Response of the Retinal Pigment Epithelium to Selective Photocoagulation

Johann Roider, MD; Norman A. Michaud, MS; Thomas J. Flotte, MD; Reginald Birngruber, PhD

• **Multiple short argon laser pulses can coagulate the retinal pigment epithelium selectively, while sparing the adjacent neural retina and choroid; in contrast, continuous-wave laser irradiation typically damages the neural retina and choroid. The healing response to selective photocoagulation of the retinal pigment epithelium was studied in rabbits during a period of 4 weeks. The lesions were never visible ophthalmoscopically. During the healing period, the epithelium was reformed by a single sheet of hypertrophic retinal pigment epithelial cells. In contrast to continuous-wave photocoagulation, only minimal inflammatory response was found. Retinal pigment epithelial cells showed clear signs of viability, eg, phagocytized outer segments. The local edema in the photoreceptor layer and subretinal space found in the early stage disappeared when the blood-retinal barrier was reestablished. The choriocapillaris remained unaffected. No subsequent damage to the photoreceptors was found. This type of photocoagulation may be useful for retinal pigment epithelium–related diseases, eg, diffuse diabetic macular edema.**
(*Arch Ophthalmol.* 1992;110:1786-1792)

The coagulative effect of laser light on retinal tissue is exploited therapeutically in the field of ophthalmology. Despite its widespread clinical use, the mechanism by which laser photocoagulation prevents visual loss from proliferative vitreoretinopathies or macular edema is not completely understood. The therapeutic benefit of retinal photocoagulation is often not caused by the immediate laser burn but by the secondary tissue response induced by the initial photocoagulation. In proliferative diabetic retinopathy, either the removal of the oxygen-consuming photoreceptors and the replacement by a low-oxygen-consuming glial scar[1] or the regeneration of the retinal pigment epithelium (RPE) causes the therapeutic effect.[2] The observation that RPE cells release inhibitors of neovascularization in vitro suggests that these cells may play an important role in the regulation of intraocular and choroidal neovascularization in vivo.[3] In central serous retinopathy and in diffuse macular edema, the restoration of the pigment epithelial barrier is thought to be stimulated[4,5] and to lead to the therapeutic effect.

Approximately 50% of the incident 514-nm light delivered by laser irradiation is absorbed in the RPE.[6] If pulse durations of 100 milliseconds or more are used, heat dissipates out of the RPE during and after the irradiation and causes damage to the adjacent choroid and neural retina. The heat flux out of the RPE is negligible if very short pulse durations are used. The upper limit of the pulse duration for a selective effect of the RPE can be estimated by the thermal relaxation time of the RPE, which is on the order of a few microseconds.[7-9] For short pulses, however, high temperatures are necessary to produce a similar thermal effect as with long pulses. Thermomechanical mechanisms, such as explosive effects, increasingly compete with pure thermally mediated effects and are clinically intolerable. To avoid the high peak temperatures necessary with a single short pulse, multiple pulses with subthreshold energies are applied, and a given number of pulses should compensate for the lower pulse energy.

Our objective was to develop a procedure to coagulate selectively the RPE while sparing the directly adjacent neural retina and choroid. The healing response of these selective RPE lesions was monitored during a period of 4 weeks.

The secondary tissue response of the RPE to continuous-wave (CW) laser irradiation has been investigated previously.[5,10-13] All of these investigations studied the tissue response when the photoreceptors, the choriocapillaris, and the RPE had been thermally damaged simultaneously. In this study, only the RPE underwent selective photocoagulation, and the surrounding tissues were spared.

MATERIALS AND METHODS

Laser System

A 20-W (multiline) CW argon laser (Innova 100, Coherent, Palo Alto, Calif) was used at a 514-nm wavelength. The argon beam was modulated externally with an acousto-optical modulator (DLM-40-V-7, Andersen Laboratories, Bloomfield, Conn). It was possible to produce any pulse duration, from 500 nanoseconds up to continuous irradiation, and any repetition rate, up to several kilohertz; any number of pulses could be delivered. The modulated beam was focused into an 80-μm fiber (numerical aperture 0.1) and delivered to a clinically used ophthalmic slit lamp (30 SL-M, Zeiss, Oberkochen, Germany) that had been developed for laser delivery. The laser beam was focused into rabbit eyes. A helium-neon laser beam was coupled into the slit lamp for aiming. The spot size (defined as the distance across the center of the beam, for which the irradiance equals $1/e^2$ of the maximum irradiance) was 170 μm in air, as measured with a photodiode array before irradiation. Detailed calculations show that the use of a plano concave contact lens in cycloplegic emmetropic rabbit eyes translates the laser focus of 170 μm into a retinal spot size of 110 μm.[14]

Laser Photocoagulation

Chinchilla gray rabbits were used because the density and location of light-absorbing pigments in the fundus are rather uniform and are similar to that of the human eye.[6] The animals were anesthetized with ketamine hydrochloride (35 mg/kg of body weight) and xylazine hydrochloride (5 mg/kg of body weight). The treatment of experimental animals in this study was in compliance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research.

To achieve selective RPE damage with multiple pulses, the pulse energy was decreased below the single-pulse threshold energy, and many pulses were used to com-

Accepted for publication April 2, 1992.

From the Wellman Laboratories of Photomedicine, Harvard Medical School, Massachusetts General Hospital, Boston (Drs Roider, Flotte, and Birngruber and Mr Michaud), and the Medical Laser Center of Lübeck (Germany), Medical University of Lübeck (Dr Birngruber). Dr Roider is now with the Department of Ophthalmology, Medical University of Lübeck. Dr Birngruber has a patent pending on some of the procedures described in this article. This patent may constitute a potential financial interest in the subject matter. The other authors have no proprietary interest in the subject matter of this article

Presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Sarasota, Fla, April 23, 1991.

Reprint requests to Medizinische Universität zu Lübeck, Klinik für Augenheilkunde, Ratzeburger Allee 160, W-2400 Lübeck 1, Germany (Dr Roider).

pensate for the lower pulse energy. The number of pulses necessary to damage the RPE was evaluated as a function of the pulse energy. Two end points for damage were chosen: the ophthalmoscopic and the fluorescein angiographic visibility of the lesion. Pulses with a duration of 5 microseconds were used at a repetition rate of 500 Hz at 514 nm. The number of pulses included 1, 25, 100, and 500. The pulse energy varied from 2 μJ to 10 μJ, limited by the maximum power of the CW laser. As a control, CW exposures were performed at 514 nm with exposure times of 50, 100, and 500 milliseconds and 1 second. Threshold curves were made for angiographically and ophthalmoscopically visible damage for all CW irradiations.

Approximately 400 CW and 800 pulsed irradiations were performed in the regio macularis of 40 eyes of 25 rabbits, and statistical analysis was performed 2 hours after exposure.

To study the healing response of the RPE after selective photocoagulation, the retinas of 18 additional chinchilla rabbits were exposed to repetitive pulsed photocoagulation. The exposure variables were as follows: the pulse duration was 5 microseconds, the repetition rate was 500 Hz, the total number of pulses delivered was 100, and the single-pulse energies were either 3 or 6 μJ. The lesions were applied to the regio macularis in a cluster of typically 50 to 100 lesions in an area of 1×2 mm for each energy. A typical irradiation pattern is shown in Fig 1.

Fundus Evaluation and Morphologic Study

Characteristics of the lesion were recorded by fundus photography and standard fluorescein angiography by injection of 10% fluorescein sodium into the ear vein at 2 hours, 1 day, 2 days, 4 days, 2 weeks, and 4 weeks. The animals were killed with an overdose of phenobarbital sodium, and the eyes were studied histologically 2 hours (n=6), 1 day (n=5), 3 days (n=3), 14 days (n=3), and 28 days (n=1) after exposure. The eyes were removed before the rabbits were killed, and step sections were examined by light microscopy and electron microscopy. Pathologic examination included 150 lesions by light microscopy, 36 lesions by transmission electron microscopy, and 41 lesions by scanning electron microscopy.

For light microscopy and transmission electron microscopy, the tissue was prepared by immersion fixation in 4% glutaraldehyde in 0.1 mol/L of cacodylate buffer, with removal of the anterior parts within 15 minutes. The eyes were fixed for 12 to 24 hours in the cold, trimmed to block size after 2% osmium fixation, dehydrated in ethanol, and embedded in epoxy resin (Epon). One-micron sections were stained with toluidine blue, and ultrathin sections were stained with uranyl acetate and Sato's lead stain. For scanning electron microscopic studies, the eyes remained on ice for 1 hour after enucleation and then were briefly fixed in 1% glutaraldehyde in 0.1 mol/L of cacodylate buffer. The neural retina was carefully removed. A second fixation in 4% glutaraldehyde in 0.1 mol/L of cacodylate buffer was followed by dehydration in a series of graded ethanols, and then the pieces of retina were prepared in a critical-point drier with the use of liquid carbon dioxide as the transition fluid. Specimens were coated with a mixture of gold and palladium.

RESULTS

Fundus Examination and Angiography

Typical fundus photographs obtained 2 hours after irradiation with repetitive 5-microsecond laser pulses are shown in Fig 1. Only five of 800 lesions produced by repetitive 5-microsecond pulses were visible ophthalmoscopically at the energy levels used. The other lesions could be detected only by angiography. This finding was independent of pulse number and pulse energy. The other lesions also were not visible during irradiation. No sign of disruptive effects, such as hemorrhage, was observed. Table 1 shows the threshold powers (ED_{50}) of the control CW exposure for fluorescein angiographically and ophthalmoscopically visible lesions. Ophthalmoscopic and angiographic thresholds differed by a factor of 2. Continuous-wave lesions, which are not ophthalmoscopically visible, may immediately turn white after 1 day. By 4 days, there was no fluorescein leakage from either pulsed or CW lesions; some of the lesions had already disappeared after 1 day.

Statistical Analysis

Statistical evaluation (performed 2 hours after exposure) of the repetitive pulsed lesions involved plotting the incidence of fluorescein angiographic visibility vs pulse energy for various pulse numbers. The angiographic or the ophthalmoscopic threshold energy (ED_{50}) at an applied number of pulses is the necessary pulse energy to achieve fluorescein angiographically visible or ophthalmoscopically visible damage with a 50% probability. The threshold energies are shown in Table 2 as a function

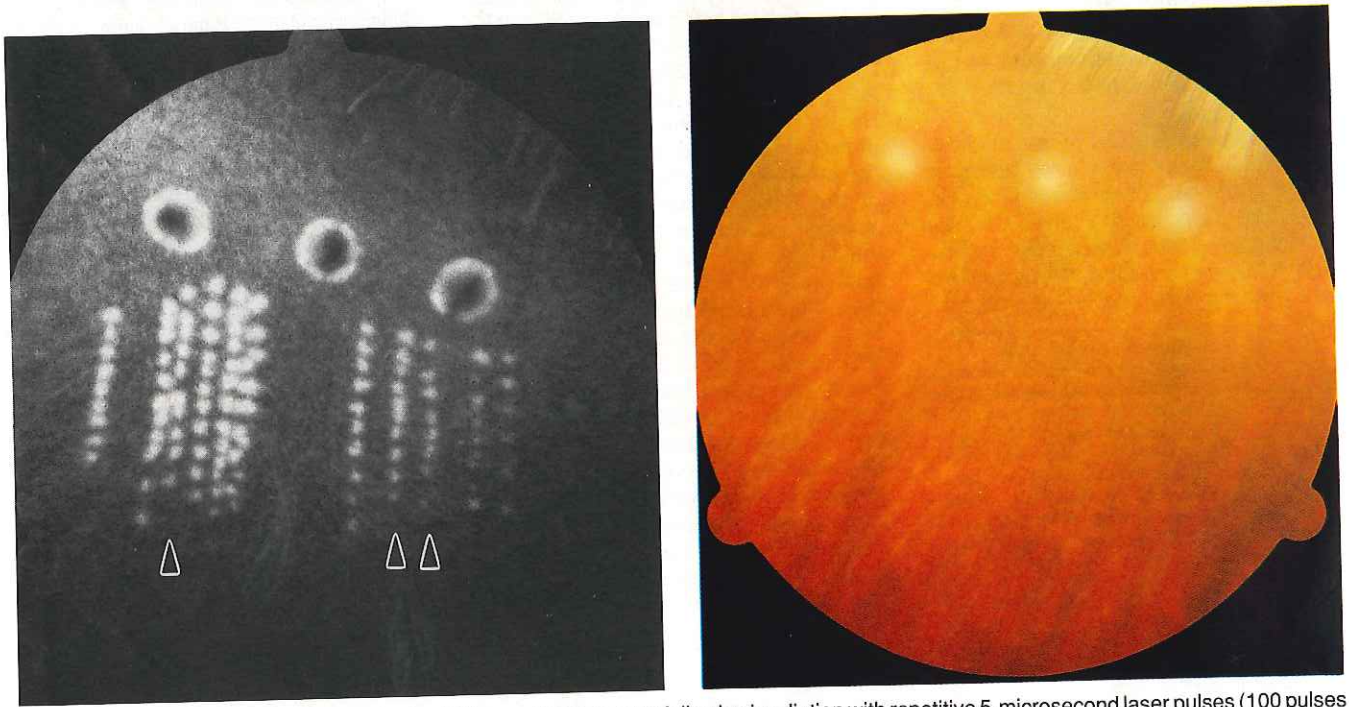

Fig 1.—Left, Angiogram of the fundus obtained 2 hours after exposure following irradiation with repetitive 5-microsecond laser pulses (100 pulses of 6 μJ, single arrowhead; 100 pulses of 3 μJ, double arrowheads). For orientation, three large marker lesions are made. Right, Corresponding fundus photograph obtained 2 hours after irradiation. None of the pulsed lesions was visible ophthalmoscopically.

of the pulse numbers. The per pulse threshold energy can be decreased by applying more pulses. Because very few of the pulsed lesions were visible ophthalmoscopically, the per pulse threshold energy for ophthalmoscopic visibility has to be higher than the maximum pulse energy used (10 μJ). It is generally possible to increase the pulse energy to increase the probability of damaging the RPE. It is also possible to achieve the same probability of fluorescein angiographically visible damage of the RPE by increasing the total number of pulses applied.

Histologic Findings

Immediate Biopsies of Pulsed and CW Lesions (2 Hours).—The primary immediate pathologic alterations after selective RPE photocoagulation, even at energy levels two to four times above the angiographic threshold, were present in the RPE with very limited damage to the neural retina and no structural damage to the choroid (Fig 2, left). The RPE showed disruption of individual cells, loss of cell membrane integrity, vacuolization of the cytoplasm, and condensation of cytoplasmic proteins. Occasional disc disorganization and vacuolization of the outer segments were noted, as was mitochondrial swelling in the inner segments (IS). At higher energy levels, scattered pyknotic nuclei were found in the outer nuclear layer (ONL) area overlying the damaged RPE (Fig 2, middle). Bruch's membrane remained unbroken at all energy levels.

The CW lesions investigated histologically were irradiated with angiographic threshold powers. Even at these power levels, damage in the neural retina and choroid caused by CW irradiation was much more extensive than that caused by pulsed irradiation. The RPE was destroyed, and major changes were also found in the neural retina and choroid. As with the pulsed lesions, RPE cells were severely damaged, with few recognizable cytoplasmic organelles remaining. Intact melanin granules were found among the debris. Most of the nuclei of the ONL appeared pyknotic (Fig 2, right), and the cytoplasm appeared vacuolated. The IS of the cells showed swollen mitochondria and cytoplasmic vacuolization. All outer segments (OS) had vesicular profiles, and many showed loss of cytoplasmic detail. In the choriocapillaris, fibrin and inflammatory cells were found within capillaries as well as in the interstitium. The blood vessels showed damaged endothelial cells and occlusion, with a mixed thrombus composed of fibrin, polymorphonuclear leukocytes, and damaged erythrocytes.

Table 1.—Angiographic and Ophthalmoscopic Threshold Powers (ED_{50}) at Different Continuous-Wave Exposure Times Needed to Achieve Fluorescein-Visible or Ophthalmoscopically Visible Damage to the Retinal Pigment Epithelium 2 Hours After Exposure*

	ED_{50}, mW	
Exposure Time, ms	Angiographic Visibility	Ophthalmoscopic Visibility
50	24	40
100	16	34
500	12	27
1 s	11	20

*ED_{50} indicates median effective dose.

Table 2.—Per Pulse Threshold Energy Needed to Achieve Fluorescein-Visible or Ophthalmoscopically Visible Damage to the Retinal Pigment Epithelium 2 Hours After Exposure by 5-Microsecond Pulses at Different Numbers of Pulses Applied at a 500-Hz Frequency*

	Per Pulse Energy (ED_{50}), μJ	
No. of Pulses	Angiographic Visibility	Ophthalmoscopic Visibility
1	5.5	>10
25	2.6	>10
100	2.0	>10
500	1.5	>10

*ED_{50} indicates median effective dose.

Healing Response of Pulsed Lesions: Early Stage (2 Hours to 3 Days).—No difference in the healing response of the RPE after selective photocoagulation was found between 3- and 6-μJ lesions. The response of the RPE starts immediately after the laser irradiation. At 2 hours after irradiation, the RPE cells adjacent to the damaged cells were extending cell processes underneath the debris toward the center of the irradiated site (Fig 3). After 1 day, these immediate neighboring RPE cells were enlarged and flattened (Fig 4). The RPE cells at the periphery of the lesions showed an increased quantity of smooth endoplasmic reticulum and appeared hyperpigmented. Basal infoldings appeared primitive. Very little phagocytosis was present. The cellular debris was present on top of the extending RPE cells. Fibrin was detectable in Bruch's membrane.

After 3 days, the irradiated site was covered by an increased number of cells (Fig 5). There was a local perturbation of the regular hexagonal RPE pattern, representing approximately three rows around the original RPE lesion. The size and height of cells inside the irradiated site varied. All of these cells showed basal infoldings, microvilli, and melanin granules (Fig 6, left). The RPE cells showed an increased amount of smooth endoplasmic reticulum and increased phagocytic activity. Phagocytized cellular debris was found intracellularly (Fig 6, right). In some irradiated areas, the basal infoldings were primitive; in other areas, they were normal. Melanin granules were found mostly in the apical portion of the cell but were sometimes dispersed throughout the entire cell. Phagocytized lamellar bodies were found inside the RPE. Tight junctions were present within 3 days (Fig 6, left). The continuity of Bruch's membrane remained unbroken. Spindle cells resembling fibroblasts were found inside Bruch's membrane.

The local retinal edema inside the ONL, IS, and OS increased for the first 3 days (Fig 7). Parts of some single damaged OS were present on top of RPE cells, although most were normally aligned and showed no morphologic damage. After 3 days, the OS appeared longer in the irradiated area (Fig 7). In the limited number of lesions examined, no inflammatory cells, such as macrophages, were found in the neural retina or in the choriocapillaris within the first 3 days. The choriocapillaris appeared unaffected.

Healing Response of Pulsed Lesions: Late Stage (2 Weeks and 4 Weeks).—Most of the cellular debris was removed by 14 days. All lesions were covered by a single layer of hypopigmented and hyperpigmented RPE cells. Some local perturbation of the hexagonal RPE pattern around and inside the original irradiation site could still be found. Many RPE cells in the original lesion site showed an increased height. The melanin granules were located in the apical part of the cell. The microvilli and the basal infoldings appeared normal. The RPE cells contained phagocytized OS (Fig 8), and some of them had changed into dark inclusion bodies. Bruch's membrane remained unbroken.

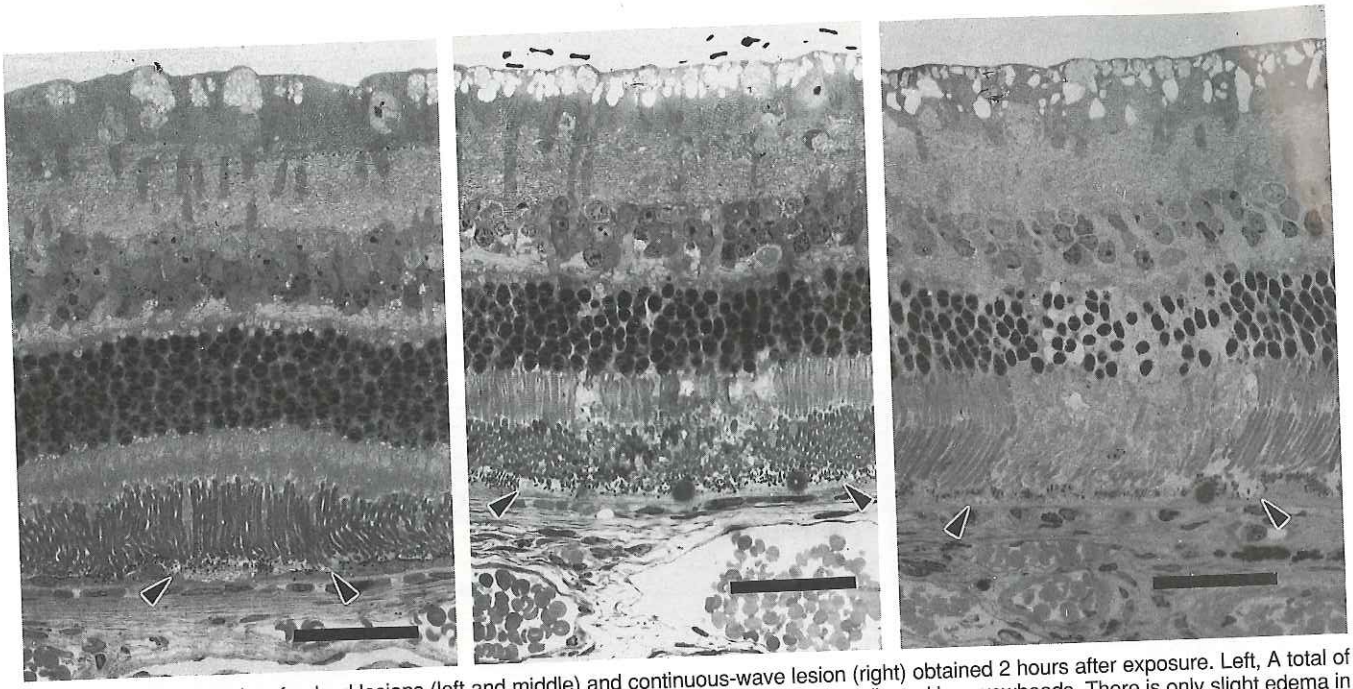

Fig 2.—Light micrographs of pulsed lesions (left and middle) and continuous-wave lesion (right) obtained 2 hours after exposure. Left, A total of 500 pulses of 3 μJ each (5 microseconds, 500 Hz); retinal pigment epithelium damage is indicated by arrowheads. There is only slight edema in the neural retina but no damage to the choroid. Middle, A total of 500 pulses of 6 μJ each (5 microseconds, 500 Hz); damage (arrowheads) is more extensive. The outer segments, inner segments, and outer nuclear layer are damaged in an irregular pattern within the lesion. Right, Continuous-wave lesion (arrowheads) at the angiographic threshold (1 second, 10 mW). The photoreceptors are uniformly destroyed. Bar indicates 40 μm.

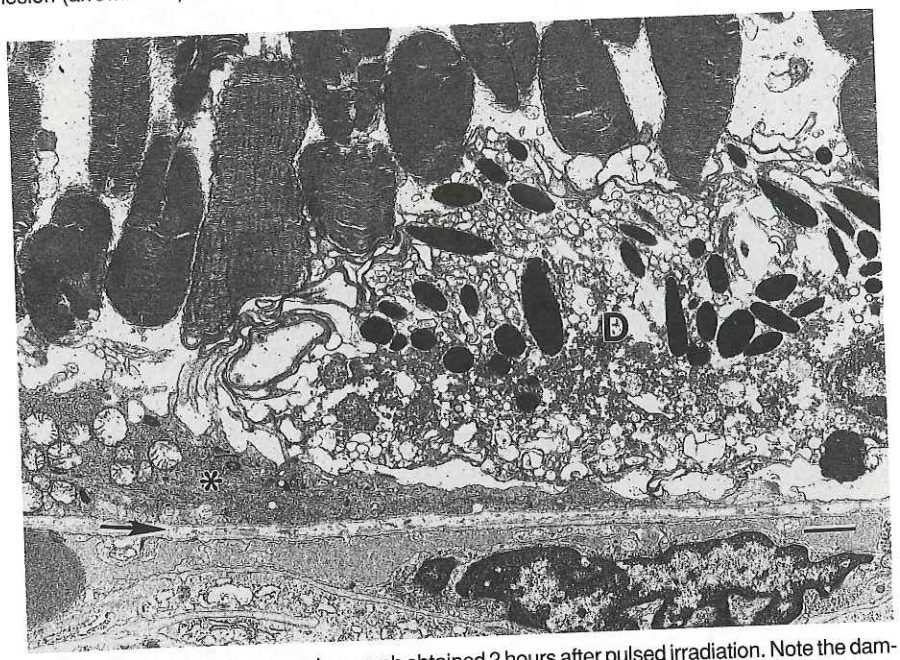

Fig 3.—Transmission electron micrograph obtained 2 hours after pulsed irradiation. Note the damaged retinal pigment epithelial (RPE) cell (D) and the adjacent RPE cell, which shows a cytoplasmic extension (asterisk) underneath the damaged RPE cell along Bruch's membrane (arrow). The choroid is not damaged, and the outer segments show minimal alterations. Bar indicates 1 μm.

Only in one rabbit, in areas where three or more original lesions were confluent, a single macrophage was occasionally seen at the interface of the OS and the regenerated RPE (Fig 9). During the late stage, no edema was present in the ONL, IS, OS, or subretinal space. All OS appeared ultrastructually normal. The density of the nuclei in the ONL appeared to be the same as in unirradiated areas. The choriocapillaris appeared unaffected and normal.

COMMENT

The in vivo observations and morphologic findings demonstrate that most of the neural retina can be spared by using repetitive microsecond pulses at low energy levels. It is impossible to reproducibly and selectively affect the RPE by using CW exposure times of 50 milliseconds to 1 second without injury to adjacent structures. The lack of ophthalmoscopic visibility of lesions produced by multiple microsecond pulses results from reduced damage to the neural retina. Ophthalmoscopic visibility and whitening indicate a change in the scattering properties of the neural retina due to cell debris.[15] The therapeutic bandwidth regarding selective RPE damage with sparing of the photoreceptors is at least a factor of 2 to 3, if 100 or more repetitive pulses are used. This has the practical advantage that the use of energies above angiographic threshold is tolerable, with no additional harm to the photoreceptors. Thus, higher energies could compensate for any energy loss due to opacities in the eye.

At all lesions investigated, the RPE barrier had been restored morphologically within 4 weeks. The cells at the lesion site closely resembled normal RPE cells, exhibiting their characteristic polarity and surface membrane specializations. The lesions were covered by a single sheet of hypertrophic RPE cells within 2 weeks. Although we did not test the function of the restored RPE cells, we inferred from its morphologic appearance, eg, phagosomes of OS, that function had returned to normal. During the repair process, the RPE cells used Bruch's membrane as a guide. This type of healing of RPE cells in a single sheet after photocoagulation has been observed in monkeys[5] and humans[10,12]

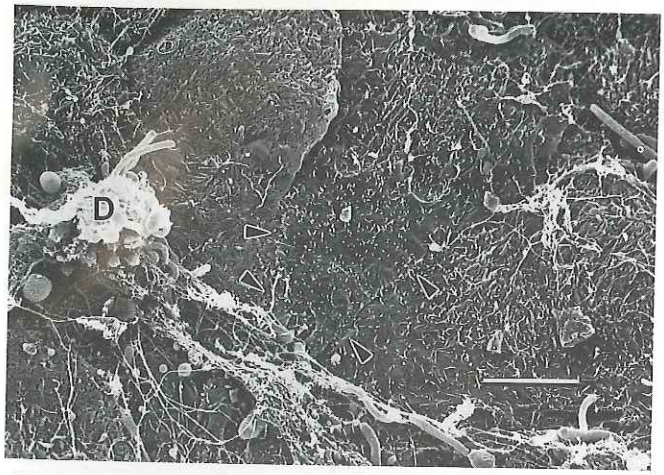

Fig 4.—Scanning electron micrograph obtained 1 day after pulsed irradiation. There is debris from the damaged cells (D). Note the cytoplasmic extensions of the surrounding cells into the area of damage. Arrowheads indicate cell boundaries. Bar indicates 10 μm.

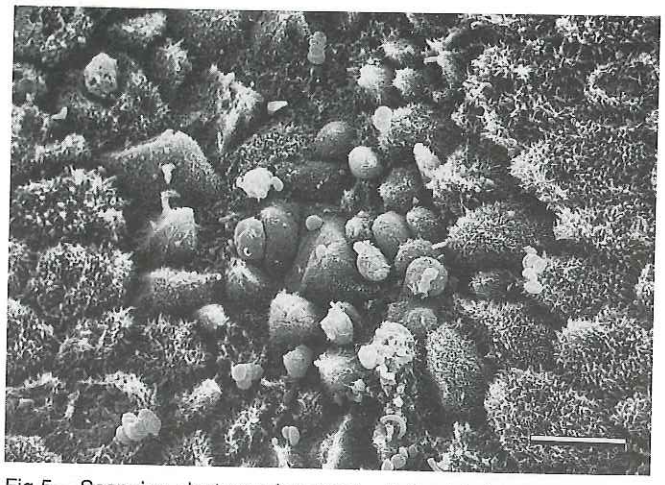

Fig 5.—Scanning electron micrograph obtained 3 days after irradiation. There is an increased density of the retinal pigment epithelial cells completely covering the damaged area. The cells have fewer microvilli, are smaller, and have altered the normal hexagonal pattern. Bar indicates 10 μm.

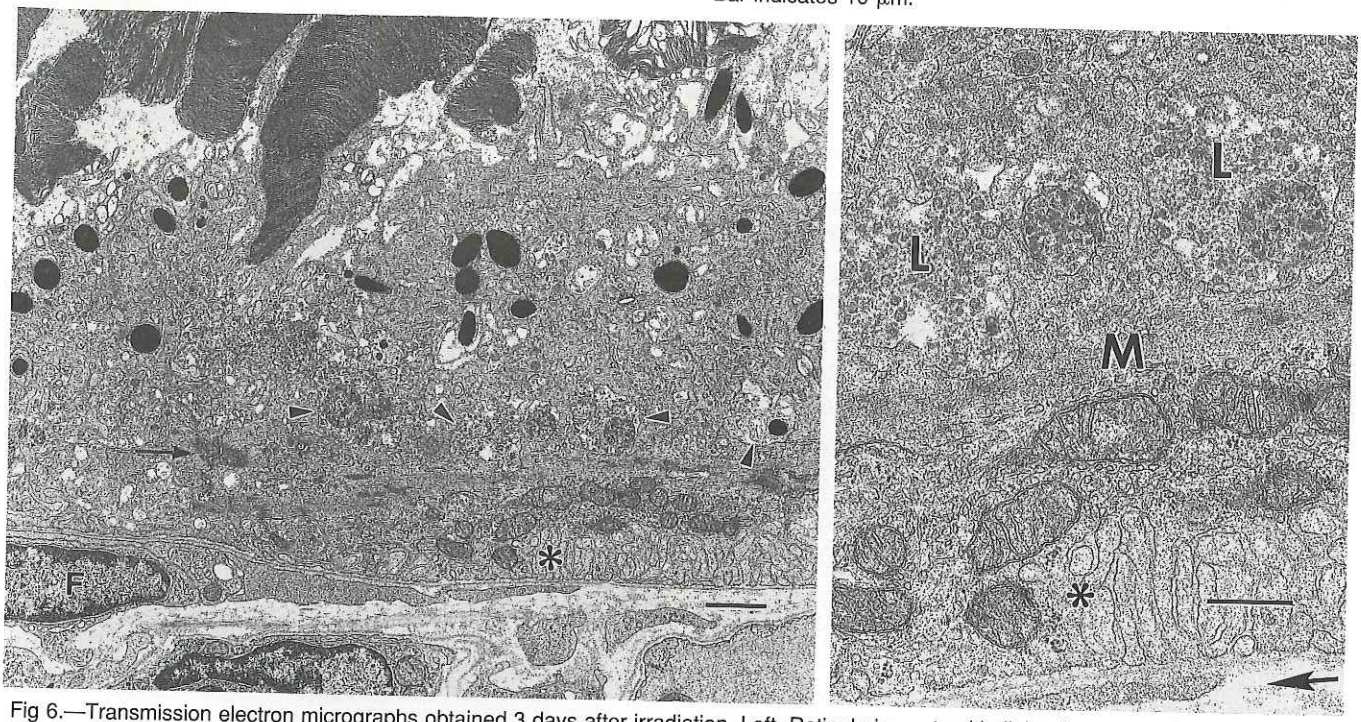

Fig 6.—Transmission electron micrographs obtained 3 days after irradiation. Left, Retinal pigment epithelial cells extend from Bruch's membrane to the outer segments and show areas of normal basal infoldings as well as other areas where the basal infoldings are primitive (asterisk). There is abundant cellular debris in lysosomes (arrowheads). Apical microvilli and single melanosomes are present. Note the spindle cells resembling fibroblasts (F) in Bruch's membrane and the intercellular junction (arrow). Bar indicates 1 μm. Right, Intermediate-power electron micrograph from Fig 6, left, showing more detail of the retinal pigment epithelial cell. Note the boundary (asterisk) between normal-appearing basal infoldings and a basal region without infoldings. There is abundant debris present in lysosomes (L). Note the bundle of microfilaments (M) present in the basal region of this cell. The bundle runs entirely within the cell and is associated with intercellular junctions. Arrow indicates Bruch's membrane. Bar indicates 0.5 μm.

after mild CW photocoagulation, often referred to as mild grade II lesions.[5] Repair of the RPE with several layers of cells, as described after heavy CW photocoagulation,[12] was not seen following pulsed irradiation.

Scanning electron microscopy demonstrated a new perspective of cells proliferating from the edge of the lesion. The re-formed RPE cells originated from the surrounding RPE by spreading and migrating; this has also been shown in organ culture.[16,17] The number of RPE cells inside the irradiated region was significantly increased after 3 days, which is highly suggestive that the RPE cells found in the irradiated sites are derived from cells at the border. It has been speculated that proliferation of the RPE cells after photocoagulation with long laser pulses (CW mode) occurs by budding.[18,19] Other studies have described RPE cell mitosis as a mechanism of replenishing the RPE after CW photocoagulation.[10,13] Incorporation of tritiated thymidine, indicating DNA synthesis and cellular proliferation, was found in the RPE cells, following xenon arc photocoagulation of the rabbit retina[20] and following retinal detachment in monkey eyes.[21] In organ cul-

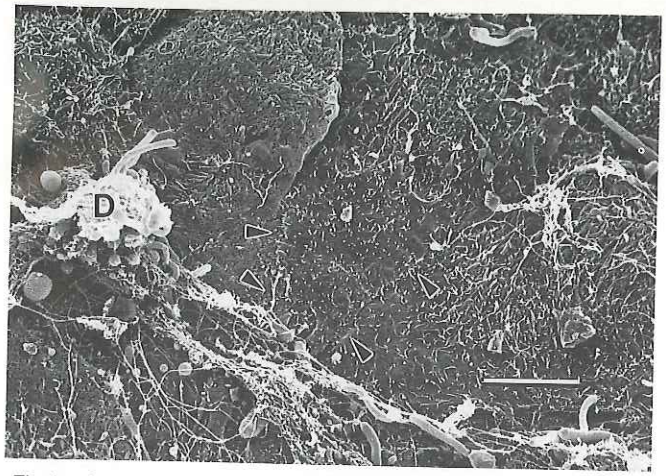

Fig 4.—Scanning electron micrograph obtained 1 day after pulsed irradiation. There is debris from the damaged cells (D). Note the cytoplasmic extensions of the surrounding cells into the area of damage. Arrowheads indicate cell boundaries. Bar indicates 10 μm.

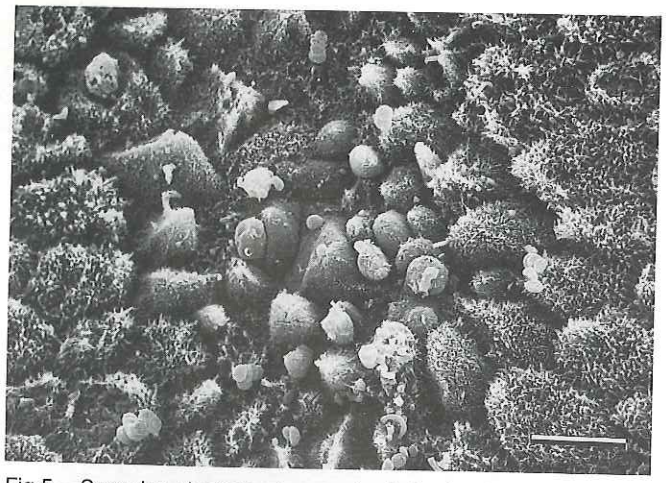

Fig 5.—Scanning electron micrograph obtained 3 days after irradiation. There is an increased density of the retinal pigment epithelial cells completely covering the damaged area. The cells have fewer microvilli, are smaller, and have altered the normal hexagonal pattern. Bar indicates 10 μm.

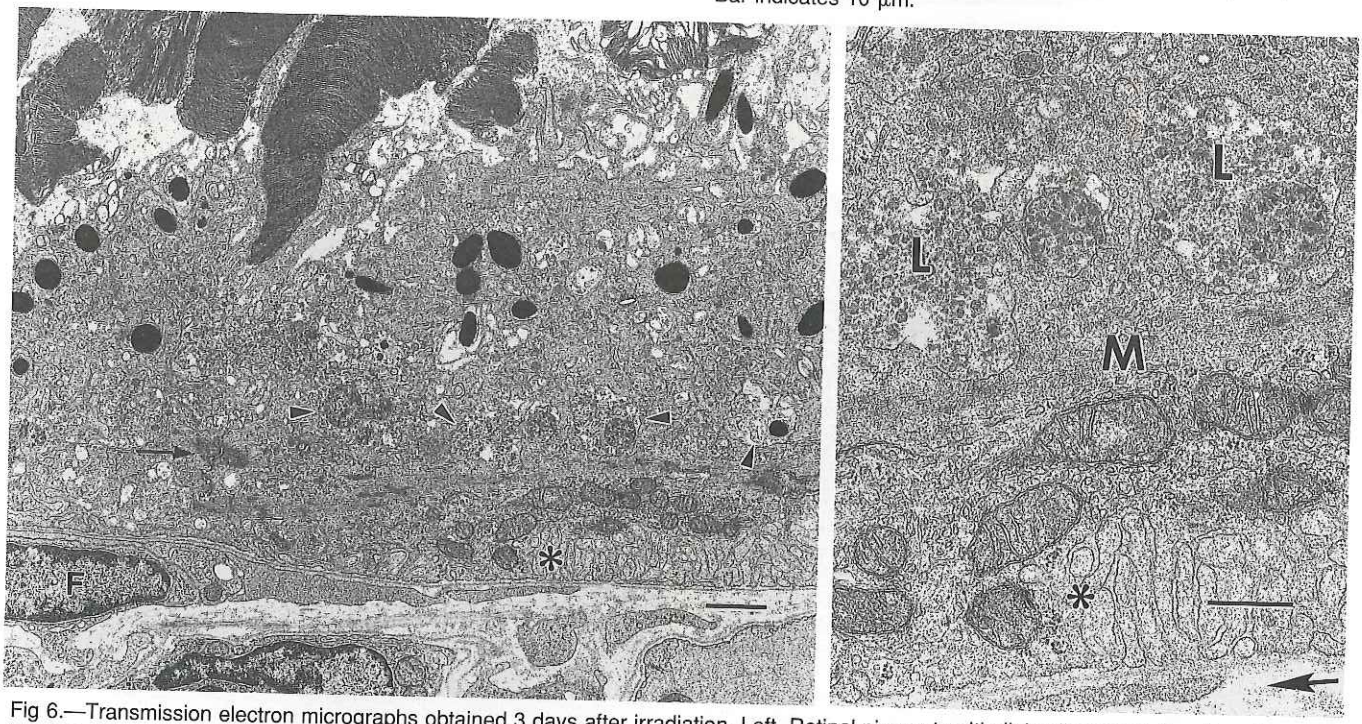

Fig 6.—Transmission electron micrographs obtained 3 days after irradiation. Left, Retinal pigment epithelial cells extend from Bruch's membrane to the outer segments and show areas of normal basal infoldings as well as other areas where the basal infoldings are primitive (asterisk). There is abundant cellular debris in lysosomes (arrowheads). Apical microvilli and single melanosomes are present. Note the spindle cells resembling fibroblasts (F) in Bruch's membrane and the intercellular junction (arrow). Bar indicates 1 μm. Right, Intermediate-power electron micrograph from Fig 6, left, showing more detail of the retinal pigment epithelial cell. Note the boundary (asterisk) between normal-appearing basal infoldings and a basal region without infoldings. There is abundant debris present in lysosomes (L). Note the bundle of microfilaments (M) present in the basal region of this cell. The bundle runs entirely within the cell and is associated with intercellular junctions. Arrow indicates Bruch's membrane. Bar indicates 0.5 μm.

after mild CW photocoagulation, often referred to as mild grade II lesions.[5] Repair of the RPE with several layers of cells, as described after heavy CW photocoagulation,[12] was not seen following pulsed irradiation.

Scanning electron microscopy demonstrated a new perspective of cells proliferating from the edge of the lesion. The re-formed RPE cells originated from the surrounding RPE by spreading and migrating; this has also been shown in organ culture.[16,17] The number of RPE cells inside the irradiated region was significantly increased after 3 days, which is highly suggestive that the RPE cells found in the irradiated sites are derived from cells at the border. It has been speculated that proliferation of the RPE cells after photocoagulation with long laser pulses (CW mode) occurs by budding.[18,19] Other studies have described RPE cell mitosis as a mechanism of replenishing the RPE after CW photocoagulation.[10,13] Incorporation of tritiated thymidine, indicating DNA synthesis and cellular proliferation, was found in the RPE cells, following xenon arc photocoagulation of the rabbit retina[20] and following retinal detachment in monkey eyes.[21] In organ cul-

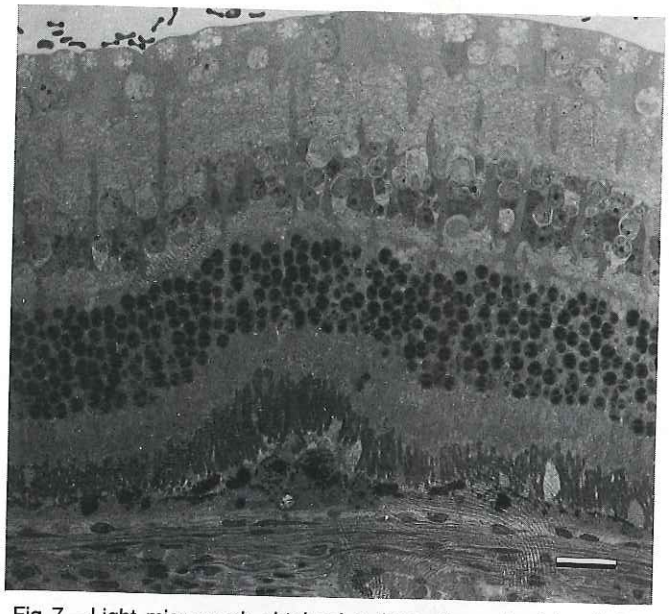

Fig 7.—Light micrograph obtained 3 days after pulsed irradiation. There is edema and thickening of the retinal pigment epithelium. Outer segments appear longer in the area above the irradiated retinal pigment epithelium. Bar indicates 20 μm.

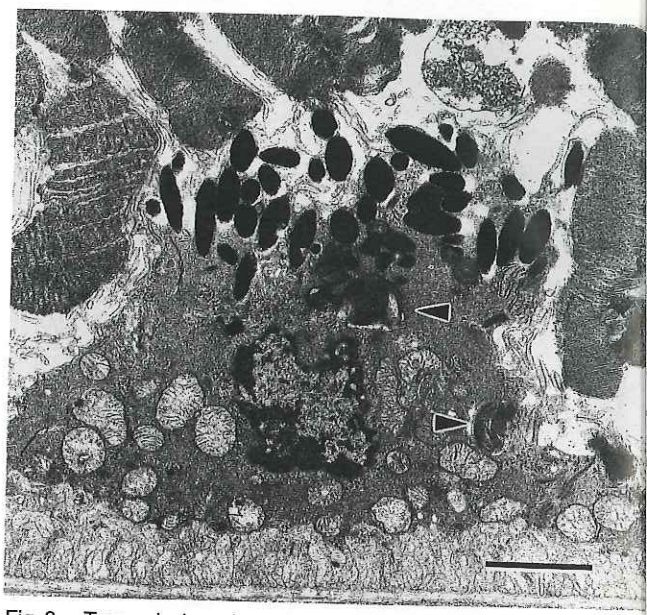

Fig 8.—Transmission electron micrograph obtained 14 days after pulsed irradiation. A single hypertrophic retinal pigment epithelial cell is illustrated, demonstrating an increased height, nearly normal basal infoldings, phagolysosomes (arrowheads), and single melanosomes. Note the membrane-bound debris in the subretinal space. Bar indicates 2 μm.

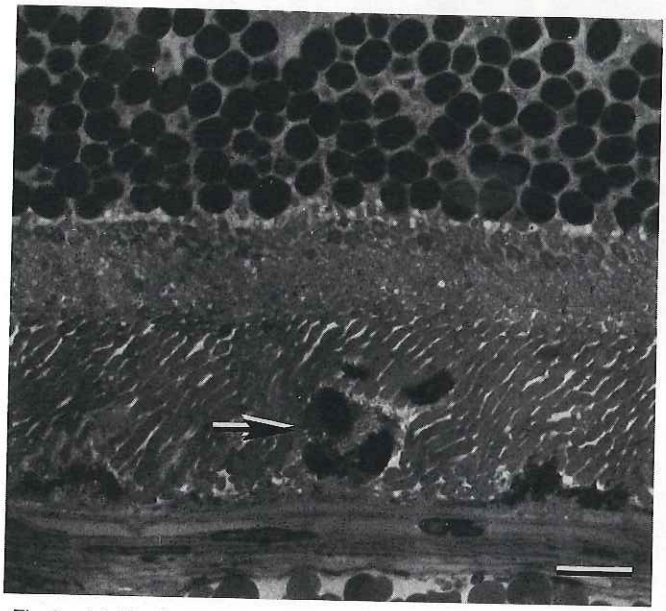

Fig 9.—Light micrograph obtained 14 days after pulsed irradiation. There is a macrophage (arrow) in an area where there was damage to several retinal pigment epithelial cells. Note the variable distribution of the melanosomes in the regenerating retinal pigment epithelium. Bar indicates 10 μm.

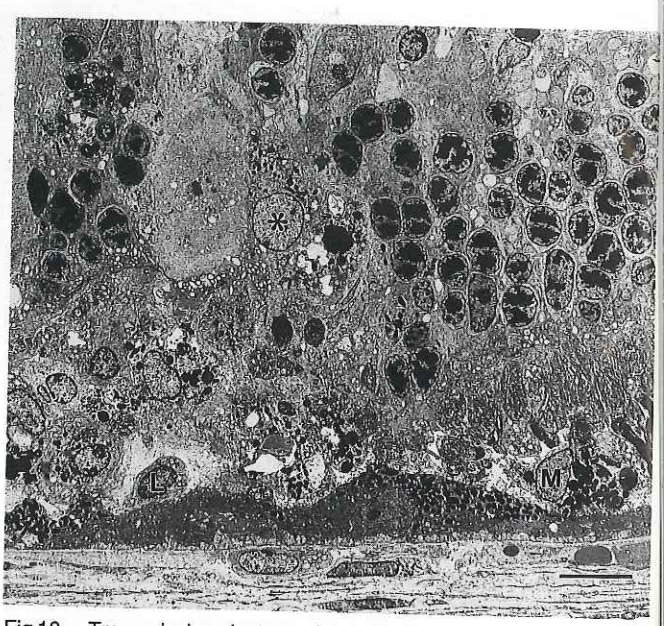

Fig 10.—Transmission electron micrograph obtained 3 days after mild continuous-wave irradiation (100 milliseconds, 20 mW, 110-μm spot size). There is extensive damage to the outer nuclear layer and outer segments. Note macrophages (M), lymphocytes (L), and pigment-containing cells with single melanosomes present in the outer nuclear layer (asterisk). The choriocapillaris is absent under the center of the irradiated area. Bar indicates 10 μm.

ture, it was found that RPE cells at the edge of an RPE wound express proliferating cell nuclear antigen, an obligatory cofactor of a DNA polymerase and a marker of cells committed to cell mitosis.[22] No clear mitotic figures were found in our study; however, the chances of seeing a mitotic figure would be expected to be small. In this study, some cells with protruding nuclei and cytoplasm consistent with the observations of Marshall et al[18] and Bülow[19] were noted.

The appearance of morphologic variations among proliferating RPE cells, such as RPE-derived macrophages, has been described in several studies as a consequence of retinal laser injury[13,18,23] or in other conditions in both human and animal eyes.[21] Phagocytic and inflammatory cells in the neural retina are regularly found in the photoreceptor layer after photocoagulation with a CW laser.[13,18,23] Figure 10 shows the tissue reaction 3 days after a mild (slightly

above angiographic threshold) CW photocoagulation argon burn (514 nm) with a single 100-millisecond pulse at 20 mW. Clear evidence of phagocytosis, eg, numerous phagocytized melanin granules, was found in cells resembling macrophages. In addition, lymphocytes were present. The number of macrophages found after selective RPE photocoagulation was minimal. In only one rabbit, we found occasional single macrophages in the OS. The integrity of the RPE was reestablished in all of our lesions, and most of the repair process was done by the RPE cells themselves.

We found clear signs of viability of the RPE cells at the irradiated site. Phagocytized lamellar bodies of the OS were regularly found in these RPE cells, which are known to actively transport fluid from the subretinal space into the choroid.[24,25] The local edema in the photoreceptor layer and subretinal space found in the early stage had disappeared in the late stage. Because no inflammatory cells were present in the early stage, the retinal edema resulted from a breakdown of the RPE barrier. The disappearance of the retinal edema in the late stage followed the reestablishment of the RPE border. The time frame of the RPE repair is in accordance with that reported in other studies about blood-retinal barrier recovery after clinically mild CW photocoagulation of the RPE, in which it was shown that the active transport for fluorescein across the blood-retinal barrier had almost completely recovered within 4 weeks.[26]

No subsequent damage to the choriocapillaris and no inflammatory cells after selective RPE photocoagulation were found during the period of investigation. Following CW photocoagulation, choriocapillaris damage is regularly found with an inflammatory response during the healing period. By experimental injection of sodium iodate, choriocapillaris atrophy in rabbits resulted from damage to RPE cells.[27] No atrophy was detected in those studies in which the RPE was viable and undamaged. In our case, where small spot sizes were used, the RPE was absent only for short periods ranging between 3 days and 2 weeks. This could explain why no signs of choriocapillaris atrophy could be found. Maybe viable RPE cells produced a vascular modulating factor that helped maintain the choriocapillaris.[27,28]

The healing process of the RPE after selective photocoagulation shows characteristics of RPE recovery as seen after therapeutic mild CW photocoagulation. One possible, yet unproved, explanation for the therapeutic effect of photocoagulation for diffuse diabetic macular edema is thought to be the result of the restoration of a new blood-retinal barrier.[4] Because after selective photocoagulation the RPE seems morphologically and functionally recovered, this treatment modality may likewise be useful for diabetic macular edema. In addition, it would eliminate the chorioretinal neovascularization occasionally found in response to CW photocoagulation.[29] Finally, selective RPE photocoagulation has the advantage that most of the photoreceptors would be spared. Paracentral scotomas observed clinically after CW photocoagulation[30] might thus be avoided.

The feature of confined thermal damage by repetitive laser pulses could make this type of photocoagulation useful in a variety of other clinical applications, eg, age-related macular degeneration, central serous retinopathy, and even diabetic retinopathy. In addition, it could also be helpful for RPE transplantation to remove the host RPE before transplantation. Repetitive pulsed photocoagulation could be used to investigate the basic mechanism of photocoagulation and to answer the question as to whether clinical photocoagulation needs the destruction of the photoreceptors, as occurs in all current types of photocoagulation procedures, or whether selective effects in the RPE are sufficient for the therapeutic effects. Further clinical investigations will now be needed to prove the significance of this type of photocoagulation.

This work was supported in part by the Office of Naval Research and Medical Free Electron Laser Program under contract N0014-86-K-0117 and by grant Ro 804/1-1 from Deutsche Forschungsgemeinschaft, Bonn, Germany (Ro 804/1-1).

The authors wish to thank Veit-Peter Gabel, MD, John Marshall, PhD, and Carmen A. Puliafito, MD, for helpful discussions.

References

1. Wolbarsht MT, Landers MB. The rationale of photocoagulation therapy for proliferative diabetic retinopathy: a review and model. *Ophthalmic Surg.* 1980;11:235-245.

2. Tso MO, Cunha-Vaz JG, Shih C, Jones CW. Clinicopathologic study of blood retinal barrier in experimental diabetes mellitus. *Arch Ophthalmol.* 1980;98:2032-2040.

3. Glaser BM, Campochiaro PA, Davis JL, Jerdan JA. Retinal pigment epithelial cells release inhibitors of neovascularization. *Ophthalmology.* 1987;94:780-784.

4. Bresnick GH. Diabetic maculopathy: a critical review highlighting diffuse macular edema. *Ophthalmology.* 1983;90:1301-1317.

5. Wallow IH. Repair of the pigment epithelial barrier following photocoagulation. *Arch Ophthalmol.* 1984;102:126-135.

6. Gabel VP, Birngruber R, Hillenkamp F. Visible and near infrared light absorption in pigment epithelium and choroid. In: Shimizu K, ed. *International Congress Series No. 450, XXIII Concilium Ophthalmologicum, Kyoto.* Princeton, NJ: Excerpta Medica; 1978:658-662.

7. Anderson R, Parrish JA. Selective photothermolysis. *Science.* 1983;220:524-527.

8. Birngruber R, Hillenkamp F, Gabel VP. Theoretical investigations of laser thermal retinal injury. *Health Phys.* 1985;48:781-796.

9. Roider J, Birngruber R. Spatial confinement of photocoagulation effects using high repetition rate laser pulses. In: *Conference on Lasers and Electro-Optics, Technical Digest Series.* Washington, DC: Optical Society of America; 1990;7:168-169.

10. Marshall J. Interactions between sensory cells, glia cells and the retinal pigment epithelium and their response to photocoagulation. *Dev Ophthalmol.* 1981;2:308-317.

11. Pollack A, Korte GE. Repair of retinal pigment epithelium and its relationship with capillary endothelium after krypton photocoagulation. *Invest Ophthalmol Vis Sci.* 1990;31:890-898.

12. Wallow I. Long term changes in photocoagulation burns. *Dev Ophthalmol.* 1981;2:318-327.

13. Wallow IH, Tso MO. Repair after xenon arc photocoagulation, II: a clinical and light microscopic study of the evolution of retinal lesions in the rhesus monkey. *Am J Ophthalmol.* 1973;75:610-626.

14. Birngruber R, Gabel VP, Hillenkamp F. Experimental studies of laser thermal retinal injury. *Health Phys.* 1983;44:519-531.

15. Yanoff M, Fine BS. *Ocular Pathology; A Text and Atlas.* 2nd ed. New York, NY: Harper & Row Publishers Inc; 1982:196-201.

16. Del Priore LV, Glaser BM, Quigley HA, Green R. Response of pig retinal pigment epithelium to laser photocoagulation in organ culture. *Arch Opthalmol.* 1989;107:119-122.

17. Hergott GJ, Sandig M, Kalnins VI. Cytoskeletal organization of migrating retinal pigment epithelial cells during wound healing in organ culture. *Cell Motil Cytoskeleton.* 1989;13:83-93.

18. Marshall J, Fankhauser F, Lotmar W, Roulier W. Pathology of short pulse retinal photocoagulations using the Goldmann contact lens. *Graefes Arch Clin Exp Ophthalmol.* 1971;182:154-169.

19. Bülow N. The process of wound healing of the avascular outer layers of the retina, light- and electron-microscopic studies on laser lesions of monkey eyes. *Acta Ophthalmol Suppl (Copenh).* 1978; 139:7-60.

20. Inomata H. Wound healing after xenon arc photocoagulation in the rabbit retina. *Ophthalmologica.* 1975;170:462-474.

21. Machemer R, Laqua H. Pigment epithelium proliferation in retinal detachment (massive periretinal proliferation). *Am J Ophthalmol.* 1975;80:1-23.

22. Hergott GJ, Gallie B, Kalnins VI. Proliferating cell nuclear antigen (PCNA) in migrating retinal pigment epithelial cells (RPE) during wound healing in organ culture. *Invest Ophthalmol Vis Sci.* 1991;32:1219. Abstract.

23. Pollack A, Korte GE, Weitzner AL, Henkind P. Ultrastructure of Bruch's membrane after krypton laser photocoagulation, I: breakdown of Bruch's membrane. *Arch Ophthalmol.* 1986;104:1372-1375.

24. Marmor MF. Control of subretinal fliud: experimental and clinical studies. *Eye.* 1990;4:340-344.

25. Miller SS, Hughes BA, Machen TE. Fluid transport across retinal pigment epithelium is inhibited by cyclic AMP. *Proc Natl Acad Sci U S A.* 1982;79:2111-2115.

26. Zweig K, Cunha-Vaz J, Peyman G, Stein M, Raichand M. Effect of argon laser photocoagulation on fluorescein transport across the blood retinal barrier. *Exp Eye Res.* 1981;32:323-329.

27. Korte GE, Reppucci V, Henkind P. RPE destruction causes choriocapillary atrophy. *Invest Ophthalmol Vis Sci.* 1984;25:1135-1144.

28. Henkind P, Gartner S. The relationship between retinal pigment epithelium and the choriocapillaris. *Trans Ophthalmol Soc U K.* 1983;103: 444-447.

29. Lewis H, Schachat A, Haiman M, et al. Choroidal neovascularization after laser photocoagulation for diabetic macular edema. *Ophthalmology.* 1990;97:503-511.

30. Olk RJ. Argon green (514 nm) versus krypton red (647 nm) modified grid laser photocoagulation for diffuse diabetic macular edema. *Ophthalmology.* 1990;97:1101-1112.

Printed and Published in the United States of America