The dose-dependent effect of epidermal growth factor (EGF) on the development of wound tensile strength following full-thickness corneal wounds was evaluated in 60 adult rabbits. One eye from each rabbit received a single 7-mm long corneal incision. After injury each rabbit was treated three times daily for 5 or 10 days with either EGF at 0.001 mg/ml (10 eyes), 0.01 mg/ml (10 eyes), 0.1 mg/ml (10 eyes), 1.0 mg/ml (15 eyes), or vehicle (15 eyes). The tensile strength of the wound was evaluated using a 5-mm wide strip of cornea mounted on a tensiometer. We found that EGF at 0.1 mg/ml and at 0.01 mg/ml increased wound strength by 100% at 5 days and by 60% at 10 days (P < 0.05 and P < 0.05). However, EGF at 0.001 mg/ml and 1.0 mg/ml appeared to have no effect on wound strength. Histologic examination of full-thickness wounds in a separate series showed an increase in wound fibroblastic response and a diminished fibrin clot at 5 days in rabbits treated with 0.1 mg/ml and 0.01 mg/ml. We conclude that EGF enhances the wound strength of full-thickness corneal wounds in a dose-dependent manner which may be explained in part by an increased fibroblastic response. Concentrations of EGF greater or less than an optimal dose may be less effective in enhancing corneal wound strength. Invest Ophthalmol Vis Sci 30:2403-2406, 1989

Epidermal growth factor (EGF) has been demonstrated in vivo to increase the development of wound tensile strength in both rabbits and primates when applied topically at a concentration of 0.5 mg/ml and 0.1 mg/ml, respectively. Histologic evaluations of these eyes suggest that EGF stimulates wound fibrosis, perhaps by inducing both increased cell proliferation and collagen biosynthesis. Recent in vitro studies have shown that the effect of EGF on fibroblast proliferation is dose-related. Concentrations outside the optimal range—high doses of EGF in particular—appear to have little or no effect on cell culture. To our knowledge the in vivo dose response of corneal wounds is unknown.

We tested topically applied EGF to full-thickness corneal wounds in rabbits to evaluate the relative effect of a 1000-fold change in EGF concentration. We also examined these wounds histologically to evaluate the effect of EGF on the epithelium, the stromal keratocytes, and the formation of fibrin plugs in full thickness corneal wounds.

**Materials and Methods.** Sixty adult, New Zealand white rabbits (2-3 kg) were used in this study, in compliance with the ARVO Resolution on the Use of Animals in Research. Each animal received a general anesthetic with intramuscular rompun (2 ml) and the eyelid was prepared with Betadine solution. Under the operating microscope, a single stab incision in one eye was made with a stainless steel blade (Beaver #65; Beaver Surgical, Waltham, MA) creating a full-thickness linear wound of 7 mm in the central cornea. The eye was then closed with five 10-0 nylon sutures. A single application of Gentamycin ophthalmic ointment with propylparaben preservative was then applied.

After wounding, each animal received one drop of either EGF in buffered phosphate vehicle or buffered phosphate vehicle alone three times per day. The EGF used in this study was biosynthetic human EGF and was kindly provided by AMGEN, Inc. (Thousand Oaks, CA). The concentrations of EGF tested were 1.0 mg/ml, 0.1 mg/ml, 0.01 mg/ml, and 0.001 mg/ml. Each concentration was tested for a treatment period of both 5 and 10 days with five animals in each group.

At the end of each treatment period, the animals were sacrificed and the wounded eyes removed. The corneal scleral rim was dissected from the globe and, with the operating microscope, a strip of cornea 5-mm-wide and 12 mm long was cut perpendicular to the wound. The sutures were carefully removed with microscopic dissection, and each strip was mounted on a tensiometer. Tension was then applied until the wound separated. The tension required to separate the wound completely was recorded on a chart.

Histologic examination of these wounds was performed in a separate series of experiments using identical techniques and concentrations. In this
series, two eyes at each concentration were treated for 5 days. The eyes were then removed, and the corneas were fixed in 4% glutaraldehyde and examined with light microscopy.

**Results.** We found that 5-mm-wide strip of cornea containing a full-thickness corneal wound from animals treated 5 days with vehicle alone required an average of 30 ± 10 g tension to rupture. However, corneas from animals treated with 0.10 mg/ml EGF three times per day required 68 ± 28 g tension to rupture, and with EGF at 0.01 mg/ml required 60 ± 22 g tension to rupture. Both of these concentrations produced a 100% increase in wound strength and were significantly different from controls (P < 0.05 respectively) in the groups treated days. EGF applied at the highest (1.0 mg/ml) or lowest (0.001 mg/ml) concentration showed no increase in wound strength over controls (Fig. 1).

In groups treated 10 days, the control wounds exposed to vehicle alone required 176 ± 46 g tension to rupture, while animals that received EGF at 0.10 mg/ml or 0.01 mg/ml increased wound strength to 237 ± 38 g and 297 ± 60 g respectively. This represented a significant increase (60%) in wound tensile strength over controls (P < 0.05). When the highest concentration (1.0 mg/ml) was applied, the wound strength was found to be 145 ± 39 g, which was less than control values. Wounds treated with EGF at the lowest concentration (0.001 mg/ml), like those in the 5-day test group, (191 ± 37 gm) were not different from control wounds (Fig. 1).

Histologic examination of the corneas in a separate series indicated that vehicle-treated eyes showed little fibroblastic response within the wound 5 days after injury (Fig. 2). Surface epithelium and fibrin appeared to fill the wound, and minimal cell reaction or fibroblastic activity was found at the wound margin. All wounds treated with either vehicle alone or with EGF showed minimal inflammatory response around the site of injury. Treatment of corneal wounds with either 0.1 mg/ml or 0.01 mg/ml EGF, however, showed an increase in the number of fibroblasts in the corneal stroma at the wound edge and within the wound. There was also a corresponding decrease in the amount of fibrin matrix found in the

![Fig. 2. Histology of full-thickness corneal wound after 5 days of control vehicle applied three times per day. Considerable fibrin remains in the wound. The fibroblastic response is modest and the wound has not drawn together.](image)
Fig. 3. Histology of corneal wound after 5 days of EGF applied at a concentration of 0.01 mg/ml. There is minimal fibrin remaining in the wound and the wound is filled with a dense fibroblastic response. There are increased fibroblasts evident in the stroma and the wound edges are drawn closer together.

wound (Fig. 3). The highest concentration, 1.0 mg/ml, showed no fibroblastic response, and retained the fibrin matrix, in the wound (Fig. 4). Moreover, keratocytes adjacent to the wound margin appeared pyknotic and fragmented.

It has been shown that the epithelium plays a substantial role in the development of wound strength in full-thickness corneal wounds and is responsive to stimulation from EGF.² It was not possible, however, to evaluate separately the role that the epithelium

Fig. 4. Histology of corneal wound after 5 days of EGF at a concentration of 1.0 mg/ml. Fibrin loosely fills the wound, and the cellular response is diminished compared to that at lower EGF concentrations. Keratocytes adjacent to the wound margin appear pyknotic and fragmented.
played in the development of wound tensile strength in this animal model, because we did not examine the wounds histologically until the fifth day, by which time all wounds were fully covered by epithelium. No metaplasia was seen in the epithelium of any of these wounds treated either with vehicle alone or with EGF.

**Discussion.** EGF has been shown to accelerate the development of tensile strength in full-thickness corneal wounds, both in the rabbit model when applied at a concentration of 0.5 mg/ml and in the primate model when applied at a concentration of 0.1 mg/ml. This accelerated healing probably represents a number of separate effects: (1) stimulation of corneal epithelial regeneration and fibronectin synthesis, and (2) activation of human corneal fibroblasts, which accelerates the incorporation of tritiated thymidine. This latter effect has been demonstrated in vitro and has been shown to be concentration-dependent: when cells are exposed to high concentrations of EGF, the incorporation of tritiated thymidine decreased. EGF has also been shown to stimulate cell proliferation in cultured endometrial carcinoma cells when applied at low concentration, and to decrease cell proliferation at a high concentration.

Our experiment supports the hypothesis that an excess concentration of EGF reduces the effects of EGF seen at lower concentrations. This reduction may result from down-regulation of the EGF receptor when cells are maximally stimulated with EGF. This down-regulation may ultimately lead to the deceleration or inhibition of the wound healing process seen in our study.

Our histologic findings show that EGF stimulation increases the number of fibroblasts adjacent to the wound and also affects the amount and density of fibrin persisting in the wound. Similar effects were previously observed in partial-thickness corneal wounds stimulated with mesodermal growth factor. When the highest concentration of EGF was tested, we found the number of fibroblasts at the wound edge was markedly diminished compared to the lower concentrations of EGF and to controls. Fibrin is deposited in the very early phase of wound healing and later is removed as healing proceeds. Therefore, the persistent fibrin seen in wounds treated for 5 days with very high and very low concentrations of EGF (1.0 mg/ml and 0.001 mg/ml) is evidence that wound healing is less advanced in these eyes compared to eyes treated with EGF at 0.1 mg/ml and 0.01 mg/ml. It appears that for EGF to accelerate wound healing effectively in full-thickness corneal wounds, it must be applied at an optimal concentration.

These wounds were closed with five interrupted 10-0 nylon sutures to simulate the closure of deliberate surgical incisions. The presence of these sutures undoubtedly influences the reparative process, and in subsequent experiments, we will eliminate the use of sutures. Every effort was made to perform the histologic examinations in areas as remote from the sutures as possible.

EGF may possibly deactivate in the presence of the preservatives benzalkonium chloride and Polyquad. In our experiments, a Gentamicin ointment, preserved with propylparaben, was applied at the end of the wounding procedure. It is possible that propylparaben also has some inhibitory effect on EGF, but this question has not yet been resolved.

**Key words:** cornea, growth factors, wound healing, fibroblasts

From *the Center for Sight, Georgetown University Medical Center, Washington, DC; †University of Arizona, Tucson, Arizona; and ‡Ohio State University, Columbus, Ohio. Presented at ARVO Annual Scientific Meeting, May 5, 1988. Supported in part by a grant from the NEI (EY-07348) and an unrestricted grant and Manpower Award from Research to Prevent Blindness Inc. Submitted for publication: October 21, 1988; accepted April 13, 1989. Reprint requests: William D. Mathers, MD, Center for Sight, Georgetown University, Washington, DC 20007.

**References**