophy of the globe, but the cornea is completely opaque. Exposure number 8 has no atrophy and a completely normal cornea. Some lenticular opacities can be seen and the animal has obvious visual loss. No exact assessment of visual loss has been attempted.

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. Figure 1 presents two views of an enucleated globe exposed to a 20 joule pulse. In the lower figure, the bulb is transilluminated from the front. Energy levels below this level result in destruction of the

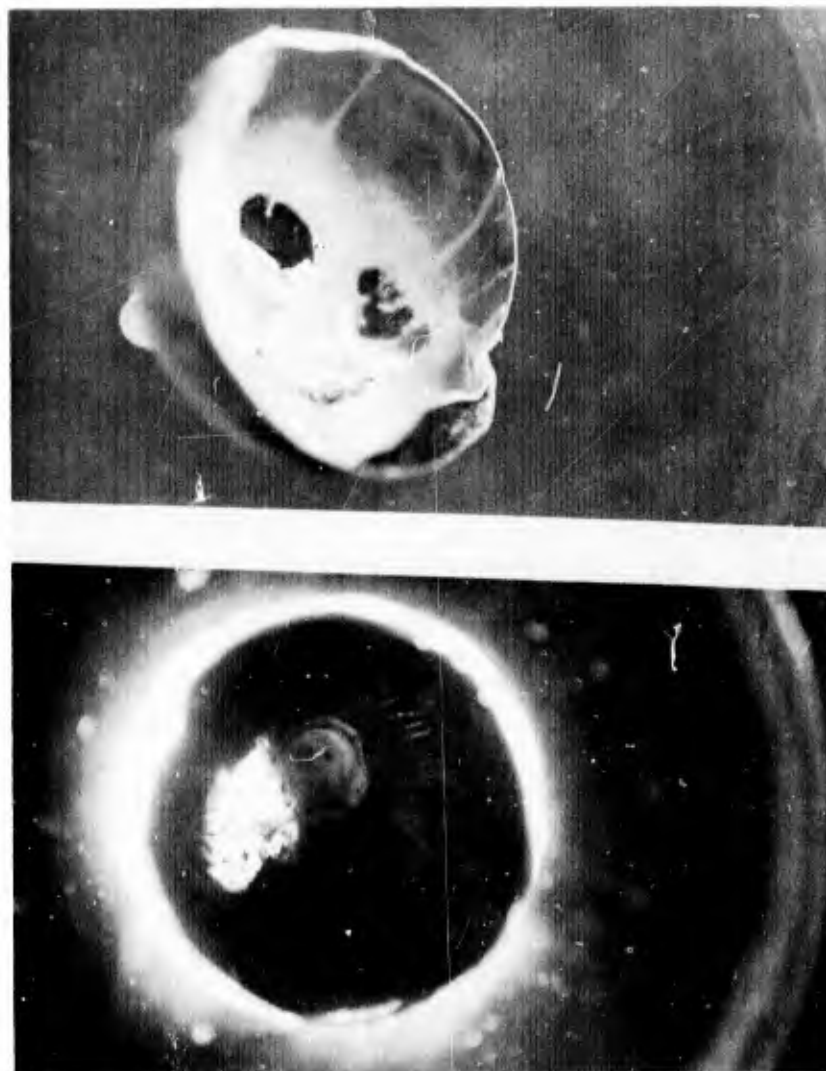


Fig. 1. Dissected globe, surface illuminated (upper) and transilluminated from the front (lower), after exposure to a 29 joule 1 msec laser flash. Maxwellian presentation.

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 the types of damage found following exposure.

TABLE I

Exposure Number	Energy in joules	Energy density in cal/cm ²	Post exposure in days	Species
1	5.0	1.53	1	Macaca cynamolgus
2	5.0	1.53	6	Macaca cynamolgus
3	5.0	1.53	23	Macaca cynamolgus
4	8.6	2.64	23	Macaca cynamolgus
5	15.0	4.59	1	Macaca cynamolgus
6	18.4	5.63	6	Macaca cynamolgus
7	60.0	18.40	19	Macaca cynamolgus
8	65.0	19.90	Surviving	Cercocebus torquatus
9	118.0	36.30	Surviving	Cercocebus torquatus
10	255.0	78.30	Surviving	Macaca cynamolgus

Exposure Number	Retinal detachment	Pigment loss in P. E.	Choroid damage	Microlesions or scattered pigment granules	Degeneration of inner & outer segments	Fibrous elements	Pycnotic Nuclei	Free blood cells	Loss of architecture
1	X	X	X	X	X		X	X	
2	X	X	X		X				
3	X	X	X	X	X	X			
4	X	X	X	X		X			
5	X	X	X	X	X	X	X	X	X
6	X	X	X	X	X	X			
7	X	X	X	X	X	X	X	X	X
8	Still surviving - large retinal lesion visible with ophthalmoscope								
9	Still surviving - cornea opaque								
10	Still surviving - cornea opaque, atrophy of the globe								

Figure 2 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 8x10" enlargements of 4x5"

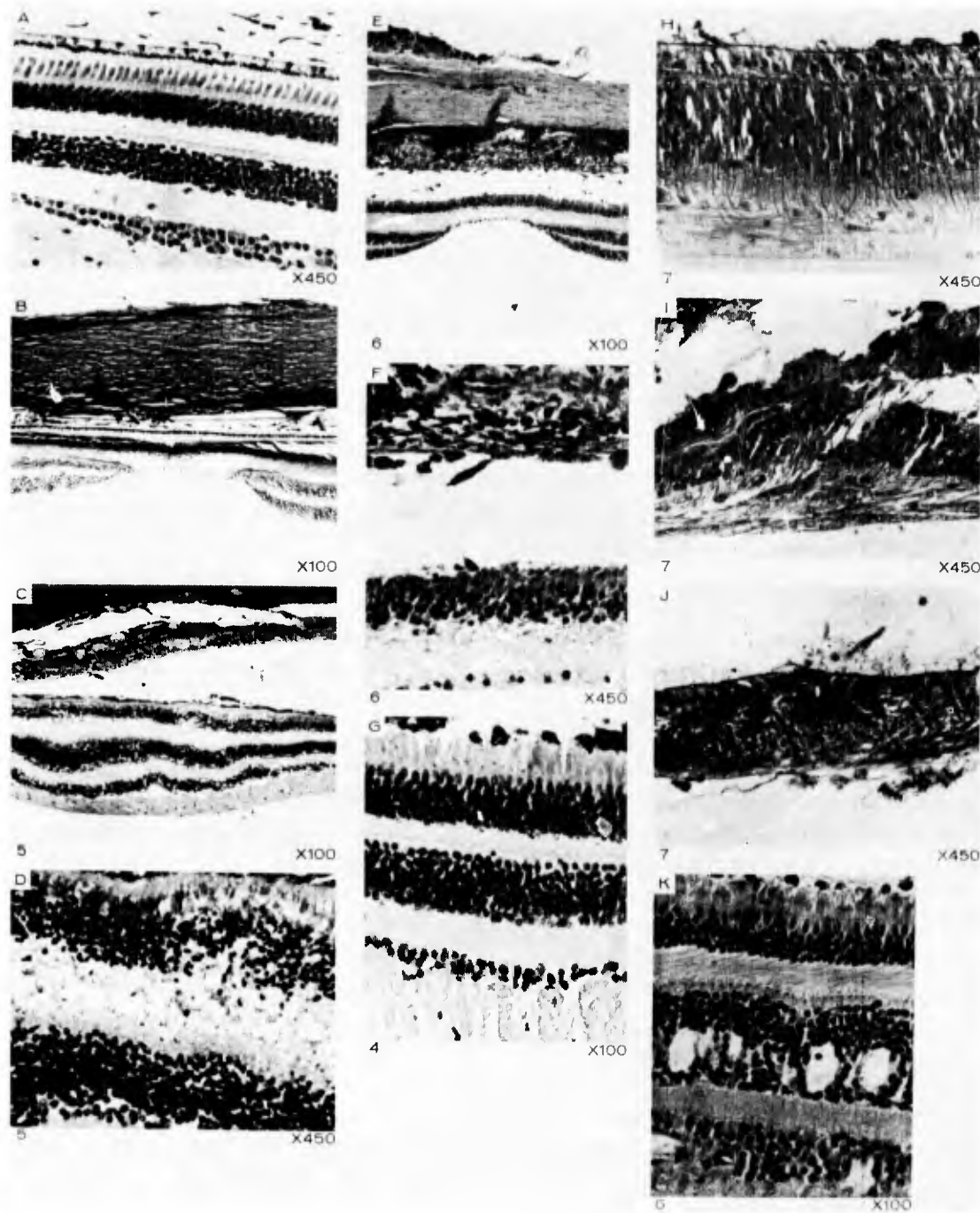


Fig. 2. Composite showing normal retina (A and B) and the damage categories listed in Table I. Sections are perpendicular to the retinal surface, and the light path is from the bottom. See text for description.

photographic plates. The true magnification of each section must take into account the photographic reduction in size for publication.

Sections A and B in Figure 2 are control sections from an unexposed Cercocebus torquatus eye. Section A is stained with gallocyanin (Lapham, 1964), and section B with Mallory-Heidenhain "azan" anilin blue (Lillie, 1954). Section C is a gallocyanin stained section of Macaca retina, exposure 5, 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 but 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 pycnotic receptor cell nuclei in the outer nuclear layer and the distorted outer plexiform layer. Section E is a gallocyanin stained section, from exposure 6, 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 hemotoxylin eosin 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 segment. 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 indicating a reduction in the density of the glial component of the retina. 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 from exposure 7 of peripheral retina. Ghost inner segments 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 K is a hemotoxylin and eosin stained section from exposure 6 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. This is taken to indicate degeneration of the Müller glial cells.

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 4000 Å would contain twice as much energy as a laser pulse of X quanta at the wavelength 8000 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 *et al* (1960) determined the percent of radiant energy absorbed in the human ocular media over the range of 3500-15,000 Å, and their data indicate that nearly 100% of the energy at 4000 Å is absorbed before it reaches the fundus. Less than 5% of the energy at 8000 Å is absorbed by the ocular media and, assuming 0% refractive loss, 95% 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 energy required to produce an ophthalmoscopically just visible lesion in the pigmented rabbit eye is something on the order of 0.85 J/cm² delivered in 200 microseconds to 0.07 J/cm² delivered in 30 nanoseconds (Ham *et al*, 1964). When these "threshold" values are converted to power density they become 4,250 watts/cm² for a 200 μ sec pulse and 2,300,000 watts/cm² for a 30 nanosecond pulse. The power densities used in the exposures reported here range from 4,250 watts/cm² to 216,600 watts/cm². Although the energy values presented at the eye are considerably higher than previously reported experimental exposures, the energy density values range only from 6.4 to 328 J/cm².

Clinical laser photocoagulators currently on the market can deliver 50 millijoules in 200 microseconds. Assuming a roughly circular area of 150 microns irradiated on the retina, this produces an energy

density of 290 joules/cm² and a power density of 1,448,000 watts/cm². These figures are well above the threshold figures reported by Ham et al (1964) and the energy density nearly equals the maximum experimental value reported here. The power density is substantially above the maximum value reported here because of the differences in pulse durations.

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 et al (1964) 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. Supra threshold 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 (Flocks and Zweng, 1964; Kapany and Peppers, 1963; Nayori et al, 1964; Zaret et al, 1963). 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 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 epithelium.

Ikeda and Foulds (1964) 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 weeks period from treatment to replacement of neural elements with glial tissue, the degenerative changes found closely parallel our findings following laser exposure. The findings of Ikeda and Foulds (1964) and the degenerative changes seen following A vitaminosis (Dowling and Gibbons, 1961) 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.

Since the advent of the photocoagulator, it has been well known that the severity of a chorioretinal burn is determined by the degree of pigmentation as well as the absolute intensity of the light. Clinical use of the photocoagulator, in fact, requires visual observation of the developing lesion (Havener, 1960) because of the variability in energy requirement to produce a satisfactory chorioretinal adhesion in different areas of the same retina. [Geeraets et al (1960) determined that the albino rabbit fundus absorbs less than 10% of the incident light at 700 m μ while the heavily pigmented rabbit fundus absorbs nearly 80% of the incident light at 700 m μ .] While many postulated effects of the unique radiation of the ruby laser are yet to be elucidated, most of the ocular effects are determined by the same parameters, i. e., rate of incident energy per unit area and degree of pigmentation that determine the severity of the lesion produced by white light photocoagulation. The primary ocular damage from laser exposure occurs in the pigment epithelium and the pigmented melanocytes of the choroid. The major retinal damage occurs as a result of thermal insult from the underlying choroid and microlesions from displaced pigment granules. Secondary retinal effects are seen peripheral to the exposed area that are attributed to loss of metabolic activity by the disruption of Bruch's membrane and vascular elements.

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13. ABSTRACT <p>Ruby laser flashes of 5-250 joules energy and 1 msec duration were focused by external optics into the eyes of seven monkeys, illuminating a retinal area of 78 sq mm. Temporary pitting of the corneal epithelium was seen at all energy levels, but cleared within 24 hours. Above 5 joules, the animals' lens were destroyed as a result of the light being focused into the lens. The retina was gray, without a light reflex, and the central area often manifested acute hemorrhage. Peripheral vessels were blackened as were the central artery and vein. At energy levels above 10 joules, large gas bubbles present in the anterior chamber precluded further gross examination.</p> <p>The major pathological changes occurred in the pigment epithelium of the retina and in the choroid, with coagulation of these tissues and displacement of melanin granules in all directions causing microlesions of the retina. Widespread retinal detachment occurred peripheral to the central burn area with subsequent dedifferentiation of the retinal neural layers to a pseudostratified epithelium. Because of the comparable energy densities used in clinical laser photocoagulations, similar pathological changes might be expected to occur in much smaller retinal areas with laser photocoagulations.</p>		

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UNCLASSIFIED
1. Photoreceptor
2. Laser
3. Vision

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RUBY LASER EFFECTS ON OCULAR STRUCTURES -
A. E. Jones and A. J. McCartney, w/tech asst
of J. E. Morrison

UNCLASSIFIED
1. Photoreceptor
2. Laser
3. Vision

Report No. 653, 7 Jan 66, 10 pp & iii - 2 illus -
1 table - DA Project No. 3A014501A71E, Unclassified Report
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focused by external optics into the eyes of seven monkeys, illumi-
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Above 5 joules, the animals' lens were destroyed as a result of the
light being focused into the lens. The retina was gray, without a
light reflex, and the central area often manifested acute hemorrhage.
Peripheral vessels were blackened as were the central artery and vein.
At energy levels above 10 joules, large gas bubbles present in the an-
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logical changes occurred in the pigment epithelium of the retina and
in the choroid, with coagulation of these tissues and displacement of
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Widespread retinal detachment occurred peripheral to the central burn

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