EPIRETINAL MEMBRANE FORMATION AFTER VITRECTOMY

WALTER H. STERN, M.D., STEVEN K. FISHER, PH.D.,
DON H. ANDERSON, PH.D., JAMES J. O'DONNELL, M.D.,
PAGE A. ERICKSON, B.A., GEOFFREY P. LEWIS, B.A.,
CHRISTOPHER J. GUERIN, B.S., GERARD A. BORGULA, B.A.,
AND MAEVE A. MCDERMOTT, F.R.C.S.

San Francisco, California

Our experimental model of epiretinal membrane formation in the rabbit eye after lensectomy and vitrectomy provides a way of studying pharmacologic and surgical approaches to inhibiting epiretinal cellular proliferation and contraction in the eye that has undergone vitrectomy.

We injected 400,000 tissue-cultured retinal pigment epithelial cells onto the retinal surface of rabbit eyes that had undergone lensectomy, vitrectomy, and fluid-gas exchange. By one week, a funnel-shaped detachment of the medullary rays had occurred in 100% of the injected eyes.

Histologically, the cells formed an epiretinal membrane by six hours after injection and caused major wrinkling of the inner retina after 24 to 48 hours. The percentage of tritiated-thymidine-labeled epiretinal cells increased dramatically 24 hours after injection and then declined. Cellular membranes bridging the optic nerve, followed by growth and contraction of the epiretinal cells on the detached internal limiting membrane, were responsible for the closed funnel appearance of the medullary rays.

Epiretinal membrane formation and membrane contraction are major complications in a variety of ocular conditions, including retinal detachment and penetrating ocular injuries. Various studies have shown that these contractile membranes are composed of many cell types and a collagenous matrix that interact with the surface of the retina.[1-5] Several animal models in which cells are injected into the intact vitreous cavity of the rabbit eye have been used to develop techniques for inhibiting intraocular cellular proliferation.[6-8]

In order to develop an experimental model that emphasized epiretinal membrane formation and membrane contraction rather than intravitreal membrane formation, we injected aphakic rabbit eyes that had undergone vitrectomy with tissue-cultured retinal pigment epithelial cells. This modification resulted in a clinical and histologic sequence of intraocular proliferation that differs substantially from that of other models. Further, it

enables us to study the pharmacologic inhibition of epiretinal membrane growth and contraction—the major obstacles to long-term retinal reattachment—in eyes that have undergone vitrectomy.

MATERIAL AND METHODS

Animals—Initially we used the B-J strain of inbred rabbits (co-efficient of inbreeding, 0.98) weighing 2.5 to 3.5 kg each because skin allografts between these animals are accepted without rejection. We wanted to obtain tissue-cultured retinal pigment epithelial cells that could be grown in large quantities and injected into any rabbit of the B-J species without fear of immune rejection. Our studies later disclosed that the injection of tissue-cultured B-J retinal pigment epithelial cells into New Zealand albino rabbits, 2.5 to 3.5 kg each in weight, produced a similar clinical and histologic result. The data for this report were derived from 57 eyes obtained from male and female rabbits, including eight eyes from the B-J strain.

Retinal pigment epithelial tissue culture—We killed albino rabbits of the B-J strain with intravenously administered sodium pentobarbital (50 mg/ml) and enucleated the eyes. We removed the anterior segments and gently detached the retina from the underlying retinal pigment epithelium. We isolated the retinal pigment epithelium from Bruch's membrane by pronase digestion and subsequent aspiration with a small Pasteur pipette. We grew retinal pigment epithelium as a monolayer cell culture in minium essential medium with 10% fetal calf serum in a 37 C, 5% carbon dioxide incubator. We passed the cultures serially and expanded the colony in additional flasks. In this experiment we used cells from passage 2 to passage 6, but most cells came from passage 5.

We obtained cells from confluent cell flasks by pronase digestion followed by washing of the cells three times with phosphate-buffered saline. We suspended the cells in phosphate-buffered saline and counted the cells in a hemocytometer. We adjusted the volume of injected cells for each experiment, which ranged from 0.1 to 0.25 ml, in order to inject the same number of cells in each experiment. Injection of 400,000 cells was found to be the minimum number of cells which produced a consistent clinical and histologic sequence of events. The data presented in this paper were obtained from eyes injected with 400,000 cells. A trypan blue test indicated that 98% of the cells were viable just before injection (measured by exclusion of dye from the cell).

Surgical technique—We sedated the rabbits with intramuscularly administered ketamine HCl (20 mg/kg of body weight) and xylazine HCl (2 mg/kg of body weight) and 0.3 ml of intramuscularly administered atropine to reduce bronchial secretions. We inserted an intravenous line and maintained anesthesia with sodium pentobarbitol (25 mg/ml) administered as needed. We also administered a retrobulbar injection of 0.5 ml of 2% lidocaine HCl. We dilated the pupil of the eye that was to be operated on with 10% phenylephrine, 1% cyclopentolate, and 1% atropine.

After the animal was anesthetized, and before we opened the eye, we injected 0.1 ml of 1:1000 adrenalin chloride into the anterior chamber to maintain pupillary dilation and induce vasoconstriction of the iris vessels, thus reducing fibrin formation in the anterior chamber. We inserted a 20-gauge pars plana cannula attached to a solution of lactated Ringer's solution through the pars plana on the temporal side of the superior rectus muscle 2 mm posterior to the corneoscleral limbus and secured the infusion cannula with a 5-0 Dacron mattress suture.

We inserted a microvitreoretinal blade through the pars plana 2 mm posterior to

the corneoscleral limbus on the side of the superior rectus muscle opposite to the infusion cannula, and directed the knife into the center of the lens. We inserted a 20-gauge Girard fragmentor into the lens and removed the lens by fragmentation and aspiration.

When most of the lens cortex had been removed, we withdrew the fragmentor and inserted a 20-gauge suction cutter (Ocutome) into the sclerotomy site to remove the remaining cortical material and anterior capsule.

We then injected 0.1 ml of 1% sodium fluorescein into the infusion line and stained the vitreous body yellow to enhance visualization of the vitreous. We placed an 8-mm self-adhering contact lens on the cornea and, using the microscope coaxial light, removed the vitreous with the suction cutter. We used gentle scleral depression over the medullary ray to bring this area into view and to bring the posterior pole towards the side opening of the suction cutter posteriorly. Throughout the procedure the stained vitreous was visible and its attachment to the medullary ray observed by the synchronous pulsing of the medullary rays when the suction-cutter port was opened.

In the initial series of experiments, we set the suction at 110 mm Hg. In the last series of ten rabbits we decreased the suction to 0 mm Hg and excised the vitreous with an infusion pressure of 30 mm Hg to obtain tissue engagement in the suction-cutter port. This procedure minimized the microscopic disruption of the photoreceptor retinal pigment epithelial interface that resulted from vigorous suction on the vitreous body which was firmly anchored to the medullary rays and retina.

When the entire vitreous body had been excised, we closed the suction-cutter sclerotomy site with an interrupted 9-0 nylon suture and closed the pars plana infusion site with the original mattress suture used to secure the infusion line. We injected 40 mg of gentamicin sulfate and 1 mg of dexamethasone phosphate subconjunctivally and placed an atropine ointment and an ointment containing polymyxin B sulfate, bacitracin zinc, neomycin sulfate, and hydrocortisone (Cortisporin) on the eye.

It was extremely important to maintain the dilation of the pupil with atropine ointment 1%, administered daily, so that the dilation was not lost during the subsequent surgical procedures.

Two weeks after the lens extraction and vitrectomy, we examined the fundi by indirect ophthalmoscopy and if we found no retinal detachment or other abnormality we injected the eyes with the cultured cells.

Injection of retinal pigment epithelial cells—Immediately after obtaining retinal pigment epithelial cells from the tissue culture flasks, we sedated the animals in the same way as for the previous procedure. We then performed a fluid-gas exchange with a 50% sulfur hexafluoride-air mixture. We inserted a beveled 20-gauge needle attached to a fluted needle holder through the clear cornea and inserted a 30-gauge disposable needle attached to the syringe containing the air-sulfur hexafluoride gas mixture through the clear cornea 180 degrees away from the first needle. The fluid-gas exchange was performed under the microscope with a Harvard stepping motor syringe drive pump under foot control to drive the syringe until the eye was free of intraocular fluid. We then withdrew the 20-gauge fluted needle and slowly injected 0.1 to 0.25 ml of phosphate-buffered saline containing the cultured retinal pigment epithelial cells through a 20-gauge needle onto the retinal surface just below the optic nerve. We maintained a normal or slightly increased intraocular pressure with a 50% sulfur hexafluoride-air mixture injected through the 30-gauge needle. We kept

the animals sedated and on their side for two hours after the cells were placed on the retina.

Seven of the eyes underwent fluid-gas exchange and injection with 0.2 ml of balanced salt solution to control for any effects caused by the intraocular injection rather than by the cells.

Clinical evaluation—The eyes were examined daily with a penlight and binocular indirect ophthalmoscopy. On some occasions a slit-lamp examination was performed as well. In selected animals, we documented the progression of the disease by color photography.

Histologic examination—We enucleated eyes two, four, six, 12, 18, and 24 hours after cell injection, daily from day 2 through day 15, and on day 35. We examined several eyes each time.

Two hours before killing the animals, we injected 100 μCi of tritiated thymidine into the vitreous cavity of the gas-filled or fluid-filled eye. At the end of the incubation period we enucleated the eyes, removed the corneas, and placed the eyes in a phosphate-buffered, 1% glutaraldehyde, 1% paraformaldehyde mixture (pH 7.1) overnight. The next morning, the specimens were rinsed in several changes of phosphate buffer for one hour. With a sharp razor blade we removed the remaining anterior segments just posterior to the iris and cut the specimen in half in a plane parallel to the medullary ray and just below the optic nerve. This provided a cross-sectional view of the eye and permitted us to observe the extent of detachment of the medullary rays and retina. We then photographed the specimens.

Next, the tissue was postfixed in 2% osmium tetroxide, washed in distilled water, dehydrated in a graded ethanol series, placed in propylene oxide, and then embedded in Araldite 6005. The tissue sections, about 60 nm thick, were stained sequentially with uranyl acetate and lead citrate and studied by electron microscopy.

Light microscopic autoradiograms were prepared in the following manner: We dipped 1-μm sections in a 1:1 solution of NTB-2; they were kept at 43 C in water, exposed in light-tight boxes for about one week (at 6 C), and developed in full-strength D-19 developer for two minutes (at 19 C). Autoradiograms were stained with a methylene blue-azure II mixture and counterstained with basic fuchsin according to the protocol of Humphrey and Pitman.[9] This staining technique allows differentiation of cellular and extracellular components. For example, collagen stains pink to red and elastin stains violet.

The percentage of tritiated thymidine-labeled retinal pigment epithelial nuclei was determined at two and six hours, one and two days, and one and two weeks after cell injection by comparing the numbers of labeled and unlabeled epiretinal cells resembling myofibroblasts. The number of cells counted in each eye ranged from 480 to 2,800. These counts were made from at least four nonoverlapping sections of the preretinal cells.

RESULTS

Control eyes—After lensectomy and vitrectomy, we could see a small amount of yellow-stained vitreous adherent to the medullary rays. The operated on eyes were minimally inflamed, had clear corneas, minimal anterior chamber flare and cells, and a clear vitreous cavity. In 50% of the eyes, one or both of the medullary rays appeared to lose their feathery appearance and to become much thinner and rolled. Approximately 2% of the operated eyes developed rhegmatogenous retinal detachments. These eyes were discarded.

Although some areas appeared normal,

there were areas of disrupted or sheared photoreceptor outer segments in all eyes. Some outer segments were absent or shorter than normal, and they often lacked proper orientation. Additionally, there were areas of shallow separation at the photoreceptor-retinal pigment epithelial interface, and mounding of the apical retinal pigment epithelial surfaces. These areas of disruption were located in the posterior retina underlying the medullary rays as well as in the peripheral retina. Decreasing the suction applied during the vitrectomy procedure decreased, but did not eliminate, the damage.

In the rabbit, retinal blood vessels are found on the vitreal surface of the retina along the medullary rays. These blood vessels are surrounded by a multilayered basal lamina produced by the capillary endothelial cells. Where blood vessels emerge onto the retinal surface, basal lamina surrounding the blood vessels merges with the internal limiting lamina of the retina. This contiguous basal lamina "isolates" the retinal blood vessels from the vitreous cavity. The basal lamina also plays a major role in the traction retinal detachments observed in this study.

We found four distinct types of extracellular material remaining adjacent to the retinal blood vessels: (1) filaments 17 to 20 nm in diameter with a banding periodicity of the same dimensions; (2) filaments 14 nm in diameter with a banding periodicity of 22 nm that sometimes aggregated into bundles of varying diameter; (3) filaments 10 nm in diameter with short, intermittent perpendicular projections from the central filament; and (4) aggregates of amorphous, floccular matrix. These four types of extracellular material were also found in the eyes injected with retinal pigment epithelial cells.

A few polymorphonuclear neutrophils and immature tissue macrophages were found within the vitreous cavity near the retinal blood vessels.

Cell-injected eyes—CLINICAL FINDINGS—Immediately after the injection of 400,000 cells into the gas-filled eye and for the next two days, there was no change in the appearance of the fundus and we found no signs of the injected cells. By the third day, however, we observed a clump of white material inferior to the optic nerve with thin white strands extending along the vascular medullary rays.

By the fourth and fifth days, the medullary rays were moderately elevated. In a few cases the elevation was asymmetric. By the sixth and seventh days the medullary rays had usually assumed a closed funnel appearance and appeared adherent to the underlying iris. During the next week the funnel appeared to close even further and a pupillary membrane that had sometimes obscured portions of the pupil by the end of the first week had often contracted considerably, allowing a better view of the fundus by the end of the second week.

It was difficult to observe retinal detachment ophthalmoscopically except for medullary ray detachment, although we could demonstrate the retinal detachment after one week by hemisecting the globe after enucleation.

With cell concentrations of less than 400,000 cells, the ophthalmoscopic findings were more variable. With 100,000 injected cells, we sometimes saw strands extending from a clump of cells below the optic disk toward the medullary rays. Variable elevation, usually of one medullary ray, occurred. With 200,000 cells the clinical time course of medullary ray elevation appeared similar during the first week to that with 400,000 cells, but the tight closed funnel appear-

Fig. 1 (Stern and associates). At 48 hours after cell injection, there is slight wrinkling of the retinal vessels of one of the medullary rays (arrow).

Fig. 2 (Stern and associates). Four days after cell injection, the medullary rays are moderately elevated.

ance was not observed until three weeks after cell injection.

GROSS ANATOMY—Examination of the hemisected specimens provided a clear view of the elevation of the medullary rays and retina. For the first two days, the vitreous cavity, medullary rays, and retina appeared to be normal. After 48 hours, in some eyes, we could see wrinkling and a slight elevation of the medullary rays (Fig. 1). During the next five days the medullary rays became progressively elevated until they were vertical by the seventh day (Figs. 2 to 4). In some cases, the elevation was asymmetric, but at least one of the medullary rays was vertically elevated by the seventh day in 100% of the eyes and both medullary rays were vertically elevated in 95%. As the medul-

lary rays became progressively elevated, they appeared to fuse with the iris and ciliary body (Fig. 5).

During the first week, the retina appeared to be flat except for the areas underlying the medullary rays. During the second week, however, the retina appeared to be elevated and tears in the retina were visible.

HISTOLOGY AND ULTRASTRUCTURE— After the retinal pigment epithelial cells were injected into the posterior vitreous cavity they dispersed along the internal limiting membrane of the retina. Two
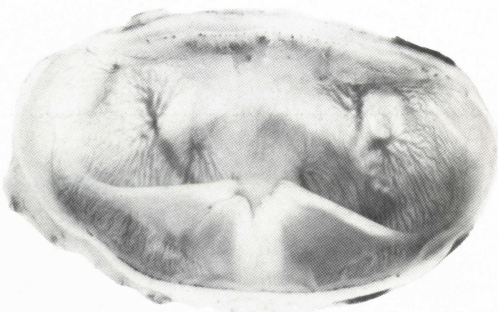

Fig. 3 (Stern and associates). Five days after cell injection, a funnel-shaped detachment of the medullary rays bordering the optic nerve has developed.
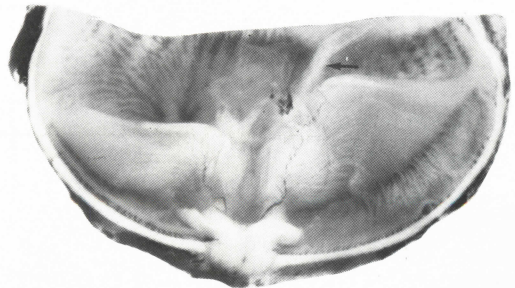

Fig. 4 (Stern and associates). Seven days after cell injection, the funnel-shaped detachment of the medullary rays has extended vertically towards the cornea. The detached internal limiting membrane extends from one of the medullary rays toward the pars plana (arrow).
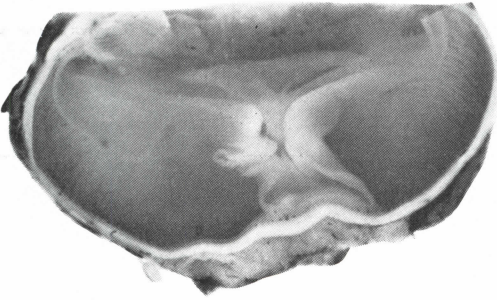
Fig. 5 (Stern and associates). Eleven days after cell injection, a funnel-shaped detachment of the medullary rays extends from beneath the iris to the optic nerve.

hours after injection, they had spread over the surface of the medullary rays and the adjacent posterior retina (Fig. 6). At this point these cells and their nuclei were oval, and some nuclei were indented. Microfilaments were uncommon. Rough endoplasmic reticulum was abundant and appeared as long or distended and irregular cisternae. The Golgi complexes were numerous. Many vesicles were adjacent to or fused with the plasma membrane. There was no basal lamina associated with the injected retinal pigment epithelial cells. Primary and secondary lysosomes were common in these cells. A few necrotic cells and cell debris were found. Polymorphonuclear neutrophils and immature tissue macrophages

were located adjacent to the retinal blood vessels. Fibrin fibrils with a banding periodicity of 23 nm were usually found scattered along the vitreal aspect of the injected retinal pigment epithelial cells, and sometimes appeared in phagosomes within the retinal pigment epithelial cells. Some extracellular fibrin was present in all of the eyes injected with retinal pigment epithelial cells.

By six hours after injection, retinal pigment epithelial cells had congregated near the retinal blood vessels and also formed a multilayered population along the detached internal limiting membrane that bridged the optic nerve. The retina was elevated on either side of the optic nerve (Fig. 7). These epiretinal cells and their nuclei were oval or spindle-shaped and some nuclei were indented (Fig. 8). Microfilament bundles with densifications were more common. The rough endoplasmic reticulum and Golgi complexes appeared to be the same as at two hours. Many vesicles were adjacent to or fused with the plasma membrane, and an interrupted basal lamina was associated with some of the cells. As before, primary and secondary lysosomes were common.

Beginning six hours after injection and continuing throughout these experiments, the retinal pigment epithelial
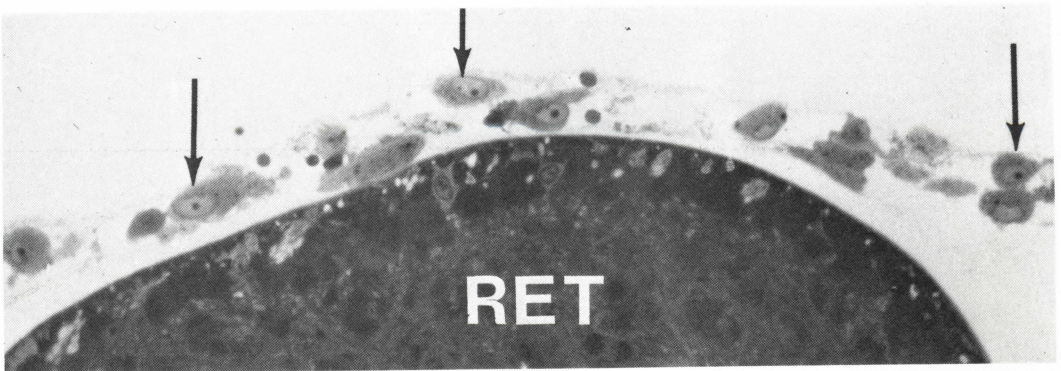

Fig. 6 (Stern and associates). The retinal pigment epithelial cells two hours after injection. The cells (arrows) are dispersed along the internal limiting membrane of the posterior retina (RET). The cells and their nuclei are oval (×380).
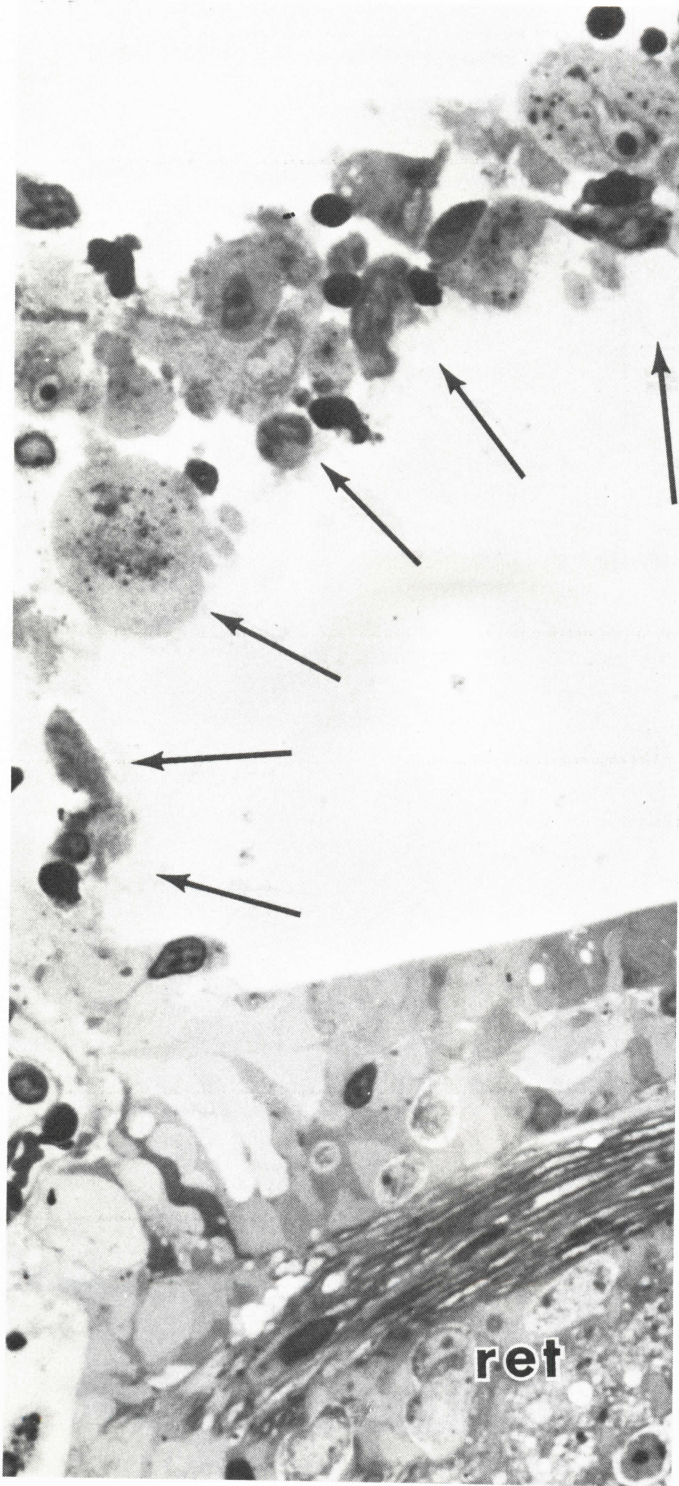
Fig. 7 (Stern and associates). High-power view of the epiretinal membrane formed by retinal pigment epithelial cells six hours after injection. The epiretinal cells interact with the detached and elevated retina on one side of the optic nerve head. The cells are multilayered along the detached internal limiting membrane (arrows). The internal limiting membrane remains contiguous with the multilayered basal lamina that surrounds the retinal blood vessels. The cells appear to interact with the retina (ret) where the retinal blood vessels protrude into the vitreous cavity (×750).
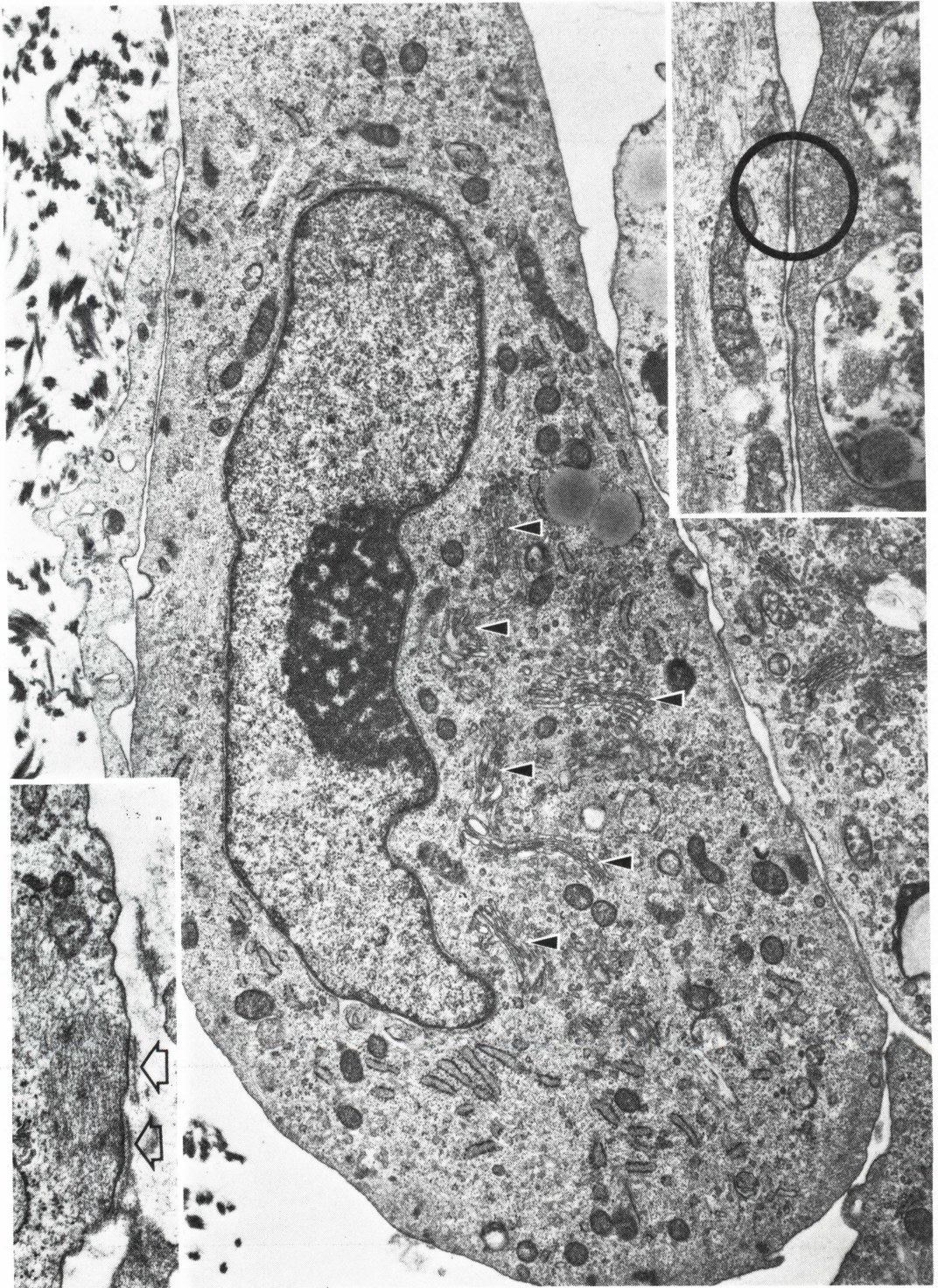
Fig. 8 (Stern and associates). Transmission electron micrograph of a retinal pigment epithelial cell in the epiretinal membrane six hours after injection. The golgi complexes (arrowheads) are numerous (×13,750). The cells are attached to each other by adherens junctions (circle, top inset) and to the extracellular matrix by hemidesmosomes (arrows, bottom inset) (×27,500).

cells attached to each other by adherens junctions and to the extracellular matrix by hemidesmosomes (Fig. 8, insets). The extracellular matrix stained positively for collagen. The amount and staining intensity of the matrix increased with time after cell injection.

Between six and 24 hours after cell injection, the internal limiting lamina detached from most of the retinal surface but remained attached to the retinal blood vessels along the medullary rays and to the nonpigmented ciliary epithelium. No detachment of the internal limiting membrane was found in control eyes injected with 0.1 ml of balanced salt at each of the time periods studied. Polymorphonuclear neutrophils and imma-

ture tissue macrophages were found adjacent to the transvitreal internal limiting membrane near the ciliary body.

Between 24 and 48 hours after cell injection, the number and population density of the epiretinal cells increased (Fig. 9). By 48 hours after cell injection, the epiretinal cells formed a multilayered population in the area of the medullary rays (Fig. 10). The posterior retina underlying this region was detached into local folds. Retinal detachments extended beyond the medullary rays. Most of the epiretinal cells and their nuclei were now spindle-shaped (Fig. 11), and many of their nuclei were indented. The cells had ultrastructural characteristics similar to those of epiretinal cells after six hours,
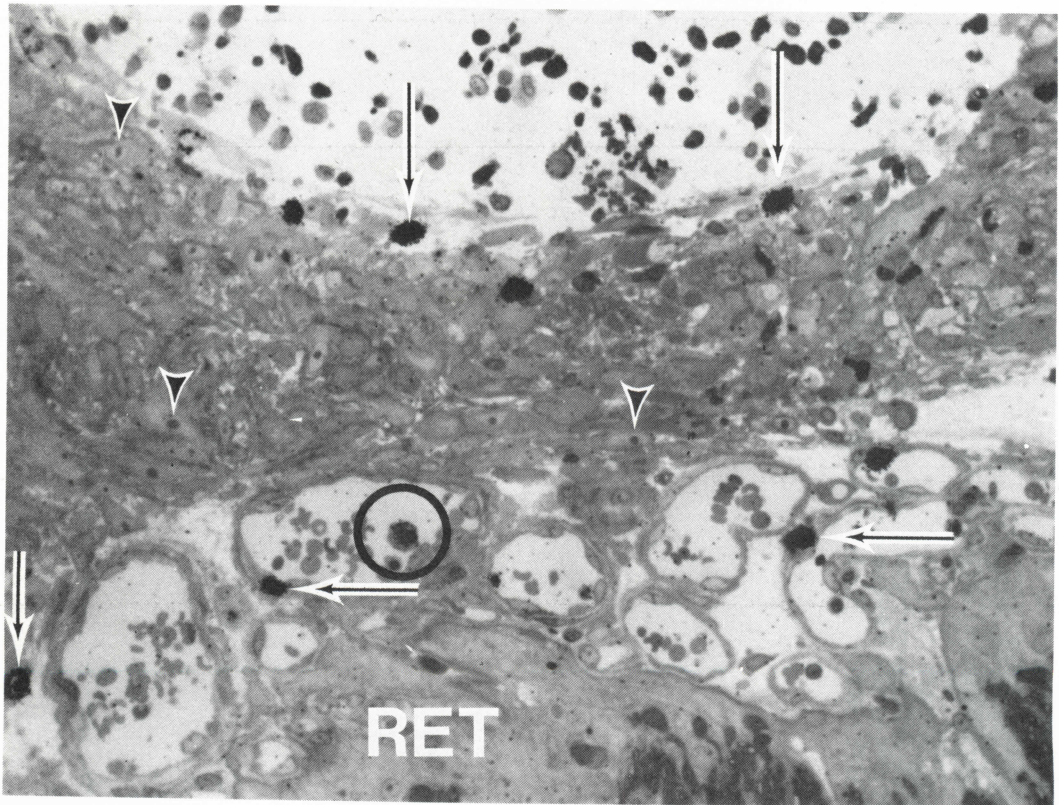

Fig. 9 (Stern and associates). Autoradiograph of the epiretinal membrane 48 hours after injection. Arrowheads, individual retinal pigment epithelial cells; vertical arrows, tritiated thymidine-labeled epiretinal cell nuclei; horizontal arrows, labeled capillary endothelial cell nuclei; circle, labeled monocyte within one of the vessels; RET, retina in the region of the nerve fiber bundles (×380).
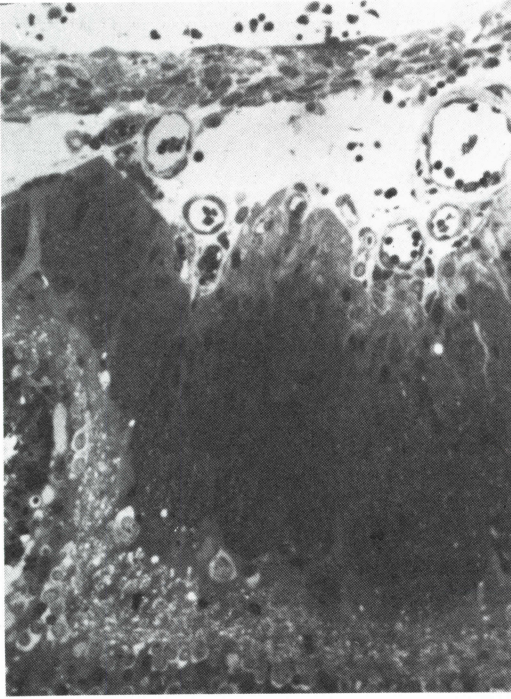
Fig. 10 (Stern and associates). The epiretinal membrane in the region of the medullary rays 48 hours after cell injection. The epiretinal membrane is multilayered and has caused considerable wrinkling of the underlying retinal surface (×190).

but had a more extensive basal lamina, more microfilaments, and fewer primary and secondary lysosomes.

Forty-eight hours after cell injection, the internal limiting membranes of the posterior retina bordering the medullary rays was sometimes broached by glial cell cytoplasmic processes. These processes protruded into the vitreous cavity but did not touch or contribute to the epiretinal membrane. The retina underlying these areas was not wrinkled into local folds. A few cells resembling fibroblasts were also found along the medullary rays two days after cell injection. These cells and their nuclei were spindle-shaped, their nuclear heterochromatin was thick and peripherally located, their Golgi complexes and endoplasmic reticulum were inconspicuous, there were no bundles of intracellular microfilaments, and the cells did not possess a basal lamina.

In some eyes, the epiretinal cells extended along the detached internal limiting membrane towards the ciliary body as soon as 48 hours after injection. The retina directly underlying the epiretinal cells was wrinkled into local folds (Fig. 12). The retina adjacent to this area was not wrinkled but was slightly detached.

One week after injection of the retinal pigment epithelial cells, the retina was pulled toward the center of the vitreous cavity.

Two weeks after injection, portions of the ciliary body and iris were located in the center of the vitreous cavity. Tearing of the detached posterior retina was extensive. The epiretinal cells remained spindle-shaped and had indented nuclei. They appeared to have more microfilaments than at earlier times. Their rough endoplasmic reticulum was organized as normal-appearing cisternae and the distended, irregularly arranged cisternae observed earlier were absent. Their Golgi complexes were less extensive and fewer vesicles were fused or adjacent to the plasma membrane. There was less basal lamina associated with the cells than at earlier times. In addition to the four components of extracellular matrix, there were filaments 30 nm in diameter that had banding periods of 20 nm. These filaments were present near the few cells that resembled fibroblasts, and absent near the cells that resembled myofibroblasts. Small retinal blood vessels projected further into the vitreous cavity than normal retinal blood vessels. These vessels were not enveloped by the multilayered basal lamina present in other nearby retinal blood vessels, or in the blood vessels of the control eyes.

Tritiated thymidine autoradiography— The percentage of epiretinal pigment epithelial cell nuclei labeled with tritiated
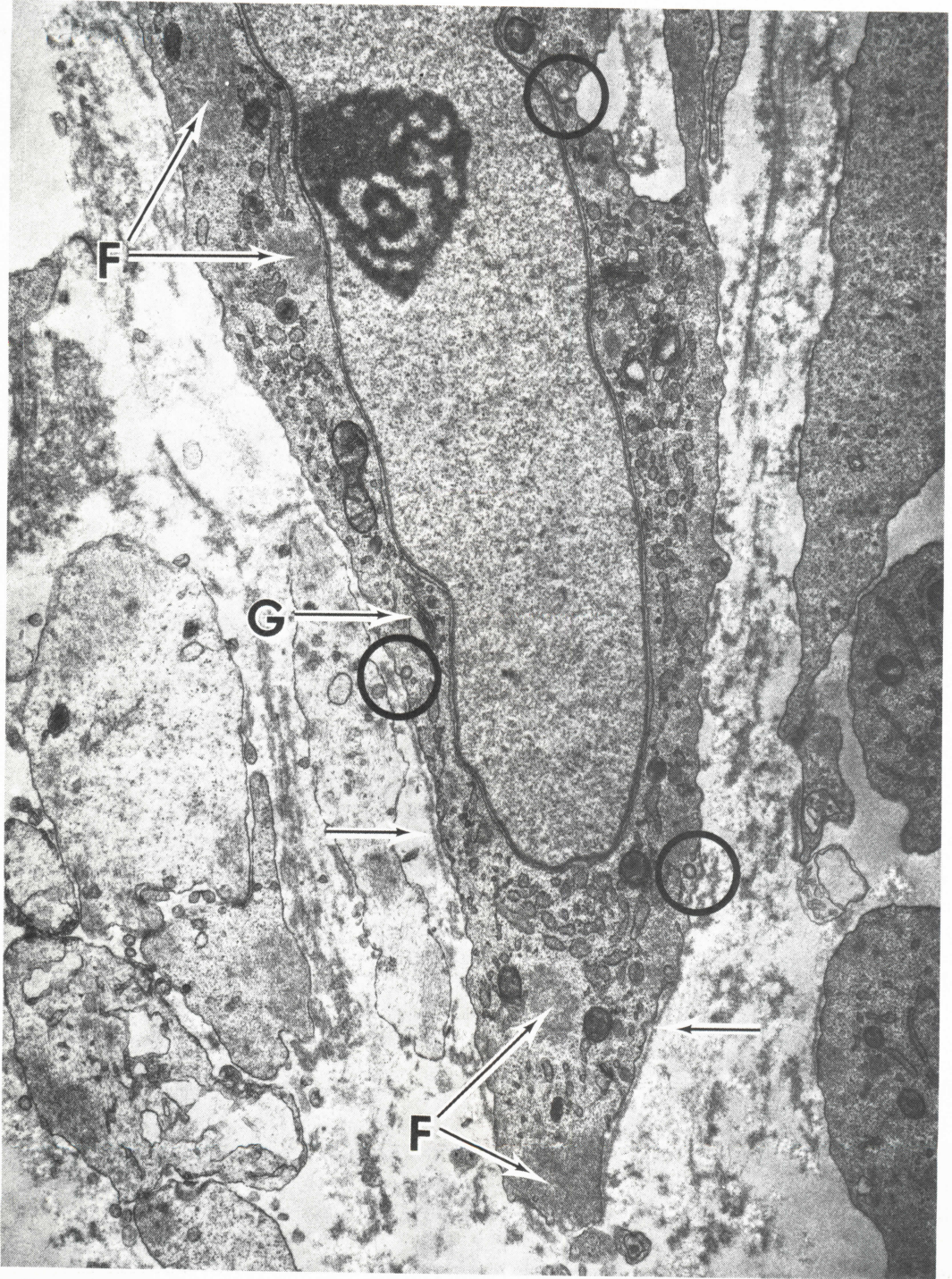
Fig. 11 (Stern and associates). Transmission electron micrograph of a retinal pigment epithelial cell 48 hours after injection. The cell and nucleus are spindle-shaped, and the nucleolus is prominent. F, intracellular microfilaments; G, a Golgi complex; circles, vesicles located adjacent to the plasma membrane; arrows, the interrupted basal lamina (×15,000).

Fig. 12 (Stern and associates). The epiretinal membrane 48 hours after injection. The cells appear as a multilayered population adjacent to the nerve fiber bundles of the posterior retina and along the posterior portion of the detached (transvitreal) internal limiting membrane. The retina (ret) is detached into local folds. srs, subretinal space (×84).
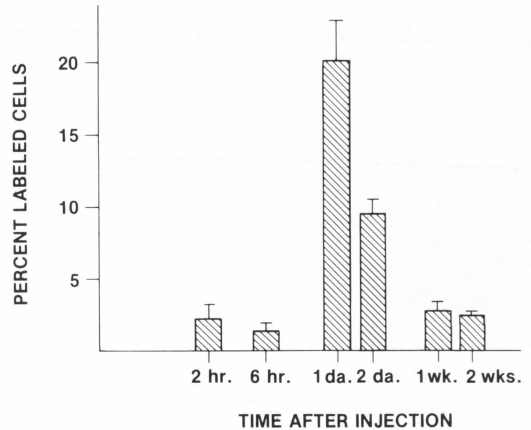

Fig. 13 (Stern and associates). Histogram relating the percentage of intravitreal retinal pigment epithelial cell nuclei labeled with tritiated thymidine to the time after injection of the cells. The two-hour, six-hour, one-week, and two-week values range from 1.3% to 2.6% and are not significantly different from each other (chi-square test). The one-day (20%) and the two-day (9.4%) values differ significantly from each other and from the earlier and later values (each comparison yields P<.001 with the lowest $\chi^2=46$).

thymidine was determined at six different times after injection of the cultured cells (Fig. 13). The two-hour, six-hour, seven-day, and 14-day values ranged from 1.3% to 2.6% but were not significantly different from each other by chi-square test. One day after injection, 20% of the cells were labeled; two days after injection 9.4% of the cells were labeled.

The one- and two-day values differed significantly and from the earlier and later values (each comparison yielded P<.001 with the lowest $\chi^2 = 46$).

Other cell nuclei labeled with tritiated thymidine included capillary endothelial cells of the retinal blood vessels, monocytes within the capillaries, retinal astrocytes, Müller cells, endogenous retinal pigment epithelial cells, and choroidal capillary endothelial cells.

DISCUSSION

Vitrectomy markedly relieves transvitreal traction resulting from intraocular cellular proliferation. Nonetheless, extensive epiretinal membrane formation remains a problem for which there is as yet no satisfactory solution.

Previous studies using intravitreal injection of fibroblasts into the rabbit eyes with an intact lens and vitreous have provided a model for studying pharmacologic therapy for intravitreal cellular pro-

liferation.[7,8] Our study differed from previous studies by providing a model for studying pharmacologic and surgical therapy for epiretinal cellular proliferation in eyes that had undergone vitrectomy.

The clinical and histologic changes caused by injecting 400,000 retinal pigment epithelial cells into an eye after vitrectomy differed significantly from those caused by injecting cells into an eye with an intact vitreous body and lens. The most prominent features of the present model were the speed and consistency with which membranes developed on the retinal surface, rather than within the vitreous, as well as the speed with which traction was exerted on the underlying retinal surface.

We can divide the histologic changes into three overlapping stages. In stage 1, at two hours, the cells were oval, loosely packed, and situated in the region of the medullary rays. During stage 2, between six and 48 hours, the epiretinal membrane became more developed, with layers of oval and spindle-shaped cells tightly packed within a collagenous matrix. Stage 3 began with detachment of the internal limiting membranes, between six and 24 hours, continued with cellular proliferation and contraction along the detached internal limiting membrane, and resulted in a funnel-shaped detachment of the medullary rays by seven days after injection and displacement of the ciliary body towards the center of the vitreous cavity.

In the present model, growth of the injected cells took place predominantly on the retinal surface rather than intravitreally. The resulting elevation of the nerve fiber bundles was the result of proliferation and contraction of cells on the internal limiting membrane, causing it to become detached and shortened, rather than of intravitreal membrane contraction. Unlike the eye with an intact vitreous body in which the injected cells appeared as a gray intravitreal cloud, the cell-injected eye showed no ophthalmoscopic evidence of the injected cells after their introduction onto the retinal surface. By the third day, however, a white preretinal mass appeared just below the optic nerve and extending along each medullary ray. Elevation of the medullary rays could be observed clinically by the third or fourth day. This rapidly progressed to a funnel-shaped detachment in 100% of the eyes by the seventh day after injection. Of the intact rabbit eyes injected with 250,000 tissue-cultured fibroblasts in previous studies, about 25% of the eyes showed evidence of pucker resulting in detachment of the medullary rays after one week, and about 60% showed combinations of puckers and detachments after two weeks. Finally, in contrast to the clinically visible inflammatory reaction that lasted one week in intact rabbit eyes, there was minimal reaction in eyes that had undergone vitrectomy and lensectomy.

Tritiated thymidine-labeling showed that a low level of proliferative activity at two and six hours after injection was followed by a burst of proliferation in the next 24 hours. At 48 hours, the rate dropped to 50% of the 24-hour value. At one and two weeks, the rate was the same as at two and six hours after injection. The rapid increase in proliferation at 24 hours might be expected after transplantation of the cultured cells to a new medium with a continuous supply of nutrients and an ensuing loss of contact inhibition. The decline in proliferation was correlated with the transition of the cells to a more differentiated state. The early extensive cellular proliferation in this model differed from that noted in previous studies in which fibroblasts were injected into the solid vitreous and

no tritiated thymidine-labeled cell nuclei were found in the vitreous until the third day after injection. This may have been the result of the easy access of the injected cells to nutrients and factors obtained from the retinal blood vessels in the eye that had undergone vitrectomy. The continued cellular proliferation in this animal model presumably resembled the clinical situation after vitrectomy for massive periretinal proliferation in which epiretinal membranes continue to form and to contract for weeks and sometimes months after surgical intervention.

Ultrastructural studies of the injected retinal pigment epithelial cells showed that they possessed all of the features of contractile myofibroblasts,[10] including spindle-shaped cell bodies, indented nuclei, extensive rough endoplasmic reticulum, prominent Golgi complexes, microfilament bundles with densification, basal lamina, and adherens junctions. The ability of retinal pigment epithelial cells to undergo transformation into cells with these characteristics as well as to participate in massive periretinal proliferation has been shown in both experimental and clinical studies.[11,12]

Rentsch[13] attributed the contractile nature of epiretinal cellular membranes to intercellular collagen as well as to the contractile nature of the involved cells. This experiment suggested that the contractile features of the membrane were related to the cellular elements because collagen production was not prominent shortly after injection but the membranes were capable of exerting remarkable traction on the underlying retina.

In an eye that has undergone vitrectomy, epiretinal membrane traction on the underlying internal limiting membrane and retina appears to occur in the following manner: The epiretinal cells are joined by adherens junctions and are attached to the extracellular matrix by hemidesmosomes. The contractile force of the epiretinal cells is transmitted via the extracellular matrix to the internal limiting membrane. The traction is transmitted to the retina where the internal limiting membrane remains adherent to the retina. In the rabbit, this occurs in the region of the medullary rays.

Recurrence of contractile epiretinal membranes after vitrectomy is presently the major obstacle to long-term retinal reattachment. Any pharmacologic therapy must be effective in both the gas-filled and the fluid-filled vitreous cavity. The rapid and reliable development of epiretinal membranes in this model should prove to be advantageous in evaluating various pharmacologic approaches to inhibiting epiretinal cellular proliferation and contractility.

REFERENCES

1. Machemer, R., and Laqua, H.: Pigment epithelium proliferation in retinal detachment (massive periretinal proliferation). Am. J. Ophthalmol. 80:1, 1975.
2. Laqua, H., and Machemer, R.: Glial cell proliferation in retinal detachment (massive periretinal proliferation). Am. J. Ophthalmol. 80:602, 1975.
3. Clarkson, J. G., Green, W. R., and Massof, D.: A histopathologic review of 168 cases of preretinal membrane. Am. J. Ophthalmol. 84:1, 1977.
4. Green, W. R., Kenyon, K. R., Michels, R. G., Gilbert, H. D., and De La Cruz, Z.: Ultrastructure of epiretinal membranes causing macular pucker after retinal re-attachment surgery. Trans. Ophthalmol. Soc. U.K. 99:65, 1979.
5. Cleary, P. E., Minckler, D. S., and Ryan, S. J.: Ultrastructure of traction retinal detachment in rhesus monkey eyes after a posterior penetrating ocular injury. Am. J. Ophthalmol. 90:829, 1980.
6. Algvere, P., and Kock, E.: Experimental fibroplasia in the rabbit vitreous. Retinal detachment induced by autologous fibroblasts. Albrecht von Graefes Arch. Klin. Exp. Ophthalmol. 199:215, 1976.
7. Sugita, G., Tano, Y., Machemer, R., Abrams, G., Claflin, A., and Fiorentino, G.: Intravitreal autotransplantation of fibroblasts. Am. J. Ophthalmol. 89:121, 1980.
8. Tano, Y., Sugita, G., Abrams, G., and Machemer, R.: Inhibition of intraocular proliferations with intravitreal corticosteroids. Am. J. Ophthalmol. 89:131, 1980.

9. Humphrey, C. D., and Pittman, F. E.: A simple methylene blue-azure II-basic fuchsin stain for epoxy-embedded tissue sections. Stain Technol. 49:9, 1974.

10. Gabbiani, G., Hirschel, B. J., Ryan, G. B., Statkov, P. R., and Majno, G.: Granulation tissue as a contractile organ. A study of structure and function. J. Exp. Med. 135:719, 1972.

11. Machemer, R., Van Horn, D., and Aaberg, T. M.: Pigment epithelial proliferation in human retinal detachment with massive periretinal proliferation. Am. J. Ophthalmol. 85:181, 1978.

12. Radtke, N. T., Tano, Y., Chandler, D., and Machemer, R.: Simulation of massive periretinal proliferation by autotransplantation of retinal pigment epithelial cells in rabbits. Am. J. Ophthalmol. 91:76, 1981.

13. Rentsch, F. J.: The ultrastructure of preretinal macular fibrosis. Albrecht von Graefes Arch. Klin. Exp. Ophthalmol. 203:321, 1977.

Policy Statement on Copyright Transfer

Ophthalmic Publishing Company

In order to continue to provide the widest possible dissemination of scientific information in ophthalmology, the Ophthalmic Publishing Company, publishers of The AMERICAN JOURNAL OF OPHTHALMOLOGY, must obtain written transfer of copyright from every author. At the time of acceptance, a request for written transfer of copyright will be sent to the corresponding author of each article. The signed transfer must be returned to:

Copyright Recorder
Ophthalmic Publishing Company
Tribune Tower, Suite 1415
435 N. Michigan Ave.
Chicago, IL 60611

The signed copyright transfer confirms that each part of this work, including text, figures, tables, and references, has been submitted solely to THE AMERICAN JOURNAL OF OPHTHALMOLOGY and has not been submitted for publication elsewhere, or published previously. This signed transfer confirms that no part of the article is subject to copyright or other rights except those of the author or authors.

No article may be published in THE AMERICAN JOURNAL OF OPHTHALMOLOGY until the company has received this signed transfer agreement.

In return for the transfer of rights, the Ophthalmic Publishing Company assumes responsibility for future reprinting of the article. As in the past, permission to reprint portions (text, tables, figures, and long quotations) or entire articles will be granted only with the approval of the authors. This approval must be obtained by whomever wishes to use the material.

FRANK W. NEWELL, M.D.
Editor-in-Chief
AMERICAN JOURNAL OF OPHTHALMOLOGY