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Histopathology of ruby and argon laser lesions in monkey and human retina
A comparative study

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The sensitivity of the monkey macula to laser radiations is well established (Frisch, Beatrice, and Holsen, 1971). Experimental studies have been carried out with ruby (Campbell, Rittler, Noyori, Swope, and Koester, 1966) and helium neon (Lappin, 1970) lasers operating at suprathreshold energy levels. These showed that the macula is damaged by levels of radiation significantly below those which are required to damage more peripheral areas (Leibowitz, Peacock, and Friedman, 1969).

The mechanism by which coherent radiation induces retinal trauma consists of a complex interaction between thermal, mechanical, and displacement effects (Marshall, 1970a). The relative importance of each of these effects is dependent both on the physical parameters of the laser beam, and the absorption characteristics of the target tissue. Friedman and Ts'o (1968) demonstrated by comparative histology that the pigment epithelial cells in the (human) fovea are smaller than those in the periphery and that each cell contains more pigment. This increase in pigment density would result in a higher absorption of laser energy in the macula than in the periphery for any given exposure. Lappin and Coogan (1970) have suggested that the high foveal sensitivity is due to this increased absorption by the pigment epithelium, and also that the reduced retinal thickness at the fovea would result in less attenuation of the incident beam as it passed through the retina, and would therefore allow more energy to reach the pigment epithelium.

Recent work with both prolonged exposures to white light (Marshall, Mellerio, and Palmer, 1972) and short pulsed exposure to incoherent monochromatic light (Sperling and Harwerth, 1971, 1972) has indicated that cones may suffer chronic damage more readily than rods. A similar effect has recently been demonstrated in a study on monkey fovea irradiated with an argon laser (488 nm), in which the authors concluded that the foveal threshold differential was due to this increased sensitivity of cones (Tso, Wallow, and Powell, 1973).

Threshold figures derived from experiments on monkeys are used when compiling data for safety levels for human exposures. These figures are amended to allow for different optical and absorption properties between the two species. There is a small difference in pigmentation between the threshold exposure levels of the rhesus monkey and the Caucasian human eye, the monkey being at greater risk (Wallow, Lund, Gabel, Birngruber, and Hillenkamp, 1974), and this has been considered to give an additional safety factor when considering safety levels of human exposure.

Clinically, the advent of reliable high energy short pulse light sources resulted in increasing numbers of workers treating lesions of the posterior pole by photocoagulation (Watzke and Snyder, 1968; Gass, 1971; Schatz and Patz, 1973; Bird, 1974). However, histological observations of such lesions are limited, as all published pathological studies of such irradiations in human maculae have been confined to ruby laser exposures (Watzke and Moore, 1972), and no comparative samples from monkey retinae have been obtained. Numerous centres are now using Argon laser systems and ophthalmoscopic observations of argon lesions in the macula suggest that the coagulation site has a different topography from the lesions produced in the peripheral retina.

Many workers have described the occurrence and absorption characteristics of a yellow pigment in the macula (Wyszecki and Stiles, 1967). Most of these studies describe the absorption curve as a biscuspid distribution with a maxima at 460 nm and a second peak at 490 nm (Ishak, 1952; Ruddock, 1963; Bone and Sparrock, 1971). It is possible that the absorption properties of this pigment could account for the ophthalmoscopically observed distribution of suprathreshold argon laser damage, and for the significantly raised macular sensitivity to laser light.

It is the purpose of the present study to examine the damage topography of argon and ruby laser

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lesions in the maculae of both human subjects and rhesus monkeys, and to attempt to relate such damage to differences in absorption properties of retinal tissue.

Method and materials

ARGON LASER EXPOSURES

Two different argon lasers were used in the present study, one for the human exposures and one for the animal experiments. However, both lasers were the same model (Coherent Radiation 800) and were operated under identical conditions. All power levels quoted are those registered on the manufacturer's monitors, and no study has been carried out on the beam energy profiles. At the power levels used in these experiments 95 per cent of the emitted energy is at 488 nm.

Exposures were delivered *via* the integral Zeiss slit-lamp system in conjunction with a Goldmann fundus contact lens. The pupils were dilated with mydrilate 1 per cent and phenylephrine 10 per cent. Single eyes were exposed in three rhesus monkeys (*Macaca mulatta*) and in three human patients before enucleation for malignant melanomas of the anterior uvea (two without posterior retinal detachment and one with a detachment which included the macula).

In each eye a series of irradiations was placed in groups of four within 1°, 2°, 3°, and 10° of visual angle from the fovea. In each group lesions were produced by power levels of 50, 100, 200, and 300 mW. A single foveal exposure of 100 mW was given in each eye. All exposures had a 50 μm spot size and a pulse duration of 0·05 seconds.

RUBY LASER EXPOSURES

The ruby laser used in this study was an experimental system with a Pockel cell and polarizer to give a 40 ns Q-switched pulse. The laser was operated in the TEM_{00} mode, and had a beam divergence of 1 mrad and an estimated retinal spot size of 30 μm. Q-switched pulses were monitored with a silicon pin photo diode (Hewlett Packard 4203) calibrated against an EG and G radiometer.

Animals exposed to this system were refracted and in each case a suitable correction for the ruby wavelength was included in the beam optics to ensure minimum spot size on the retina. These animals were premedicated with phencyclidine hydrochloride (3 mg/kg intramuscularly), and then anaesthetized with intravenous sodium pentobarbitone. Maximum mydriasis was effected by alternate instillation of 10 per cent phenylephrine and 1 per cent cyclopentolate.

Six monkey eyes were given macular and paramacular exposures with energy values ranging between 0·4 and 10·0 μJ.

HISTOLOGY AND ELECTRON MICROSCOPY

In both humans and monkeys enucleation was carried out under deep anaesthesia and after a corneal incision the eyes were immediately immersed in 100 ml fixative. This initial solution contained 3 per cent gluteraldehyde buffered in 0·1 M sodium cacodylate containing 10 mg/ml calcium chloride and with a final pH 7·4. The eyes were progressively dissected in this solution. After a total fixation period of 45 minutes, the individual lesions were identified and measured. Their location was recorded both as a function of their distance from the fovea, and as a function of their relative position to each other. In some cases the lesions were photographed on a Wild M 20 microscope using incident illumination and a ×5 objective. Lesions were isolated with a surrounding area of unirradiated tissue by microdissection under a dissecting microscope. Each sample was cut in such a fashion that its orientation could be determined during ultramicrotomy. Tissue was washed briefly in 0·1 M sodium cacodylate buffer containing 7·5 per cent sucrose, and post-fixed for 1 hour in 2 per cent osmium tetroxide buffered in 0·1 M sodium cacodylate. Samples were dehydrated through a graded series of concentrations of ethanol in water and embedded in Epon *via* epoxypropane.

For light microscopy 1 μm sections were cut on glass knives mounted in a Huxley Mark I ultra-microtome and were stained with alcoholic toluidine blue (Meek, 1963). A chuck adaptor was used so that once mounted the blocks could be cut on several microtomes without re-trimming. Sections for electron microscopy were cut using diamond knives in a Reichert OMU_3 ultra microtome. These sections were mounted on uncoated copper grids and stained with uranylacetate followed by lead hydroxide. All sections were viewed in an AEI 801 electron microscope operating at 60 kv and were recorded on Ilford EM6 plates.

Results

At a distance greater than 2° from fixation the fundus lesion caused by argon laser photocoagulation could be observed at the level of the pigment epithelium. However, within 2° of the fovea a second lesion was seen in the inner retina and this inner lesion appeared to become progressively larger as the fovea was approached. Foveal burns appeared as a single full-thickness lesion involving the pigment epithelium and retina. This pattern of lesions has been a constant clinical observation. Inner retinal lesions have been seen further than 2° from the fovea in clinical practice with high intensity burns which are commonly used in treating macular disease. In contrast, damage only at the level of the pigment epithelium could be observed in response to the ruby laser, regardless of the position on the fundus of the exposure.

The above described topography could be seen in the fixed eyes during isolation of the lesions under a dissecting microscope, and by motion parallax the two retinal damage planes could clearly be discriminated in the argon lesion. In most of these lesions the pigment epithelial damage plane had the larger diameter of reaction. However, in one monkey, the reverse situation was found, and in this case all lesions showed an inner retinal reaction four

or five diameters of that of the pigment epithelial coagulation. In all cases the intensity of the inner coagulation increased as distance from the fovea decreased. In the more severe lesions intraretinal pale streaks could be seen extending radially away from the fovea in both the fibre layer of Henle and the nerve fibre layer (Fig. 1).

Intraretinal damage was not observed in any exposure to the ruby laser.

LIGHT MICROSCOPY

The light microscopic sections of argon lesions showed differences in damage topography which varied with both energy input and lesion location on the fundus. Comparable results were obtained from both human and monkey retinae in terms of damage distribution, but there were differences in the retinal sensitivity between the two species.

It was not possible to identify induced lesions in the retina of the eye with posterior retinal detachment as the retina was in a degenerate condition. This eye was therefore excluded from the results.

All ruby lesions showed damage associated solely with the pigment epithelium. The severity of these changes increased with increasingly energetic exposures.

Argon laser lesions

In the monkey retina 50 mW lesions positioned 2° or more from the macula appeared to be similar to 100 mW lesions induced in corresponding positions in the human retina (Fig. 2). These lesions showed vacuolation and pyknosis in both pigment epithelial cells and receptor cells, but no damage could be detected in the inner retinal layers. The epithelial cells had either shrunken or swollen outlines and all showed disorientation of their apical surfaces. The overlying receptor cells also showed disorientation of their outer segments which were radially displaced from the lesion centre. In the inner segments of these displaced receptors the mitochondria were vacuolated (Figs 2*c*, 2*d*). The nuclei of many receptor cells in the irradiated area were pyknotic and the densely staining degenerate fibres of these cells could be seen traversing the fibre layer of Henle and terminating in degenerate synaptic pedicles. These degenerate fibres demonstrated the degree of receptor synaptic displacement in and around the macula. Not all receptor cells in the irradiated area showed these degenerate changes and in the outer plexiform layer normal synaptic pedicles could be seen adjacent to those of damaged cells.

Within a 2° radius of the fovea a 50 mW input energy produced a lesion which showed a marked change in the retinal damage (Fig. 3). In these lesions, the damage to the outer retinal layers was less severe. The pigment epithelial cells still showed displacement of their apical surfaces, but were less vacuolated and did not show the large volume changes seen in the more peripheral lesions. The irradiated receptor cells again showed the radial displacement of their outer limbs, but few had vacuolation in their inner segments. In these lesions very few cells showed pyknotic nuclei or degeneration in the fibre layer of Henle. All lesions in this location in both monkey and human exposures showed vacuolation damage to the inner retinal layers. The locus of this damage varied both with the site of the lesion within the central area and from one eye to another, but in most cases it was centred on the inner nuclear layer. Serial sections were cut through several examples of this type of lesion and in all cases the two damaged areas were seen to be discrete.

Increase in input energy within this 4° field resulted in increasing trauma at both damage sites. Lesions produced by 100 mW showed severely

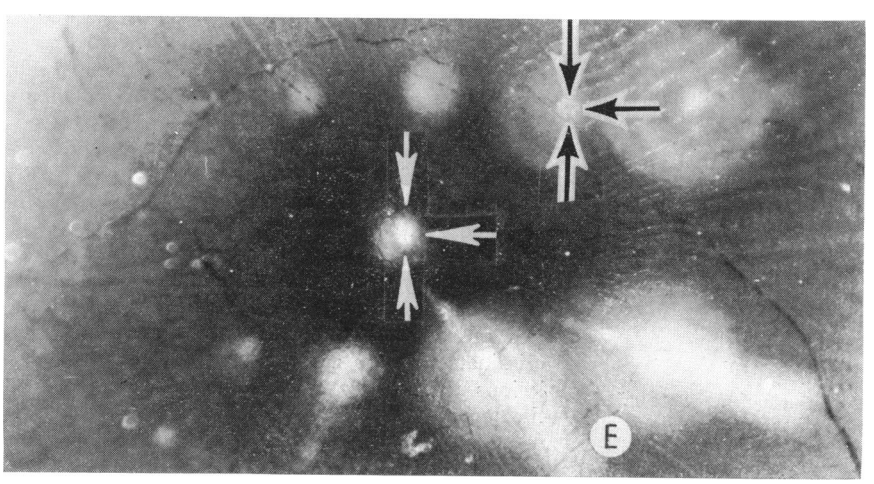

FIG. 1 *Photomicrograph of isolated monkey macula showing part of typical grid of argon laser lesions. Note two levels of damage in retina (arrowed). Exudate can be seen trapped within fibre layer of Henle (E).* ×95

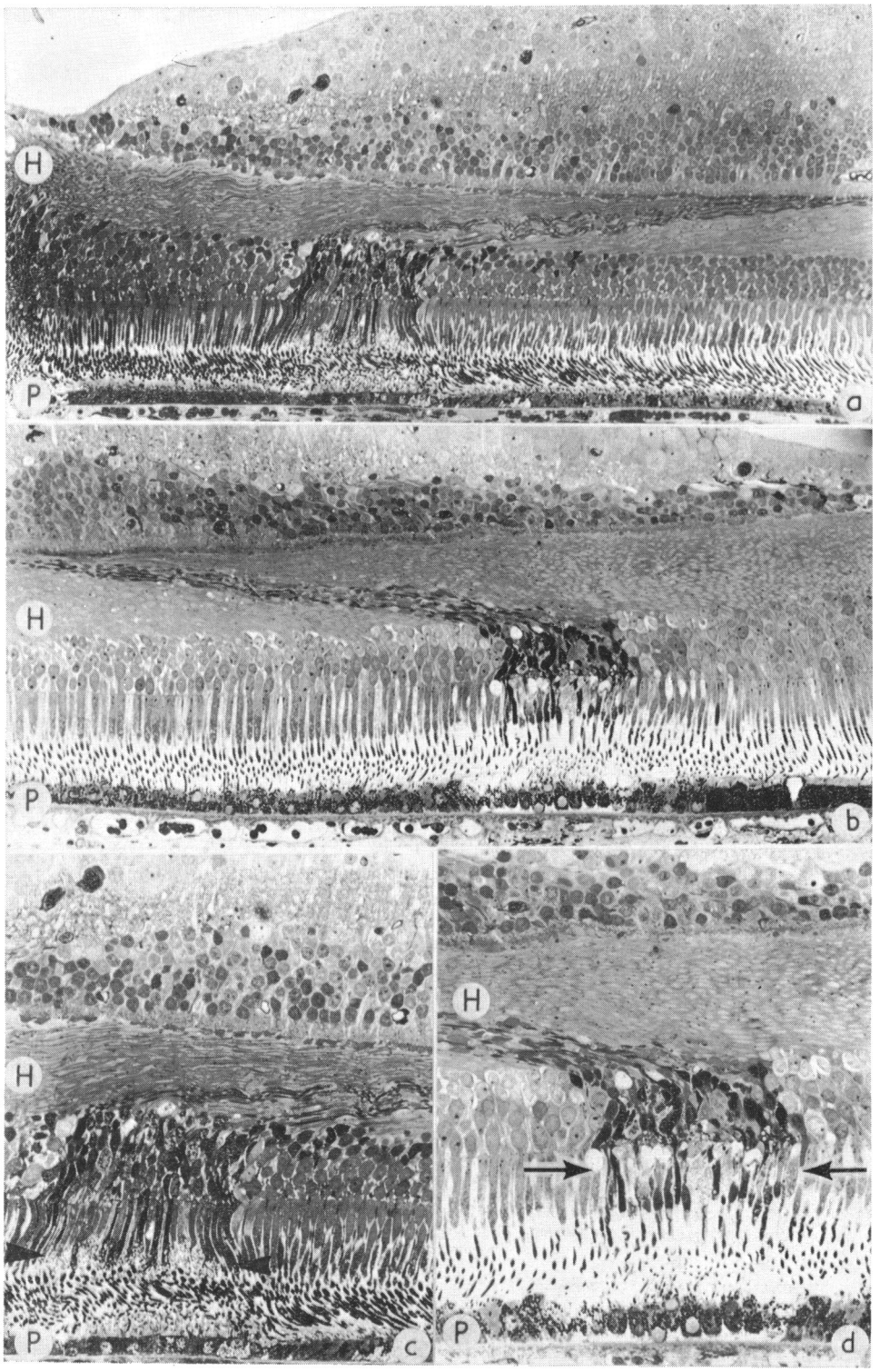

FIG. 2 *Photomicrographs of argon laser lesions induced at a distance greater than 2° from fovea by exposures of 50 mW in monkey (a and c) and of 100 mW in human (b and d). Pigment epithelial cells (P) are shrunken within irradiated area, and overlying receptor cells have vacuolated inner segments (arrowed), pyknotic nuclei, and abnormally dense inner connecting fibres (H). Degree of displacement facilitated by these connecting fibres within fibre layer of Henle can easily be determined.* a *and* b × 450; c *and* d × 600

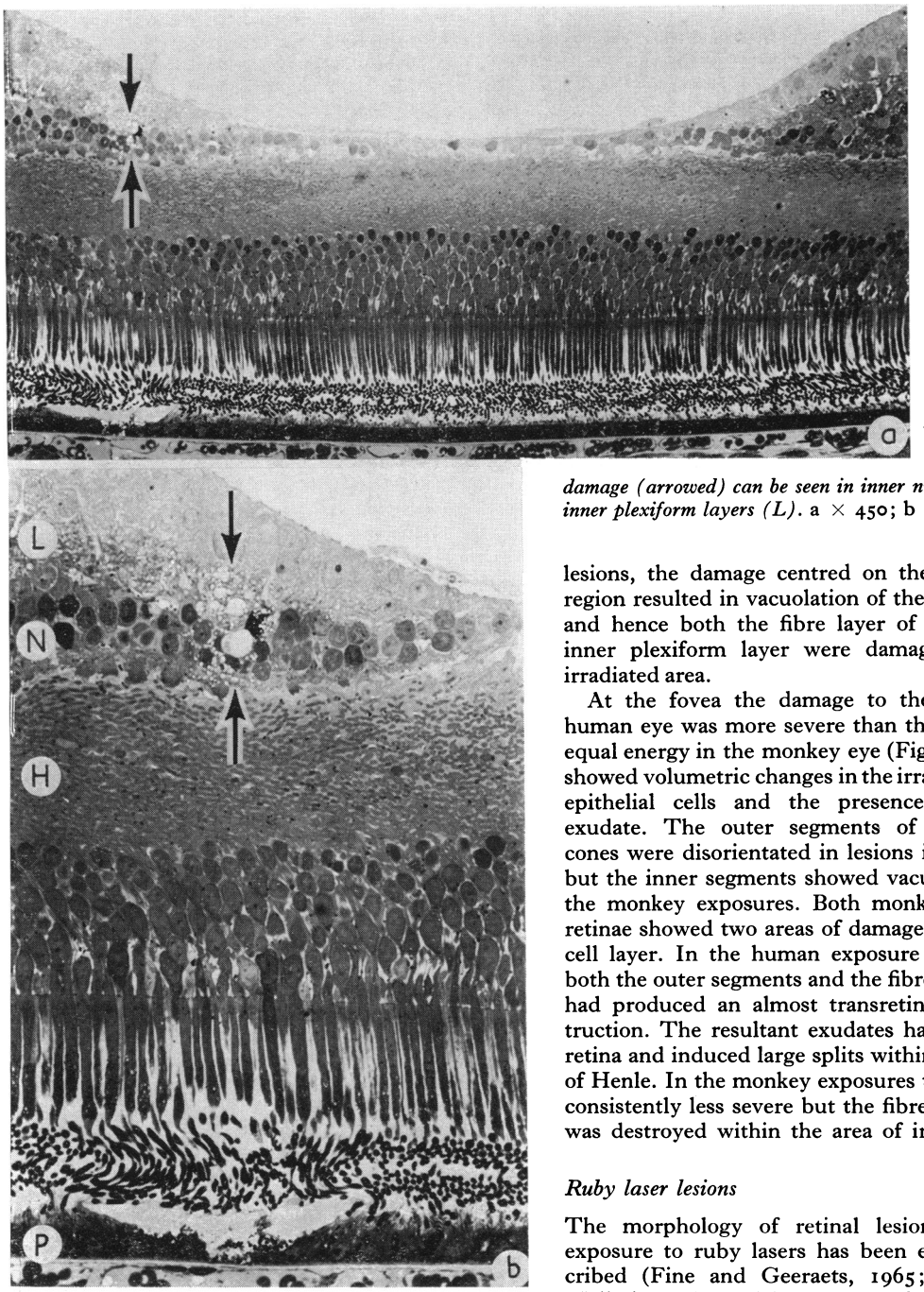

FIG. 3 *Photomicrographs of 50 mW argon laser lesion within 1° of monkey fovea. Note less damage to pigment epithelium and overlying receptors than is seen in equally energetic but more peripheral lesion (Fig. 2a, c). Vacuolation damage (arrowed) can be seen in inner nuclear (N) and inner plexiform layers (L).* a × 450; b × 900

vacuolated and volumetrically distorted pigment epithelial cells and degenerate and pyknotic receptors (Fig. 4). Degenerating fibres could be seen traversing the fibre layer of Henle which terminated in synapses which were 350 to 500 μm away from the irradiated area. However, in many of these lesions, the damage centred on the inner nuclear region resulted in vacuolation of the adjacent layers and hence both the fibre layer of Henle and the inner plexiform layer were damaged within the irradiated area.

At the fovea the damage to the retina in the human eye was more severe than that produced by equal energy in the monkey eye (Fig. 5). All lesions showed volumetric changes in the irradiated pigment epithelial cells and the presence of subretinal exudate. The outer segments of slender foveal cones were disorientated in lesions in both species, but the inner segments showed vacuolation only in the monkey exposures. Both monkey and human retinae showed two areas of damage in the receptor cell layer. In the human exposure the damage to both the outer segments and the fibre layer of Henle had produced an almost transretinal area of destruction. The resultant exudates had detached the retina and induced large splits within the fibre layer of Henle. In the monkey exposures the damage was consistently less severe but the fibre layer of Henle was destroyed within the area of irradiation.

Ruby laser lesions

The morphology of retinal lesions induced by exposure to ruby lasers has been extensively described (Fine and Geeraets, 1965; Marshall and Mellerio, 1967, 1968). However, few workers have studied such exposures in the macula or fovea (Wolbarsht, Fligsten, and Hayes, 1965; Blair and Gass, 1972) and for this reason we will describe lesions produced only within this area.

Typical ruby laser lesions (Fig. 6*a*) show the characteristic disturbances seen in more peripheral

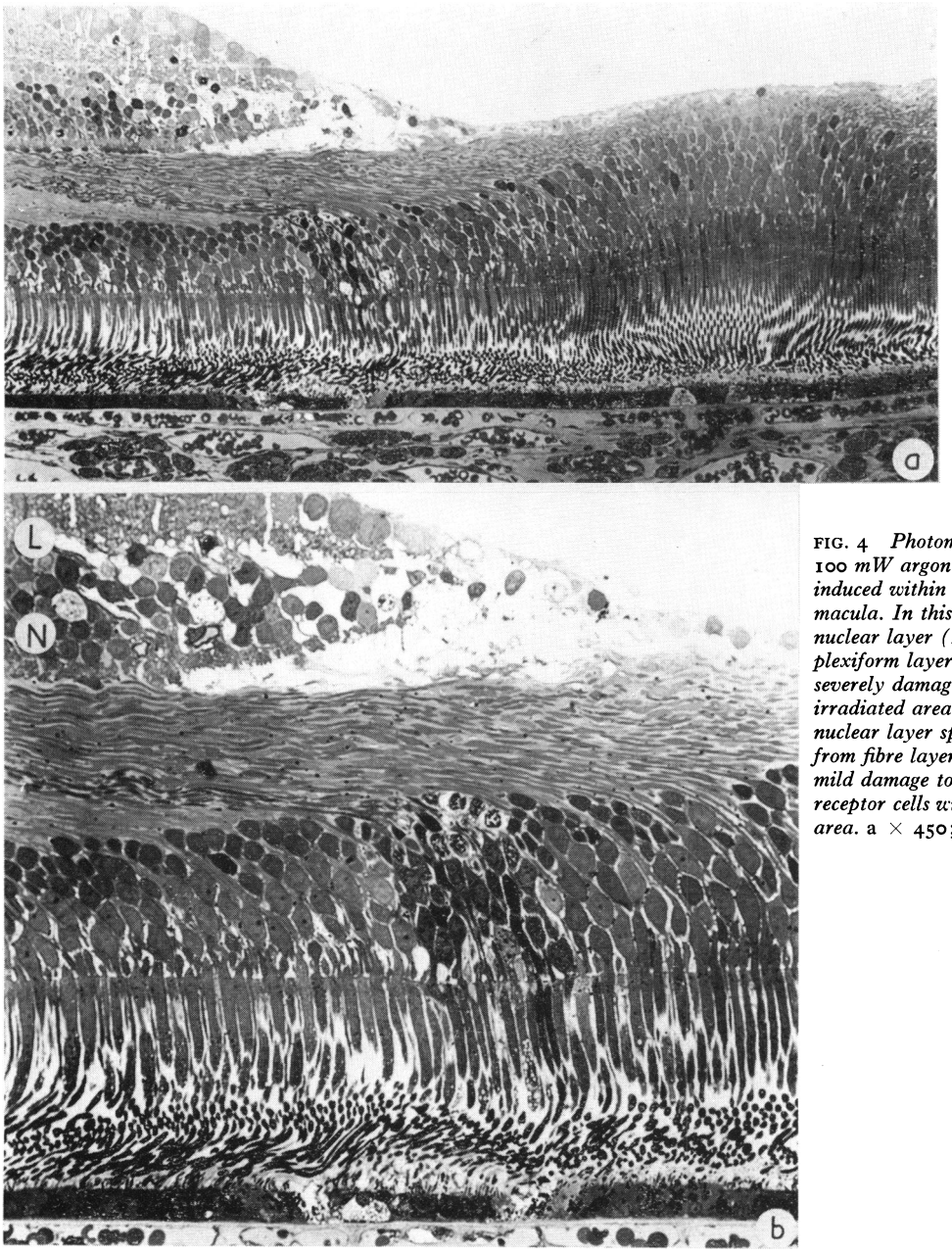

FIG. 4 *Photomicrographs of a 100 mW argon laser lesion induced within 1° of monkey macula. In this lesion inner nuclear layer (N) and inner plexiform layer (L) have been severely damaged within irradiated area resulting in inner nuclear layer splitting away from fibre layer of Henle. Note mild damage to outer parts of receptor cells within irradiated area.* a × 450; b × 900

lesions (Marshall, 1970a). The retinal pigment epithelium is disrupted and vacuolated and both the outer and inner segments of the overlying receptor cells are damaged. As was noted in argon lesions both normal and pyknotic receptor nuclei can be observed throughout the irradiated area. The interconnecting fibres of these damaged receptor cells can be seen as densely staining or vacuolated fibres traversing the fibre layer of Henle (Fig. 6). The damage to these fibres is related to the reaction at the pigment epithelium since it arises as a result of intracellular degeneration within individual receptor cells. The isolated areas of damage to the inner retinal layers in argon lesions were not observed in any of the ruby laser exposures. The severe disruption of the fibre layer of Henle that was seen in argon lesions (Fig. 5) was not seen in ruby lesions even when exposure energies were increased to

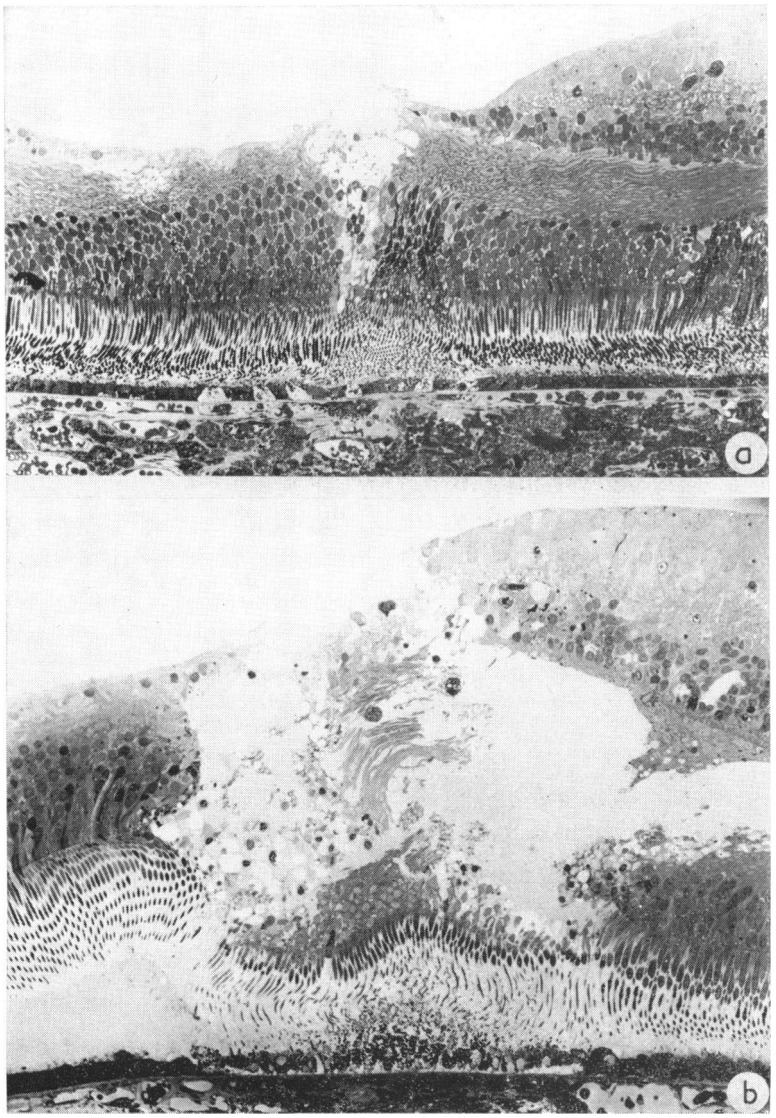

FIG. 5 *Photomicrographs of foveal lesions produced by* 100 *mW argon laser exposures in retinae of* (a) *monkey and* (b) *human. In both species fibre layer of Henle is destroyed within irradiated area. Note greater severity of inner retinal damage in human exposure.* × 350

produce a breakdown of the pigment epithelium and Bruch's membrane sufficient to cause a subretinal haemorrhage (Fig. 6*b*).

ELECTRON MICROSCOPY

The electron microscopy of ruby laser lesions has been previously described (Marshall, 1970a).

The argon exposures showed ultrastructural damage in all lesions in both species.

Argon laser lesions

A comparative analysis of pigment epithelium from a nonirradiated area and that from a low energy exposure (50 mW) may be made from Fig. 7. Within the lesion area Bruch's membrane appears to be structurally unaffected by the irradiation, but it has a higher stain affinity and therefore greater electron density. In the basal zone of the pigment epithelial cells the convoluted border with Bruch's membrane is lost and so also are the mitochondria. Within this area of the cell is a coagulum with a similar appearance to that of the intraphotoreceptor material. These cells have also lost all Golgi bodies and both rough and smooth endoplasmic reticulum. In some cells dense particles of a similar size and periodicity to those of ribosomes can be seen associated with the apical villi. The previously noted vacuolation of these cells is seen to be orientated around the melanin granules and also around larger

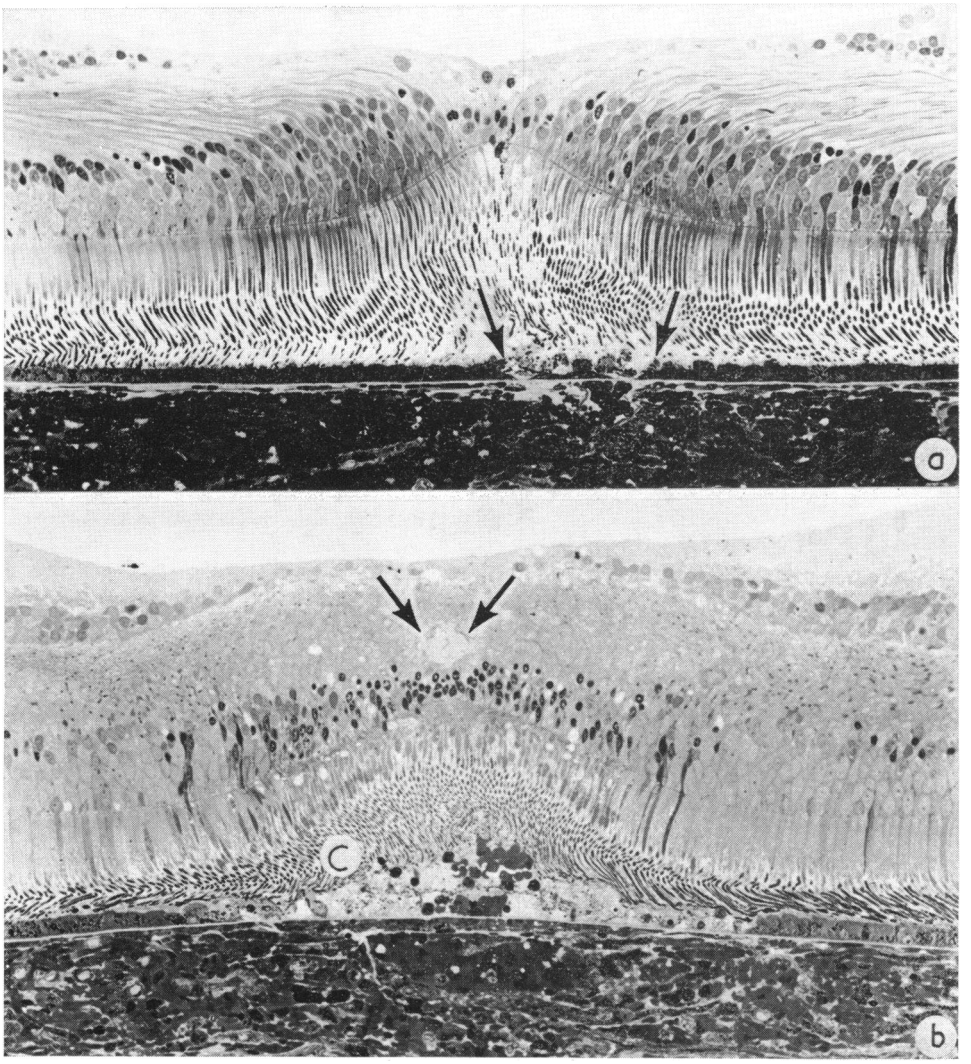

FIG. 6 *Photomicrographs of monkey fovea showing lesions produced by ruby laser.*
(a) *Low energy lesion, showing damage to pigment epithelium (arrowed) and slight damage to overlying receptor cells.*
(b) *High energy lesion, showing destruction of pigment epithelium and subretinal haemorrhage* (c). *Split within fibre layer of Henle (arrowed) results from displacement of outer retinal layers.* × 400

and less electron dense bodies. These inclusions are more numerous in the human pigment epithelium and appear to be of two different types. Some have a morphology similar to pigment granules but at a high magnification many show the characteristics of phagosomes.

With higher energy densities (200 mW) Bruch's membrane still remains structurally intact but all trace of pigment epithelial cell outlines are lost and their cell cytoplasm degenerates to small condensations of densely staining material (Fig. 8). The melanin granules and the other electron dense bodies can still be seen and areas of chromatin can occasionally be identified.

A common finding in these exposures was that the transition between irradiation damaged and undamaged pigment epithelial cells was abrupt and the boundary could be seen between adjacent cells.

In all lesions damage could be seen to the distal receptor cell outer segments, which are in close association with the pigment granules in the pigment epithelial cell apical villi. The damage can be seen as a tubular or vesicular breakdown of the membranes of the photoreceptor discs (Fig. 9). The degree and extent of this breakdown again varied with both energy density and retinal position. High-energy peripheral lesions showed a continuous damage zone extending along the receptor from the pigment epithelial end. In the more central lesions vesicular damage could be seen at both ends of the outer segment with a normal intermediate zone. In cells towards the edge of the lesions little vesicular degeneration was observed but many cells showed displacement of their disc membranes.

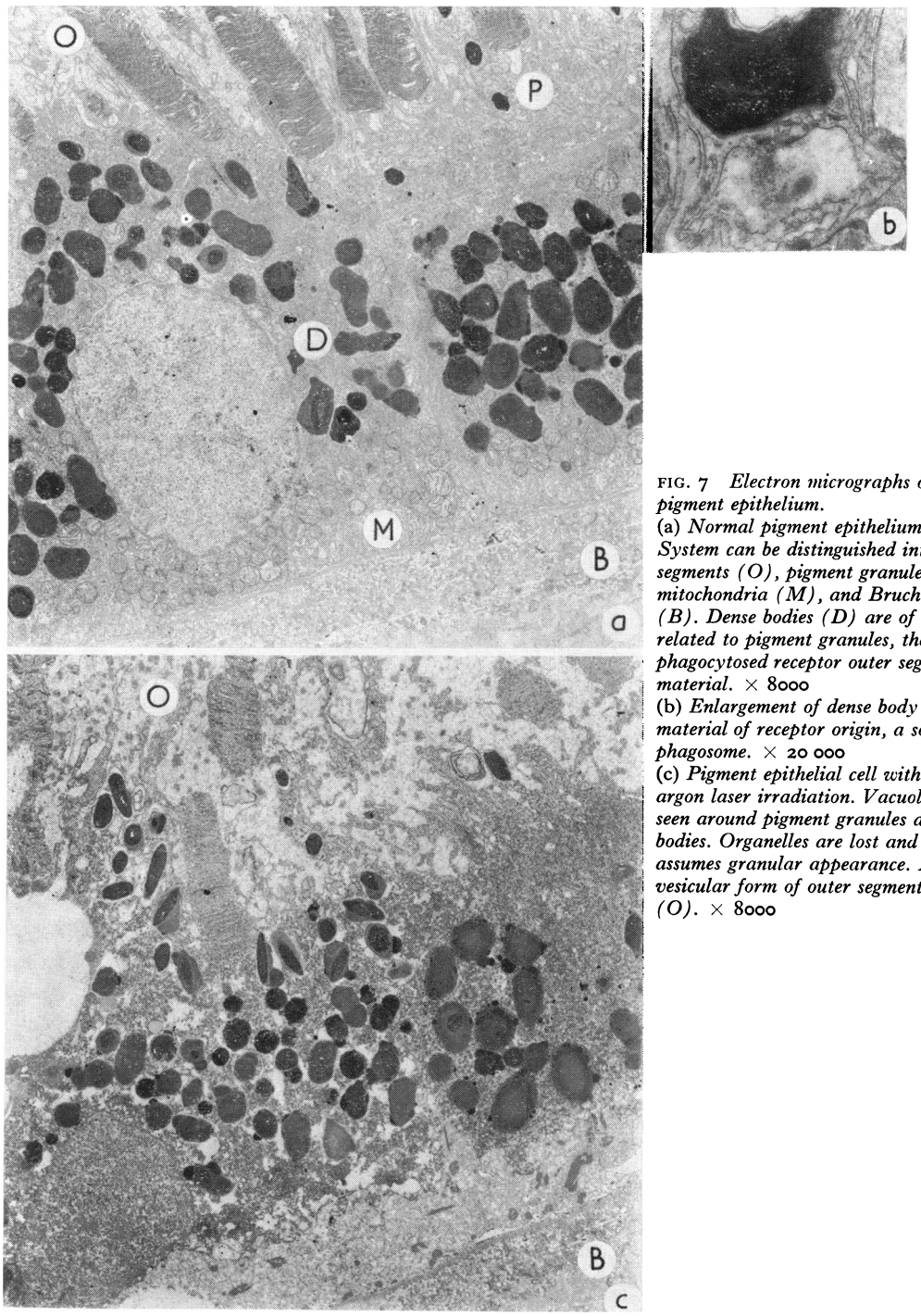

FIG. 7 *Electron micrographs of human pigment epithelium.*
(a) *Normal pigment epithelium from fovea. System can be distinguished into outer segments (O), pigment granules (P), basal mitochondria (M), and Bruch's membrane (B). Dense bodies (D) are of two types, one related to pigment granules, the other to phagocytosed receptor outer segment material.* × 8000
(b) *Enlargement of dense body containing material of receptor origin, a so-called phagosome.* × 20 000
(c) *Pigment epithelial cell within low energy argon laser irradiation. Vacuolation can be seen around pigment granules and dense bodies. Organelles are lost and cytoplasm assumes granular appearance. Note degenerate vesicular form of outer segment membranes (O).* × 8000

In the low-energy lesions (50 mW) the inner segments of the receptor cells showed an abnormally granular and densely staining cytoplasm. The mitochondria in most cells in this region were vacuolated and had lost the regular orientation of their cristea (Fig. 10). These changes were most marked in the monkey exposures. In high-energy exposures the inner segments in both species showed

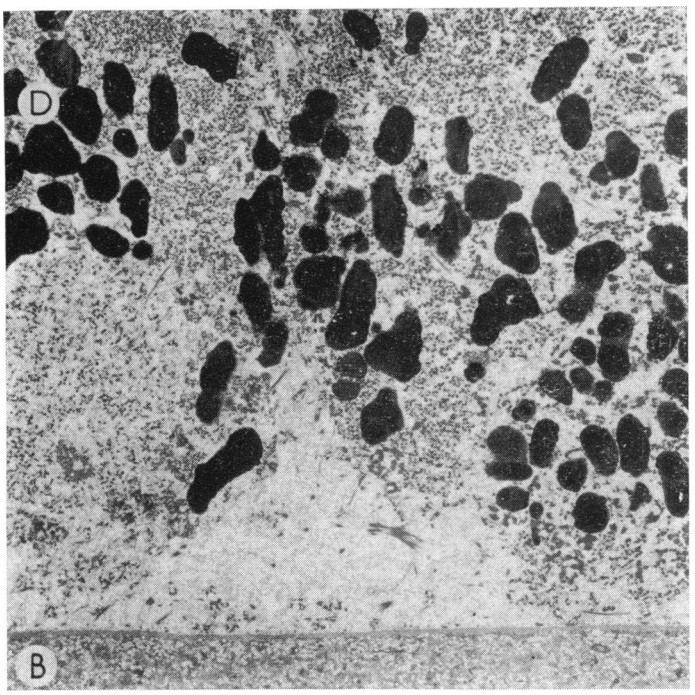

FIG. 8 *Electron micrograph of pigment epithelial cell from centre of* 100 *mW argon laser lesion produced more than* 2° *from fovea. Entire cytoplasm of cell is coagulated into dense granular appearance. Bruch's membrane (B); electron dense bodies (D).* × 8000

the granular breakdown described for the pigment epithelium (Fig. 8).

Two forms of damage could be seen in the receptor nuclear layer. In some cells the nuclei were shrunken within a corresponding shrunken nuclear membrane, while in other cells the chromatin had condensed and pulled away from the nuclear membrane. In all lesions some nuclei within the irradiated area had a normal morphology.

Two types of damage were seen in the nerve fibre layer of Henle. Fibres originating from receptor cells damaged by energy transfer from the pigment epithelium showed vacuolation of their mitochondria and their cytoplasm was abnormally dense (Fig. 11). This same staining reaction could be seen where these fibres terminate in their receptor pedicles and again the mitochondria in this region were damaged. The synaptic vesicles are swollen and irregular and few synaptic ribbons can be seen. In many cases the synaptic clefts have opened to an abnormal width. Transynaptic degeneration was not observed in any lesion. In peripheral lesions the outer plexiform layer was the innermost layer of retina to show damage. The variability in receptor cell responses to laser damage was clearly demonstrated by the fact that the number of pyknotic internal receptor fibres seen in the fibre layer of Henle was fewer than the total number of receptor cells irradiated. In parafoveal and foveal lesions, a second and separate zone of damage occurred involving the inner fibres of Henle's layer, which showed specific changes in the microtubules which were abnormally electron dense (Fig. 11).

The vacuolation damage to the inner retinal layers in more central lesions resulted in large areas of tissue loss. The degree of vacuolation could be related to distance from the absorption centre. For example, cells on the edge of the lesion had microvacuolation of mitochondria and other organelle systems, while more central cells had lost all their cytoplasmic components and were merely distinguished by their cell membranes (Fig. 12). In the most central areas large splits could be seen containing cell debris. In some cases the granular degenerative changes previously described for high-energy damage to pigment epithelial cells could be seen.

Associated with the vacuolation damage to the inner retinal layers was the abnormal electron dense staining of areas of Müller's fibres. This reaction appeared to result from degenerative changes within individual fibres. All the processes of a damaged fibre showed degenerative changes in all retinal layers (Fig. 13). The authors have observed similar changes in Müller's fibres situated around retinal vessels that have been irradiated with argon laser light in an attempt to induce vessel occlusion.

In summary we have observed the following:

1. The site of retinal damage is dependent upon the location of the lesion within the fundus and the wavelength of the incident energy.

It has been shown that, in both human and monkey,

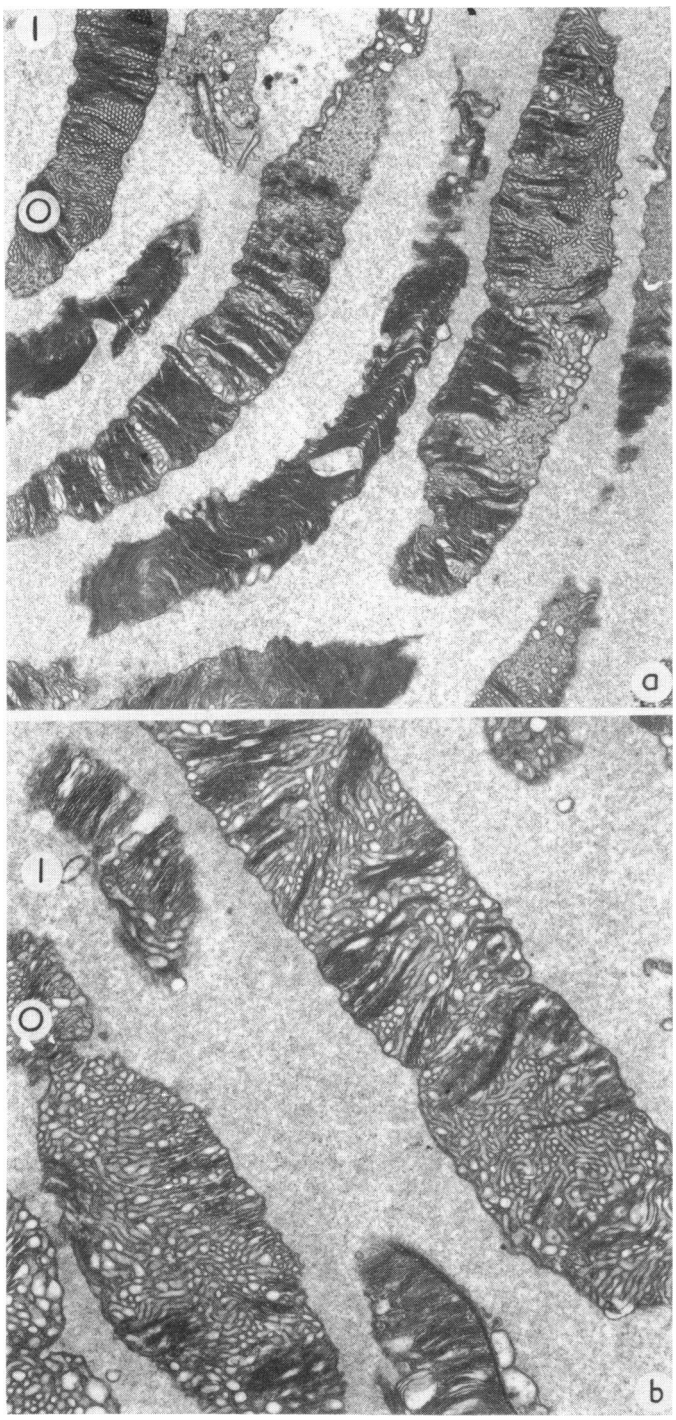

FIG. 9 *Electron micrographs of outer segments of argon laser irradiated receptor cells in* (a) *monkey and* (b) *human retina. Disc membranes are degenerate and have fused to form vesicules and tubules. Outer segments (O); intrareceptor matrix (I).* a × 8000; b × 15 000

inner retinal damage occurs at the macula independent of outer retinal damage in argon laser lesions (488 nm) but not in ruby laser lesions (694·3 nm). 2. The severity of the retinal damage, which is dependent upon density at the absorbing site, varies from one part of the retina to another and varies with species.

When comparing macular lesions produced by

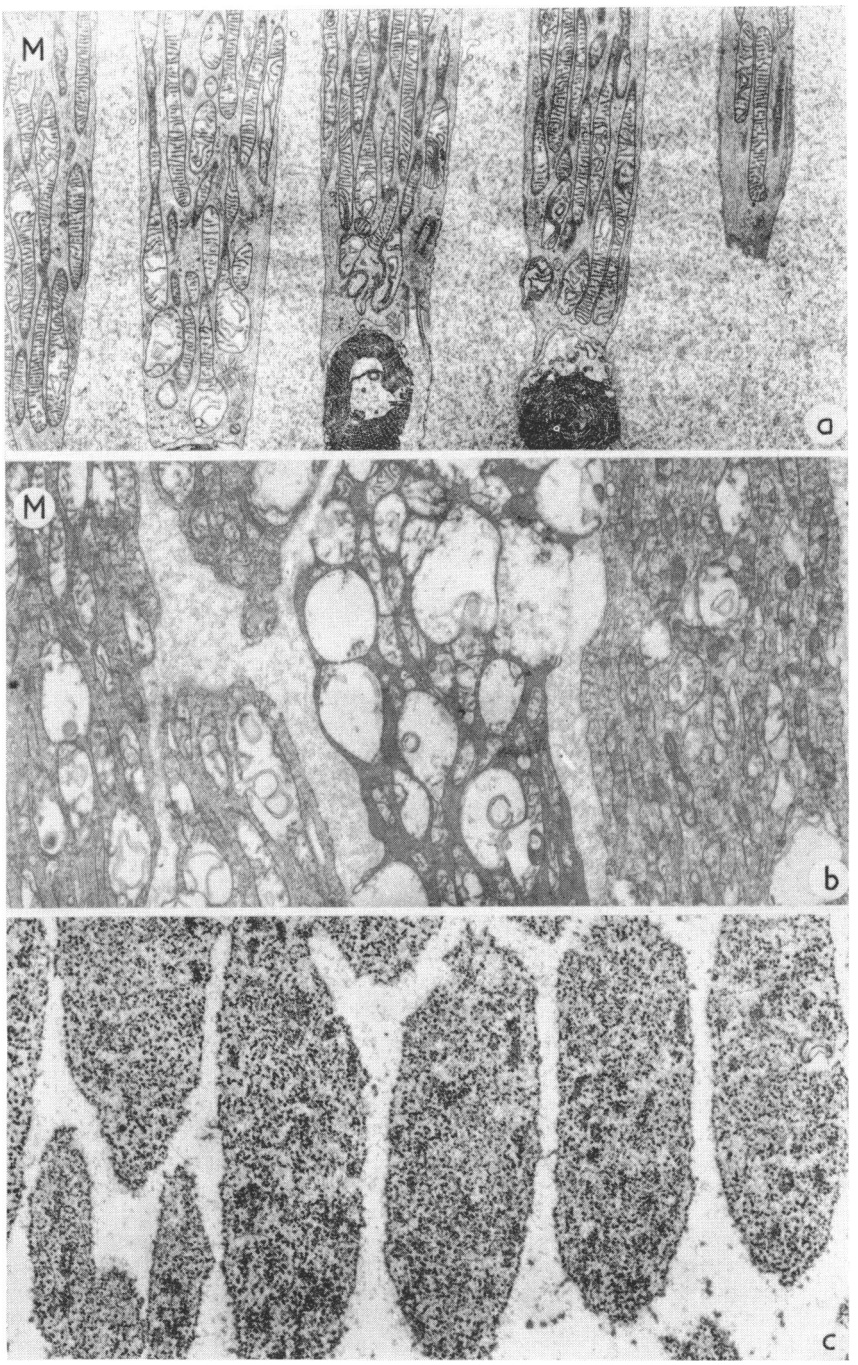

FIG. 10 *Electron micrographs of inner segments of foveal cones.*
(a) *Normal human inner segments from nonirradiated area showing mitochondria (M).* × 7000
(b) *Vacuolated inner segments from area of monkey retina irradiated by low-power argon laser exposure. Note vacuolation appears mainly intramitochondrial.* × 7000
(c) *Inner segments of human cones from within high energy argon laser induced lesion, showing characteristic dense granular appearance of severely coagulated material.* × 7000

argon laser radiation of equal energy it has been shown that inner retinal damage is more severe in humans than in monkeys. In both species the outer retinal damage was greater in parafoveal than in foveal lesions and this disparity was also greater in humans than in monkeys.

Discussion

The results described in the present paper show that, when the macular region of the retina is exposed to suprathreshold doses of argon laser light (488 nm), two discrete areas of damage occur, one situated in the outer retinal layers and the other in

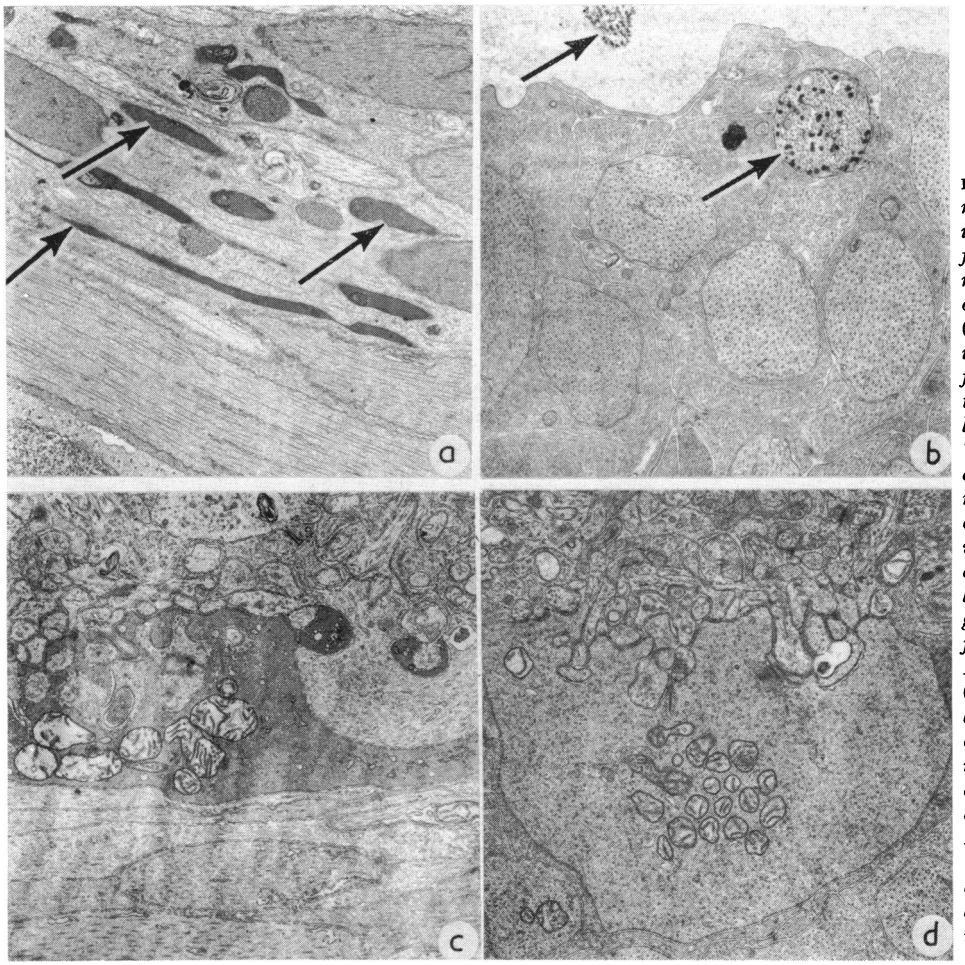

FIG. 11 *Electron micrographs of inner connecting fibres* (a, b) *and receptor synapses of cone cells.*
(a) *Electron dense inner connecting fibres (arrowed) traversing fibre layer of Henle. These fibres emanate from receptor cells the outer segments of which have been damaged by argon laser irradiation greater than 2° from fovea (see Fig. 2).* × 6000
(b) *Interface between fibre layer of Henle and intraretinal split in argon laser exposure in fovea. In some fibres (arrowed) electron dense staining can be seen over microtubules.* × 10 000
(c) *Damage cone pedicle with electron dense staining properties. Pedicles with these staining characteristics are seen displaced up to* 500 μm *away from irradiated area (see Fig. 2). Note vacuolated and degenerate mitochondria.* × 10 000
(d) *Normal cone pedicle.* × 10 000

the inner retinal layers. In contrast, when this area is exposed to ruby laser radiation (694·3 nm), only a single area of damage is seen, which is associated with the pigment epithelium.

The damage to biological systems from exposure to low-power laser systems results from the forces generated by the thermal degradation of the incident energy absorbed in the target tissue. The amount of energy absorbed is a function of the wavelength of the incident radiation and the absorption characteristics of the irradiated media. In all previous studies of laser damage to the retina, with the exception of Wolbarsht and others (1965), the site of retinal absorption has been considered to be the pigment epithelium. For this reason most of the studies concerned with high-energy light and the absorption characteristics of the retina have concentrated on the absorption properties of the pigment epithelium (Geeraets, Williams, Chan, Ham, Guerry, and Schmidt, 1960, 1962; Geeraets and Berry, 1968). These studies describe the variation in absorption of the pigment epithelium and choroid of both human and monkey eyes as a function of wavelength. They showed that both species have a maximal absorption of 70 per cent of incident light, although the band width was narrower in humans (500–600 nm) than in monkeys (450–850 nm). However, these authors have not measured the absorption of the isolated pigment epithelium, nor do they give the variation in pigment density of this layer as a function of fundal position. A knowledge of both of these parameters is important in considering the interaction between laser light and the retina, since analysis of the generation and propagation of a thermal front cannot be done without full knowledge of the dimen-

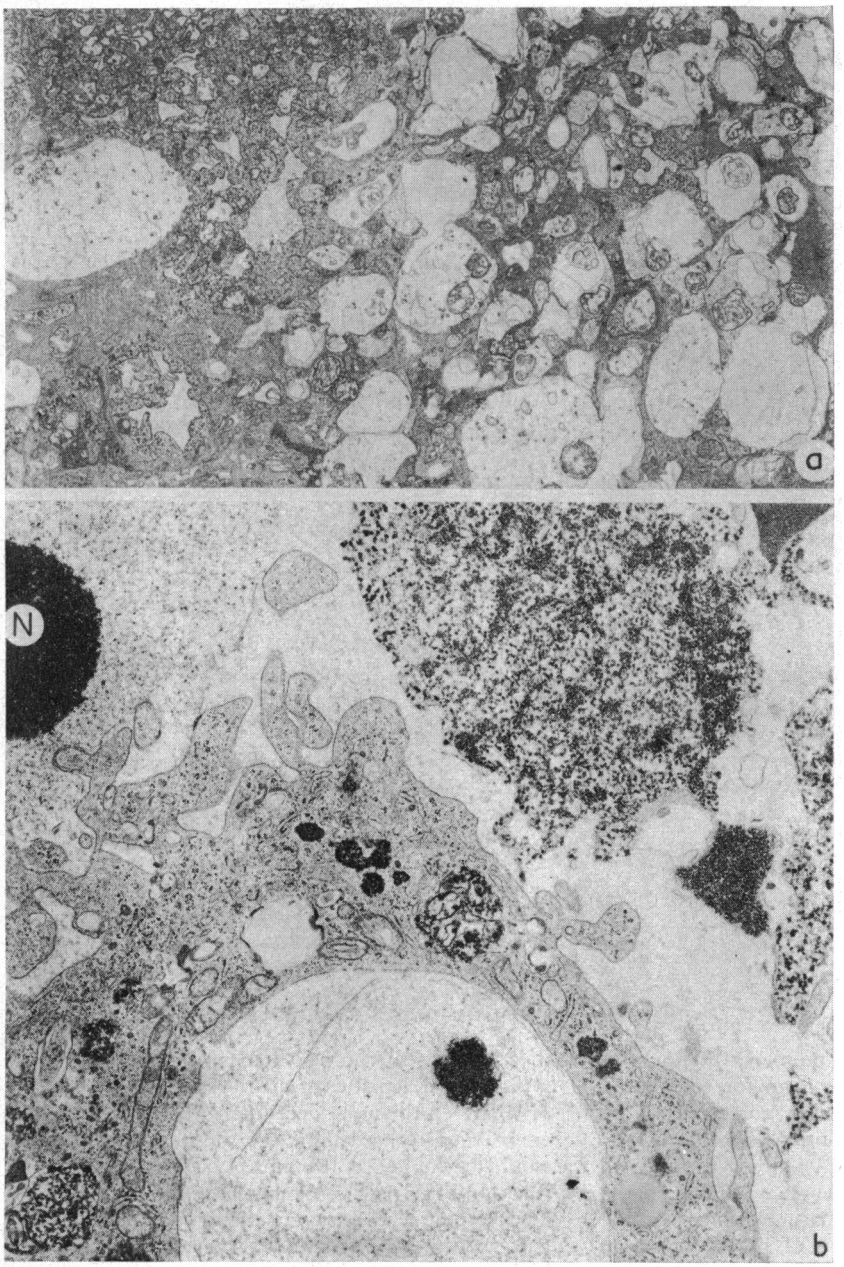

FIG. 12 *Electron micrographs of areas of inner retinal damage produced by argon laser exposure in macula.*
(a) *Vacuolation damage to inner plexiform layer of human retina.* × 6000
(b) *Coagulation damage to inner nuclear layer of monkey retina. Note condensed chromatin (N) of severely damaged cell which has lost both nuclear and cell membranes.* × 10 000

sions of the layer responsible for the primary absorption of energy. The final tissue temperature is related to the absorbed energy density, that is the energy absorbed/unit volume. Thus, if the average figures obtained for the optical density of both the pigment epithelium and the choroid are applied in calculations for generation of temperature changes in laser lesions, the dimensions of the depth of absorption should include both these layers. However, it can be seen from Figs 2 to 6 that insufficient energy passes through the pigment epithelium to cause damage as a result of absorption in the melanin of the choroidal melanocytes. In the past several authors (Vos, 1962; Ham, Williams, Mueller, Guerry, Clarke, and Geeraets, 1966) have considered the absorption of laser light to occur throughout the pigment epithelium of the monkey retina. This layer is approximately 10 μm thick, but Fig. 3 shows that the pigment granules are confined to the apical portion of the cell in a band about

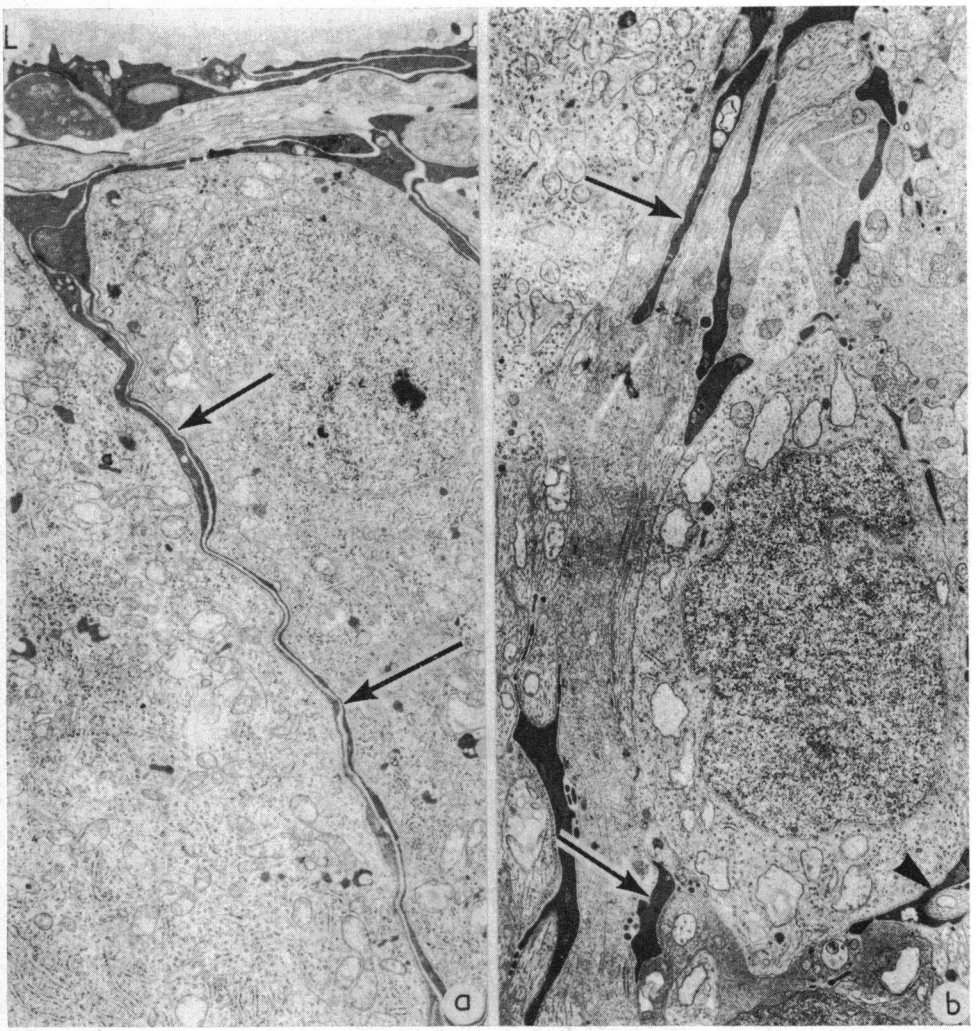

FIG. 13 *Electron micrographs of Müller's fibres damaged by argon laser exposures.*
(a) *Electron dense staining of Müller's fibre elements associated with inner limiting membrane (L), and ganglion cell layer (arrowed).* × 8000
(b) *Damaged Müller's fibres (arrowed) traversing inner nuclear layer.* × 8000

1·5 μm in depth. Thus, in the monkey, the figure of 1·5 μm should be used as the term for calculating absorbed energy density, and a corrected figure must be obtained for the optical density of the isolated pigment epithelium before meaningful calculations can be made on the nature of the thermal front generated in laser lesions. In the human retina the distribution of pigmentation in the pigment epithelium of the macular region is seen to be quite different (Fig. 7). Many of the electron dense bodies deep in the cell cytoplasm have the characteristics of degenerating or developing pigment granules, being rounder and less uniformly dense than those in the apical portion of the cell (Moyer, 1961). In this region of the human retina the pigment epithelial cells are approximately 10–15 μm in depth, and in this case absorption must take place throughout the epithelium so that more damage is seen in the middle and basal zones of these cells in the human than in the monkey. Even if the optical densities of the monkey and the human pigment epithelium were identical, the absorbed energy density and therefore the peak temperature would be higher in the monkey. The localized absorption of energy in the apical region of the monkey epithelial cell would inevitably modify the pattern of pigment epithelial cell damage, and the higher temperature generated would cause an increase in the severity of receptor cell damage.

Pigment granules is the term used by Hogan, Alvarado, and Weddell (1971) for all the electron dense bodies in the human pigment epithelium. However, high-power observation during the present study has shown some of these inclusions to have a membrane structure with a periodicity similar to that of phagosomes (Fig. 7*b*). The work of Young (1965) has shown that the disc membranes of rod outer segment of many species including the

monkey (Young, 1971) are continually being synthesized in the region of the inner segment, and spent discs are continually being phagocytosed by the pigment epithelium (Young and Bok, 1969; Marshall and Ansell, 1971). The phagocytic capacity of the pigment epithelium may be utilized in cases of retinal trauma, where these cells engulf increasing amounts of retinal debris (Marshall, 1970b). In recent studies of prolonged exposure to conventional light sources, cones were found to degenerate and cone 'phagosomes' were found in the retinal pigment epithelium (Marshall and others, 1972). At the fovea there is an exclusively cone population, but the cones have rod-shaped outer segments. It has been shown that under certain pathological conditions cones can regenerate their outer segments (Kroll and Machemer, 1969), and that the pigment epithelium is capable of phagocytosing cone outer segment material. Furthermore, in the pigment epithelium of the human fovea, we find phagosome-like inclusions. These findings suggest that the outer segments of foveal cones are continuously resynthesized like those of rods. This must await further experimental studies for corroboration (Hogan, 1972; Hogan, Wood, and Steinberg, 1974).

The vacuolation and organelle damage to the pigment epithelium in mild argon coagulations are similar to those described for ruby and helium neon laser lesions. However, the dense granular appearance seen in all cells in high-energy exposures (Figs 8 and 10*c*) is characteristic of argon laser exposures (Lerche, 1973; Tso and others, 1973). The appearance of similar damage associated with ribosomes in the apical region of epithelial cells in a low-energy lesion (Fig. 7*c*) suggests that the granular particles may be the degeneration product of coagulated proteins.

The vesicular breakdown of the disc membranes of the receptor outer segments (Fig. 9) seems to be an extension of a condition sometimes seen in normal receptor cells and more commonly in older eyes. This vesiculation should not be confused with that described by Tormey (1964), as it is independent of the method of fixation. In rods vesicular breakdown occurs in the outer segment and involves one or two discs only. In cones many of the disc membranes in the region of the cilium show this type of membrane fusion. It is most unlikely that the receptor breakdown seen in the present study results from direct absorption of laser light by the visual pigments within the disc membranes. This change is not specific and is common to many types of retinal disorder, caused by heat, prolonged exposure to light (Kuwabara and Gorn, 1968), and osmotic shock (Heller, Ostwald, and Bok, 1971), and it is also seen in genetically-determined retinal degeneration (Dowling and Sidman, 1962).

Studies on membrane fusion have shown that both heat (Lucy, 1970) and vitamin A (Dingle, 1961) have membranolytic properties. There is clear evidence of the presence of both of these mechanisms and of membranolytic enzymes within the pigment epithelium. It is possible, therefore, that receptor membrane fusion occurs as a direct result of heating, or of increased vitamin A concentrations due to imbalance in the visual cycle induced by excessive stimulation. The breakdown may result from the release of lytic enzymes into the subretinal space by damaged pigment epithelial cells. Evidence of this later mechanism has been found to be associated with the complex membrane fusion of outer segment discs in rats suffering from retinitis pigmentosa (Ansell and Marshall, 1974). We consider the disc breakdown in the suprathreshold argon lesions described in this study to be due to the action of heat. However, we do not propose that this agent acts on the discs directly, but rather that the degeneration arises as a result of the loss of some central metabolic control system which under normal conditions actively prevents disc membrane fusion.

The second type of damage seen in receptor outer segments is the whorl type disorientation of the disc membranes. This disorientation probably indicates a less severe insult, and has been previously described in subthreshold exposure to a Q-switched ruby laser (Adams, Beatrice, and Bedell, 1972). In many cases these 'whorls' are seen in cones in lesions months or even years old (Marshall and Mellerio, 1971).

One puzzling aspect of the response of receptor cells to laser irradiation is the discontinuous distribution of damaged cells within the irradiated area. In the area of retina irradiated by a 50 μm beam, the receptor population would be approximately 650 cells, yet in a 100 mW irradiation only about 60 per cent of the cells within this area will be sufficiently damaged to show nuclear pyknosis. Undamaged cells are distributed throughout the lesion. Because of the high receptor density, some cells with central nuclei may well have outer segments towards the periphery of the lesion and may thus experience a lower thermal insult on account of the gaussian energy distribution within the irradiating beam. However, this could not explain the large number of unaffected nuclei that are seen in each lesion. The distribution of nuclear pyknosis of receptor cells suggests either that individual cells have a differential thermal tolerance or that the thermal front does not reach the nuclear layer, in which case the nuclear response arises as a result of some critical volume of the inner or outer segment of the cell being exposed to an intolerably high temperature.

Differential receptor responses to argon laser injury have been reported in a study on monkey

retinae (Tso and others, 1973). These workers showed that, within a period of 5 months after exposure, cones but not rods were lost from areas of retina irradiated by up to 50 mW. We have recently observed similar results with an infrared laser (1060 nm) which suggests that cone loss in these studies cannot be due to excessive light absorption within the receptor as is suspected with prolonged exposure cone degenerations (Marshall and others, 1972) and the pulsed exposure work of Sperling and Harwerth (1971). Thus cone loss in laser lesions must indicate either that cones have a lower thermal tolerance, or that the limited renewal systems in these receptors are incapable of replacing large volumes of damaged outer segment within periods of up to 6 months. We have found no evidence of differential damage in acute lesions, and therefore conclude that the inadequate cone outer segment renewal system accounts for the apparent cone sensitivity to laser damage. However, such a rod/cone differential could not possibly explain the discontinuous nature of receptor cell damage in the macula.

A strong indication that pyknotic damage in one part of a cell may be due to remote insult to the cell and that this may occur rapidly, is seen in the degeneration of the inner receptor fibres traversing the fibre layer of Henle (Fig. 2). Clearly, if this damage arose in response to the passage of an elevated thermal front, one would expect pyknosis of those fibres immediately above the area of pigment epithelial damage. The angular displacement that is seen in damaged fibres must therefore indicate a process of rapid intracellular degeneration (Fig. 2).

Primary damage caused by the absorption of laser light in the neural retina has been described in only one previous study (Wolbarsht and others, 1965). These authors compared the retinal pathology of lesions produced in the fovea of monkey by two short pulse laser irradiations—ruby (694·3 nm) and neodymium (1060 nm)—and claimed that more damage occurs in the neural retina with the infrared laser. They attributed this differential damage distribution to absorption within the inner retinal layers (Geeraets and others, 1960). The work of Geeraets demonstrates that the absorption of light at 1060 nm in the human ocular media is indeed 55 per cent higher than that at 694·3. However, this figure is a function of the thickness of these components. If 55 per cent more energy is absorbed throughout the 15 mm of ocular media, this would result in a very low absorbed energy density. The more significant finding (Geeraets and others, 1960) is that the figures for retinal and pigment epithelial absorption at 1060 nm vary between approximately 5 per cent in an albino to about 45 per cent in an average pigmented fundus. From these figures it would appear that most energy is absorbed by the epithelial melanin resulting in high absorbed energy density and that absorption at this site would be the most important in the production of retinal damage. It has not been our experience that the morphology of lesions induced in the peripheral retina by ruby or neodymium lasers differ from each other even at threshold levels (Marshall, 1970a). If significant intraretinal absorption was occurring, the magnitude of this effect should be highest in the paramacular region where the retina is thickest. We consider that the effects described by Wolbarsht and others (1965) may be due to differential absorbed energy densities at the pigment epithelium, produced by the different input energies used for each of the laser wavelengths.

In the present study using argon laser radiation we have found discrete areas of damage in the inner retinal layers and that the intensity of this damage varies with distance from the macula. Reports describing argon exposures in the peripheral retina show no indication of this second area of damage. In previous experiments on the macula, using two laser systems emitting red light, ruby (694·3) (Blair and Gass, 1972) and helium neon (633·0) (Ham, Geeraets, Mueller, Williams, Clarke, and Cleary, 1970), no such damage has been described and our present results are in agreement with these studies. All damage from the ruby laser is associated with the pigment epithelium.

If it is accepted that the site of primary damage to a biological system is coincident or adjacent to the site of absorption of the incident radiation, then (Figs 3 to 6) there exists a pigmented system in the neural retina, which is localized around the macula and absorbs more radiation at 488 nm than at 633 or 694·3 nm. The macular pigment has these characteristics and at the fovea would absorb approximately 70 per cent of incident radiation at 488 nm. The concentration of this pigment decreases with distance from the fovea (Ruddock, 1963). The Table shows the calculated relative absorption of both argon and ruby laser radiation by the macular pigment (Ruddock, 1963) and the pigment epithelium (Geeraets and Berry, 1968) for representative exposures in the present study. It should be noted however that macular pigmentation varies both between individuals (Bone and Sparrock, 1971) and between races (Ishak, 1952). We have not been able to obtain figures for either absorption or distribution of this pigment in the monkey retina.

The Table shows that, within a 1° field centred on the fovea, extremely high intraretinal absorption would occur at 488 nm and that these energy levels would result in damage in this absorbing layer. However, it should also be apparent that this intraretinal absorption would so attenuate the transmitted energy that little would be available for absorption within the pigment epithelium. This

Table *Differential absorption of argon (488 nm) and ruby (694 nm) laser radiation in human retinal tissue at various distances from centre of fovea*

Exposure (Incident power mW)	*Calculated absorbed power mW*									
	Neural retina (macular pigment)					*Pigment epithelium (melanin)*				
	488 *(Argon)*				694 *(Ruby)*	488 *(Argon)*				694 *(Ruby)*
	1°	2°	3°	10°	*Throughout*	1°	2°	3°	4°	*Throughout*
50	35	18	4	<1	<1	11	24	35	38	35
100	70	35	8	<1	<1	22	48	70	76	70
200	140	70	17	<1	<1	44	96	140	152	140
300	210	106	25	<1	<1	66	144	210	228	210

absorption differential is clearly demonstrated by retinal change. In Fig. 2 no intraretinal damage occurs despite marked changes in the pigment epithelium and receptor cells, but in Fig. 4, where there is inner retinal damage, the outer layers are little changed. The lack of attenuation of ruby laser radiation by absorption in the inner retina results in sufficient energy passing through the neural retina at the fovea to produce a subretinal haemorrhage, but only secondary degeneration damage is seen in the fibre layer of Henle (Fig. 6).

In all long pulse lesions, the tissue damage may be related to the thermal front generated by degradation of the absorbed incident energy. The magnitude and rate of propagation of this front will depend on the absorbed energy density which is a function of the thickness of the absorbing layer. In short pulse lesions, the tissue reaction may be complicated by the induction of rapid state changes within the absorbing cell. Such rapid state changes may be explosive and may in turn result in the propagation of acoustic waves. However, both types of lesion show maximum damage to the cells which absorb the incident energy and both show severity of damage to vary as a function of the absorbed energy density (Marshall, 1970a). For this reason it would be useful to identify the cell population which contains the macular pigment.

A major specialization of the foveal cones is the development of long inner receptor fibres forming the fibre layer of Henle. Brindley (1970) suggests that, if this layer contained the macular pigment, and if this pigment were dichroic, it would be ideally suited to produce the entoptic phenomenon of Haidinger's brushes. However, this is not a unique explanation, because the structure of the brushes would also be produced if the radially arranged fibres of Henle were merely polarizing structures. The colouration of the brushes would be explained by the presence of a yellow filter at any position in the retina distal to the outer segments. Such a filter would produce the yellow brushes, and possibly induce the blue brushes by contrast.

Our results do not discriminate between these alternative distributions. Thus, if Brindley is correct, and absorption occurs in the fibre layer of Henle, then the observed damage could be explained by assuming that the nuclei in the inner nuclear layer were more sensitive to heat damage than the inner connecting fibres. The observed damage is then analogous to the damage seen in receptor cells resulting from absorption within the adjacent pigment epithelium. Alternatively, our results can also be explained by assuming a diffuse distribution of macular pigment since we have observed damage to be concentrated within the fibre layer of Henle only at the foveola (Fig. 5), but further from the fovea maximal damage occurs in the inner nuclear and inner plexiform layers (Figs 3 and 4). From this distribution it would seem either that pigment is present in each of these retinal layers, or that it is contained within a particular cell type which occurs in each of these cell layers, namely the Müller's fibres. However, it is doubtful that the diffuse structure of Müller's fibres would result in sufficiently high absorbed energy densities to produce tissue damage, and we have never observed primary damage associated with the terminal expansions of these structures at the inner limiting membrane.

If the radial array of the inner connecting fibres within the fibre layer of Henle is responsible for Haidinger's brushes, it is difficult to explain why this phenomenon is best demonstrated using blue light unless it is assumed either that the pigment and the polarizer are coincident or that the polarizer is proximal to the light source. Such considerations would imply that the macular pigment is contained with the fibre layer of Henle.

Both the above-described distributions of macu-

lar pigment in retinal neurones could account for the recently reported degenerative changes in the inner retinal layers of monkeys 1 year after being exposed to an argon laser (Frisch, Shawaluk, and Adams, 1974). These authors seem to be unaware of the existence of a pigmented system in the inner retinal layers since they tried to relate the loss of neurones after exposures within 3° of the fovea to damage from thermal degradation of energy absorbed at the pigment epithelium. They suggest that, in continuous wave exposures, the retina shrinks in the area of irradiation and the inner retinal layers are thus exposed to a higher temperature than previously suggested (Geeraets, Ham, Williams, Mueller, Burkhart, Guerry, and Vos, 1965). It was also inferred that the ganglion cells are sensitive to elevated temperature. Using this thermal model, they speculate that the human nerve fibre bundle may not be damaged as seriously as that of the monkey during argon laser exposures. Our observations are to the contrary; equally energetic exposures of the inner retina of the human macula shows more damage than that of the monkey.

To relate the results of argon exposure in the monkey to those in the human, the respective retinal flux densities must be considered. Under our experimental conditions any differences in flux densities can be related only to different transmission properties of the ocular media in the two species. Published figures for human and monkey eyes show transmission properties to be identical at the argon wavelength (Geeraets and Berry, 1968). However, the transmission of light through the lens decreases with ageing (Weale, 1973). All our human subjects were over 40 years of age, so that the total energy reaching the retina may well have been lower in the human exposures than in those in the monkey.

We appreciate that these data are collected from few human eyes, and that our argon exposure energies are well above threshold levels. However, we think our results should be considered when examining codes of practice for laser workers. Obviously we should like more data from the maculae of both primates and humans, and from energy values nearer threshold before a detailed analysis of the dangers of neural retinal absorption can be defined. Most laser safety codes are based on experimental data obtained from monkey irradiations, and there is at least a factor of 10 between the recommended maximum permissible exposure and the level at which ophthalmoscopic damage occurs in these animals. In all codes, throughout the visible region of the spectrum, the absorption of laser light is assumed to take place in the melanin of the pigment epithelium, and absorbed energy densities have, therefore, been calculated solely on this basis. The pigmentation in the rhesus monkey eye is on average about twice as dense as that of a Caucasian, thus for most laser irradiations, the monkey eye requires less energetic exposures to produce threshold damage. Hence, when these threshold exposure energies determined empirically in the monkey are applied to man, there is a small additional safety factor. If the neural retinal damage we have shown (Figs 3, 4, and 5) still occurs at threshold exposure levels, then for argon irradiation of the fovea this small additional safety factor would be removed, and might even be reversed.

These observations are also important to the therapeutic use of argon laser. Successful treatment of disciform macular lesions by argon laser photocoagulation depends upon complete obliteration of the subretinal neovascular tissue in the foveal and parafoveal regions. Retinal absorption of energy in the foveal area, and the subsequent reduction of incident energy at the pigment epithelium which has been demonstrated in this paper, would prevent adequate treatment of subretinal blood vessels in the central area. This probably accounts for the poor therapeutic results obtained in this disease by early workers and for this reason only those lesions which are 200 μm or more from the fovea will be amenable to treatment. Since this disease mainly affects the central fundus, this will reduce the percentage of cases amenable to treatment. Furthermore, inner retinal damage due to retinal uptake of energy in the foveal and parafoveal regions may result in poor vision through foveal denervation.

We think that the threshold for argon laser damage to the macula requires further investigation, and that some attempt should be made to determine the functional differences inherent in lesions produced in different retinal layers.

Summary

Suprathreshold fundus lesions produced by ruby and argon laser photocoagulation were studied within 24 hours by light and electron microscopy. It was shown that damage was maximal in the outer retina in all ruby laser lesions and extramacular argon laser lesions. In both monkey and human, inner retinal damage occurred independently of outer retinal damage in macular lesions produced by the argon laser. In lesions produced by equal energy, inner retinal damage was more severe in humans than in monkeys. In both species outer retinal damage was less severe in the foveal than the parafoveal region and this disparity was greater in humans than in monkeys.

These findings are important to the therapeutic use of argon laser energy for macular disease. In particular, absorption of energy in the inner retina reduces the energy available in the treatment of subretinal lesions in the foveal area, and causes unwanted

neuroretinal damage. The higher sensitivity to argon laser irradiation of the human fovea compared with the monkey fovea, has not been appreciated when defining laser safety limits.

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