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LASER EXPOSURES IN THE MACULAS OF HUMAN VOLUNTEERS

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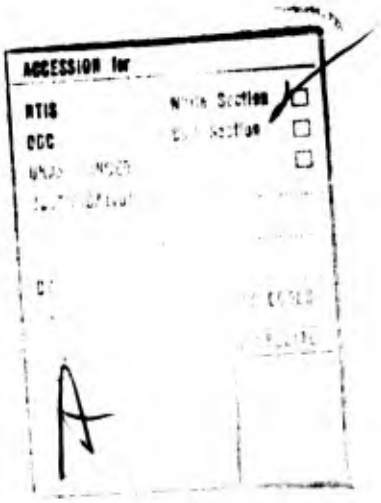
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SUMMARY

Patients whose eyes require enucleation for medical reasons (ocular melanoma) have been exposed to focused argon laser beams in a regular pattern of locations in the eye, especially in the macula, including the fovea. The power levels have been suprathreshold to just produce instantaneous lesions. The ophthalmoscopic appearance of the resultant lesions has been correlated with the loss of function and with the subsequent histological examination. An argon laser lesion in the macula may have greater functional impairment than a comparable ruby laser lesion. In lesions outside the macula the damage is confined to the retinal pigment epithelium and adjacent structures. In markedly suprathresnold lesions the outer segments of the rods were less disturbed than the inner nuclear and plexiform layers. Within the macula there are usually two damage locations separated by unchanged layers of the retina. In the pigment epithelium in which the damage was the same or less than in lesions outside the macula, there was a larger area of damage in the outer plexiform layer. Foveal lesions had greater damage to the fiber layer of Henle (outer plexiform layer) than in adjacent areas of the macula at comparable power levels. The pigment epithelium underlying the fovea had minimal or no damage since the macular pigment in the outer plexiform layer of the fovea may be present in a heavier concentration than in other portions of the macula.

A continuous line of argon laser exposures placed to form a closed square within the retina of owl monkeys will produce complete degeneration of the enclosed area. These exposures were markedly suprathreshold

and the lesion appeared to be continuous. Histological examination showed no permanent impairment of the choroidal circulation in enclosed areas although minor damage to some small vessels could be observed. Histological examinations taken at different times post irradiation show that degeneration of the retina begins with the photoreceptor layer and progresses across the inner layers. In the end stage complete atrophy of the retina was seen. Only a very thin tissue remained containing unidentifiable cells and exhibiting marked gliosis.

A previous report [24] has reviewed the literature and summarized our work to the end of 1972. In previous work we found that minimal lesions outside of the fovea could not be detected functionally. Superthreshold lesions outside of the macula and large enough to be used as marker lesions produced small scotomas which could be located easily when the projection in the visual space was accurately calculated. In general they would escape detection in a routine screening type examination. The patient could not detect them until they were pointed out to him.

Exposures at approximately 1/3 of the 50% level for macular burns did not produce a visible lesion in the fovea nor was there any functional effect. In every case that we could find a functional defect there was a lesion visible in the fovea, both by funduscopic examination and histology. Functional loss from rather large foveal burns would degrade vision to approximately 20/40 from original 20/20.

Laser Injury in the Macula

Earlier attempts to explain retinal damage resulting from laser exposure involved mainly thermal mechanisms. Vos[22] assumed uniform absorption of the laser energy by the pigment epithelium. He related the experimental data available to him at that time to a thermal model in which steam formation was the main mechanism for injury. Functional

damage may also occur at levels of irradiation too low to produce steam. In these cases the energy deposition or power level need only be sufficient to reach the temperature between 45 and 60°C. Vos assumed that exposures longer than 20 milliseconds were required to achieve thermal equilibrium in the retina. More recent experiments indicate that observable injury in the retina can result from nanosecond pulses whose energy is too small to cause the large temperature rises required by Vos's model.[4,5,12,13] Visible damage may result from a 5°C or less temperature rise. Later models[11,12] attempted to account for this discrepancy by considering the fine structure of the retina, assuming the melanin granules to be localized absorbers. More refined versions of these models have been proposed which incorporate two quantum excitation, Raman and Brillouin scattering, shock waves,[4,5] frequency doubling, reradiation by black body emission, and most recently, electron avalanche. Shock waves and electron avalanche are the current top contenders as models in the nanosecond pulse range, i.e., while in the intermediate pulse range, millisecond to 10 seconds, a purely thermal model appears to satisfy most of the data. When the exposure is longer than 10 sec the damage data seems to fit a photochemical model.[21] The damage from long exposures seems to be determined by the total energy. The spectral effectiveness of the exposure closely approximates the absorption spectrum of rhodopsin.

None of the above models distinguish between the macula and other parts of the retina. This may reflect a certain bias in the data. Either animals lacking a macula were used (rabbits, owl monkeys,

etc.) or where the test eyes had a macula (rhesus monkeys and human volunteers,[9,17,18,19] for example) most of the test exposures were in the non-macular regions of the retina.[2,14]

In the thermal model which applies to the intermediate range of exposures (microsecond to seconds) the energy is absorbed by the melanin pigment granules in the pigment epithelium. Heat diffuses from the pigment granules into the neural retina. Only in the millisecond to second range does this absorption produce thermal equilibrium. For shorter pulses a complicated and dissimilar time temperature dependence exists for each cell within the retina. The absorption of the melanin granules within the visible range (400-700 nm) is quite high.[12] This absorption does not change much as a function of wavelength. In the infrared region the granules absorb only slightly but do scatter to a high degree. This transition from absorption to scattering occurs in the near infrared--700 to 800 nm.

An examination of the anatomy of the retina indicates that absorption by the yellow macular pigment should modify this thermal model picture. The absorption spectra of the macular pigment is shown in Figure 1.[16] In the blue end of the spectrum the macular pigment could absorb a significant fraction of the radiant energy before it reaches the pigment granules in the pigment epithelium. However the macular pigment should absorb very little red light. The exact location of the macular pigment within the retina is not known with certainty. Several sites have been proposed. Fine, on the basis of an electronmicroscopic study, identified some fine granules in the ganglion cell layer as the macular pigment.[10]

However, other empirical evidence, such as the radial polarization centered around the fovea (as shown by the Haidinger brush effect) which is a characteristic of the macular pigment indicates the fiber layer of Henle as a more probable site. The fiber layer of Henle is the fiber bundle radiating from the fovea which forms the outer plexiform layer of the macula.

The purpose of the present series of experiments was to determine if absorption by the macular pigment plays a significant role in laser damage to the retina. One possible implication of such an effect would be to change the energy required for threshold lesions in the blue end of the spectrum as compared with other wavelengths.[15,18] Absorption by the macular pigment could also change the character of the lesion as compared with other wavelengths. Absorption by the macular pigment could also change the character of the lesion as compared with the classical type of threshold lesion formed at the junction of pigment epithelium and the photoreceptor outer segments. For simplicity's sake we have restricted our studies of this problem to the millisecond to second time domain in which a purely thermal model of damage can be used.

In the present series of experiments, the eyes were exposed to focused argon laser beams in the paramacular and macular regions. In each eye the macular exposures usually included one in the fovea. The resulting lesions were examined ophthalmoscopically as well as histologically. It was obvious from these examinations that the macular pigment has a marked effect on the type of lesion produced within the macula. This suggests that a different model is required for laser damage within the macula and furthermore that the appearance (both ophthalmoscopically and

histologically) of a macular lesion will be highly dependent upon the wavelength of the laser exposure.

Since the previous report the eyes of seven additional patients have been studied. During the present reporting period a difference in the funduscopic appearance of the lesions in the paramacular and macular regions was studied intensively.

In connection with the development of the histological techniques a number of experiments were carried out in owl monkeys (Aotus trivirgatus) and rhesus monkeys. In these monkeys squares of extensive continuous lesions were produced in the retina by an argon laser. Fluorescein angiography indicated minimal interruptions to the retinal circulation in the center of the lesions immediately after laser exposure. However, no clear indication of sustained break in the integrity of the choroidal circulation could be obtained. At various times after placing the lesions histological sections were taken. In the irradiated areas there was complete destruction of the retina and scarring of the superficial choroid. Although some subadjacent small choroidal vessels were plugged, most of the vessels appeared normal. In the center of the ring, the retina was thin and showed extensive gliosis. The retinal atrophy appeared to begin with the photoreceptors and later extended into the retinal nuclear layers. This was followed by severe atrophy of the retinal nerve fiber layer. The underlying choroid appeared unaffected.

Methods

A Coherent Radiation laser photocoagulator Model 800 was used for all exposures. This consists of an argon laser (normally 1 watt output)

coupled to a Zeiss photo slit lamp. The laser coagulator has been modified to give a digital reading in milliwatts for the output power level. The argon laser plasma tube exit window is imaged on the retina through the slit lamp optics and a flat surfaced truncated cone designed to neutralize the corneal refraction. This flat faced cone is similar to the axial (or nonreflecting) pathway of the Goldman three-mirror lens system.[7,8] In this position a flat-surfaced truncated cone is placed over the cornea in the direct optical pathway.[24] This lens replaces the corneal refracting surface with a flat front surface and allows the image of the laser exit mirror, the viewing light, and the aiming light to be projected and focused in the plane of the retina simultaneously.

In all cases the accommodation of the lens was paralyzed and the iris was dilated with Mydriacyl. Fundus photography and fluorescein angiography were done routinely before and after all laser exposure procedures.

Special Procedure for Owl Monkeys

Both eyes in fourteen owl monkeys were used. However, in a given monkey both eyes were not always exposed on the same day.

Ketamine anesthesia (Ketalar) was used in all cases. In an occasional animal a retrobulbar injection of xylocaine was used to aid in stabilizing the eye. A corneal contact lens similar to that used on human volunteers was used to allow visualization of the fundus. Mydriacyl was used to paralyze the accommodation reflex and produce dilation of the iris. Fundus photography, occasionally accompanied by fluorescein angiography, followed the laser exposures. The animals were maintained for varying periods, ranging from 2 days to 2 weeks following laser exposure and then sacrificed to allow histological examination. In general, sacrifice

was accompanied by perfusion of the head through the carotid artery with a glutaraldehyde solution using the method of Yanoff et al.[27]

In conducting the research on owl monkeys described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as set forth by the Committee on the Guide for Laboratory Animal Facilities and Care sponsored by the Institute of Laboratory and Animal Resources and the National Academy of Sciences-National Research Council.

Results: Case Descriptions

Case #1

This 61-year-old white male was admitted to the Duke University Eye Center for an evaluation of a mass in the left eye at the posterior pole. Best corrected vision was 20/60 in the left eye and 20/40 in the right eye. An ophthalmological examination showed a mottled subretinal mass elevating the retina inferior to the fovea and extending toward the optic disc. The visual field had a superior arch cut in the visual field to approximately 5° from the fovea. Following this examination a series of laser burns was placed around and through the left macula. Approximately 37 exposures were made with the argon laser. The laser exposures were made in a spoke-like pattern with six arms centered on the fovea. A 50 micron retinal spot size was used with a 0.2 sec duration. Four power levels were used: 25, 50, 100, 200 milliwatts, all at 0.2 sec duration. A large marker burn was made at the end of each spoke. No burn was made in the fovea. In addition there were several exposures in the disc at 100 milliwatts, 100 micron spot size, 0.5 sec duration.

All lesions in the macular region appeared through the ophthalmoscope as if they were elevated. The macular lesions seem to be in the neural retina, while non-macular lesions seemed to be on or in the pigment epithelium. Immediately following the exposures the visual acuity was recorded as unchanged. The patient could not discern any of the laser burns unless they were especially searched for in a tangent screen examination. Subsequent histological examination showed the mass to be spindle B type malignant melanoma. However, histological examination of the area in which laser exposures were made was not possible. This portion of the retina deteriorated during the processing. Possibly this was due to the extensive detachment of the retina over the tumor extending into the laser exposed area during the histological processing procedure.

Case #2

This 52-year-old white male was admitted to the Duke University Eye Center for diagnosis of a mass in the upper portion of the fundus of the right eye. Upon examination he had a best corrected visual acuity of 20/25 in the right eye and 20/20 in the left. There was some blood within the vitreous and lying on the retina over an elevated mass superior to the macula. Approximately 100 laser exposures were made in a cross-shaped pattern on the retina with the center at the fovea. The vertical arm was approximately 60-70 milliwatts, 0.2 sec and a 100 micron retinal spot size. The vertical meridian produced light, small lesions. As in Case #1, the macular lesions appeared much more superficial than those in the paramacula and periphery. The exposures in the horizontal meridian were at 55 milliwatts, 0.2 sec and a 100 micron retinal spot size. The horizontal exposures did not produce much reaction or edema

in the macular region although most of the exposures farther out in the temporal part of the retina were followed by some edema. The exposures produced no change in the visual acuity as measured 15 minutes after the last exposure. The patient could see the exposures as small nonadapting lights, each like the remainder of a small flash bulb. Approximately 24 hours later the same report was given by the patient. The following day the eye was enucleated. Histological examination showed that the mass was either an amelanotic melanoma or a metastatic neoplasm. No clear retinal sections could be obtained in the macular region. The retina was extensively detached and the fovea could not be located.

Case #3

This was a 52-year-old white female who was admitted to the Duke Eye Center for a diagnosis concerning a mass in her left eye. At the time of examination the best corrected visual acuity in the normal right eye was 20/20 and in the left eye, 20/60. An examination of the left eye showed a hard dome-shaped mass nasal to the disc and an area of mottled pigmentation inferior to the optic disc. Accompanying this was a large temporal field defect corresponding to the position of the dome-shaped mass. Following the examination a series of laser burns was placed horizontally across the left macula. Vertical markings were made superior and inferior to the fovea. Seventy-six exposures were made with a 100 micron spot size, 0.2 sec duration, and 60 milliwatts power. Subsequent to the laser exposure the patient had no subjective change in her vision. Eighteen hours after the laser exposure visual acuity in the control eye (right) was 20/20 and the exposed (left) eye 20/40. At

both 72 and 96 hours post-exposure, the vision in the exposed (left) eye was 20/50. At 120 hours after laser exposure vision in the exposed (left) eye was 20/40. The visual field, tested by tangent screen, revealed an isolated para-central scotoma, and large temporal field cut similar to those shown before laser exposure. Fluorescein angiography following laser exposure showed a staining pattern typical of laser lesions with a rim of fluorescence appearing initially around the dark hypofluorescent lesion spot followed by staining of the entire lesion and adjacent areas. Approximately 138 hours after laser exposure an erucleation was performed. Subsequent histological examination showed a melanoma of the choroid, spindle B type, at the location of the mass and a granulomatous chorioretinitis. In the histological examination all laser lesions showed involvement of the retinal pigment epithelium. In the macular region adjacent to the fovea there was also damage of the outer nuclear and outer plexiform layers. In the fovea there was extensive damage to the inner layers of the retina including the nuclear layer and the fiber layer of Henle. No clear lesion was seen in the retinal pigment epithelium. There was a serous detachment of the part of the macula which included the fovea.

Case #4

This was a 62-year-old white female who came to the Duke Eye Center for examination of a mass in her right eye. Funduscopic examination showed a pigmented, subretinal mass inferior and temporal to the optic disc in the right eye. The macula appeared normal. The best corrected vision was 20/20 in each eye.

Subsequent to this examination a horizontal series of laser exposures was placed across the right macula with vertical markers superior and inferior to the fovea. All exposures used a 100 micron retinal spot size, 0.2 sec duration and 70 milliwatts power. Each exposure produced a lesion which appeared immediately after the exposure. Subsequent to laser exposure, the patient was conscious of a horizontal line obscuring the vision in her right eye. Four hours after the laser exposure the visual acuity was 20/400 in the exposed (right) eye and 20/20 in the control (left) eye. Eighteen hours after laser exposure vision was 20/80 in the exposed (right) eye, and 48 hours after laser exposure vision was 20/70. Tangent screen visual field examination at the time showed a horizontal central scotoma. Fluorescein angiography at that time showed a typical staining pattern as described for Case #3. Approximately 64 hours after photocoagulation an enucleation was done.

The histological examination showed that the mass was a melanoma of the choroid, spindle B type. All lesions showed extensive damage to the retinal pigment epithelium. The majority involved the inner retina also in the area around the macula and in the macula itself. The fovea had a large lesion in the neural retina with moderate disruption of the pigment epithelium. A typical macular lesion eccentric to the fovea was similar to that shown in Figure 3. Although the retinal pigment epithelium is only moderately affected, the inner retina is much more heavily involved. The photoreceptors and the fiber layer of Henle and the inner limiting membrane are all quite extensively damaged. The damage in the neural retina does not seem to be continuous with the damage in the pigment epithelium. It is almost as if this is another damage site in addition to that in the retinal pigment epithelium.

Case #5

This was a 43-year-old white female who was admitted to the Duke University Eye Center. At the time of her examination she had a best corrected visual acuity of 20/20 in both eyes. Funduscopic examination showed a large fleshy mass inferior and temporal to the macula in the right eye. The macula, however, appeared normal. Immediately subsequent to this examination a horizontal series of laser exposures was placed across the right macula with vertical markers superior and inferior to the fovea. Several markers were also placed around the periphery of the macula. Thirty-six laser exposures were made using a 100 micron retinal spot size, 0.2 sec duration and 100 milliwatt power. After each laser exposure a lesion was immediately visible. Immediately following the complete series of laser exposures the patient noted a horizontal line obscuring her vision in the right eye, approximately in the position that the laser exposures occupied in the visual field. One hour after laser exposure the visual acuity was 20/60 in the right eye. At this time a tangent screen visual field test showed a horizontal central scotoma corresponding to the laser lesions and a superior nasal defect corresponding to the position of the large fleshy mass seen in the funduscopic examination. At 15 hours after laser exposure vision remained unchanged at 20/60 in the right eye. Immediately subsequent to this enucleation was performed. Histological examination showed that the large fleshy mass was a melanoma of the choroid, spindle B type.

A histological examination was also available for the laser exposed portion of the retina. All laser lesions showed some disruption of the retinal pigment epithelium. In the paramacular region, the retinal

pigment epithelium was often quite seriously damaged. Figure 2 is a histological section from the retina immediately adjacent to the macula. This shows rather extensive damage of the pigment epithelium without any noticeable damage to the inner layers of the retina. In the macular region exclusive of the fovea, both the retinal pigment epithelium and the outer plexiform layer were damaged. The layers, however, between the outer plexiform layer and the pigment epithelium did not appear to be damaged as extensively as either the pigment epithelium or the outer plexiform layer. Figure 3 shows a histological section from a typical area in the macula near the clivis. In this section the disruption of both the pigment epithelium and the outer plexiform layer are notable. The areas surrounding them are only slightly damaged. Indeed, there is definitely an undamaged zone in the outer nuclear layer including the proximal part of the outer segments of the photoreceptors which divide the two damage zones--the pigment epithelium and the outer plexiform layer. The damage in the fovea in this eye was similar to that shown in Figure 4. In the foveal region the neural retina was almost completely destroyed while the retinal pigment epithelium was only mildly damaged.

Case #6

This 45 year old white female was admitted to the Duke Eye Center for diagnosis concerning a probable melanoma in the right eye. At the time of examination the best corrected vision was 20/40, with central black area in her vision. Approximately 85 laser exposueres were placed in a 6-sided spoke-like pattern centered on the fovea. Each exposure was 0.2 sec, 250 mW, 500 micron spot size. Around the spoke-like

pattern were an additional group of 500 micron lesions placed to prevent the retina from detaching temporal to the disc. Approximately three days later the eye was enucleated. Subsequent histological examination showed a tumor composed predominantly of B-type sindle cells with fine nucleoli and abundant melanin. There was no extension of the tumor into the retina and sclera. The retina appeared to be detached in the region of the fovea. Figure 4 shows the wide separation due to detachment by proteinaceous fluid between the pigment epithelium and the foveal neural retina. The laser lesion in the neural retina is clearly separated by a millimeter or more from the pigment epithelium, thus obviating any possibility of heat flow from the pigment epithelium as a causative factor in the damage shown in the fiber layer of Henle. Also the pigment epithelium appeared to be normal, as if the exposure was subthreshold for injury in that part of the retina.

Case #7

This 59 year old white male was admitted to the Duke Eye Center for examination for possible melanoma of the ciliary body in the right eye. Funduscopic examination showed ciliary body mass at approximately 11 o'clock in the right eye with a heavy pigment concentration throughout the trabecular meshwork. Intraocular tension was 42, vision was 20/25. Subsequent to this examination a series of 71 exposures of argon laser were placed in the right eye in a grid pattern approximately four wide and 20 high, centered on the fovea. Exposures were 500 micron spot size, 217 mW and 0.2 sec. Histological examination showed malignant melanoma of the epithelial type with invasion into the trabecular meshwork and Schlemm's

canal. Enucleation was performed approximately 24 hours following laser exposure. Damage in the central macula was seen in the pigment epithelium layer as well as the inner portion of the outer plexiform layer. It was particularly noticed that in lesions on the periphery of the macula (where the ganglion cell layer was one or two cells thick) the damage was only in the pigment epithelium. All inner layers of the retina were undisturbed, including the outer plexiform layer. In the typical lesion in this area the pigment epithelium was heavily damaged, while the overlying retina was essentially normal, as is shown in Figure 2.

Results: Macular Lesions in Human Volunteers

A comparison of the energy levels required to make lesions in the paramacular, macular, and foveal regions does not indicate that there is a large systematic difference between these areas in their susceptibility to laser damage. Previous reports[18] have indicated that in the rhesus monkey there is a two-fold difference in threshold between the macula, including the fovea, and other parts of the retina. Our data is not sufficient to allow statistical analysis. However the differences shown previously are consistent with our findings. Certainly the difference is not larger. In the histological examination, all non-macular laser lesions showed involvement of the retinal pigment epithelium. In the macular region adjacent to the fovea there was also damage of the outer nuclear and outer plexiform layers. In the fovea there was extensive destruction of the outer layers of the retina including the nuclear layer and the fiber layer of Henle. The damage was often minimal or absent in the retinal pigment epithelium underlying the fovea.

In the paramacular region, the retinal pigment epithelium was often quite seriously damaged, without any noticeable damage to the inner layers of the retina. This is shown in Figure 2, which is a section from the retina on the edge of the macula. There is rather extensive damage of the pigment epithelium while inner layers of the retina still appear relatively normal. In the more central macular region exclusive of the fovea, both the retinal pigment epithelium and the outer plexiform layer were damaged. The layers, however, between the outer plexiform layer and the pigment epithelium did not appear to be damaged as extensively as either the pigment epithelium or the outer plexiform layer. Figure 3 shows a histological section from a typical area in the macula. In this section the disruption of both the pigment epithelium and the outer plexiform layer are notable. The areas surrounding them are only slightly damaged. The damage in the neural retina does not seem to be continuous with the damage in the pigment epithelium. It is as if this is another damage site in addition to that in the retinal pigment epithelium. Indeed, there is definitely a normal portion of the retina, including the outer nuclear layer and proximal parts of the photoreceptor outer segments, which divides the two damage zones--the pigment epithelium and the outer plexiform layer.

In the foveal region, the neural retina was almost completely destroyed while the retinal pigment epithelium was often only slightly damaged. A lesion in the fovea is shown in Figure 4. The retina was detached previous to the time of the laser exposure by leakage secondary to an intraocular melanoma. The photoreceptors and the fiber layer of Henle are quite extensively damaged, while no damage of the pigment epithelium is seen.

Owl Monkey Retinal Damage

In the monkeys, degeneration of a portion of unirradiated retina was found adjacent to that marked by suprathreshold lesions. In each eye a large square on the retina (approximately 2 millimeters in diameter) was delineated by a series of adjacent laser exposures, 500 microns in size and approximately 1/2 second exposure duration. Each part of the outline of the square was exposed twice. In most eyes the threshold for damage was approximately 10-20 milliwatts depending on the spot size. For a 500 micron spot it was about 20 milliwatts. The exposure levels used to outline the test square were thus all suprathreshold. Occasionally an exposure level as high as 100 milliwatts was used when it was necessary to reburn a partially exposed area to ensure continuity of the lesions around the square.

Figure 5 shows a typical photograph of the posterior pole of an owl monkey eye two hours after laser exposure. The laser lesions are outlined by fluorescein staining. As mentioned above the square is approximately 2 millimeters on the retina. The absence of bleeding, and the clarity of the vitreous in front of the lesion site are usual. The appearance of blood in the vitreous is infrequent and confined to small streamers which clear rapidly.

Sections taken one day later after the laser exposure show complete disruption in the area of the lesion. A typical section is shown in Figure 6A. Adjacent to the lesion a rather large disruption of the photoreceptor portion of the retina can be seen on the inside of the square. In the area adjacent to the lesion on the outside of the square the retina appears to be completely normal. Close examination of the

site of the lesion showed marked disruption of the pigment epithelium and extensive gliosis and inflammation within all layers of the retina with one exception. The outer segments and in some cases the inner segments also of the photoreceptors are preserved almost completely. Under high magnification extremely regular orientation of the outer segments was observed within a circumscribed area similar to that shown in Figure 6A. This was seen in all animals, and those isolated pockets were also found several weeks after laser exposure.

Figure 6B shows sections taken from another animal which was sacrificed two weeks after laser exposure. There is necrosis in all layers of the retina inside the square. Indeed, within the square, the normal retina has almost completely vanished. Outside of the square the retina is normal and appears unchanged. Within the square the underlying choroid does not appear to be seriously damaged. Most vessels are patent, although careful searching does reveal a few small vessels which may be plugged.

Fluorescein angiography taken after laser exposure (shown in Figure 5) indicated a normal retinal circulation inside the square immediately following laser exposure. If the circulation of the retina is compromised on any large scale as a result of the pattern of laser exposures, it is only a transient phenomena, at most lasting only during the time of the laser exposure.

Macular Lesions

We have noted that power levels for comparable suprathreshold laser lesions in the macula and the retina outside the macula are approximately

the same. This was especially noticeable if the lesions were comparable in terms of latency of appearance and apparent size, as both the ophthalmoscopic and histological appearances were different.

The macular lesions have an ophthalmoscopic appearance different from lesions in other parts of the retina. They appear to be located at a level within the retina that is well above the pigment epithelium. Stereoscopic viewing through a slit lamp used as a biomicroscope gives the best visualization of this phenomenon. This is a clear indication that the histological changes are not postmortem artifacts as these changes correlate with the *in vivo* appearance. Indeed it was the ophthalmoscopic examination that suggested that histological pictures would differ for macular and non-macular lesions. Bowbyes *et al.*[1] and Marshall *et al.*[15] noted that argon laser radiation produced threshold lesions in the macula which differ in appearance and location from those in other parts of the retina. Threshold lesions in the macula showed damage in the inner plexiform layer and the inner nuclear layer. Marshall *et al.*[15] did not mention damage in the outer plexiform layer or fiber layer of Henle near the fovea. Because pigmentation in the macula differs from individual to individual, it may be that these differences could be large enough to be responsible for the differences in the type of lesion that we saw as compared with Marshall *et al.*[15] and Bowbyes *et al.*[1]

In the paramacular region the lesion is typical of those previously described following laser exposure in the visible spectrum. The main damage is in the pigment epithelium layer. The degree of damage in the other layers of the retina seems dependent upon the proximity to the

pigment epithelium, although the mechanism of damage depends upon the power level and exposure duration. In some time domains simple heat flow from the pigment epithelium is evidently the cause of damage. Short exposure durations and high power levels produce damage more consistent with some form of mechanical disruption. Rapid heating of the pigment granules may lead to steam formation[12,22] or ejection of melanin granules and other tissue fragments from the pigment epithelium.[4] As we have demonstrated, within the macula, argon laser exposure damage is also mediated by the absorption of energy in the macular pigment.

The location of the macular pigment has not been definitively established yet. Fine[10] has suggested that the macular pigment is present in the ganglion cell cytoplasm as submicroscopic pigment particles or lipoidal granules. However, the laser damage which we find in the macula is mainly in the outer plexiform layer, occasionally spilling over into the outer nuclear layer and bipolar layer. This seems to indicate that the greatest concentration of the macular pigment is in the outer plexiform layer, if indeed this is not its sole location. As shown in Figure 4, we find two sites for damage in the macula--the pigment epithelium and the outer plexiform layer. Within the foveal region the macular pigment concentration appears to be much heavier, since comparable laser exposures produce much greater damage in this region (as shown in Figure 5). The neural retina is often literally blasted apart at exposure levels which produce much less extensive damage in adjacent macular areas.

The phenomena known as Haidinger's brushes is another reason for supposing that the pigment is in the outer plexiform layer. This entopic effect is due to the macula acting as an analyzer for polarized

light.[3,6,16] It preferentially absorbs one plane of polarized blue light. The absorbed plane of polarization of the analyzer changes in a radial arrangement around the fovea.[16,20] The outer plexiform layer or fiber layer of Henle is the only macular structure arranged radially within the macula.[26] This suggests that the plane of polarization in the macular pigment arises from its association with this fiber layer of Henle. It is this radial arrangement of these fibers that gives the macular pigment the characteristics of a polarizer arranged radially about the fovea.

The extensive literature on the macular pigment has been reviewed by Wald.[23] What is known of the nature of the macular pigment, which is lutein, a xanthophyll carotenoid, does not explain its association with the fiber layer of Henle preferentially to other retinal locations. However the nature of our laser lesions are consistent with the location of the macular pigment in this layer, or at least it is more concentrated here than in any other layer of the retina.

It is also of interest to note that suprathreshold lesions from argon laser exposures in the macula can be visualized by the subject. That is, they produce a noticeable scotoma in visual space. However, our earlier studies indicate that similar ruby laser lesions were not so visible or so disturbing.[25] It may be that disturbances in the fiber layer of Henle, as produced by the argon laser, degrade vision generally by interrupting fibers which project to an increased area of photoreceptors. That is, the interruption of the projecting fiber helps make the functional disturbance appear to be larger than the actual area destroyed.

Rowe and Rockwell,[19] who also exposed human volunteers to argon laser radiation, did not mention any difference in appearance of macular

versus non-macular lesions. However, only a few of their exposures were in the macular region, and none of these eyes with the macular exposures were subjected to histological examination. They did, however, notice one additional effect. The power levels for argon laser threshold lesions were higher than they calculated, even after making corrections for the absorption of the macular pigment and the pigment epithelium for the argon laser wavelength. They attributed this discrepancy to absorption by the yellow lens pigment. The density of the lens pigment was especially marked in some of their subjects. None of the subjects that we used had markedly yellowed lenses. It is also true that their patient population, i.e. diabetic patients, may have more lens yellowing than other subjects. This point, therefore, probably needs further investigation.

PART II. OWL MONKEY EXPERIMENTS

The experiments on the owl monkeys, designed to assist in the development of histological procedures, had a rather unexpected finding. A large square was burned into the fundus in an effort to prevent artifactual retinal detachment during the histological preparation of the material. Permanent interruption of the choroidal circulation by lesions placed in a square on the retina should certainly be accompanied by degeneration of the overlying retina, at the very least in the outermost layers. However, in the experiments described in this report only the moderate types of interruption could have happened. Fluorescein angiography and subsequent histological examination demonstrated that the retinal circulation within the square was almost normal subsequent to laser exposure. The histological examination showed that all of the larger choroidal vessels were patent, as were most of the smaller ones. An

occasional sclerosed vessel was seen but this minimal effect seems to be an unlikely cause for the ensuing complete destruction of the retina. The sequence of the degeneration of the normal retina was unmistakable. It was obvious that the outer layer segments of rods and cones disappeared first, followed by their cell bodies. Adjacent layers degenerated in turn back through the retina until all organized layers within the retina were affected. After a few weeks only a disordered, very thin mass of cells remained. Outside the square the retina was essentially normal in all respects. The demarcation between normal and degenerated retina was quite sharp with the laser lesion as the boundary. As mentioned above, the histological examination showed an occasional minor occlusion in the choroidal circulation within the square. However, immediately following the laser exposure there may well have been transient spasms in many, if not all, of the vessels supplying the area within the square of laser lesions.

It may be that a bright photic stimulus must accompany any interruption of the choroidal circulation. In our experiments there was both scattered light from the laser, and the rather bright examining light on the retina within the square outlined by the laser lesions. Perhaps this high photic stimulation could overstress the retina while the choroidal circulation is interrupted. The photic stress alone did not produce injury for the retina outside the square was exposed to the same light levels, and yet in all respects remained normal.

Injuries of this type introduce an added feature to the problem of laser safety. Although such degeneration of the retina within a circumscribed laser exposure region has not been observed in humans,

the possibility that large portions of the retina can be annihilated by laser exposure around the periphery must be kept in mind in cases of chronic or repeated laser exposure, even in the peripheral portions of the retina. The possibility of large scale degenerations appears remote as exposures similar to this have been used therapeutically in diabetic retinopathy without any evidence of central degeneration. However, no documentation exists for the degeneration of a completely circumscribed ring of markedly superthreshold exposures around the macula or for any area between the macula and the disc, either of which could seriously compromise visual function.

Conclusions

1. Differences between lesion formation following laser exposure in the macula and other parts of the retina have been examined and the following features noted:

a. Argon laser exposures on the human macula produce lesions which differ in ophthalmoscopic and histological appearance from lesions outside the macula. The radiation is absorbed by the macular pigment in the outer plexiform layer and causes damage in that layer.

b. The lesion in the fovea is much more apparent than in other parts of the macula from comparable laser exposures. We conclude that the macular pigment is more concentrated in the fovea than in other parts of the retina.

2. In the retinal lesions in owl monkeys and humans the outer segments of the receptors appear often to be less affected than any other cells or cellular parts adjacent to the pigment epithelium. This is evident in cases where lesions were made both within the macula and

outside. This is especially noticeable in markedly suprathreshold lesions where the outer segments appear to be preserved even though much of the rest of the retina was quite deranged. This preservation of the outer segments is compatible with a mechanism of heat fixation.

3. A continuous ring of argon laser lesions placed around a patch of retina in the fundus of an owl monkey eye produces atrophy of the inner retinal area even though the inner portion of the retina is not exposed directly to laser radiation. The degeneration of the retina begins with the outer segments of the receptors and proceeds with time through all the inner layers until only a thin layer of glial cells remains.

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Figure 1. Absorption spectrum of macular pigment (adapted from Naylor and Stanforth).[16] The absorption spectrum of xanthophyll (or lutein) extracted from the macular region in alcohol. The absorption spectrum of the macula *in vivo* and *in vitro* appears to be the same. In the macula this pigment shows a radial pattern of polarization but there is no dichroism. The peaks at 450 nm and 480 nm agree with the psychophysical experiments of Naylor and Stanforth.[16]

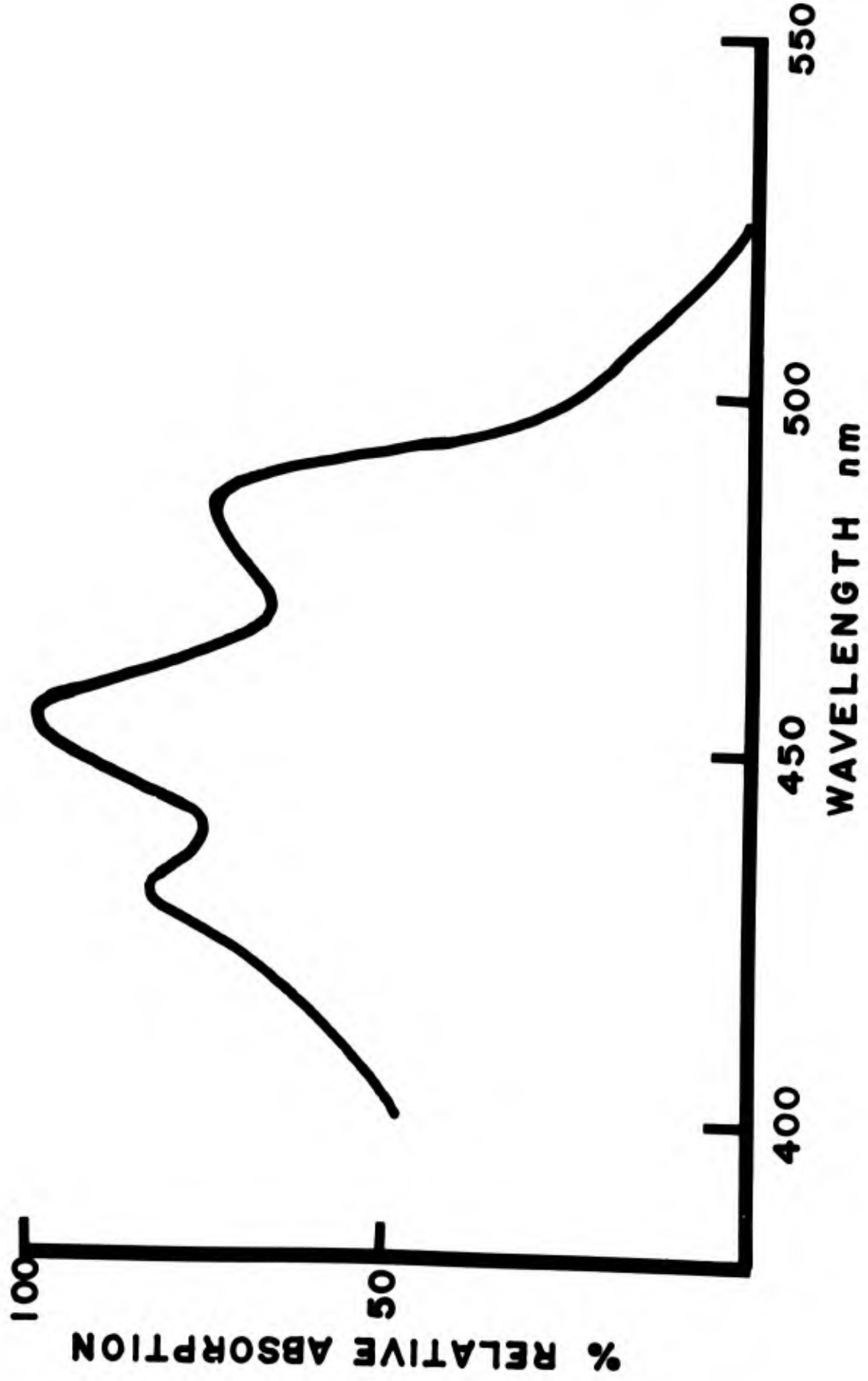

% RELATIVE ABSORPTION
100
50
400
450
500
550
WAVELENGTH nm

Figure 2. Lesion in the human retina resulting from an argon laser exposure on the edge of the macula as indicated by the thin ganglion cell layer. There is marked disturbance of the pigment epithelium layer and some derangement of the outer segments and of the outer nuclear layer. The inner layers of the retina appear to be undamaged. This lesion resulted from a 0.2 sec exposure at 60 milliwatts into the eye with a retinal spot size of 100 microns. It was well above threshold and was visible immediately after exposure (case #7).

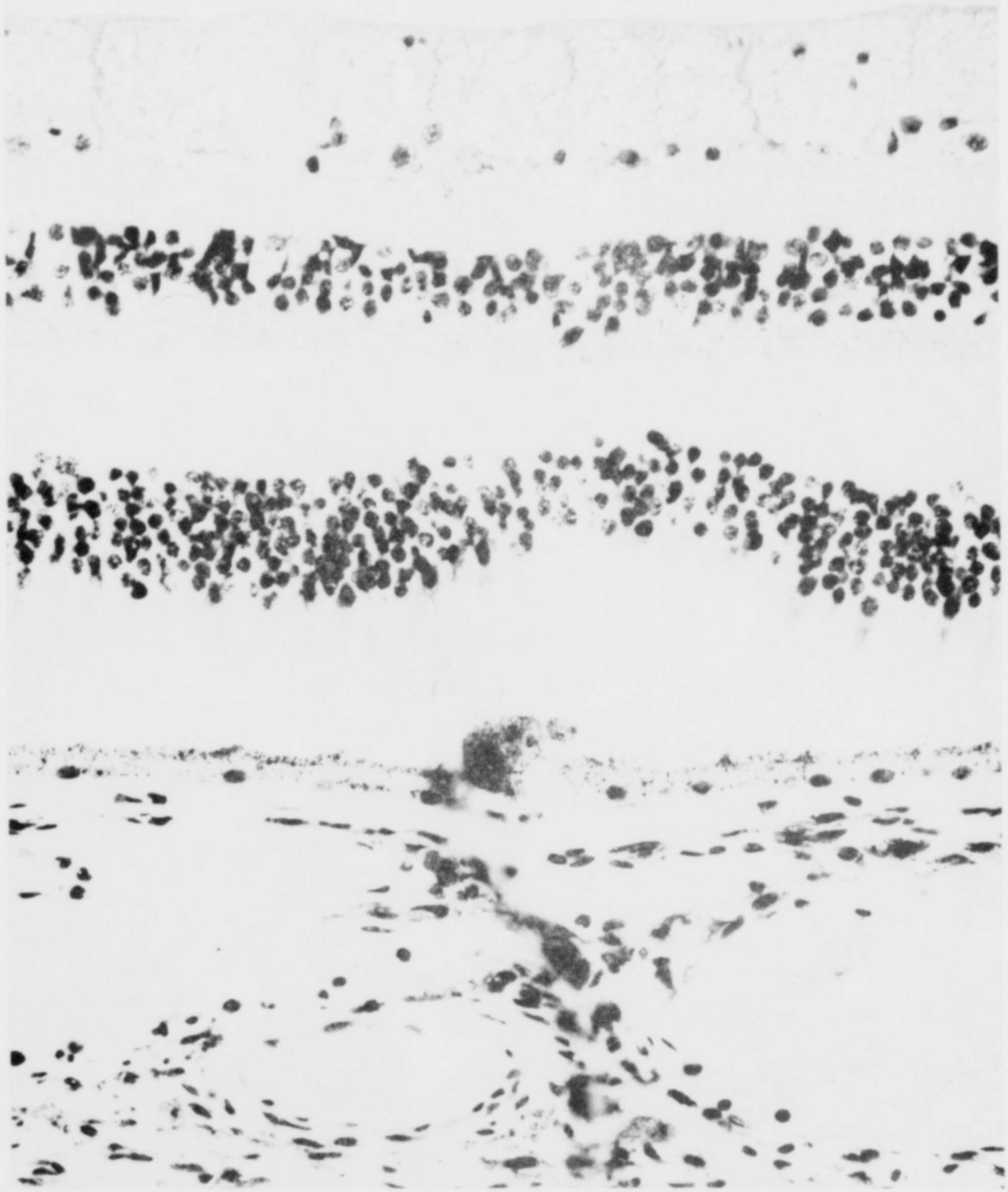


Figure 3. Lesion in the human retina resulting from argon laser exposure. The exposure duration was 0.2 sec, at 150 milliwatts with a retinal spot size of 100 microns. This lesion is in the macula on the edge of the clivus. There is some derangement of the pigment epithelium. The outer segments of the receptors and the outer nuclear layer next to them do not appear to be markedly deranged. The innermost portion of the outer nuclear layer and the neighboring outer plexiform layer are markedly disturbed. The damage in the outer plexiform layer is quite extensive and can be traced laterally for a wider range than any noticeable damage in the underlying pigment epithelium. The inner layers of the retina do not appear to be changed (case #5).

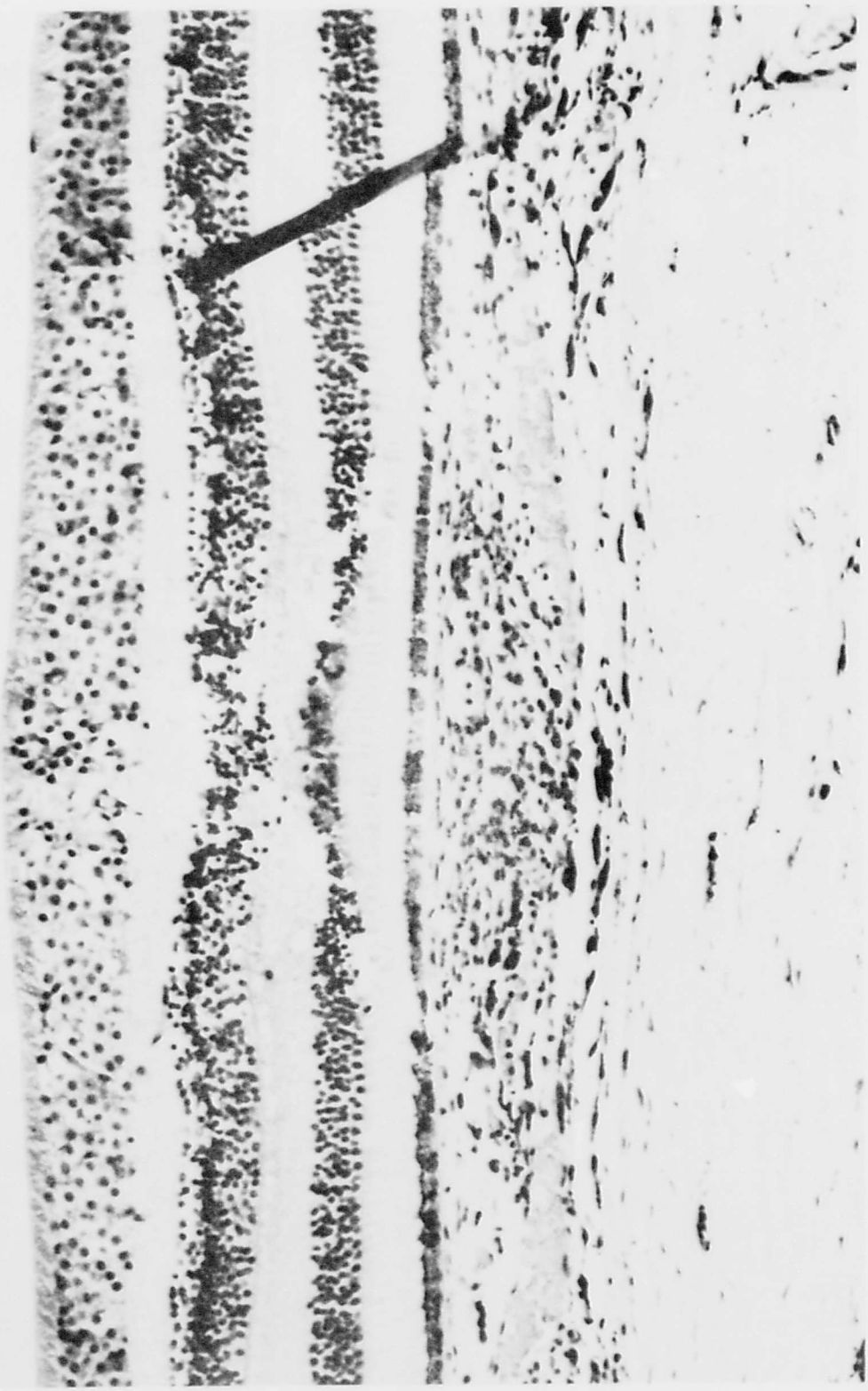


Figure 4. Damage in the human fovea from an argon laser exposure. The retina was detached in the entire macular region previous to the time of the exposure. The exposure was for 0.2 sec at 257 milliwatts and 500 micron retinal spot size. There is no derangement of the underlying pigment epithelium but in the outer nuclear layer the cell bodies of the foveal cones have almost been obliterated. Cells in the adjacent retina which had been detached for the same period of time but not exposed to laser radiation appear to be quite normal. The retina had been detached by serous fluid exudates secondary to an ocular melanoma. The proteinaceous serous fluid was preserved during the fixative process and is continuous from the outer segments of the receptors to the pigment epithelium. Presumably there could be little heat transfer at the power levels used between the pigment epithelium and the foveal region due to the separation by the serous fluid. The lesion was visible immediately and by both direct visualization and stereophotography appeared to be at a higher level than lesions in the undetached part of the retina. This indicates that the immediately visible part of the lesion was in the neural retina and did not spread from the pigment epithelium (case #6).

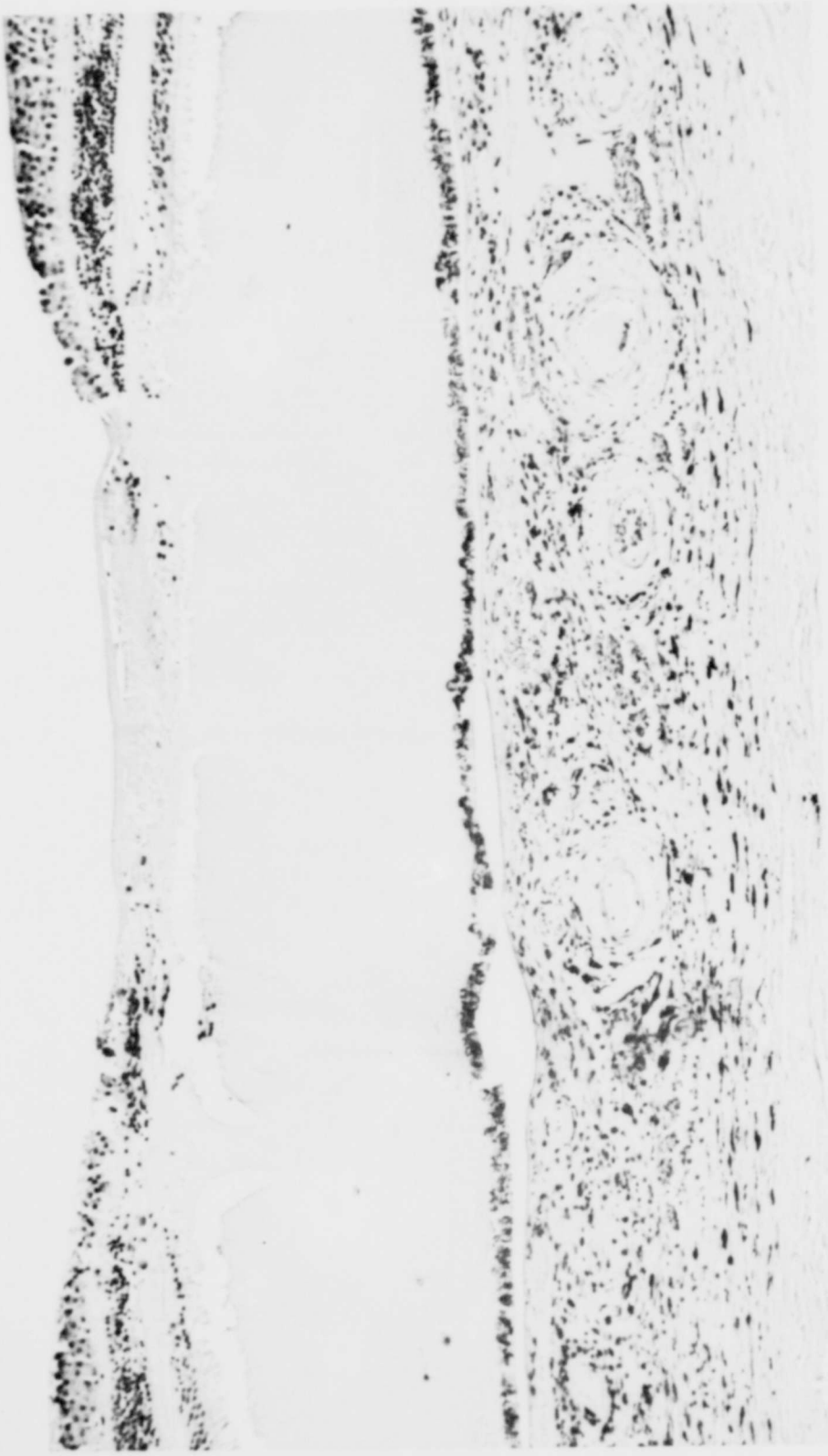


Figure 5. Fluorescein angiogram of owl monkey fundus. The square is approximately 2 mm on the retina. The square was formed by a series of adjacent argon laser exposures with a retinal spot size of 500 microns, 0.5 sec duration and initially 20 milliwatts into the eye. Each part was exposed twice with superthreshold levels. The second exposure was often at a considerably higher power level to produce a visible increase in width of the burned area. A late phase of the fluorescein angiograph has been chosen to show staining of the choroid and leakage in the retina to outline the lesions. The circulation within the retina enclosed by the square appears to be normal.

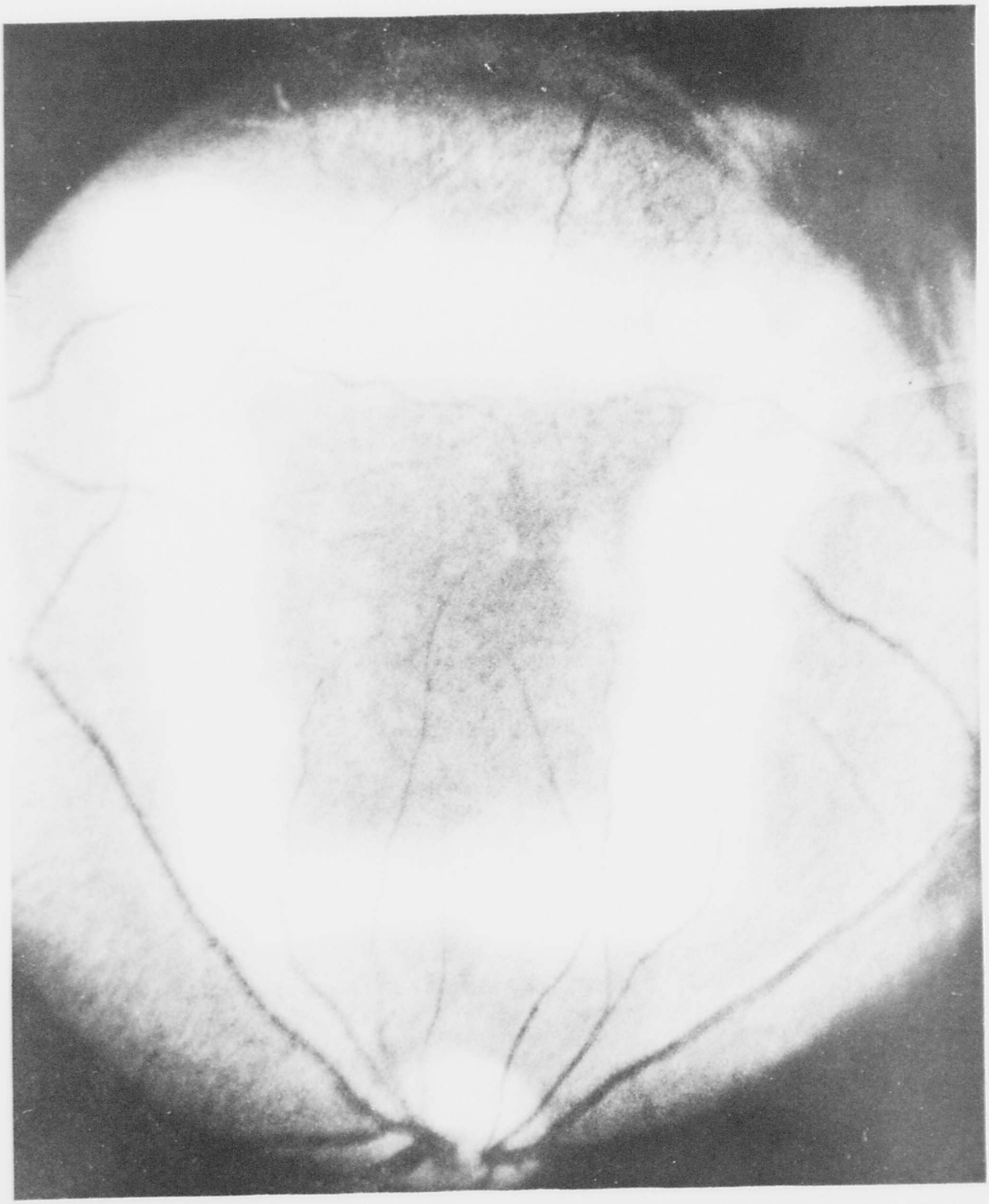
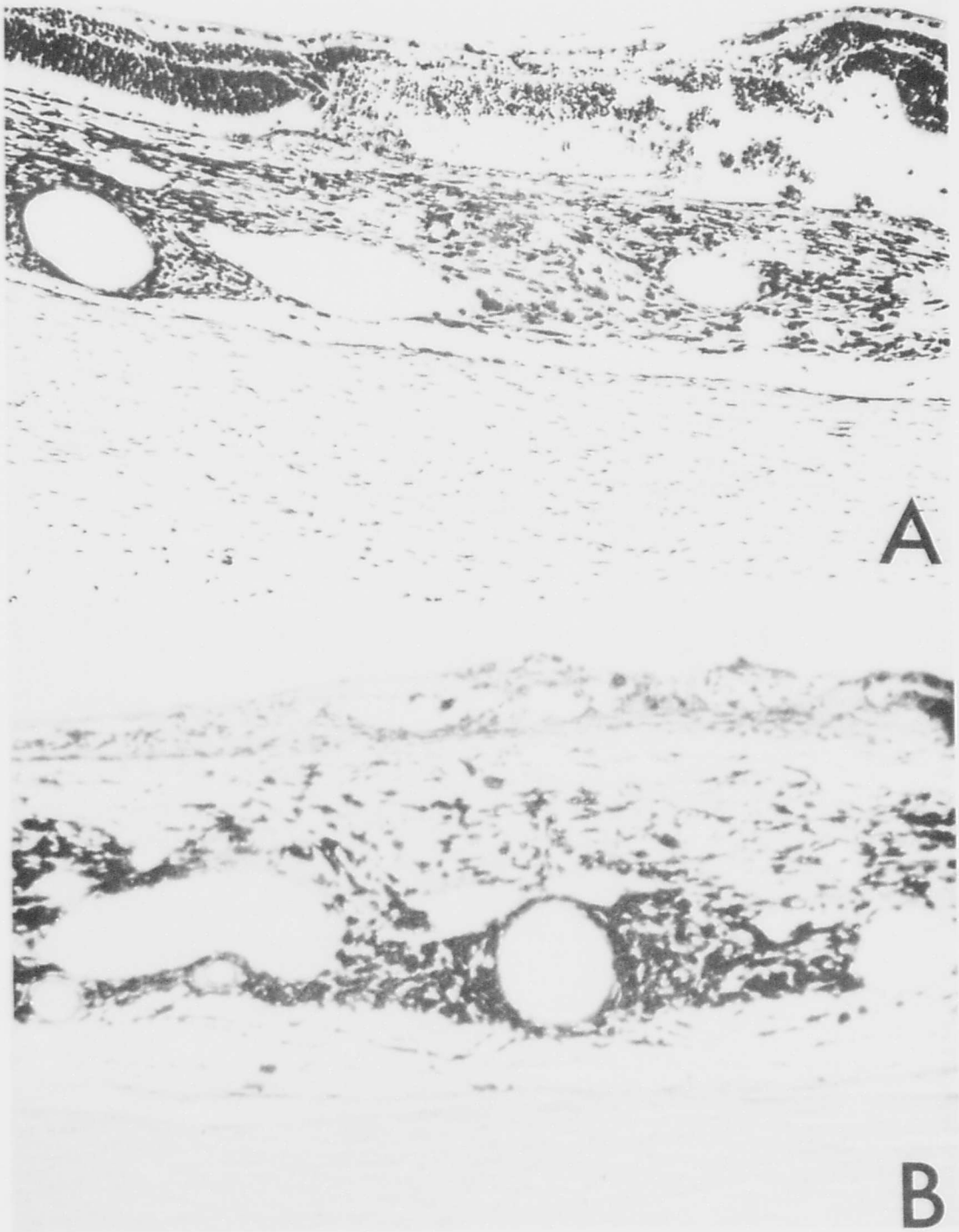


Figure 6. Cross sections of owl monkey retina following argon laser exposure. In each case the pattern of argon laser exposures is in a square as shown in Figure 5.

Part A shows a lesion and portions of adjacent unexposed retinas both inside (right) and outside (left) the laser exposure square. The section was taken approximately one day after laser exposure. The unexposed retina outside the square appears to be normal. In the retina inside the square the rod outer segments are quite disorganized, although the inner layers of the retina do not appear to be changed markedly yet. It should be noted that within the lesion site there is an area in which the outer segments of the distal portions of the receptors appears well preserved, although the inner nuclear layer and the rest of the normal cells in the retina have disappeared. This may be due to heat fixation of the outer segments and possibly the inner segments also by the laser exposure.

Part B is a section of the retina inside the square about one month after laser exposure. Although the portion of the retina within the square has completely degenerated, the retina adjacent to the lesion on the outside of the square is still completely normal.


A
B

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