Choroidal Microvascular Repair after Argon Laser Photocoagulation

Ultrastructural Observations

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Acute laser injury to the tapetum of the feline retina produces thrombosis of the choriocapillaris. Early changes are characterized by the appearance of platelet-fibrin thrombi within capillary loops and disruption of endothelial integrity. By 4 days, thrombi have disappeared, and the endothelium shows regenerative changes. No endothelial cell mitotic activity is seen. The endothelial cytoplasm becomes plump, and there is a loss of the fenestrations adjacent to Bruch's membrane. By 10–20 days, the capillary structure shows gradual restoration. At 30 days, endothelial cell fenestrae are clearly evident adjacent to Bruch's membrane. The reparative process in this model appears to evolve as a result of thrombolysis and endothelial cell activation. Invest Ophthalmol Vis Sci 25:1019–1026, 1984

The immediate response of the normal choriocapillaris to laser photocoagulation is vessel wall damage and thrombosis. These changes have not been investigated systematically, since most studies have been concerned with events that occur soon after laser injury[1-3] or cursorily in experiments of long-term studies of retinal repair.[4-6] In a previous study from this laboratory using scanning electron microscopy of vascular casts of eyes following retinal photocoagulation, we demonstrated that there is restoration of circulation through the choriocapillaris within 30 days.[7] The purpose of this study was to evaluate systematically the ultrastructural changes that lead to revascularization of the occluded choriocapillaris utilizing transmission electron microscopy.

Materials and Methods

Healthy, adult domestic cats, each weighing 2–4 kg were used in this study. Utilization of animals in this study conforms to the ARVO Resolution on Use of Animals in Research. Prior to photocoagulation of the retina, each cat was anesthesized with intramuscular injections of ketamine HCl (30 mg/kg of body weight) and atropine (0.3 mg/kg of body weight). After dilating the pupils with 1% tropicamide and 2.5% phenylephrine HCl, argon laser photocoagulation was administered to the region of the retinal tapetum. As defined in our previous study,[7] lesions of moderate intensity were produced with 400 mW power, 0.2 sec duration, and 500 spot size exposures. An average of 100 burns were created in each eye, using a scatter technique. One of the investigators (JMR) performed the retinal photocoagulation in all animals. Immediately after photocoagulation, the burns appeared pale tan in color, were uniform in size, and were spaced equidistantly. Specimens were prepared for transmission electron microscopy at one day, 2 days, 4 days, 10 days, 20 days, and 30 days after photocoagulation. The animals were anesthetized with ketamine HCl (30 mg/kg of body weight) and acepromazine maleate (1.0 mg/kg of body weight), both administered intramuscularly. Both common carotid arteries and jugular veins were exposed, and the arteries cannulated with a 22-gauge plastic cannula. When the arterial cannulation was complete, the jugular veins were incised. Arterial perfusion then was begun with 2% phosphate buffered glutaraldehyde at a gravity flow rate of 25 cc/min. After enucleation, the anterior segment was removed by a circumferential pars plana incision, and the posterior segment was immersed immediately in a solution of 2% phosphate buffered glutaraldehyde. The tissues were processed subsequently for electron microscopy utilizing standard techniques. One animal was used for each time interval studied and approximately 10 lesions were evaluated from each eye by both light

Fig. 1. Control. Light microscopic appearance of the nondamaged eye. The choriocapillaris is present as a thin row of capillaries between the retina and tapetal area of the feline eye. The normal arrangement of capillary loop, morphology of Bruch's membrane, and RPE is shown (×9,720).

microscopy (to select areas for thin sections) and electron microscopy.

Results

Nondamaged Controls

Nondamaged vessels consisted of oval to elongated capillary loops bordered by a thin, slightly separated, basement membrane (Fig. 1). Adjacent to Bruch's membrane, the capillary endothelial cytoplasm was attenuated. Fenestrations were present between segments of endothelial cytoplasm. The endothelium adjacent to the tapetum was thicker and contained few fenestrations. Tight junctions were prominent. Retinal pigment epithelial cells (RPE) were in contact with

Fig. 2. A, Capillaries occluded by platelet-fibrin thrombi dominate the early phase of injury. Three such capillaries are shown (×2,070).
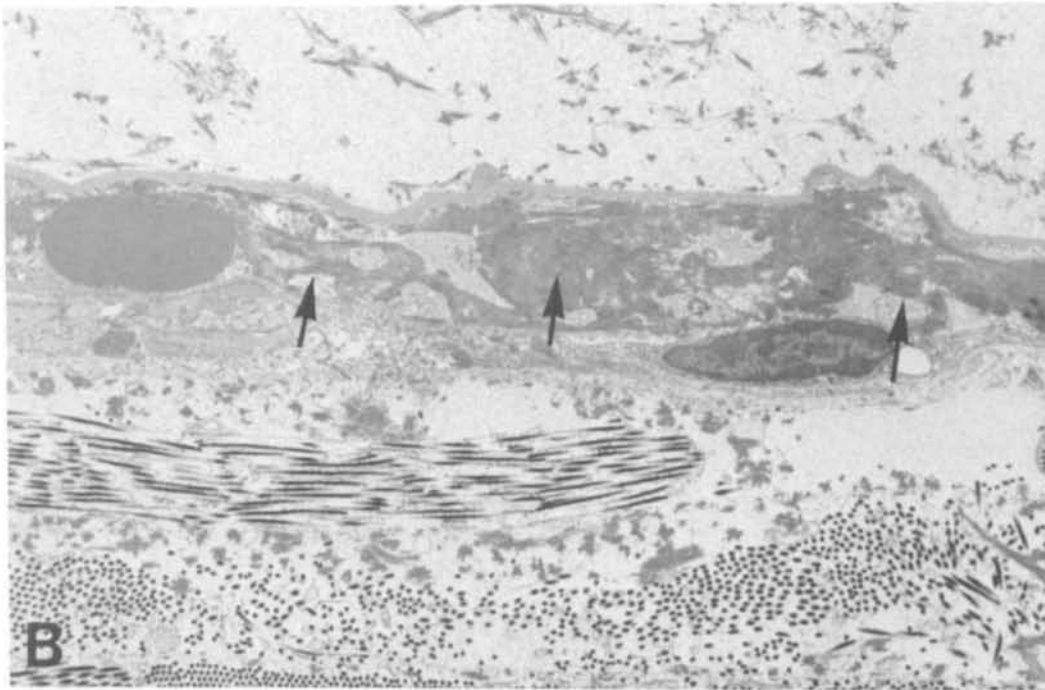
Fig. 2. B, A higher magnification. Fibrin strands are indicated by arrows. The normal cellular architecture is disrupted (×5,100).

Bruch's membrane by thin cytoplasmic processes. The portion of the vessel adjacent to the tapetum was bordered by a thin, basal lamina that, in some instances, appeared to be duplicated. There was loose connective tissue adjacent to the basal lamina.

One To Two Days Postphotocoagulation

Numerous thrombosed capillaries were present in areas of maximal injury (Figs. 2A–C). The occluded choriocapillaris contained fibrin, platelets, and occa-
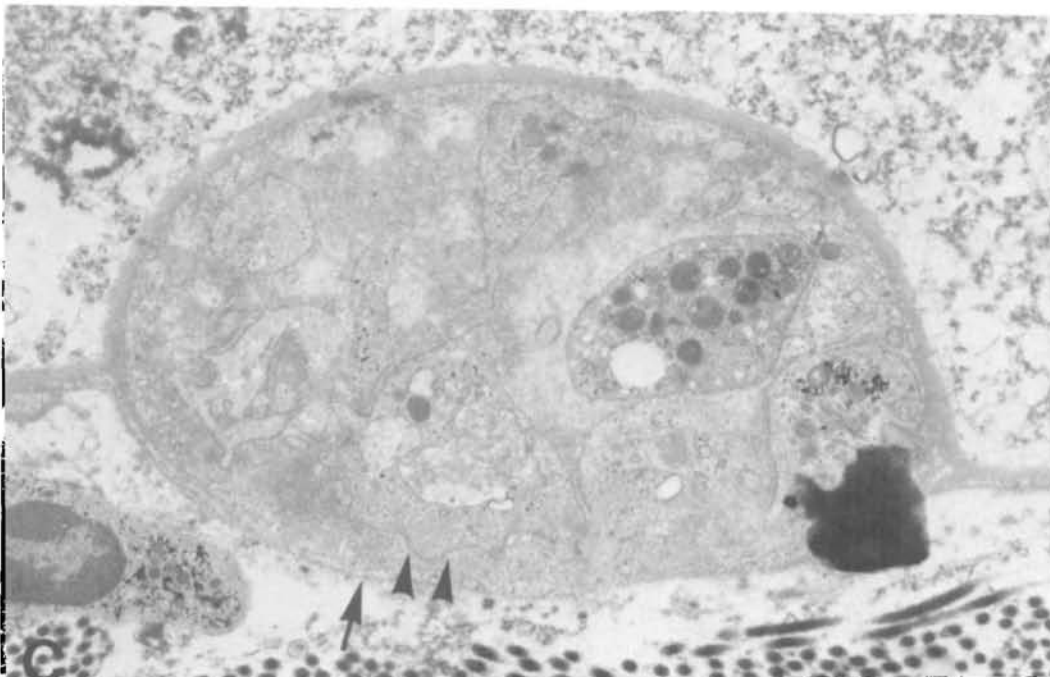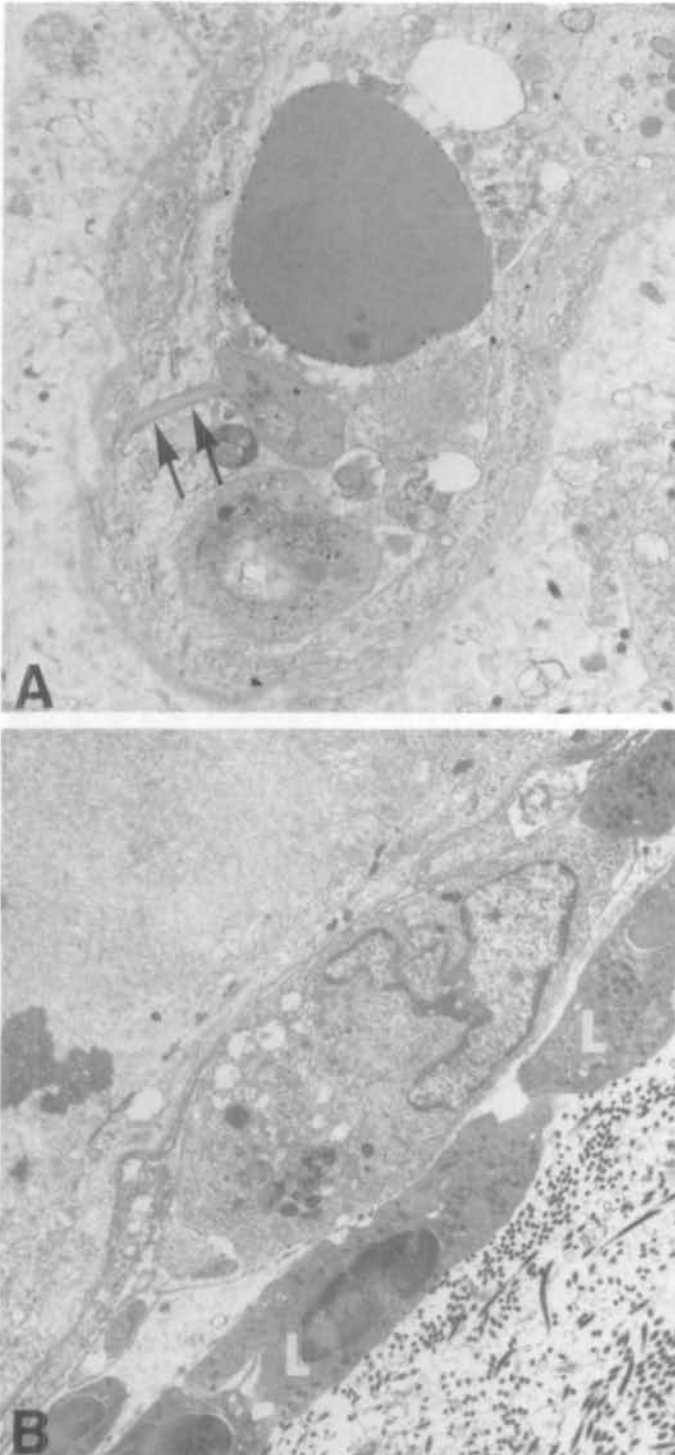

Fig. 2. C, A faint basement membrane remains in some areas (arrow). Endothelial cells are detached from the basement membrane and appear to be swollen (arrow heads). Platelets have attached to some of these areas (arrow heads) (×12,960).

Fig. 3. Forty-eight hours. The capillary outline remains virtually intact. Platelets continue to be present in the areas of damage. Platelet pseudopods (arrow) were occasionally present in gaps between endothelial cells, **A.** Leukocytes (L) were evident in areas adjacent to damaged capillaries, **B.** (A: ×7,200; B: ×3,800).

sional erythrocytes. Endothelial cells lining the capillaries were contracted and partially detached from the poorly defined basement membrane. Tight junctions were absent and gaps were present between endothelial cells. In some areas, platelets were present between endothelial cells and in contact with subendothelial structures (Fig. 3). Although the platelets were

altered in shape and pseudopods were prominent, most contained characteristic granules. Bruch's membrane was intact, although the surface was mildly irregular. In most areas, the choriocapillaris basement membrane was fragmented.

Cells comprising the retina were coagulated. Strands of fibrin were present in this area. The tapetum was

Fig. 4. Four days. Endothelial cells are present in regenerating capillaries. Prominent junctional complexes are evident (arrow). Fenestrae are absent within the mildly plump endothelial cytoplasm adjacent to Bruch's membrane (×10,800).
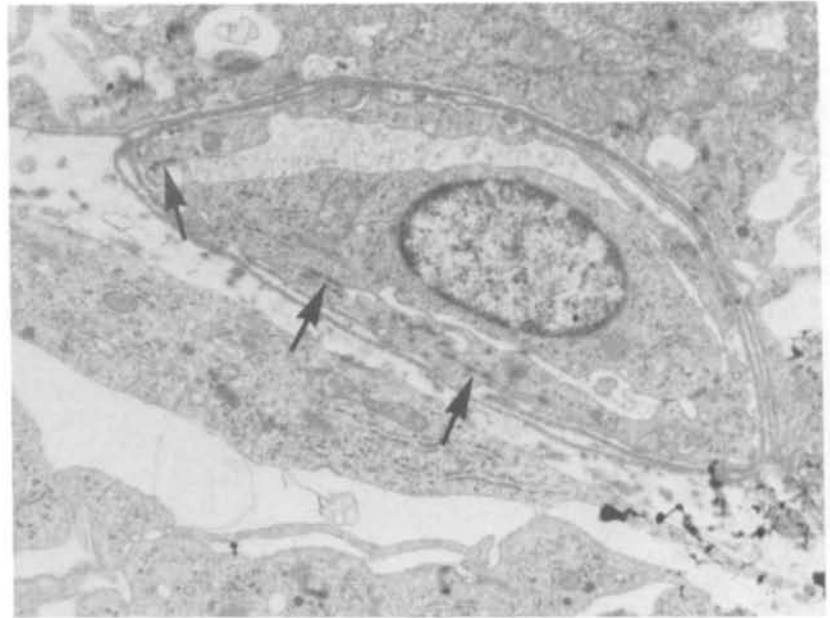
edematous and contained disorganized collagen fibrils. Fibrin strands were present in edematous spaces between the tapetum and the remaining basal lamina surrounding the choriocapillaris. Although pre- and postcapillary vessels were abnormal, vessels deep within the tapetum were unaffected (not shown).

Four Days Postphotocoagulation

At four days, the most striking finding was the absence of platelet–fibrin thrombi within capillary lumina. The endothelial cells lining these capillaries were plump and contained collections of polyribosomes and slightly enlarged mitochondria (Fig. 4). The endothelial fenestrations adjacent to Bruch's membrane were absent. Tight junctions connected the endothelial cells. Enlarged endothelial nuclei were present. The endothelium forming the vascular wall opposite Bruch's membrane was also plump, with prominent, tight junctions. Some cells contained surface microvillous processes. No mitoses were evident in the endothelial cells. The basement membrane was slightly altered and contained focal discontinuities (not shown). Normal splitting of the basement membrane was accentuated and appeared duplicated in some areas. In most areas of severe damage, the RPE cytoplasmic processes adjacent to Bruch's membrane were absent (Fig. 5). This was most prominent in intracapillary areas. The RPE cells contained lysosomes, nondilated profiles of rough endoplasmic reticulum, and elongated to oval-shaped mitochondria. Golgi complexes were present in some cells. The photoreceptor cell layer was disrupted totally or absent in areas of severe injury (not shown). Macrophages, leukocytes, and degenerating cellular products were a constant finding.

Ten Days Postphotocoagulation

Although occlusive thrombi were absent at this stage, an occasional, intact platelet was evident in some cap-
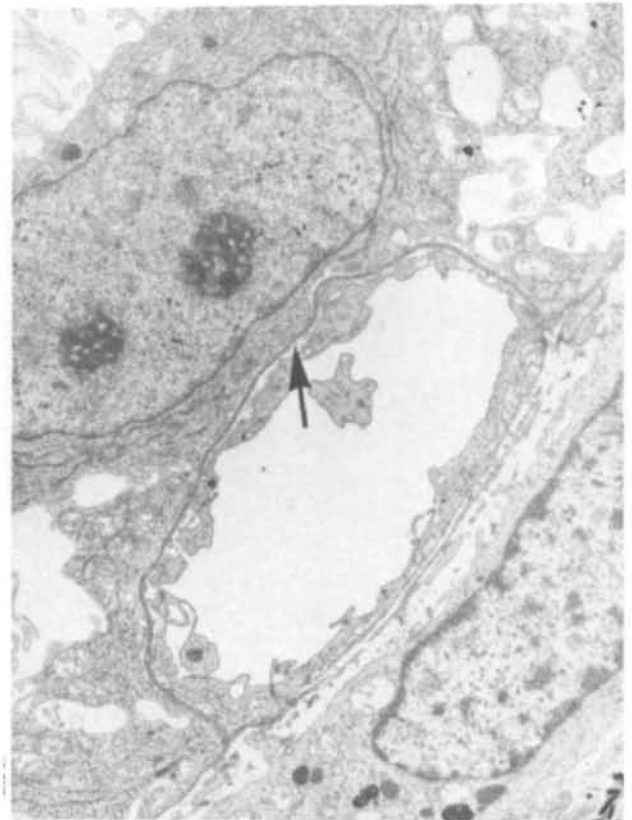
Fig. 5. Four days. Altered endothelial morphology is clearly evident in this regenerating capillary. Cytoplasmic processes normally present in RPE cells are absent at this stage of repair. Bruch's membrane appears thinned (arrow) (×7,800).
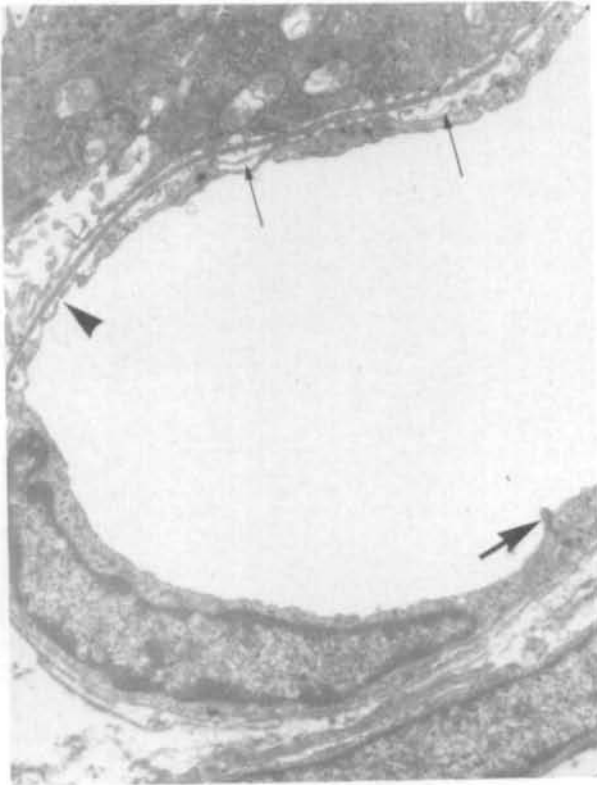
Fig. 6. Ten days. Attempted reorganization of the RPE and Bruch's membrane is evident at this stage. The capillary endothelium adjacent to this area is slightly attenuated and definite fenestrae are seen (arrow heads). Duplicated basement membrane (smaller arrow) and a tight junction also is seen (larger arrow) (×9,000).
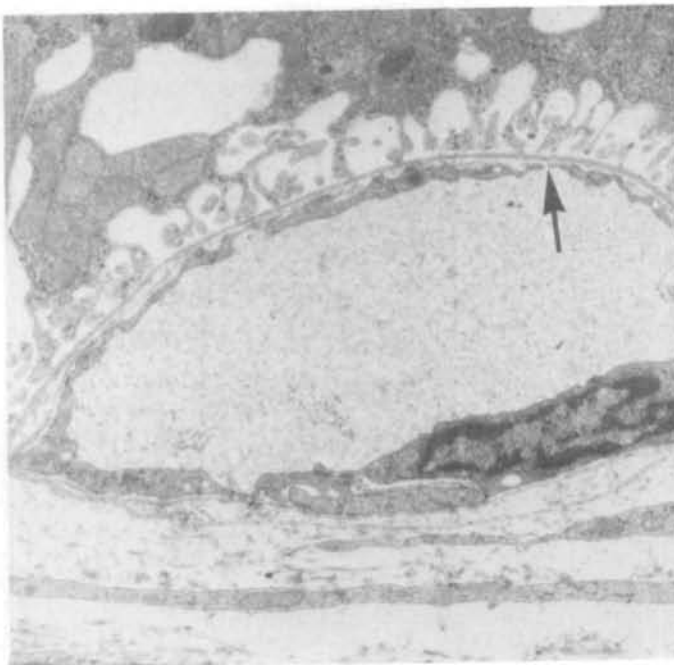
was lined by nonfenestrated endothelium. These areas were joined by well-formed, tight junctions. The basal lamina was thin and indistinct with areas of duplication. The RPE cells had regained some of the cytoplasmic extensions that normally attach to Bruch's membrane. The cell cytoplasm contained the organelles described previously. The retinal pigment epithelial cell nuclei were slightly irregular, and the nuclear chromatin was finely granular. Degenerating photoreceptor cells were prominent. The tapetum also was damaged, showing clearly degenerated cell products (not shown).

Twenty Days Postphotocoagulation

The ultrastructural findings at this point were similar to those described at 10 days. The normal fenestrae observed in the capillary endothelium adjacent to Bruch's membrane continued to be partially absent. Retinal pigment epithelial cell cytoplasmic processes were focally absent. The tapetum was not restored completely at this stage, and inflammatory cells and macrophages were seen.

Thirty Days Postphotocoagulation

At 30 days, the injured choriocapillaris was almost normal (Fig. 7). The endothelial cytoplasm adjacent to Bruch's membrane was focally fenestrated. However, focal solid areas continued to be present at this stage of repair. A thin basal lamina was present around capillaries. Numerous segments of smooth, endoplasmic reticulum were evident in the cytoplasm of RPE cells. Vacuoles and lysosomes also were present in retinal pigment epithelial cells. Total collapse of the retina

illaries. There was partial restoration of fenestrae, although portions of plump endothelial cells also could be seen (Fig. 6). The wall opposite Bruch's membrane

Fig. 7. Thirty days. The capillary lumen is widely patent and the endothelial cytoplasm is thinned and focally fenestrated (arrow). Abnormalities remain in the relationship between the RPE and Bruch's membrane. The latter continues to be reduced in width (×11,960).

with loss of the photoreceptor cell layer in areas of maximal injury was a prominent finding at this stage.

Discussion

In this study, laser injury to the choriocapillaris produced microthrombi that initially occluded the vessels. The cat tapetum, consisting of multilayered rows of cells that reflect incoming light lies between the choriocapillaris and the large choroidal vessels. Uveal pigment is only present in the large choroidal vessels, and RPE cells in the tapetal area contain no pigment granules.[8] Therefore, damage to the choriocapillaris in this experimental model was due to energy absorption by red blood cell hemoglobin, not by pigmented cells and tissues within the vicinity of vessels. Within 2–4 days, the thrombi were no longer present and endothelial cell proliferative activity was the prominent finding. At 30 days, almost normal appearing capillaries were present in the areas of previous damage. Mechanisms that may be responsible for such changes include thrombolysis and fibrinolysis, recanalization, and neovascularization.

One well-studied model of ocular neovascularization involves the studies of corneal neovascularization by McCracken[9] and Schoefl.[10] McCracken examined the pattern of corneal neovascularization following silver-nitrate application. Leukocytes were evident within vascular lumina immediately after injury. By 24 hr, mitotic activity was present in both endothelial cells and pericytes. The authors concluded that there was a possible relationship between leukocytic infiltration and vascularization. McCracken also observed that neovascularity resulted from an outgrowth of sprouts from existing capillaries and postcapillary venules. This observation was confirmed by Schoefl[10] who studied growing capillaries in muscle wounds and after silver nitrate injury to the cornea. Platelets were present in the lumina of vascular sprouts. Fibrin and leukocytes were absent. Endothelial growth activity was a constant finding in the early stages of capillary growth. With increased growth, cytoplasmic processes penetrated the basement membrane and appeared on the abluminal surface. Although we found increased endothelial activity and platelets in areas of damage, we saw no other evidence of neovascularization, for example, vascular sprouting or cytoplasmic penetration of the basement membrane. However, this may, in part, be a reflection of the morphologic differences between the choriocapillaris and corneal capillaries.

One model of subretinal neovascularization involves the use of an argon laser high-energy density beam by Ryan.[11] Histopathologic specimens in that study showed disruption of Bruch's membrane with an overlying area of vascular proliferation that included a prominent fibrous component. We found no discontinuities in Bruch's membrane and no fibrosis in the area of damage.

Studies on the recanalization of thrombi indicate that endothelial cells grow along clefts and spaces within the thrombus. Within a short period, these cells completely line the channels.[11] More recent data has shown that, following a well-defined injury to endothelial cells lining a vessel, the adjacent cells proliferate to repair the defect.[12]

Our findings support the concept that resumption of (capillary) vascular integrity appears to result from thrombolysis and proliferation of endothelial cells within the confines of the basement membrane. However, in our study, the entire vascular integrity was restored without actual recanalization; the development of endothelial cell growth along slit-like spaces within the thrombus.

Results from this study showed that when increased endothelial activity was present in the capillaries, platelet–fibrin thrombi were absent. This initially appears to be a combination of enhanced fibrinolysis and thrombolysis within the occluded capillary and subsequent endothelial proliferation to repair the defect. As suggested by others, when the injury does not completely destroy the basic structure of the capillary, both processes may be operable.[13,14] Further, the presence of platelet and endothelial-derived growth factors also may aid in endothelial growth in these damaged vessels.[14]

The role of the revascularized choriocapillaris following extensive retinal photocoagulation used for proliferative retinopathies requires further study. Also, in the management of subretinal neovascularization where the principal aim is to produce permanent occlusion of the vessels, further investigation is needed. The manipulation or alteration of the clotting and thrombolytic processes may prove beneficial in providing more permanent obstruction of vessels following photocoagulation.

Key words: choriocapillaris, photocoagulation, thrombosis, cats, vascular injection

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