Regeneration of retinal limiting membranes after chorioretinal biopsy in dogs. Ian J. Constable, Robert Horne, Douglas H. Slatter, Graeme H. Chester, and Richard L. Cooper. After chorioretinal biopsy in dogs, obtained without vitreous loss, accessory glial cells and fibroblasts filled the defect between cortical vitreous and bare sclera. The proliferating glial cells laid down a new basement membrane which was complete both on the internal and external aspects of the defect. After biopsy complicated by vitreous loss, however, cellular proliferation failed to reestablish complete internal and external limiting membranes, and new formed fibroblastic tissue extended into the vitreous. To our knowledge, this is the first report to describe effective regeneration of the internal limiting membrane of the retina in any species. These findings suggest that when vitreous loss can be avoided, healed chorioretinal biopsies are unlikely to lead to delayed pathological complications.

The proliferation of retinal glial cells through breaks in the inner limiting membrane (ILM) of the retina is a problem of fundamental clinical importance in premacular fibrosis and in massive preretinal proliferation associated with retinal detachment. These frequently disastrous examples of inappropriate wound healing on the retinal surface are thought to occur because of breaks in the inner retinal surface. Implicit in this hypothesis is the concept that the ILM cannot be readily repaired, so that cells proliferating at the site of the break are not sealed off from the preretinal area. Previous studies in rabbits and cats, in which the ILM and the inner half of the retina were disrupted by a scratch under microscopic control, showed a healing reaction characterized by proliferation of glial cells into the wound and onto the retinal surface. No evidence of regeneration of the ILM was reported in these studies. Several of the human studies cited above and one report after retinal injury in the rabbit described attempts at basement membrane regeneration by the proliferating glial cells but no orderly repair of the ILM of the retina.

The development of a method of full-thickness chorioretinal biopsy under a scleral flap has provided another model for examination of retinal wound healing. This study reports the orderly repair of both inner and outer limiting membranes after full-thickness chorioretinal biopsy in eyes in which vitreous has not been grossly disrupted, and compares the results after biopsy in eyes in which vitreous was displaced.

Materials and methods. Forty adult dogs were subjected to one chorioretinal biopsy (30 dogs) or two chorioretinal biopsies (10 dogs) under controlled hypotensive anesthesia as previously described. Electron microscopy showed that a full-thickness biopsy including the ILM of retina was obtained in each case. A detailed record was kept as to whether significant vitreous loss occurred, and the eyes were followed by regular clinical examination, including fundus photography, for periods of up to 6 months. Eight eyes with 10 healed chorioretinal biopsies were selected for this study from the larger experimental population without regard for randomization. All eyes were clinically free of inflammation or other complications and healed biopsy sites were visible on indirect ophthalmoscopy. The animals were anesthetized by intravenous sodium pentothal and maintained after intubation with nitrous oxide, oxygen, and halothane in a semi-closed circuit. The eyes were enucleated and immediately fixed by partial vitreous exchange with 2.5% glutaraldehyde, buffered with cacodylate. The animals were then killed with an overdose of intravenous pentobarbital sodium. The individual healed biopsy sites were excised and prepared for light and electron microscopy. Light microscopic sections were cut from standard paraffin blocks and stained with hematoxylin and eosin. Specimens for electron microscopy were embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate and examined with a Siemens EM 200 microscope.

Results. The wound healing response of the retina, choroid, and sclera in each case depended on whether there had been vitreous loss at the time of chorioretinal biopsy.

Full-thickness chorioretinal biopsy repair in the absence of vitreous loss. In all six specimens examined in this group, the biopsy site healed without clinical or histological evidence of complications (Fig. 1). There was no evidence of proliferation of scar tissue into the vitreous or onto the retinal surface. No signs of retinal traction surrounding the biopsy site were seen. The scleral flap healed without overgrowth into the chorioretinal defect, and an incomplete layer of fibroblasts was found on the inner surface of the flap. An orderly layer of glial cells grew in from the edge of the retinal

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Fig. 1. Healed chorioretinal biopsy (below) shows no preretinal strands of scar tissue nor retinal traction. This biopsy was done 6 months previously. No vitreous was lost. Histology (above) shows healed biopsy site with intact inner limiting membrane. (×125.)
wound, completely covering the inner aspect of the defect. These cells were characterized by cytoplasmic microtubules and basal lamina formation. The layer of glial cells was two to five cells in thickness at the center of the defect (Fig. 2). In each case, a continuous basement membrane was laid down on both the inner and outer surface of the glial sheet. These basement membranes could be traced to the internal and external limiting membranes of the retina at the edge of the wound.

Repair of chorioretinal biopsy in the presence of vitreous loss. In each of the four eyes examined, strands of scar tissue were visible clinically extending into the superficial vitreous (Fig. 3). There were no signs of vascularization of this scar tissue nor of tractional distortion or detachment of the surrounding retina. These scar tissue strands grew within two months and did not change thereafter during the period of observation. On electron microscopy, these biopsy sites showed a disorganized process of repair. Within the strands of scar tissue invading the superficial vitreous, there was a large number of glial cells characterized by densely packed cytoplasmic filaments and microtubules (Fig. 4). These proliferating glial cells were found adjacent to and often completely enveloped by prominent strands of basal lamina. Only on the surface of an individual scar tissue stalk was this basal lamina laid down with any degree of regularity. Within the invading scar tissue stalk, there were also large clumps of collagen fibrils characterized by 60 nm macroperiodicity and measuring 40 to 80 nm in diameter. Also interspersed in these stalks were multiple patches of fine vitreous-type fibrils 10 to 20 nm in diameter (Fig. 4). Although typical fibroblasts were seen within the scar tissue, pigment containing cells from either retinal pigment epithelium or choroid did not appear to play a major role in the invading tissue.

Discussion. A number of authors have noted that in humans, glial cells proliferating in preretinal membranes lay down strands of basement membrane. No such basement membrane deposition was reported after partial-thickness ret-
Fig. 3. Healed biopsy site (below) six months after vitreous loss shows tissue strands (*FT*) invading the vitreous cavity. Histology (above) shows stalk of invading tissue (*FT*) which contains fibroblasts, glial cells, and collagen. (*×80.*)
Fig. 4. Electron micrograph from invading fibrous tissue strand. A glial cell is enveloped by basement membrane. V, Vitreous type fibrils; C, collagen; F, fibroblast. (x21,600.)

inal scratches in rabbits or cats. However, new but disorganized strips of basement membrane were reported after injury to the rabbit retina in an earlier paper. The present observations after full-thickness excision of choroid and retina in dogs clearly demonstrate that new basement membrane is laid down by glial cell proliferation after this type of injury. If the vitreous surface is not ruptured by the biopsy technique, these reparative glial cells grow in orderly fashion across the defect and lay down complete inner and outer limiting membranes.

If, however, during the excision of choroid and retina the vitreous is damaged and partly lost, the reparative process is disorganized. Invading strands of tissue include glial cells, fibroblasts, and thick collagen fibrils which are foreign to the vitreous cavity. Although the glial cells lay down multiple basement membranes in an attempt to seal off the wave of proliferating tissue, they do not succeed. This is presumably because the advancing edge of proliferating tissue has a ready-made scaffolding in the form of the displaced vitreous fibrils on which to advance. It is well known from clinical experience with vitrectomy that the absence of a scaffolding on which the cells may grow will prevent invasion towards the centre of that cavity but not along the retinal surface.

We have shown previously in both dogs and humans that it is possible to biopsy choroid and retina without losing vitreous. That the inner limiting membrane may regenerate in orderly fashion in this situation suggests that delayed complications such as preretinal scar tissue formation or retinal detachment might not occur after uncomplicated retinal biopsy.

From the University of Western Australia, Murdoch University and Royal Perth Hospital, Perth, Australia. Supported by Australian Foundation for the Prevention of Blindness (W.A. Division) Inc., Lions Save-Sight Foundation (W.A.) Inc., Royal Perth Hospital Research Centre and National Health and Medical Research Council of Australia. Submitted for publication Aug. 6, 1980. Reprint requests: Professor I. J. Constable, Uni-

An adult male, domestic short-haired cat with generalized retinal atrophy was found to have a 60-fold increase in plasma ornithine and ornithinuria. Ornithine-ß-aminotransferase activity was undetectable in its tissues and in its cultured skin fibroblasts. This feline condition is thus analogous to gyrate atrophy of the choroid and retina in humans.

Gyrate atrophy of the choroid and retina (GA) is a rare, inherited form of human chorioretinal degeneration. In addition to the characteristic appearance of the fundus, patients with GA have 10- to 15-fold elevations in plasma ornithine concentration and overflow ornithinuria. The primary defect has been shown to be a deficiency of the mitochondrial matrix enzyme ornithine-ß-aminotransferase (OAT). The present report describes a cat with clinical and biochemical abnormalities analogous to GA in humans.

The cat was an adult male, domestic short-haired cat of uncertain age and ancestry that, with the exception of a vision problem, was in apparent good health. It received a standard commercial cat food diet. It was presented for evaluation of suspected blindness and was found to have bilateral generalized retinal atrophy. Retinal thinning and vascular attenuation were present diffusely over both fundi. Cataracts were not present. A complete blood count and serum chemistries were all within normal limits; however, amino acid screening of his urine revealed ornithinuria and resulted in the studies described below. Attempts to breed this cat were unsuccessful due to behavioral abnormalities, and several months after his initial presentation it became severely dehydrated and died despite several days of intensive care.

Significant postmortem findings were limited to mild testicular atrophy and retinal atrophy. Throughout the fundus, pigment epithelial and photoreceptor cells were missing, which brought the inner retinal layers into close apposition to the tapetal or choroid layers depending on the retinal location (Fig. 1, C). Inner retinal layer organization was variable. In the nontapetal zone adjacent to the disc an extremely thin, disorganized, and gliosed neural retina was present, whereas the tapetal zone of the posterior pole had distinct inner nuclear, plexiform, and nerve fiber layers (Fig. 1B). More peripherally, however, there was complete loss of inner retinal layer organization (Fig. 1B). A distinct and prominent choriocapillaris layer was not present. Instead, isolated segments of choriocapillaris existed between the atrophic retina and the choroid. This was most prominent in the tapetal zone where the perforating tapetal capillaries that normally give rise to the choriocapillaris were absent or greatly reduced in number (Fig. 1B).

Plasma and urine were collected from the cat on...