The fact that IDU at frequent application impairs corneal wound healing resulted in the general belief that it would delay wound healing even when used only a few times a day. This misconception has resulted in withholding IDU post-operatively in patients with previous history of herpetic keratitis, in which corticosteroids had to be used to reduce inflammation. Since both toxicity and therapeutic effect follow dose-response relationships and the effect of therapeutic and toxic ranges may differ, it may be possible to arrive at a toxic level, particularly when a drug like an antimetabolite is used. On the other hand, it is also possible to arrive at a regimen which provides the desired therapeutic effect and the least toxic side effect. For example, when treating herpetic keratitis, particularly in cases where only epithelial lesions are present, stromal wound healing is of little importance. In contrast, if epithelial healing is delayed, a significant correlate of the use of this medication would result. However, IDU has never been found to significantly decrease epithelial wound healing. Its effect in retarding stromal wound healing in this clinical situation where no stromal wounds are present somehow seems less important. Of even greater importance is the fact that it is in cases where stromal wounds are present such as after cataract extraction or penetrating keratoplasty in eyes with previous herpetic infections or chronic use of corticosteroids where the application of IDU four times a day may prevent herpetic infections. On the basis of this study, it appears that the present strength of IDU drug, 0.1 per cent, is just strong enough to be used for prevention of herpetic keratitis in such eyes without impairing corneal wound healing.

The fact that trifluorothymidine has a more striking effect on wound healing is not surprising since it is so much more effective than IDU. It may be necessary to consider this effect in its clinical use, however. After all, it should be expected that any drug which has an effect on viral DNA synthesis might also affect the healing process.

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REFERENCES


Kinetics of corneal epithelial regeneration. II. Epidermal growth factor and topical corticosteroids. PATRICK C. HO* AND JAMES H. ELLIOTT.

The kinetics of rabbit corneal epithelium regeneration were studied to determine if topical corticosteroid has an adverse effect on corneal epithelial wound healing, and if epidermal growth factor (EGF) can abrogate any adverse effect of topical corticosteroid. Healing of standardized 7 mm.
central corneal epithelial wounds was determined by serial standardized color photography of the fluorescein-stained defects and planimetry of the projected photographs. It has been found that topical application of 16 drops per day of vehicle or Decadron decreased the epithelial healing rate as compared to saline drops four times daily. Decadron 0.1 per cent given hourly (16 drops daily) was no more detrimental to corneal epithelial healing rate than the vehicle similarly applied. EGF exhibited no capacity to alter the corneal epithelial healing rate when hourly drops of either the vehicle or Decadron 0.1 per cent were given. Under the conditions of these experiments, no adverse effect on corneal epithelial healing rate could be attributed to Decadron 0.1 per cent.

Epidermal growth factor (EGF), first isolated by Cohen1 from mice submaxillary glands, has been shown to enhance the healing of experimental corneal epithelial wounds.2-4 Recently, in a kinetic study of corneal epithelial regeneration and EGF, Ho and colleagues1 have quantitated this accelerating effect of EGF on corneal epithelium regeneration. The present studies are undertaken, employing the same quantitative kinetic technique used previously4 to determine if topical corticosteroids have an adverse effect on corneal epithelial regeneration, and to determine if EGF can abrogate any adverse effect of topical corticosteroids on corneal epithelial regeneration.

Material and method.

Epidermal growth factor.* EGF was isolated from the submaxillary glands of adult male mice and purified by the new procedure of Savage and

*EGF was kindly supplied by Dr. S. Cohen at Vanderbilt University School of Medicine.
Corticosteroid. Decadron (Merck, Sharp, & Dohme) sterile ophthalmic solutions consisting of 0.1 per cent dexamethasone sodium phosphate in a vehicle mixture was used. The vehicle mixture alone was employed as control.

Experimental animals. Thirty-five pigmented adult rabbits of both sexes, weighing 3 to 5 kilograms each, were screened with a slit lamp bilaterally to detect any pre-existing eye disease. Only rabbits with both eyes free of disease were used in the experiments.

Kinetic studies. Rabbits were randomly divided into four groups and only one eye, chosen randomly, of each animal was used: (1) Group I, 10 rabbits receiving the vehicle; (2) Group II, 10 rabbits receiving Decadron 0.1 per cent; (3) Group III, 8 rabbits receiving the vehicle and EGF, 0.5 mg. per milliliter; and (4) Group IV, 7 rabbits receiving Decadron 0.1 per cent and EGF, 0.5 mg. per milliliter.

The experimental procedure for each rabbit was performed in a similar manner to the method described in details by Ho and co-workers. Standardized 7 mm. diameter central corneal epithelial wound was produced under a binocular dissecting microscope using a No. 15 Bard-Parker blade. Special efforts were made to prevent damage to the corneal stroma. Immediately after wound production, a drop of EGF solution (0.5 mg. per milliliter) was instilled into the cul-de-sac of animals in Group III and Group IV; and two more drops of EGF solution were applied at four-hour intervals on the same day starting from recovery from anesthesia. Also, at the completion of wound production, one drop of either Decadron 0.1 per cent or the vehicle was applied, respectively, to all animals in the four groups. Eight more drops of either Decadron 0.1 per cent or the vehicle were applied at hourly intervals on the same day starting from recovery from anesthesia. On the following days, EGF drops were given four times daily at hourly intervals to animals in Groups II and IV, and Decadron 0.1 per cent and the vehicle were given sixteen times daily at hourly intervals to animals in Groups II and IV, and Groups I and III, respectively, until complete healing of the epithelial wound. The progressive decrease in area of central corneal epithelial wounds was determined by serial standardized color photography of the fluorescein-stained defects four times daily; planimetry of the projected photographs; and the conversion of the projected areas to the actual size of the corneal wounds in square millimeters.

By plotting the serial wound area against the time the photographs were taken, a healing curve for each animal was obtained. Slopes, representing the healing rates of corneal epithelial wounds were calculated for all the healing curves individually, and the results were averaged for all the animals within each of the four experimental groups. Also, immediately upon complete absence of fluorescein staining of the corneal epithelium, the animals were killed. The experimental eyes were enucleated, stained with hematoxylin and eosin (H & E), and prepared for histologic examination.

Results. Thirty-five healing curves were obtained each consisting of thirteen to fourteen experimental coordinate points on the average. The initial area of central corneal wounds ranged from 42.9 square millimeters to 60.5 square millimeters and healing time spanned from 60 to 210 hours. All thirty-five healing curves appeared linear on the average. The progressive decrease in area of central corneal epithelial wounds is represented by the rate of decrease in area of epithelial wound in Table I. The linear correlation coefficient for all healing curves ranged from 0.99 to 0.78. Chi-square test indicated that within each of the four experimental groups, there was no statistically significant difference in healing rates between the groups. The mean rate of decrease in epithelial wound area for saline control four times a day is 0.78 ± 0.11 square millimeter per hour.

### Table I. Rate of decrease in area of epithelial wound (square millimeters per hour)*

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Times a day</th>
<th>Mean ± S.D.</th>
<th>P-values (saline control four times a day)§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Vehicle†</td>
<td>4 times a day</td>
<td>0.52 ± 0.20</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Decadron 0.1%†</td>
<td>4 times a day</td>
<td>0.43 ± 0.24</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Group II</td>
<td>Vehicle†</td>
<td>4 times a day</td>
<td>0.43 ± 0.12</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Decadron 0.1%†</td>
<td>4 times a day</td>
<td>0.60 ± 0.09</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Group III</td>
<td>Vehicle†</td>
<td>4 times a day</td>
<td>0.31 (0.85)</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Decadron 0.1%†</td>
<td>4 times a day</td>
<td>0.30 (0.97)</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Group IV</td>
<td>Vehicle†</td>
<td>4 times a day</td>
<td>0.28 (0.98)</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Decadron 0.1%†</td>
<td>4 times a day</td>
<td>0.49 (0.98)</td>
<td>P &gt; 0.05</td>
</tr>
</tbody>
</table>

*Each value listed represents the healing rate obtained from an animal.
†Given one drop hourly for 16 hours a day.
§Mean rate of decrease in epithelial wound area for saline control four times a day is 0.78 ± 0.11 square millimeter per hour.

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1 The vehicle consisted of an aqueous solution of creatinine, sodium citrate, sodium borate, polysorbate 80, sodium hydroxide, and water. Sodium bisulfite 0.32 per cent, phenylethanol 0.25 per cent, and benzalkonium chloride 0.02 per cent added as preservatives.
no significant correlation between the healing rate and the initial area of corneal wound (p > 0.01).

The mean rates of decrease in epithelial wound area were also calculated for each of the four groups of animals. As indicated in Table I, p-values obtained from two sample t-tests revealed that the difference in the mean healing rates between the vehicle treated (Group I), and any one of the other three groups was statistically insignificant at the 0.05 level. Analysis of variance of the rates of epithelial healing with the four groups of animals also showed no statistical significance at the 0.05 level.

Histologic examination of all experimental eyes enucleated immediately after complete absence of fluorescein staining revealed no sign of inflammation in the limbus, cornea, trabeculum, iris, and ciliary body. In the eyes from Group I and Group II animals, the corneal epithelium was two to three cell layers in thickness while in the EGF-treated groups, the corneal epithelium was five to six layers in Group IV eyes, and in four of the eight experimental eyes from Group III. In the remaining four eyes from Group III, the corneal epithelium was about ten layers in thickness. In these four eyes from Group III besides hyperplasia, hypertrophy of corneal epithelial cells was also noted. The correlation between the number of regenerated epithelial cell layers and the total number of EGF drops applied could not be ascertained. Other than the number of epithelial cell layers, there was no observable difference in eyes from the four groups.

Discussion. In the present study, the production of corneal epithelial wounds and the determination of epithelial regeneration were performed under exactly similar physical circumstances and with similar technique as described in a previous publication. The data was processed and presented in kinetic form in which the quantitative parameter for epithelial regeneration was healing rate (decrease in wound area per hour). As indicated by the p-values at the end of Table I, the mean healing rates of the four groups in this study were compared to the mean rate of decrease in epithelial wound area (0.78 ± 0.11 square millimeter per hour) of Group A animals treated with saline topically, four drops per day, as reported previously. The mean rates of epithelial healing in these experiments were, without exception, significantly slower than that obtained when saline drops were topically applied four times daily.

Our data suggested that topical application of 16 drops per day of vehicle or Decadron decreased the epithelial healing rate as compared to saline drops four times daily. Rabbits in Groups I and II received sixteen topical drops daily while those in Groups III and IV received a total of twenty daily drops. There was statistical significance at the 0.001 level in the difference between the mean healing rate of Group I animals treated with sixteen daily drops of vehicle, and the mean healing rate of animals with four drops of saline a day.

It was interesting to note that Decadron 0.1 per cent given hourly and sixteen times daily, was no more detrimental to corneal epithelial healing rate than the vehicle similarly applied. This was demonstrated by the fact that the mean epithelial healing rate from Group II rabbits was not significantly different at the 0.05 level when compared to the mean healing rate from Group I rabbits treated with the vehicle, whereas when contrasted with the mean rate of healing from rabbits given saline four drops daily, the difference was statistically significant at the 0.001 level.

It was found that EGF exhibited no capacity to alter the corneal epithelial healing rate when hourly drops of either the vehicle or Decadron 0.1 per cent were given. No statistical significance was revealed by analysis of variance of the healing rate from the four groups, and by two sample t-tests of the four mean healing rates. The histologic finding that the regenerated corneal epithelium in the EGF-treated eyes was twice the thickness of the regenerated epithelium in the non-EGF-treated eyes confirmed the observation reported by various investigators that EGF induced corneal epithelium proliferation. This property of EGF persisted in the presence of Decadron 0.1 per cent and the vehicle. However, despite this enhancing effect of EGF on corneal epithelium, EGF displayed no tendency to nullify the decreased epithelial healing rate caused by the topical application of sixteen hourly drops per day.

Under the conditions of our experiments, no adverse effect of Decadron 0.1 per cent on corneal epithelium healing rate, when compared to vehicle, could be demonstrated. The insult of hourly topical drops caused a significant deleterious effect on corneal epithelial regeneration.

Decadron vehicle was supplied by Merck, Sharp and Dohme, West Point, Pa.

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*Student Scholarship Awardee of the Seeing Eye, Inc., Morristown, N. J.

Key words: Decadron, vehicle, epidermal growth factor, central corneal epithelial wound, healing rate, hourly topical drops.

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