Experimental macular edema after lens extraction

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Macular edema has been observed frequently in man after cataract extraction, but pathogenic mechanisms remain unclear. Seven eyes of four young adult rhesus monkeys underwent lens extraction. The retinas and maculas of these eyes were examined by ophthalmoscopy, fundus photography, fluorescein angiography, light and electron microscopy, and the horseradish peroxidase tracer technique. In the macular region, the blood-retinal barrier at the retinal pigment epithelium was broken in each of the seven eyes, and the blood-retinal barrier at the retinal vasculature was disrupted in three of the seven eyes. All three eyes had had vitreous loss during lens extraction. Horseradish peroxidase was observed both intracellularly and extracellularly in the maculae. In contrast, the blood-retinal barrier at both the retinal pigment epithelium and the retinal vasculature of the peripheral retina in most eyes was intact. We conclude that macular edema secondary to lens extraction is due to disruption of the blood-retinal barrier at the levels of the retinal vasculature and the retinal pigment epithelium.

Key words: macular edema, lens extraction, blood-retinal barrier, retinal pigment epithelium, retinal blood vessels, horseradish peroxidase.

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Macular edema is a common complication after intraocular surgery. Forty to 60 percent of eyes after cataract operation were reported to develop macular edema.1-3 Macular edema has also been described after penetrating keratoplasty, retinal detachment, glaucoma filtering procedures, and pars plana vitrectomy.4-6 The ophthalmoscopic changes range from macular thickening with no clinically visible capillary leakage to severe vascular leakage that gives a petaloid appearance in the macular region by fluorescein angiography. In the majority of patients, the disease is self-limiting with spontaneous resolution.

The pathogenesis of this syndrome remains unclear. This is in part due to the fact that rarely does a patient with this condition require enucleation,7,8 and so pathologic material that is suitably fixed for detailed light and electron microscopic study remains rarely available. To investigate the pathogenetic mechanisms of this condition, we developed an experimental model in the rhesus monkey. In this report we describe the clinical findings and the light and electron microscopic changes in the macula of rhesus monkeys that had undergone lens extraction. The vascular permeability of the maculae was examined clinically by fluorescein angiography and morphologically by the horseradish peroxidase tracer technique.

Methods and materials

The lenses were extracted from seven eyes of four young, healthy, adult rhesus monkeys under general anesthesia with sodium pentobarbital. The fundus of each animal was examined by ophthalmoscopy, fundus photography, and fluorescein angiography before and after surgery and at least once just before enucleation. The pupil was dilated with 1 percent atropine before and after surgery. Lens extraction was performed with a cryoextractor after sector iridectomy and instillation of alpha-chymotrypsin into the anterior chamber. Three operative techniques were used to insure a graded degree of injury to the macula. One eye underwent an extracapsular lens extraction with vitreous loss; two eyes had intracapsular lens extraction with vitreous loss, and four eyes had uneventful intracapsular lens extraction.

The eyes were enucleated at 6 hours and 2, 3, 5, 10, 15, and 30 days after surgery. Thirty minutes before enucleation each animal received an intravenous injection of horseradish peroxidase at a dose level of 200 mg./kg. of body weight. The tissue was fixed promptly after enucleation and processed for the localization of the horseradish peroxidase.9 The tissue was postfixed in Dalton's chrome osmium solution, dehydrated in graded alcohols, and embedded in Epon. Sections (2 μ) were studied both before and after staining with toluidine blue. The electron microscopic sections were also studied unstained and after double staining with uranyl acetate and lead citrate.

Results

Clinical course. The postoperative clinical course was uneventful in all eyes. In all eyes the anterior chamber was formed and contained a few cells and flare, and the vitreous bulged anteriorly through the dilated pupil. Eyes that had vitreous loss showed some pupillary distortion and vitreous adherence to the wound. In these eyes, the vitreous was frequently mildly cloudy and stringy with scattered pigment granules and cells. No eye showed a vitreous traction band attached to the macula. The maculae were examined by slit lamp and direct and indirect ophthalmoscope, and all appeared to be mildly thickened. On fluorescein angiograms taken in all eyes immediately after surgery up to 30 days after operation, there was no evidence of leakage from the retinal or the choroidal circulation (Fig. 1), even in photographs taken 30 to 60 minutes after dye injection.

Morphologic study of retinal vasculature. In four of the seven eyes, morphologic examination of the retinal blood vessels by light and electron microscopy showed no abnormality, and no leakage of tracer substance from these vessels was observed. Three of the seven eyes, however, demonstrated extravasation of the tracer material from the retinal blood vessels at the macular region. All three eyes had had vitreous loss during surgery. The most extensive leakage of tracer appeared around the retinal venules in the nerve fiber layer.
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Fig. 1. Fluorescein angiograms of the fundus of a rhesus monkey 14 days after intracapsular lens extraction with vitreous loss. There is no evidence of leakage from retinal or choroidal circulation. Diffuse leakage of horseradish peroxidase from the retinal blood vessels (Figs. 3 and 4) and from the retinal pigment epithelium (Fig. 8) was observed in this eye. This eye was enucleated 24 hours after these angiograms.

Fig. 2. Unstained Epon-embedded section of the macula of a rhesus monkey 2 days after extracapsular lens extraction with vitreous loss. Horseradish peroxidase leaked extensively from the retinal vessels (V). Most of the extravasated tracer (arrows) was noted in the interstitial space on the posterior aspect of the retinal blood vessels facing Bruch's membrane. The tracer also leaked through the retinal pigment epithelium, extending along the subretinal space. (×165.)

(Fig. 2). The tracer material was more abundant in the extravascular interstitial space on the side of the blood vessel, away from the internal limiting membrane. The extravasated tracer rarely extended beyond the middle limiting membrane. Fluorescein angiography of these eyes before sacrifice failed to show evidence of fluorescein leakage, even though there was extensive leakage of horseradish peroxidase noted by light and electron microscopy.

By electron microscopy the leakage of tracer was observed in three forms. (1) Some blood vessels showed an increased...
Fig. 3. Unstained electron microscopic section of a retinal capillary in the macular region of an eye, 15 days after lens extraction. Fluorescein angiogram (Fig. 1) taken 24 hours after operation failed to show evidence of vascular leakage. A large number of pinocytic vesicles (black single arrows) carried tracer material in the endothelial cells. Both the basement membrane of the capillary (double arrows) and the extravascular interstitial space (black and white arrows) were heavily permeated by tracer. RBC, Red blood cell; L, lumen. (x18,000.)

Fig. 4. Unstained electron microscopic section of a retinal capillary in the macular region of an eye 15 days after lens extraction. The tracer material diffusely permeated the cytoplasm of an endothelial cell (E1), the basement membrane of the capillary (double arrows), and the extravascular interstitial space (white arrow). In contrast, the tracer material was limited to pinocytic vesicles (black single arrows) in the cytoplasm of endothelial cell (E2). L, Lumen. (x13,500.)

number of pinocytic vesicles in the cytoplasm of the endothelial cells (Fig. 3). The basement membrane of these vessels was infiltrated with tracer material. (2) Other blood vessels showed diffuse staining with horseradish peroxidase in the cytoplasm of the endothelial cells (Fig. 4). The basement membrane and interstitial space around these vessels were also permeated with tracer material. (3) More uncommonly, some endothelial cells manifested disrupted cell junctions so that tracer material was observed continuously from the lumen of the blood vessels to the extravascular interstitial space (Fig. 5).

Morphologic study of retinal pigment epithelium. Disruption of blood-retinal barrier at the retinal pigment epithelium...
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Fig. 6. Top, Unstained Epon-embedded section of the retinal pigment epithelium in the macular region 2 days after lens extraction. The widened intercellular space (arrows) between retinal pigment epithelial cells was permeated by tracer material. (x440.) Bottom, Numerous lateral infoldings (large arrow) permeated by horseradish peroxidase were present between adjacent retinal pigment epithelial cells. No tracer was noted anterior to the cell junction (double arrows). BM, Bruch’s membrane; N, nucleus of retinal pigment epithelial cell. (x9,000.)

was observed in the macula of all seven eyes. The disruption of the barrier took one of two forms—intercellular or intracellular leakage.

In some retinal pigment epithelial cells, the lateral plasma membrane developed many infoldings. Between these infoldings, the extracellular space was much widened and filled with tracer material (Fig. 6). The cell junctions between adjacent pigment epithelial cells were frequently competent, and the tracer material did not reach the subretinal space. In other areas, the cell junctions between adjacent pigment epithelial cells were disrupted and tracer material could be seen extending from Bruch’s membrane between adjacent pigment epithelial cells into the subretinal space, and surrounding the outer segments of photoreceptor cells (Fig. 7).

In other eyes, the plasma membrane of the retinal pigment epithelium appeared decompensated, and the tracer material was visible within the cytoplasm of pigment epithelial cells (Fig. 8). Frequently, the nuclear membrane remained intact, and the tracer material did not penetrate into the nuclei of these pigment epithelial cells. In focal area the tracer material was also observed in the subretinal space adjacent to these cells (Fig. 9).

After the tracer material reached the subretinal space, it spread either extracellularly or intracellularly. Extracellularly,
Fig. 8. Top, Horseradish peroxidase permeated the cytoplasm of three retinal pigment epithelial cells (arrows). No tracer material was present in the subretinal space. (Unstained, Epon-embedded section; x350.) Bottom, Tracer material diffusely permeated the cytoplasm of endothelial cell E1. The cytoplasm of endothelial cell E2 was free of tracer material. The cell junction (double arrows) remained competent, and no tracer material was present anterior to the cell junction. N, Nucleus; BM, Bruch’s membrane. (x7,200.)

the tracer material extended between adjacent outer segments and inner segments. It penetrated through the external limiting membrane and spread in the extracellular space in the outer nuclear layer and the outer plexiform layers (Figs. 10 and 11). The tracer did not extend beyond the middle limiting membrane.

In some eyes, the tracer also spread by the intracellular route. Photoreceptor ele-
ments, particularly the cone cells, were permeated by the tracer material from the subretinal space, and the tracer passed proximally through the inner segment to the perikaryon and extended along the cone pedicles (Figs. 12 and 13). A series of prominent cone pedicles heavily stained with tracer material could be seen in the outer plexiform layer under these circumstances (Figs. 12 and 14). We observed no transsynaptic passage of the tracer material.

At the foveas of some eyes, the retina was shallowly detached (Fig. 15), and tracer material was not observed in the pigment epithelium at the area where the retina had detached. Nevertheless, in the subretinal space, immediately abutting the detached retina, the tracer material was observed surrounding the outer segments of the photoreceptor cells. Some of the photoreceptor cells were permeated by the tracer material and were stained in the inner segments, the perikaryon, and the Henle's fiber layer. Cystoid accumulation of fluid in the Henle's layer was not seen in any of the maculae studied.

Sections were prepared from the periphery of each eye (Fig. 16) to compare with the macula. Retina in the periphery rarely showed leakage of tracer from either the pigment epithelium or the retinal vasculature even though diffuse leakage was observed in the macula of the same eye.

Discussion

This experiment demonstrated the vulnerability of the blood-retinal barrier at the macular region after the surgical procedure of lens extraction. Whether the operation was extracapsular lens extraction with vitreous loss, intracapsular lens extraction with vitreous loss, or intracapsular lens extraction without vitreous loss, the blood-retinal barrier was disrupted in every eye. This observation agreed with clinical reports that a high percentage
of patients (40 to 60 percent) who underwent cataract extraction developed macular edema. An even higher percentage of patients probably had disruption of the blood-retinal barrier at the macula after cataract extraction but edema was so mild that the condition was not observed clinically.

While all the eyes in this experiment developed disruption of blood-retinal barrier at the level of the retinal pigment epithelium, only three showed disruption of the barrier at the level of the retinal vasculature. This seemed to suggest that the blood-retinal barrier at the retinal pigment epithelium was more vulnerable than that at the retinal vasculature. Our findings agreed with those of Laties and Rapoport, who showed that, under osmotic stress, the disruption of the blood-retinal barrier at the retinal pigment epithelium appeared widespread while the barrier of the retinal vessels remained intact.

Disruption of the blood-retinal barrier in the three eyes was associated with vitreous loss. Vitreous loss apparently aggravated the disruption of the blood-retinal barrier to an extent that it involved not only the retinal pigment epithelium but also the retinal blood vessels. These
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Fig. 15. Fovea from an eye that had intracapsular lens extraction with vitreous loss 10 days before enucleation. Ophthalmoscopic examination of this fovea before enucleation only showed mild thickening. Histologically, the fovea was shallowly (about 100 μ) detached. Horseradish peroxidase densely infiltrated Bruch's membrane and the cytoplasm of the retinal pigment epithelial cells (arrow) immediately adjacent to the fovea detachment. At the area of detachment, the tracer was absent from Bruch's membrane and the retinal pigment epithelium but was observed in the subretinal space, surrounding the photoreceptor elements. The tracer material extended intracellularly and extracellularly to the outer nuclear and outer plexiform layer. (Toluidine blue, Epon-embedded section; ×94.)

findings agreed with clinical observations that patients with vitreous loss are more prone to macular edema after operation.

In this experiment we compared the blood-retinal barrier at the periphery of the retina with that at the macula. The disruption of the barrier was much more extensive in the macular region; only minimal disturbance was observed in the periphery of the fundus. The blood-retinal barrier at both the retinal pigment epithelium and the retinal vasculature of the peripheral retina was rarely disrupted. The reason for the vulnerability of the blood-retinal barrier at the macula was not known, but it was possible that the short posterior ciliary arteries, which supply the macula, branch abruptly into the choriocapillaris, resulting in high pressure, rapid blood flow, and, consequently, increased vulnerability of the blood-retinal barrier.

We performed trabeculectomy on rhesus monkeys and observed similar disruption of blood-retinal barrier at the retinal vasculature and at the retinal pigment epithelium. These findings supported the concept that the occurrence of macular edema after intraocular surgery is not unique to lens extraction, but rather a more general phenomenon that occurs after various forms of intraocular surgery.

The retinal blood vessels which were associated with disruption of the blood-retinal barrier appeared to undergo three types of alteration: (1) increasing pinocytotic vesicles in the cytoplasm, (2) decompensation of the plasma membrane

Fig. 16. Peripheral retina of the same eye as Fig. 2. Note the absence of leakage of tracer from the retinal vessels. Only one pigment epithelial cell (arrow) showed mild infiltration of tracer material into its cytoplasm. (Unstained, Epon-embedded section; ×165.)
with infiltration of tracer material into the cytoplasm and subsequently into the extracellular space, and (3) disruption of cell junctions and leakage of tracer material into the extracellular space. Deciding which was the predominant mechanism in this pathologic process was difficult, however. All three pathologic changes may have contributed to the eventual disruption of the barrier. These alterations appeared to be different from those observed in dogs with diabetic retinopathy and disruption of the blood-retinal barrier. In the latter condition, the major pathogenetic mechanism was reported to be incompetence of cell junctions of the endothelial cells, through which tracer leaked into the extracellular space.

The disruption of the blood-retinal barrier at the retinal pigment epithelium took one of two forms: (1) passage between cells and (2) passage through cells. Disruption of the cell junction between cells was not frequently observed. More commonly, disruption of the barrier was observed in the area where the retinal pigment epithelium was decompensated and tracer material passed into the cytoplasm of the pigment epithelial cells and subsequently into the subretinal space. We believe that the permeation of the cytoplasm of the retinal pigment epithelium by tracer was not an artifact of preparation because this was not observed in our control animals. Furthermore, this alteration was associated with infiltration of the tracer into the subretinal space and retina. Although the plasmalemma of the retinal pigment epithelium was decompensated, the nuclear membrane invariably remained competent and the tracer material was not seen within the nuclei. This seemed to suggest that these cells, although decompensated, were not necrotic and were capable of reparative process.

In this experiment, despite extensive disruption of the blood-retinal barrier and decompensation of the retinal pigment epithelium, these cells showed no evidence of proliferative reaction. In contrast, in a previous study of mild photocoagulation lesions, acute necrosis of the pigment epithelium was followed with reparative proliferation within 6 days after photocoagulation and Bruch's membrane was quickly relined with epithelial cells. Acute necrosis of the pigment epithelium appeared to excite rapid reparative proliferation and subsequent reformation of the blood-retinal barrier. This reparative reaction provides a rationale for the treatment of central serous choroidopathy by mild photocoagulation which inflicts acute necrosis of the decompensating retinal pigment epithelium in this disease. A proliferative reaction of these cells is induced to seal the disruption of the blood-retinal barrier.

The spread of the tracer material from the subretinal space followed extracellular as well as intracellular routes. Some tracer material passed along the intercellular space between the photoreceptor cells through the external limiting membrane into the outer plexiform layer. As anticipated, the zonula adherens of the outer limiting membrane did not form a barrier to the passage of the tracer material. Furthermore, some of the tracer infiltrated the inner and outer segment of the photoreceptor cells, passed through the perikaryon of the cell, and extended to the cone pedicles. The cone cells appeared to be selectively involved. Whether this intracellular permeation of tracer was a passive or an active process could not be determined in this experiment. Nevertheless, this experiment demonstrated an extracellular and an intracellular route of passage of proteinaceous material from the subretinal space to the outer plexiform layer. In some retinal diseases, such as retinal degeneration over malignant melanoma or long-standing retinal detachment, proteinaceous fluid accumulates in the outer plexiform layer of the retina to form cystoid degeneration. These routes of intercellular and intracellular permeation through the outer nuclear layer may facilitate accumulation of material in the...
outer plexiform layer and cyst formation. One of the typical pathologic changes in Irvine-Gass-Norton syndrome consists of fluid accumulation in the outer plexiform layer and appears to parallel findings in our experimental model.

The disruption of the blood-retinal barrier, so obvious on light and electron microscopy by the horseradish peroxidase tracer technique, was not seen by fluorescein angiography. Horseradish peroxidase may be a more sensitive detector of blood-retinal barrier disruption than sodium fluorescein. The molecular weight of sodium fluorescein is 376; that of horseradish peroxidase is 40,000. Although a considerable concentration of sodium fluorescein is necessary before the dye can be visualized by the fundus camera, the reaction product after the histochemical reaction for the detection of the horseradish peroxidase is so large that minimal leakage is detectable. Cunha-Vaz and associates, using vitreous fluorophotometry, detected leakage of sodium fluorescein from the retinal vasculature in patients with diabetic retinopathy even though the fluorescein angiograms were reported to be normal. The leakage from the retinal vasculature and the retinal pigment epithelium in this experiment might be demonstrable by vitreous fluorophotometry with sodium fluorescein as the tracer.

The typical fluorescein angiographic characteristic of macular edema after lens extraction is leakage of fluorescein from the perifoveal capillaries. Most investigators did not observe leakage at the retinal pigment epithelium. In our experimental model, however, leakage at the retinal pigment epithelium was more frequent and more prominent than leakage at the retinal vasculature. These alterations were demonstrated only by the horseradish peroxidase tracer technique and not by fluorescein angiography. Baltazis, Theodossiadis, and Velissaropoulos, however, observed leakage from the choriocapillaris in some patients with the Irvine-Gass-Norton syndrome.

None of the animals in our series developed typical cystoid macular changes, either clinically or pathologically. Since all of our animals were young and healthy, the pigment epithelial cells and retinal vasculature may have recovered and no cystoid macular changes resulted. Nevertheless, in patients who have generalized vascular diseases or other metabolic disorders, their capability for regeneration may not be adequate, and these are the patients who develop prolonged macular edema. Some of these patients eventually recover, and the disease in most patients is self limiting. In other patients the macular edema progresses to cystoid degeneration. So the primary defect in cystoid macular edema after cataract extraction may be in the failure of recovery of the vulnerable blood-barrier at the macula, which is probably disrupted, as demonstrated in our experiment, after every cataract extraction.

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Aldose reductase in retinal mural cells

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Cultured mural cells (intramural pericytes) from adult rhesus retinal capillaries were ex-
amined for the presence of the sorbitol pathway. A radioimmunoassay for human aldose
reductase, cross-reactive with rhesus lens aldose reductase, showed the presence of this en-
zyme in our cultured cells. Mural cells grown in culture media containing normal (10 mM)
and high (40 mM) levels of glucose were examined for polyol accumulation by gas-liquid
chromatography. Cells incubated in high glucose medium showed a threefold increase in
sorbitol concentration over cells grown at low glucose levels. After 30 days in high glucose
medium, mural cells formed dense multilayered areas with extensive cellular debris. These
findings suggest the presence of the sorbitol pathway in cultured retinal mural cells and
cellular degeneration in high glucose medium; this may have possible implications in the
pathogenesis of diabetic retinopathy.

Key words: mural cells, intramural pericytes, retina, tissues culture, aldose reductase,
sorbitol pathway, diabetic retinopathy.

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