Yonsei Medical Journal
Vol. 36, No. 3, 1995

Changes in Adhesive Force between the Retina and the Retinal Pigment Epithelium by Laser Photocoagulation in Rabbits

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A closed eyeball model was designed to estimate the chorioretinal adhesion of the laser-photocoagulated region. We used it to measure the duration of development of retinal detachment during vitrectomy before and after killing the test rabbits. During testing, negative pressure was applied into the vitreous cavity of the pigmented rabbits. Laser burns were produced in the posterior retina by exposure to an argon blue-green laser beam with a focus diameter of 200 μm of 0.1 to 0.2 second duration and 150 to 250 mW intensity. One hour and one, two, five, seven and fourteen days following laser photocoagulation, vitrectomy was done with a cutting rate of 500 per minute, aspiration pressure of 50 mmHg and infusion pressure of 55.2 mmHg. After core vitrectomy, the rabbit was killed with an intravenous bolus of 100 mg sodium pentobarbital solution. After killing the rabbit, the vitreous cavity was continuously aspirated under the pressure of 25 mmHg while the infusion was stopped. The changes of the fundus, especially development of retinal detachment, were observed in the laser-treated and untreated regions before and after killing the rabbit. When retinal detachment was noted anywhere before killing the rabbit, this postmortem change was not observed. One hour following laser photocoagulation, the laser-trteated retina was detached during core vitrectomy before killing the rabbit, and the untreated area was not detached. One day following photocoagultion, the retina was intact before killing the rabbit. After killing the rabbit, the laser-treated retina was detached in four minutes and the untreated retina in 18 minutes postmortem. Two days following photocoagulation, the retina was intact in both regions before killing the rabbit, however after killing the rabbit, the untreated retina was detached in 18 minutes postmortem and the laser-treated retina was remained attached until 32 minutes when corneal haziness occurred and further observation was not possible. After the fifth, seventh and fourteenth days, before killing the rabbit, the retina was intact anywhere and the laser-treated retina was remained attached after 40 minutes postmortem until corneal haziness occurred and further observation was not possible, while the untreated retina was detached at 18 minutes postmortem. These data suggest that laser photocoagulation can induce a stronger chorioretinal adhesion than normal adhesion in two days in rabbits.

Key Words: Laser photocoagulation, chorioretinal adhesion, vitrectomy, aspiration, retinal detachment

Received March 17, 1995
Accepted June 26, 1995
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This paper was presented in part at the annual meeting of the Association for Research in Vision and Ophthalmology, Sarasota, Florida, U.S.A., May 3-8, 1992
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Since the introduction of laser photocoagulation by Meyer-Schwickerath (1960), the clinical application of this treatment has developed rapidly. It has been used widely for various chorioretinal pathologic conditions including certain types of lattice degeneration, retinal tears or small focal retinal detachments. Although there is little information available regarding the development and degree of enhanced adhesion following laser photocoagulation, retinopexy using laser photocoagulation has taken the place of the deathermy or cryotherapy. It causes less

breakdown of the blood-ocular barrier (Jaccoma 1985), less release of chemoattractants for the retinal pigment epithelial cells (Campochiaro *et al.*, 1984; Campochiaro *et al.* 1985), less dispersion of the retinal pigment epithelial cells and less damage to the choroid and sclera (Curtin *et al.* 1966; Santos *et al.* 1966). Also, laser photocoagulation makes it easier to control the location and density of the reaction, provides chorioretinal adhesion more rapidly than diathermy or cryotherapy (Zauberman 1969; Yoon and Marmor 1988), and minimizes the risk of subsequent redetachment.

Once the retina is exposed to laser photocoagulation, the chorioretinal adhesive force is weakened rapidly, then revovers its normal force and becomes several times stronger than this normal force over several weeks after laser photocoagulation (Zauberman 1969; Folk *et al.* 1989; Kain 1984). This eventually strong chorioretinal adhesion is probably caused by the scar tissue formed between the neural retina and the choroid in the later stage of reaction (Santos *et al.* 1966; Kissen *et al.* 1961; Lavyel 1963; Powell *et al.* 1971).

Although several reports about the chorioretinal adhesion after laser photocoagulation have been published, we are unaware of detailed information concerning the recovery of the chorioretinal adhesive force in the photocoagulated retina. The major reason for this lack of detailed information regarding the development of the optimal adhesion following laser photocoagulation has been the lack of an experimental method sensitive enough to perform an accurate assessment of the changes in chorioretinal adhesion of the photocoagulated region. We designed a new closed eyeball model, nontraumatic to the choriod and retina, to estimate the chorioretinal adhesion of the photocoagulated region by measuring the time when the postmortem retinal detachment developed while negative pressure was applied into the vitreous cavity.

MATERIALS AND METHODS

All animals used in this study were treated in accordance with the "Guiding Principles in the Care and Use of Animals" approved by the American Physiological Society.

Laser photocoagulation

Six pigmented rabbits, with normal fundi (6 eyes), weighing 2.5~3.0 Kg were prepared. After the pupils were dilated with several drops of 2.5 % phenylephrine HCl (Mydfrin, Alcon laboratories), and 1% tropicamide (Mydriacyl, Alcon laboratories) Ocular solutions, the rabbits were anesthesized with an intravenous injection of 20 mg/Kg of body weight of sodium pentobarbital solution (Entobar, Hanlim pharmacy). The peripheral fundus where the instruments would be inserted for vitrectomy was exposed prophylactically to an argon laser to prevent retinal detachment at entry sites. The laser photocoagulation was performed by exposures of a blue-green argon laser (920 Argon/Dye, Coherent medical) at a wavelength of 488~512 nm with a focus diameter of 200 μm of 0.1 and 0.2 second duration and 200 mW intensity one burn-size apart from each other using slit-lamp delivery system.

Two weeks after the prophylactic laser photocoagulation, the dense chorioretinal laser scarring on the peripheral retina at the entry site for vitrectomy was confirmed. The posterior portion of the fundus was exposed to a blue-green argon laser. Moderate laser burns were produced by exposure to a laser beam with a focus diameter of 200 μm of 0.1 to 0.2 second duration and 150 to 250 mW intensity. Laser photocoagulation was performed with two-hundred laser exposures, each one burn-width apart from the preceding one.

Closed vitrectomy and aspiration system producing postmortem retinal detachments

One hour and one, two, five, seven and fourteen days following laser photocoagulation, the fundi were examined by indirect ophthalmoscopy and color photography. After the rabbits were anesthesized with an intravenous injection of 20 mg/kg of body weight of sodium pentobarbital solution, the rabbit eyes were draped for three-port, closed vitrectomy (using the SITE TXR, SITE microsurgical systems) with a cutting rate of 500 per minute and aspiration pres-

sure of 50 mmHg. The conjunctiva and Tenon's capsule were dissected with sharp scissors, and the sclera was supported by a Flieringer's ring fixed in the equatorial zone in order to prevent ocular collapse during application of the negative pressure into the vitreous cavity. An infusion cannula was fixed at a sclerotomy site 3 mm apart from the limbus in the inferior temporal direction. An illuminating probe and a vitreous cutter were inserted chrough other sclerotomies made in both horizontal directions. The vitreous gel was cut and removed while the balanced salt solution Plus (BSSP, Alcon laboratories) was infused into the vitreous cavity through the infusion cannula. The height of infusion was kept at 75 cm above the eyeball (55.2 mmHg). After the whole vitreous cavity was replaced with the BSSP, the rabbit was killed with an intravenous bolus of 100 mg sodium pentobarbital solution. As soon as the pulsation stopped, the vitreous cavity was aspirated continuously without cutting under a pressure of 25 mmHg while the infusion cannula was closed. The opening of an aspirator tip was positioned in the center of the vitreous cavity and directed toward the optic disc so that negative pressure affected the whole retina evenly during continuous aspiration (25 mmHg)(Fig. 1).

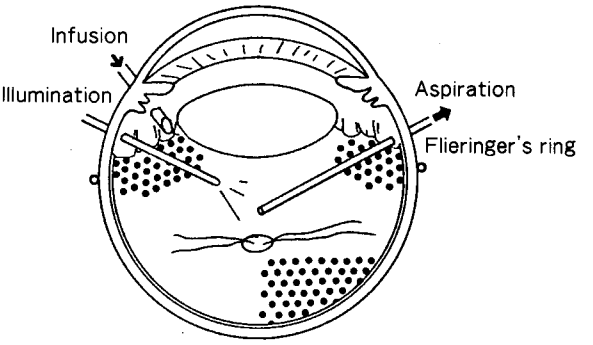

Fig. 1. 3 port vitrectomy procedure with Flieringer's ring after laser photocoagulation.

Measurement of chorioretinal adhesion

To determine the time when the lesion first showed enhanced adhesion, the changes of the fundus, especially development of retinal detachment, were observed in both the laser-treated and untreated regions during aspiration. At this time the infusion was stopped in one hour and one, two, five, seven and fourteen days following laser photocoagulation. The development of retinal detachment was measured as to be the duration measured with a stopwatch during core vitrectomy before and after killing the rabbit.

Table 1. Duration to development of postmortem retinal detachment following laser photocoagulation

After laser photocoagulation	Untreated region	Laser-treated region
one hour	Not detached (antemortem)*	1 minute (antemortem)
first day	18 minutes	4 minutes
second day	18 minutes 50 seconds	32 minutes+
fifth day	18 minutes	45 minutes+
seventh day	18 minutes 30 seconds	52 mintues+
fourteenth day	18 mintuds	38 minutes+

*The further observation was interrupted by the bullous retinal detachment in the laser-treated region.

+not detached until the corneal haziness prevented from further observation.

RESULTS

One hour following laser photocoagulation

An immediate whitish reaction appeared in the photocoagulated protion of the fundus. The central zone of photocoagulation spot was densely whitish and slightly elevated. The periphery was slightly grayish-white and had a blurred margin (Fig. 2A).

During core vitrectomy and before killing the rabbit, the focally laser-treated retina was detached and elevated toward the opening of a vitreous cutter one minute after starting the vitrectomy, while the untreated retina was not detached (Fig. 2B, Table 1).

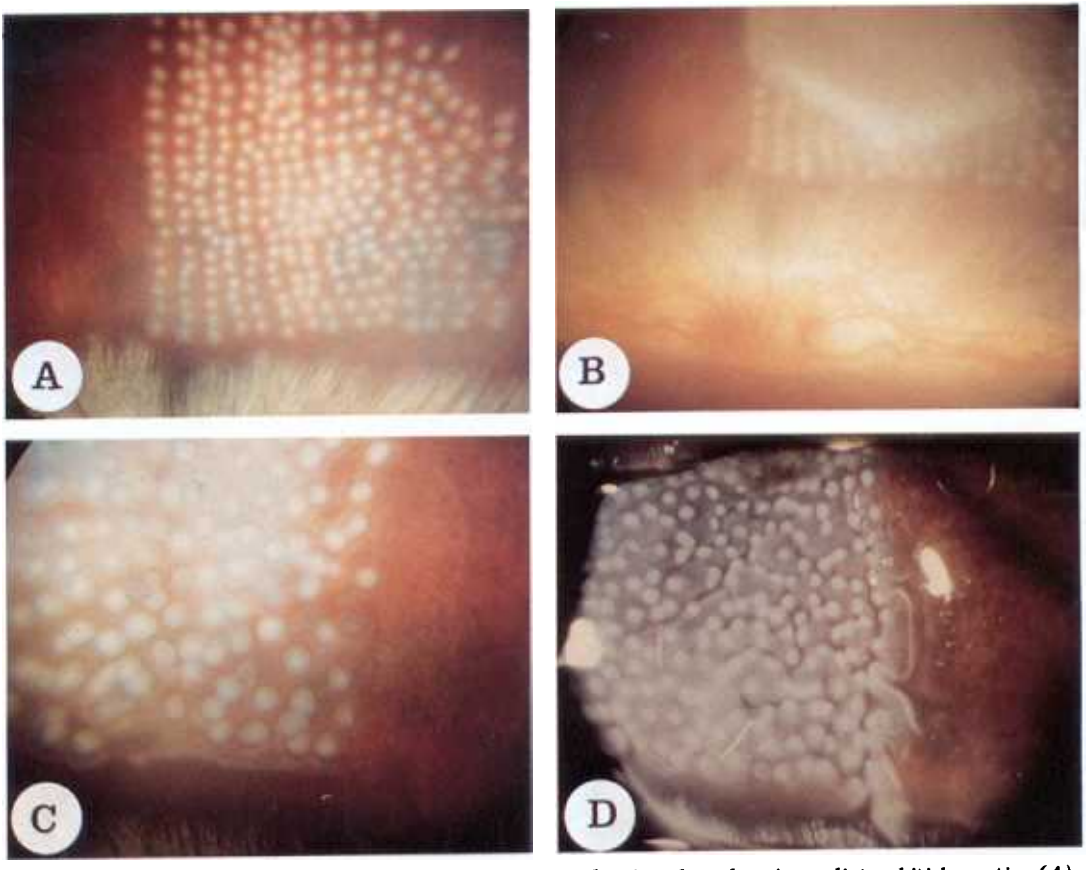

Fig. 2. One hour following laser photocoagulation, the laser-treated retina showed an immediate whitish reaction (A) and retinal detachment one minute after staring vitrectomy, while the untreated retina was not detached until vitrectomy was finished (B). After one day, the laser-treated retina showed a whitish, less elevated reaction (C) and became detached from the choroid in four minutes postmortem applying the aspiration pressure of 25 mmHg after vitrectomy, while the untreated retina was still attached to the choroid for eighteen minutes, then detached (D). After two days, mild pigmention without elevation appeared in the laser-treated region (E). The laser-treated region was not detached until 32 minutes after death when further examination was not possible (F). After the fifth day of laser photocoagulation, the laser-treated region, where dark pigmentation and cicatrical changes were found (G), was maintained without retinal detachment until 40 minutes after death (H).

One day following laser photocoagulation

The whitish burn reaction continued, but the lesion was less elevated and the margin was less blurred than at one hour following laser photocoagulation (Fig. 2C). The retina remained undetached in the laser-treated as well as in the untreated regions during vitrectomy before killing the rabbit.

The rabbit was killed with an intravenous bolus of 100 mg of sodium pentobarbital solution immediately after completion of the vitrectomy. The laser-treated retina became detached during application of an aspiration pressure of 25 mmHg at four minutes postmortem, while the untreated retina remained attached until eighteen minutes, when it was detached (Fig. 2D, Table 1).

Two days following laser photocoagulation

Mild pigmention appeared and the margin of laser burns was more distinct and the elevation

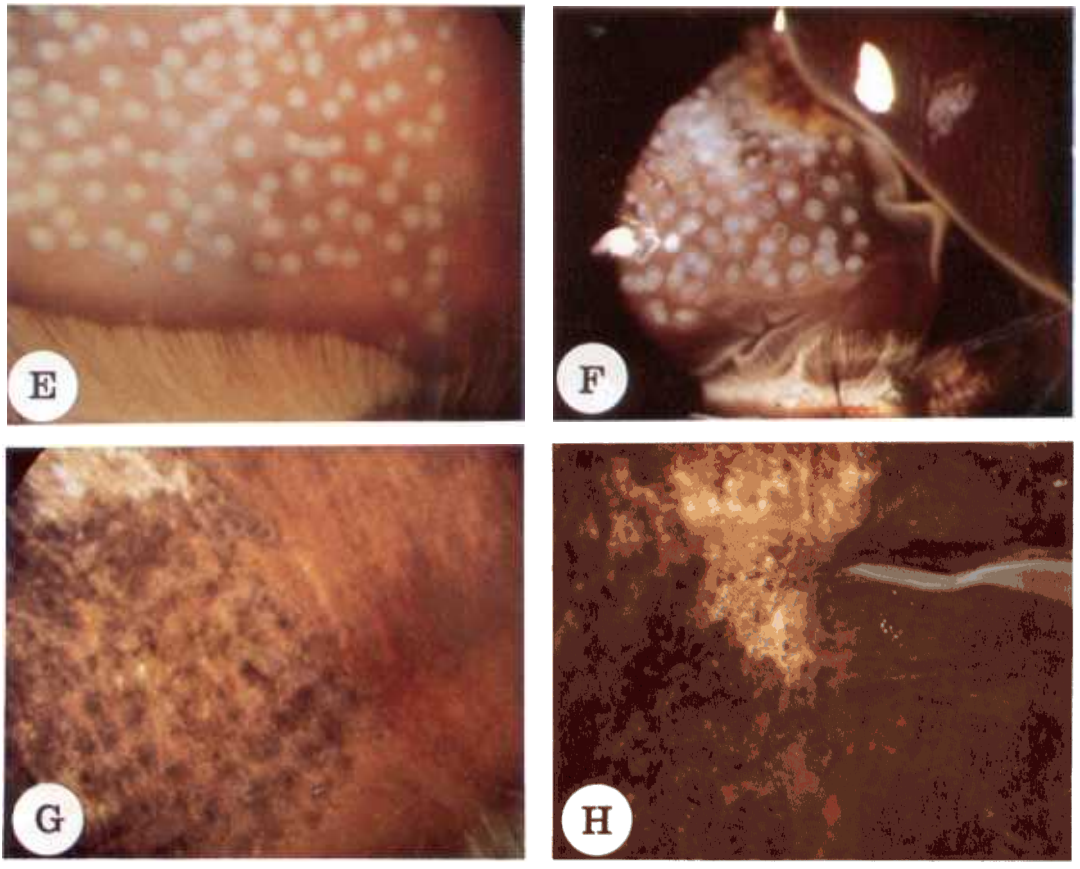

Fig. 2. Continued.

of the laser-treated region disappeared two days after laser photocoagulation (Fig. 2E). During vitrectomy but before killing the rabbit, the retina was still undetached anywhere. The retinal detachment developed in the untreated region eighteen minutes and fifty seconds postmortem, while the retina in the laser-treated region was not detached until 32 minutes after death when further examination was not possible due to the corneal haziness (Fig. 2F, Table 1).

Five, seven and fourteen days following laser photocoagulation

Dark pigmentation and cicatrical changes were found in the laser-treated regions after the fifth, seventh and fourteenth days of laser photocoagulation (Fig. 2G). During vitrectomy before killing the rabit, the retina was not detached anywhere. The retinal detachment developed in the untreated region in eighteen minutes after death. The laser-treated region did not show any evidence of retinal detachment until 40 minutes after death when corneal haziness occurred and further observation was not possible (Fig. 2H, Table 1).

DISCUSSION

Various physiologic and metabolic factors, such as hydrostatic, osmotic and metabolic factors including fluid transport, serve to keep the retina in place against the retinal pigment epithelium and help to move the detached retina back into attached position, where adhesive

properties of the interphotoreceptor matrix take an important role. However, the adhesive strength may not return immediately to normal in some pathologic conditions. Indeed, the recent experiments showed that adhesive strength was still subnormal one month after spontaneous reattachment of the retina (Yoon and Marmor 1988). When anatomic interdigitation remains abnormal, diathermy, cryotherapy or laser photocoagulation are used to induce scarring between the retina and retinal pigment epithelium. However, many studies indicate that several days are required before a strong chorioretinal adhesion is produced by any of these methods (Zauberman 1969; Kain 1985). Several histlogic studies provide some indirect evidence that laser photocoagulation may induce the chorioretinal adhesion earlier than other methods (Curtin *et al.* 1966; Yoon and Marmor 1988; Folk *et al.* 1980), although it is not clear when the more-than-normal adhesion appears after treatment.

There were several investigations to measure the chorioretinal adhesion after laser photocoagulation. However, several variants, such as the postmortem changes and the tissue damage from the vigorous manipulation of the eyeballs were ignored in all the studies where the animals were killed, the eyes were enucleated and the retina and choroid were dissected vigorously to assess the adhesion after laser photocoagulation. The results were various in accordance to the methods of the experiments. In 1969, Zauberman measured the tensile strength using 5 mm-wide strips of tissue made after enucleation of cats' eyes and said that it was much lower than normal until 2 days and slightly higher than normal, seven days following laser photocoagulation. In 1984, Kain produced retinal detachment by injecting Ringer's solution from a micropipette into the retinal pigment epithelial space and reported that the chorioretinal adhesion was reduced only within the first few days following photocoagulation, returned to normal by the third day and became enhanced at the fourth day. In 1988, Yoon and Marmor observed that the adhesive force measured by peeling the retina in 5 mm-wide strips of enucleated tissues was reduced 50% at 8 hours but increased beyond normal by 24 hours and remained twice normal between three days and four weeks following laser photocoagulation. They believed that the early reduced adhesion could be due to a combination of photoreceptor degeneration and damage to the RPE transport mechanisms, a delay to resynthesize matrix components and reestablish outer segment sheathing by the RPE microvilli. In 1989, Folk *et al.* claimed that both argon and krypton laser photocoagulation caused adhesion between the retina and the retinal pigment epithelium, but this postmortem adhesion in the untreated retina had been found to be very low in their histopathologic examination within 24 hours of treatment.

We were aware of the necessity of an accurate measurement of the chorioretinal adhesion and designed this study to assess the changes fo the adhesive force by laser photocoagulation. The medium laser reaction was obtained from about 200 exposures of an argon blue-green beam with a wave-length of 488~512 nm and a spot-diameter of 200 μm in the posterior fundus behind the equator. According to Dr. Kain's report (1984), medium and heavy intensities showing greater whitening and leading to pigmentation both subsequently provided a considerable increase in adhesion. In this study using vitrectomy and the aspiration system, tissue damage was avoided by minimal manipulation of the retina and choroid during surgery. A Flieringer's ring was fixed in the sclera in front of the equator to prevent the incidental collapse of the eyeball during procedures such as vitrectomy or aspiration. The balanced salt solution Plus used in our study was thought to cause the least damage to the ocular tissue during infusion (Burke *et al.* 1981; Moorhead *et al.* 1979). The instruments including a vitreous cutter and a light pipe were inserted through the sclera of the corresponding retina which has been treated with laser photocoagulation two weeks before vitrectomy. Core vitrectomy was done with height of infusion at 75 cm (55.2 mmHg) and aspiration pressure of 50 mmHg.

One hour following laser photocoagulation, the edematous laser-treated retina was detached from the choroid and elevated toward the opening of a vitreous cutter as vitrectomy progressed while the rabbit was alive. On the other hand, the normal untreated retina was not affected by

the aspiration used in this procedure. The breakdown of the blood-retinal barrier may have been a main mechanism of the phenomenon (Jaccoma *et al.* 1985; Kissen *et al.* 1961). The main histopathologic finding of this period was the formation of the proteinaceous coagulum between the edematous and damaged retina and choroid, which was composed of the debris of the photoreceptors, Bruch's membrane and the inner choroidal layer (Yoon and Marmor 1988; Powell *et al.* 1971). Powell *et al.* (1971) took fluorescein angiography after laser photocoagulation in rhesus monkeys and belived that acute pooling of fluorescein in the laser treated region occurred probably due to leakage of the dye from damaged choroidal vessels. Eventually, the speculated chorioretinal adhesion was much less than normal in the laser-treated region, one hour following laser photocoagulation.

One day following laser photocoagulation, no change was found during vitrectomy with aspiration pressure of 50 mmHg. The laser-treated retina was detached in four minutes postmortem with aspiration pressure of 25 mmHg, while the normal untreated region showed retinal detachment in 18 minutes postmortem. It means the chorioretinal adhesion was still less than normal in spite of a gradual return of adhesion until one day fllowing laser photocoagulation. This reulst conflicted with those of Yoon and Marmor (1988) or Flk *et al.* (1989). They emphasized the early increase of the chorioretinal adhesive force within 24 hours following laser photocoagulation, although many previous reports said the later increase of the force occurred within three or seven days after treatment. However, the chorioretinal adhesion was still less than normal in our observation, one day following laser photocoagulation.

Two days after laser photocoagulation, an interesting finding which had not be mentioned in other studies was observed. The laser-treated region was slightly grayish because dispersed pigments were easily visible due to a decrease of vacuolization and coagulation necrosis which was observed in histopatholgic studies at this period. We could see that the chorioretinal adhesion was maintained longer than normal in the laser-treated region of this period. During postmortem continuous apsiration of 25 mmHg, the untreated normal retina was detached at eighteen minutes while the laser-treated retina was not detached until thiry-two minutes. In histologic examination by Curtin *et al.* (1966), the retina showed marked necrosis of its inner layers, but the rod and cone nuclei still clinged to the pigment epithelium. According to Dr. Kissen *et al.* (1961), there were karyorrhexis of the nuclei, accumulation of fibrin beneath the retina around the burn, deposition of considerable amounts of celluar debris in the depth of the burn and many exudate-filled space. The retinal pigment epithelial layer began to demonstrate moderate pigment migration. And he said that although the fibrin deposits were still evident in retinal lesions three days postphotocoagulation, retinal elements had begun to settle down on the choroid. Anyway, our results are in discord with the early adhesion within 24 hours advocated by Yoon and Marmor (1988) or Folk *et al.* (1989) or the later increase of adhesion within three to seven days after laser photocoagulation. A few investigators found that adhesion was reduced during the first few days following laser photocoagulation and exceeded normal as scar tissue began to form (Yoon and Marmor 1988). And pigment clumping was considered as a sign of increase of the adhesion in some studies (Lyvyel 1963; Powell *et al.* 1971). However, formation of a protenaceous coagulum within the retina and choroid was a main histologic finding of the second day after laser photocoagulation, which was followed by cicatrical adhesion much later (Curtin *et al.* 1966; Yoon and Marmor 1988; Folk *et al.* 1989; Powell *et al.* 1971). Therefore, this strong adhesion may have been due to other unknown mechanisms, aside from scar formation. One possibility is a certain glue-like effect of the tissue condensation especially around the retinal pigment epithelial layer which is caused by direct laser burns, although it is not known how this condensation affects the chorioretinal adhesion.

In the laser-treated region five, seven and fourteen days following laser photocoagulation, dark pigmentation and cicatrical changes were noted. The laser-induced adhesion was too strong so that the applied negative pressure could not produce retinal detachment by the time the corneal opacity and ocular shrinkage

prevented further observation, while the untreated normal retina was detached easily eighteen minutes postmortem. Histologic studies showed healing of the burns; that is, fibroblastic proliferation, caused the late strong chorioretinal adhesion between the retina and the choroid and scattered clumps of pigment in the retina (Santos 1966; Kain 1984; Kissen *et al.* 1961; Lyvyel 1963; Powell *et al.* 1971). We could already see the more-than-normal adhesion at two days following laser photocoagulation when pigment clumping and scar formation were not obvious in histologic sections. Therefore, they are not an important clue of the increased chorioretinal adhesion in the acute stage following laser photocoagulation.

After laser photocoagulation of the intact retina, the adhesive force was reduced very low within one hour, but increased beyond normal by 2 days and remained more than normal until two weeks. These data suggest that laser photocoagulation may be acutely beneficial in the prevention or management of retinal tears and detachments; however, avoidance of vigorous excercises may be neccessary at least two days after laser photocoagulation until enhanced adhesion is obtained.

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