Figure 2. Central cornea of a patient with traumatic rupture of the eye 13 months after laser in situ keratomileusis. Note the granular staining of hemoglobin particles below the flap and the piling up of these particles at the interface (arrow), with few particles in the overlying flap (Masson trichrome, original magnification ×10).

Choroidal Neovascular Membranes Treated With Photodynamic Therapy

The Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group

The authors have no relevant financial interest in this article.

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sion identified at baseline. At the 12-month follow-up visit, 46% of the verteporfin-treated eyes vs 71.1% of the placebo-treated eyes had documented growth of the CNVM on fluorescein angiography.1,2

We describe the clinical course and histopathologic findings in 5 patients with predominately classic subfoveal CNVM secondary to ARMD who had progression of classic CNVM, despite treatment with verteporfin PDT (Figure 1). These patients underwent submacular surgery with excision of their CNVM. The excised membranes were examined using light and transmission electron microscopy.

Report of Cases. The patients' clinical history, surgical report, and fluorescein angiograms were reviewed. Visual acuity was measured using an illuminated Early Treatment of Diabetic Retinopathy Study chart. The logarithm of the minimal angle of resolution (logMAR) units were calculated by obtaining the logarithm of the reciprocal of the Snellen visual acuity. The logMAR values were then converted back into Snellen visual acuity to report outcomes. The greatest linear dimension and 2-dimensional size of the CNVM lesion on digital fluorescein angiography were measured using the IMAGEnet (TOPCON America Corp, Paramus, NJ) measure function. These results are found in the Table.

After signing an informed consent, patients underwent a conventional 3-port pars plana vitrectomy. In 1 case (patient 4), the posterior hyaloid was bimanually separated from the optic nerve and retina. The patients’ most recent fluorescein angiograms were used to determine a suitable location for the retinotomy, usually temporal to the macula in an area devoid of large retinal vessels. A subretinal pic was used to create a retinotomy, lyse any retinchoroidal vessels or adhesions, and gently elevate the edge of the membrane from the underlying tissue. With the intraocular pressure elevated to 60 mm Hg, horizontal subretinal forceps were used to grasp the subretinal membrane and slowly deliver it through the retinotomy. The intraocular pressure was then slowly lowered back to normal while the macula was inspected for any subretinal bleeding. The membrane was removed from the eye, placed on a dry sponge, and placed in fixative. The orientation of the specimen was not specified in any of the cases. Each patient underwent a 360° scleral depressed examination with an indirect ophthalmoscope before having a complete fluid-air exchange performed.

Four (patients 1 through 4) of the 5 patients had their excised CNVM fixed in 10% formalin for paraffin embedding and light microscopy. In 1 case (patient 5), the CNVM was fixed in 2.5% glutaraldehyde and 2% formaldehyde in 0.1M cacodylate buffer and embedded in EPON for 1-µm sectioning and transmission electron microscopy.

The patients ranged in age from 75 to 86 years. They all had predominately classic subfoveal CNVM secondary to ARMD. Their mean initial visual acuity was 20/300, greatest linear dimension was 2.68 mm, and CNVM lesion size was 3312 µm². They received intravenous verteporfin (6 mg/m² of body surface area) over a 10-minute period. Five minutes after the end of the infusion, a 689-nm laser was applied over the entire lesion for 83 seconds. At the first 3-month follow-up examination, the CNVM had grown in all of the cases and measured on average 8454 µm². Four (patients 1, 3, 4, and 5) of the 5 patients had repeated verteporfin PDT, while 1 underwent submacular surgery and excision of the CNVM. At the subsequent 3-month follow-up visit, the mean size of the PDT-treated CNVM was 11 310 µm². Patient 1 demonstrated retraction of the membrane at one edge and growth at another edge, with an overall decrease in the size of the membrane. At this point, the remaining patients elected to undergo submacular surgery with CNVM excision. All of these patients underwent submacular surgery 3 to 4 months after their last PDT treatment (Table).

During pars plana vitrectomy, 4 (patients 1, 2, 3, and 5) of the 5 patients had a posterior vitreous detachment present, and 1 required peeling of the posterior hyaloid. In 2 cases (patients 1 and 4), the CNVM was stiff and enlarged the retinotomy on extraction through the retina. None of the patients had laser photocoagulation of the edges of the retinotomy site, and all of these sites remained flat after surgery. No other complications such as subretinal hemorrhage, peripheral retinal tear, or retinal detachment were identified.

Histopathologic Findings. A light micrograph from case 4 of the central aspect of the CNVM demonstrated large red blood cell–filled vessels within a thick, predominantly fibrous membrane (Figure 2A). In contrast, the peripheral edges of the CNVM were filled with smaller red blood cell–filled vessels within a thin, more cellular membrane (Figure 2B). Among all cases, retinal pig-
ment epithelial (RPE) cells were seen on 1 side of the membranes and were sometimes found layered within a membrane. Patient 5 revealed a single layer of cuboidal, slightly pigmented, polarized RPE cells covering the presumed neurosensory retinal surface of the membrane (Figure 3). None of the membranes had evidence of giant cells.

A transmission electron micrograph from patient 5 revealed a stroma containing abundant collagen fibrils and fibroblasts with dilated rough endoplasmic reticulum and nuclei with dispersed chromatin (Figure 4A). Myofibroblasts with hemidesmosomes and pigment-laden macrophages with filopodia were present, as well as inflammatory cells such as lymphocytes. Proliferating RPE cells were rare in the stroma of this CNVM. Capillary endothelial cells and adjacent pericytes sometimes shared the same redundant basement membrane (Figure 4B). In addition, larger vessels surrounded by smooth muscle cells were identified. These vessels were filled with numerous enlarged endothelial cells that severely reduced or eliminated the vessel lumen (Figure 4C).

One side of the CNVM had multiple layers of RPE cells filled

### Table: Patient Data

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<th>Patient No.</th>
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<th>Lesion 1 Size, µm²</th>
<th>PDT 1 Date</th>
<th>Visual Acuity 2</th>
<th>Lesion 2 Size, µm²</th>
<th>PDT 2 Date</th>
<th>Visual Acuity 3</th>
<th>Lesion 3 Size, µm²</th>
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<td>Mean</td>
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</table>

**Abbreviations:** GLD, greatest linear dimension; NA, not applicable; PDT, photodynamic therapy.
with vesicles, dilated rough endoplasmic reticulum, and irregular pigment granules. Long-spaced collagen, an RPE basement membrane (Figure 5A), and, occasionally, capillaries were identified in the subepithelial space. This was considered to be the native RPE layer that covered the Bruch membrane, although no collagenous or elastic components of the Bruch membrane or choroidal vessels were identified. Lightly pigmented cuboidal cells covered the side of the membrane presumably in contact with the neurosensory retina. These cells possessed apical stunted microvillous processes, tight junctions, single melanosomes and melanosomes jacketed by lipofuscin, basally concentrated mitochondria, crude basal infoldings, and a rudimentary basal lamina consistent with polarized RPE cells (Figure 5B).

Comment. We describe 5 patients who had recurrent growth of a classic subfoveal CNVM associated with ARMD, despite treatment with verteporfin PDT. Three (patients 2, 3, and 5) of the 5 patients had initial visual acuity worse than 20/200, the lower limit in the Treatment of Age-Related Macular Degeneration With Photodynamic Therapy study. Growth of the CNVM was associated with worsening vision. Pars plana vitrectomy and excision of the CNVM were performed in all of these patients. In 2 of the patients (patients 1 and 4), the membranes were stiff and produced a moderate enlargement of the retinotomy site as they were extricated from the subretinal space. No other complications were associated with surgery. The mean postoperative visual acuity was 20/500, after a mean of 4 months’ follow-up.

Fluorescein angiography in all of these patients revealed persistent growth of the CNVM beyond the area of the lesion documented on baseline angiography. The central body of the membrane tended to stain on the late frames of the angiogram, suggesting the presence of fibrous tissue. In contrast, the edges of the enlarging membrane were often hyperfluorescent early and leaked late in the angiogram, consistent with actively growing, highly permeable vessels.

The fluorescein angiographic findings in these patients are consistent with the histopathologic analyses of the CNVM. The central area of the membranes was thick and fibrous, while the edges of the membrane were diaphanous and cellular. Furthermore, the central area of the membrane often harbored large red blood cell–filled vessels, while the periphery of the membrane was usually filled with smaller red blood cell–filled vessels.

Ghazi et al3 found histologic evidence of endothelial degeneration and vascular occlusion in a surgically excised membrane 27 days after PDT treatment. In contrast, we did not find any evidence of acute vascular thrombosis or injury in our membranes excised more than 90 days after PDT treatment. This difference may be due to a longer interval between PDT and surgical excision, resulting in more mature CNVMs.

Our surgically excised CNVM specimens showed evidence of vascular repair. Capillaries with reduplication of the basement membrane were seen. This finding has also been documented in surgically excised CNVM not previously treated with PDT, in CNVM previously treated with PDT 27 days before surgery, and in cynomolgus monkey CNVM and normal choriocapillaris previously treated with PDT. This may be consistent with a significant antecedent vascular injury such as PDT and a subsequent endothelial revascularization of the ghost vessel, leading to reduplication of the capillary basement membrane. Larger vessels surrounded by smooth muscle cells had numerous swollen endothelial cells that narrowed the vascular lumen. Al-
though similar endothelial cells have
been described within large vessels
surrounded by smooth muscle in
CNVM, we believe that the num-
ber of vessels and the degree of lu-
men narrowing present are remark-
able. This finding may be consistent
with endothelial cell revasculariza-
tion within larger, more mature ves-
sels 3 months after PDT-induced en-
dothelial damage.

Retinal pigment epithelial cells
were intimately associated with all
of these membranes. As seen in other
histologic studies, the RPE cells
were found predominantly on 1 side
of the CNVM but were also found
layered within the membranes. It is
difficult to orient surgical speci-
mens of subretinal CNVM using
only light microscopy. In patient 5,
ultrastructural analysis revealed
long-spacing collagen associated
with the presumed native RPE cells.

The CNVM in this patient appears
to have grown over the native RPE
cells in a fashion consistent with a
type 2 membrane.

In patient 5, a single layer of
pigmented polarized RPE cells with
apical tight junctions, apical micro-
villi, basally concentrated mitochon-
dria, and a rudimentary basement
membrane covered the neurosen-
sory retinal surface of the mem-
brane. Encapsulation of the CNVM
by RPE cells has been seen in ani-
mal models of CNVM treated with
PDT and has been reported in sur-
gically excised CNVM in patients
with ARMD. Our findings in pa-
tient 5 indicate that RPE cells in pa-
tients with ARMD are capable of cov-
ering the neurosensory surface of a
PDT-treated CNVM.

Taken together, we speculate
that PDT-induced occlusion of
CNVM vessels, and possibly the un-
derlying choriocapillaris, result in
a subretinal environment that is
more hypoxic than it is at baseline.
Some vessels may not recover from
this vascular insult, while others,
spurred on by such vascular media-
tors as vascular endothelial growth
factor, may be able to revascular-
ize themselves via endothelial cell
proliferation. Larger caliber ves-
sels, with more rapid blood flow,
may be more resistant to the injuri-
ous effects of PDT. This would be
consistent with the clinical finding
that small CNVMs have better out-
comes after PDT compared with
larger CNVMs.

Patients treated with vertepor-
fin and PDT for subfoveal, predomi-
nantly classic CNVM secondary to
ARMD may progress, despite re-
peated PDT treatment. Progressive
growth of the CNVM is associated
with loss of vision. Choroidal neo-

Figure 5. Transmission electron micrographs from patient 5. A, Native retinal pigment epithelial cell (PE) with long-spacing collagen (asterisk), basally located
mitochondria (m), and a basement membrane (arrowheads). No evidence of other layers of Bruch membrane or choroid was identified (bar=3 µm). Insert, Higher
magnification of the long-spacing collagen (bar=0.5 µm). B, Proliferating retinal PEs covering the neurosensory retinal surface of the choroidal neovascular
membrane. There are single apical melanosomes (asterisk), melanosomes jacketed by lipofuscin (ml), stunted microvilli (arrowhead), basally located
mitochondria (m), and early basement membrane formation (long arrow). Stacked parallel lamellae consisting of rough endoplasmic reticulum (short arrow) are
also seen within these cells (bar=2 µm). Insert, Higher magnification of a lipofuscin granule containing various melanosomes (bar=1 µm).
vascular membranes previously treated with PDT appear to be composed of large vessels within a predominately fibrous body and smaller vessels in the more cellular periphery of the CNVM. In some cases, RPE cells may cover the neurosensory retinal surface of PDT-treated CNVM.

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