increase in posterior chamber aqueous humor ascorbate, indicative of decreased entry of water into the posterior chamber. Thus, the effect of vanadate may be presumed to be largely due to an effect on aqueous humor production.

\[(\text{Na}^+\text{K}^-)\text{ATPase}\] activity is present in ciliary epithelium.\(^7\) Inhibitors of this enzyme, such as ouabain given systemically, lower the rate of aqueous humor formation in cats and humans.\(^7\) Vanadate is also a potent inhibitor of \[(\text{Na}^+\text{K}^-)\text{ATPase}\], which acts in a fashion different from the cardiac glycosides.\(^8\) Although vanadate appears to have little or no effect on human intraocular pressure when administered topically in aqueous solutions,\(^3\) formulations to enhance penetration, as in the current monkey study, may produce positive results. Ocular penetration and \[(\text{Na}^+\text{K}^-)\text{ATPase}\] studies currently are being performed to elucidate the vanadate mode of action.

Vanadate also is noted to stimulate adenylate cyclase activity in isolated membrane preparations.\(^9\) Preliminary experiments indicate that vanadate also stimulates monkey ciliary body-iris adenylate cyclase in vitro (unpublished data, T. Mittag). Cyclic AMP and its analogs are reported to increase outflow facility.\(^10\) The absence of increased outflow facility and the elevation of aqueous humor cyclic AMP in rabbits after topical administration of vanadate\(^3\) implies that in rabbits the ocular effect of vanadate is not related to adenylate cyclase stimulation. However, the recent report\(^11\) that forskolin, an agent that stimulates adenylate cyclase directly without affecting the cell membrane receptors that are coupled to the cyclase enzyme, lowers intraocular pressure in rabbits, monkeys, and humans and reduces aqueous flow in rabbits suggests that reduction of aqueous humor formation may be another mechanism whereby stimulation of this enzyme could affect intraocular pressure.

**Key words:** vanadate, intraocular pressure, monkey, aqueous humor flow, outflow facility

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**References**


**Neovascularization of the Iris: An Experimental Model in Cats**

Einar Stefansson, Maurice B. Landers III, Myron L. Wolbarsht, and Gordon K. Klintworth

Neovascularization of the iris was induced in cats by removing the vitreous and lens and creating a rhegmatogenous retinal detachment. The presence of new blood vessels on the anterior surface of the iris was verified from the second month onward by slit lamp examination, as well as by light microscopy six to twelve months after the operation. Control eyes undergoing vitrectomy and lensectomy, but without retinal detachment, did not develop rubeosis iridis. This model may allow investigation into causes and therapy of rubeosis iridis. Invest Ophthalmol Vis Sci 25:361-364, 1984

The introduction of vitrectomy for the treatment of vitreal and retinal complications of diabetes mellitus and other conditions has rekindled interest in iris neovascularization, since vitrectomy apparently predis-
poses to rubeosis iridis.\textsuperscript{1,2} Up to 23\% of diabetics undergoing vitrectomy have been reported to develop iris neovascularization within 6 months of the operation and, if the lens is removed with the vitreous, the likelihood of iris neovascularization is even greater (45\%).\textsuperscript{2} Should a retinal detachment complicate the vitrectomy, rubeosis iridis almost always occurs.\textsuperscript{2,3}

This study extends these observations on the association of rubeosis iridis with vitrectomy, lensectomy, and retinal detachment by reproducing the phenomenon experimentally in cats.

**Materials and Methods.** Healthy adult domestic cats (3–5 kg) of either sex were used in the study. The eyes were divided into three groups: eyes in which a total rhegmatogenous retinal detachment was induced after vitrectomy and lensectomy (Group 1), eyes that had the vitreous and lens removed, but without a retinal detachment (Group 2); and eyes that underwent no ocular operations (Group 3). Three cats died during the observation period and were not included in the study. The final count in the experimental groups was eight eyes in Group 1, nine in Group 2, and five eyes in Group 3.

**Surgical Procedures:** The cats were anesthetized with intramuscular ketamine hydrochloride (approximately 30 mg/kg; Ketaset\textsuperscript{a}, Bristol Laboratories, NY) and given an intramuscular injection of atropine sulfate (0.05 mg/kg, Eli Lilly Co., IN). Full dilatation of the pupils was achieved by applying a few drops of tropicamide (0.25\%, Alcon Laboratories, TX) and phenylephrine 5\% (Cooper Vision Pharmaceuticals, Puerto Rico) to the corneas. A lateral canthotomy was performed on each animal under an operating microscope and sutures were placed in the conjunctiva to immobilize the eye. The conjunctiva was cut exposing the sclera, which was incised laterally 6 mm behind the corneoscleral limbus overlying the pars plana. The tip of a phacoemulsification probe (Phacoemulsification unit, Alcon Laboratories, TX) was inserted into the crystalline lens, which was emulsified and washed out through the pars plana incision.

Each vitrectomy was performed under aseptic conditions through the pars plana using the Visc X (Cintext, MA) with lactated Ringer’s solution containing glucose (1 g/l) and gentamycin (Garamycin\textsuperscript{b} 40 mg/l) as irrigation fluid. The lens capsule was removed with the vitrectomy instrument. When indicated by the experimental protocol (Group 1), a retinal detachment was produced by gently nicking the posterior retina with the vitrectomy probe to create a 2 to 4 mm wide hole and injecting fluid underneath the retina. At the end of each operation, the scleral and conjunctival wounds were closed with sterile 7-0 polyglycolic acid suture (Dexon\textsuperscript{c}, Davis and Geck, Inc., Puerto Rico) and 20 mg gentamycin (Garamycin\textsuperscript{b}) was injected subconjunctivally. The canthotomy was closed with sterile 5-0 nylon (Dermalon\textsuperscript{c}, Davis Geck, Inc., Puerto Rico) and the eye covered with an antibiotic ophthalmic ointment (Garamycin\textsuperscript{b}, Schering).

**Clinical Observations:** The eyes were examined at the slit lamp (Haag-Streit, Switzerland) preoperatively and at regular intervals up to one year after the surgical procedures by one examiner (MBL) and all histologic evaluations were made by one ophthalmic pathologist (GKK) without prior knowledge of the clinical impression. In both the clinical and histologic evaluation, an arbitrary scale of “no—questionable—mild—moderate—marked” rubeosis iridis was created. Two eyes in Group 1 are still being followed clinically to see whether they will develop neovascular glaucoma.

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**Histologic Studies:** At the end of each experiment, 6–12 months postoperatively, the eyes were enucleated and fixed in 10\% phosphate-buffered formalin (pH 6.9–7.1). For light microscopy, the eyes were cut horizontally, embedded in paraplast, and stained with hematoxylin and eosin.

**Results. Clinical Observations:** All eight eyes with retinal detachment that underwent vitrectomy and lensectomy (Group 1) developed iris neovascularization as determined with the naked eye and by slit lamp
evaluation. While the time course of rubeosis iridis was not investigated precisely in this study it appeared clinically between 1 and 2 months postoperatively and was obvious for the next 4–10 months until the cats were killed. As a rule, minimal flare was seen in the aqueous humor in the eyes with neovascularization of the iris on slit lamp examination.

Neither of the control groups (Groups 2 and 3) developed rubeosis iridis (Fig. 1). Intraocular pressures were not elevated in any of the eyes of this study at the end of the experiments.

Light Microscopy: Light microscopy disclosed new vessels on the surface of the iris (Fig. 2) in all of the eyes with retinal detachment, vitrectomy, and lensectomy (Group 1), but such neovascularization was not noted in either of the control groups (Groups 2 and 3). In the eyes with rubeosis iridis (Group 1) that were processed for light microscopy the surgical aphakia and rhegmatogenous retinal detachment was confirmed histopathologically. Each of these eyes also contained an infiltration of lymphocytes, plasma cells, and macrophages in the iris stroma, on the anterior surface of the iris, as well as in the angle of the anterior chamber. A chronic inflammatory cell infiltrate was prominent in the rubeotic eyes, with the exception of one eye, which also manifested less iris neovascularization than the others. Only occasional lymphocytes and plasma cells were seen in the iris of cats that underwent vitrectomy and lensectomy (Group 2) but not in the normal eyes (Group 3) (Fig. 2).

The angle of the anterior chamber was unremarkable in histologic sections of all of the control eyes (Groups 2 and 3) and most of the eyes in Group 1. The angle of the anterior chamber was partially occluded by fibrovascular tissue containing lymphocytes, plasma cells, and macrophages with prominent iris neovascularization in two eyes in Group 1. Ectropion uveae was noted in some eyes (Fig. 3).

Discussion. The iris neovascularization observed in cats after vitrectomy, lensectomy, and retinal detachment not only resembles human rubeosis iridis both clinically and histologically, but also was developed under circumstances known to cause rubeosis iridis in humans.2,5,6

While the pathogenesis of rubeosis iridis in the present model still needs to be elucidated, current data
suggest at least two lines of inquiry worthy of experimental investigation in this model. It is noteworthy that eyes developing rubeosis iridis also manifest an infiltration of lymphocytes, plasma cells, and macrophages. Since neovascularization is an established consequence of inflammation in several settings, including corneal neovascularization and granulation tissue, similar mechanisms may be involved in rubeosis iridis in this model.

Hypoxia has been incriminated in the pathogenesis of neovascularization in many normal and pathologic states, including neovascularization of the iris. As shown previously, the oxygen tension in the anterior chamber in cat eyes after vitrectomy and lensectomy is significantly lower than normal, and this may contribute to the development of iris neovascularization. In theory, retinal detachment should make the eye even more hypoxic. This model should allow an investigation into the roles of inflammation, hypoxia, and other conditions in the pathogenesis of rubeosis iridis, as well as evaluation of therapy of iris neovascularization.

Key words: rubeosis iridis, neovascularization, vitrectomy, lensectomy, retinal detachment, neovascular glaucoma, hypoxia, diabetic eye disease

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References

Blood Vascular Abnormalities in the Degenerative Mouse Retina (C57BL/6J-rd le)

Michael T. Matthes* and Dean Bok

The authors have used a light microscopic horseradish peroxidase technique to demonstrate the arborization of blood vessels in mice homozygous for retinal degeneration and their normal heterozygous littermates. The results indicate a paucity of blood vessels in the homozygous animals as early as 14 days of postnatal age. The blood vessel deficiency at this early time coincides with degeneration of the photoreceptor cells and occurs at the approximate age when blood vessels in the normal mouse retina have reached maturity. After photoreceptor degeneration is complete, total blood vessel length per unit area continues to decrease from about one half of normal at the earlier ages to less than one third the amount at 1 yr and after. Invest Ophthalmol Vis Sci 25:364–369, 1984

We have examined the retinal vascular arborization in the C57BL/6J-rd le mouse with retinal degeneration. Using the light microscopic horseradish peroxidase (LM–HRP) method of Raviola and Fredro for whole mount visualization of the retina's blood vessels, we have shown a difference in the length of patent blood vessels in the mouse homozygous for retinal degen-