A study was made in rabbits of the effect of topical dexamethasone ointment, .05%, and either subconjunctival or intraperitoneal D-penicillamine on the duration of function of glaucoma filtering surgery. The drugs were tested alone and in combination. Intraperitoneal D-penicillamine was not effective, either alone or combined with topical dexamethasone. Topical dexamethasone ointment alone and subconjunctival D-penicillamine alone significantly prolonged the duration of function of the filter surgery; however, when combined, no additional effect was observed. It was concluded that the institution of therapy with both drugs immediately after glaucoma filtering surgery was not the optimal temporal sequence for combining the two modes of therapy. Invest Ophthalmol Vis Sci 27:1755–1757, 1986

It is known that many glaucoma filtering procedures fail because of the fibroblastic proliferation which occurs at the conjunctival scleral interface after surgery.1 Others have interfered with fibroblastic proliferation by injecting an antimetabolite, 5-fluorouracil subconjunctivally after filtering surgery to prevent filter failure in primates2 and in glaucoma patients,3 and this drug is now in clinical trials. This study was designed to determine if two drugs, D-penicillamine, a collagen cross-linking inhibitor, and dexamethasone ointment, an anti-inflammatory agent, in different combinations and methods of administration, would prolong the potency of a thermal sclerostomy in the albino rabbit by inhibiting wound healing.

Materials and Methods. All procedures described herein conform to the ARVO Resolution on the Use of Animals in Research.

The surgical procedure involves the electrocauterity technique of Praeziosi,4 but under a scleral flap. White albino rabbits weighing between 2.5 and 4.0 kg were operated on by one surgeon (LJBMcG). The rabbits were anesthetized with intramuscular ketamine, 35 mg/kg, and intramuscular xylazine, 5 mg/kg, and surgery was performed on one randomly chosen eye of each animal. A limbal based conjunctival flap was formed in the superior temporal quadrant, away from the nictitating membrane. A one-half thickness scleral flap with dimensions of 2 mm × 5 mm was made with a beaver blade under the operating microscope. A ¼ mm × 3 mm thermal sclerostomy was performed upon the scleral flap with a hand-held thermal cautery. A peripherial iridectomy was performed, and the scleral flap covered the filtration site, but was not sutured. The conjunctival flap was sutured with 6-0 plain catgut in a running fashion. After the procedure, the anterior chamber was always formed, and a conjunctival bleb had formed spontaneously. The postoperative therapeutic regimen began immediately after surgery. Both the operated eye and its contralateral control eye received identical medications.

Pharmaceutically pure D-penicillamine was obtained from Merck, Sharp, and Dohme, West Point, Pennsylvania, and was prepared as a 5% solution for intraperitoneal injection and as a 10% solution (maximum solubility) for subconjunctival injection. Solutions were made fresh weekly with distilled water (pH 7.0), and kept under refrigeration. Five milliliters of the 5% solution were used for each intraperitoneal injection. Two milliliters of the 10% solution were used for each subconjunctival injection.

After topical proparacaine, the subconjunctival injections were given 180° directly opposite the filtration site in the inferior fornix once daily for 5 consecutive days.

Dexamethasone phosphate 0.05% sterile ophthalmic ointment (Merck, Sharp, & Dohme, West Point, PA) was applied as a one-half inch ribbon to the inferior fornices bilaterally twice daily until filter failure.

Intraocular pressures were taken three times weekly with an Alcon pneumonometer (Fort Worth, TX). Filter failure was defined by the observance of a difference in intraocular pressure between the operated and control eye of 2 mm Hg or less on two successive measurement days. Coincident with this time, the filtering bleb, which was present the first day postoperatively, was no longer evident.

As shown in Figure 1, there were six groups, totalling 49 rabbits, which were treated with combinations of D-penicillamine and dexamethasone ointment.

Atropine sulfate, 1%, sterile ophthalmic ointment (Allergan, Irvine, CA) was given to all animals bilaterally, daily, for 1 week. Animals were assigned to treatment groups by random number generation, which accounted for the variability in the number of rabbits in each group.

Tonography was performed on both eyes of 18 rabbits, 3 from each group, 14 days after surgery, at a time when the intraocular pressure was appreciably lower in the operated eye in comparison to the control eye. An Alcon tonography unit was used with topical proparacaine anesthesia for 4 min readings in cloth-wrapped unanesthetized rabbits.

Results. Figure 1 demonstrates the results obtained in the six groups of rabbits. All rabbits received 1% atropine ointment bilaterally, daily for the first week of the study. The additional medications which they
Fig. 1. Duration of filtration in days vs treatment. All groups, including the controls, received 1% atropine ointment bilaterally daily for 1 week postoperatively. Additional medications received by groups 2–6 are shown by “X.” D-penicillamine I.P. was administered intraperitoneally as 5 ml of a 5% solution twice daily until filter failure. D-penicillamine Subconjunctivally was administered at least 2 ml of a 10% solution bilaterally daily for 5 days. Decadron ointment, 1% was administered bilaterally twice daily until filter failure.

received are shown in the figure. Group I, which received no additional medications, is the control group, in which the function was for a mean ± S.D. of 23.2 ± 7.5 days. All groups which received topical dexamethasone ointment, Groups 3, 4, and 6, showed durations of filtration that were greater than those of the control group; however, only groups 4 and 6 showed a statistically significant increase in the duration of filtration. Group 5, which did not receive topical dexamethasone ointment, but received subconjunctival D-penicillamine, also showed a duration of filtration that was statistically significantly greater than the control group. Although both topical dexamethasone alone (Group 4) and subconjunctival D-penicillamine alone (Group 5) resulted in increased durations of filtration, the combination of subconjunctival D-penicillamine and topical dexamethasone ointment (Group 6) showed no further increase in the duration of filtration. Intraperitoneal D-penicillamine, daily, alone showed no increase in the duration of filtration (Group 2). When topical dexamethasone ointment was added to intraperitoneal D-penicillamine (Group 3), there was an increase in the duration of filtration which was not statistically significant.

The mean outflow facility of the unoperated eyes was .36 ± 0.7 μl/min/mm Hg and the mean outflow facility of the operated eyes was .66 ± .13 μl/min/mm Hg. This was statistically significant (P < .01, paired t-test).

There were few complications of the surgery or the treatment regimens. Two rabbits which developed hyphemas greater than 50% at the time of surgery were excluded from the study. Postoperatively, there were no additional hyphemas, nor were there any flat anterior chambers or infections. The intraperitoneal D-penicillamine caused systemic lathyrogenic effects in all ten rabbits receiving this treatment; however, it seemed to have no effect on filtration function. Subconjunctival D-penicillamine, which did increase filtration function, had no observable systemic or ocular adverse effects. Seven rabbits which died during the study were excluded from the analysis. These rabbits were evenly distributed among the control and treatment groups; therefore, the deaths did not seem to be related to any form of treatment.

**Discussion.** Filter function was defined as an IOP in the operated eye of more than 2 mm Hg lower than the unoperated eye. Though it is possible that the postoperative decrease in IOP might have been due to causes other than aqueous humor filtration, the increased outflow facility found by tonography in these operated eyes indicated that functioning filters were present.

It is known that steroids delay wound healing and inhibit the formation of granulation tissue by decreasing the influx of inflammatory cells. This impairment is dose related. A recent prospective randomized study on the use of postoperative corticosteroids for trabeculectomy showed a statistically greater success rate in those patients receiving topical, but not systemic, steroids.

Steroids are only effective in decreasing fibrosis early in the wound healing process, and not when granulation tissue is already present. A previous report of the effect of topical cortisol on the operated rabbit eye showed a decrease in sceral and epithelial healing. In another study of trephine incisions in rabbits, it was found that cortisone administered intramuscularly, but not subconjunctivally, after surgery was effective in decreasing the degree of fibrosis.

D-penicillamine can be beneficial in ocular wound healing because it interrupts collagen synthesis, and because it possesses anti-inflammatory activity. D-penicillamine causes metabolic changes in collagen that alter its biochemical solubility. Newly formed, more soluble collagen can then delay wound healing.

Intraperitoneal D-penicillamine was not effective in prolonging the function of the filters. Topical dexamethasone ointment was additive to intraperitoneal D-penicillamine, although the number of rabbits was too small to demonstrate statistical significance. It appears that the effect of the combination was simply due to the dexamethasone alone.

It was concluded that topical dexamethasone ointment alone or D-penicillamine given subconjunctivally...
The biologic effect of 5β-dihydrocortisol on collagen synthesis and the patency of a thermal sclerostomy in non-glaucomatous albino rabbits.

Topical dexamethasone ointment was not additive to subconjunctival D-penicillamine, although each drug alone caused a significant increase in the duration of filter function. The reason for this lack in additivity is unclear. Perhaps the two drugs would have been more additive if subconjunctival D-penicillamine had been given several days later, at a more beneficial time in the sequence of scar tissue formation. D-penicillamine acts by preventing the cross linkages which are needed to form mature collagen after new tropocollagen has been secreted extracellularly. If some retardation of the surgically induced influx of inflammatory cells can be accomplished with topical dexamethasone,3 and if subsequent inflammatory-cells-turned-fibroblasts and migrated fibroblasts can be blocked from producing new extracellular matrix collagen by D-penicillamine, perhaps the scarring process can be foiled at two steps rather than one. Clearly, in this study, the optimum method of combining these two drugs was not found.

Key words: collagen, D-penicillamine, dexamethasone, sclerostomy, outflow facility, intraocular pressure, filtering surgery

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References


Potentiation of Collagen Synthesis in Explants of the Rabbit Eye by 5β-Dihydrocortisol

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The biologic effect of 5β-dihydrocortisol on collagen synthesis was evaluated. The metabolite was found to potentiate subthreshold levels of dexamethasone in increasing 3H-proline incorporation in cells of the outflow region of the rabbit. Digestion of the tissue with highly purified collagenase indicated that the 3H-proline was incorporated into collagen type protein. This study demonstrates another biologic activity of 5β-dihydrocortisol, a metabolite found to accumulate in cells cultured from trabeculectomy specimens from patients with primary open angle glaucoma. Invest Ophthalmol Vis Sci 27:1757–1760, 1986

Topical ocular administration of 5β-dihydrocortisol, a cortisol metabolite, has been shown to potentiate the dexamethasone and cortisol induced high-affinity nuclear binding of the glucocorticoid receptor in the rabbit iris-ciliary body, an early and necessary event in steroid hormone action. In addition, this metabolite was found to potentiate the dexamethasone-induced elevation of intraocular pressure (IOP) in young rabbits. The present study utilizes 3H-proline incorporation to demonstrate an additional glucocorticoid potentiating activity of 5β-dihydrocortisol. Dexamethasone has been previously found to increase the incorporation of 3H-proline in the outflow pathway cells of the rabbit.

Materials and Methods. Eyes from young albino rabbits (<2 Kg) were enucleated and rinsed in sterile phosphate buffered saline. The anterior segment was then removed by cutting the sclera 2 mm posterior of the limbus. The central region of the cornea was removed. The tissue was then sectioned radially into slices 1–2 mm thick. The tissue slices contained the outflow region, adjacent sclera, cornea, and iris-ciliary body. The explants were incubated at 37°C in 1 ml of Dulbecco's modified Eagle medium supplemented with...