A comparison of corneal epithelium regeneration in normal and buphthalmic rabbits

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A large globe and corneal clouding form an early finding in infantile buphthalmia in both rabbits and human subjects which is frequently associated with fragility and sloughing of the corneal epithelium. The severity of the lesions increases with the severity of the buphthalmia. This study was designed to determine comparatively if the severity of buphthalmia is reflected in the ability of corneal epithelium to regenerate. The epithelium was scraped from 34 rabbit corneas representing five categories of eyes: two normal groups and three buphthalmic. The daily rate of epithelial regeneration was calculated by planimetric measurements taken from enlarged photographs of fluorescein-stained eyes. The actual area of corneal epithelium regenerated daily by each eye is presented in tabular form. When these data were subjected to statistical analysis it was found that in rabbit eyes there is no relation between the severity of buphthalmia and the ability of the eye to regenerate corneal epithelium. Extremely buphthalmic eyes can regenerate as efficiently as the eyes of genetically normal animals from the same colony. There does appear to be a direct relation between the severity of the buphthalmia and the looseness of the regenerated epithelium. It is proposed that this looseness of the buphthalmic corneal epithelium may be related to a deficiency in the basement membrane.

Key words: buphthalmos, corneal epithelium regeneration, corneal staining, fluorescein stain, photography, time factors, anterior keratectomy, corneal basement membrane, adhesion, corneal epithelium, corneal stroma, statistical study, rabbits.

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Congenital glaucoma (buphthalmia) in its extreme manifestation is associated with an enlargement of the entire globe, especially the anterior third of the eye. The cornea gradually becomes opaque and edematous with a progressive fragility and sloughing of its epithelium. The increase in corneal fragility and cornification was described in the rabbit by Fox and Babino in the Jackson Laboratory and was further elaborated by Babino and Fox and Sheppard, Shanklin, and Fox.
pard and Shanklin extended the observations on this group of animals by describing five discrete stages which characterize the progressive increase in epithelial fragility in the buphthalmic rabbit cornea.

The remarkable regenerative and healing capacity of the vertebrate corneal epithelium has been known for many years and is well documented (for review of older literature see Arey and Covode; for review of more recent work see Khodadoust, Silverstein, Kenyon, and Dowling). Buschke showed that various drugs administered either systemically or topically can affect the rate of corneal regeneration as can temperature variations. No reports have been found of the effects of disease on the rate of corneal epithelium regeneration.

In view of the profound effect that buphthalmia has on the cornea, it is the purpose of this paper to determine how the varying degrees of buphthalmia affected the rate of corneal epithelium regeneration as compared to the regeneration rate in normal animals.

Materials and methods

**Material.** Eighteen rabbits were used in the study: 5 New Zealand Whites (hereafter designated Local Normals) were obtained from a local supplier and 13 animals were obtained from The Jackson Laboratory, Bar Harbor, Maine. These 13 consisted of 3 normal rabbits (+/+) (hereafter designated JAX normals) and ten buphthalmics (bu/bu). The buphthalmic animals were classified as **Predisposed** (3 animals); **Mild** (3 animals), and **Extreme** (4 animals).

The criteria used in classifying the above buphthalmic animals were:

- **Predisposed.** The progeny were from a mating of both buphthalmic parents bu/bu × bu/bu but showing no clinical manifestations of buphthalmia. Ocular pressure was normal to palpation (pressure was not taken with the mercury manometer on any animals in this study for fear of disturbing the epithelium or causing possible intraocular inflammation due to trauma). The cornea was clear, the angle open, and the fundus within normal limits. Pap and Giemsa stains showed few cornified epithelial cells with some pyknotic nuclei and occasional non-nucleated cells. Diagnosis: Bu + 1 from epithelial examination.*

- **Mild.** Pressure was normal to palpation and there were whorls of corneal opacity and circum-corneal injection. The angle was wide, the fundus was seen with difficulty, the nerve as pale with grayish optic atrophy, and there was early cupping. Pupillary reactions were sluggish. Epithelial smears: both Pap and Giemsa stains showed many cornified, vacuolated, and non-nucleated epithelial cells. Diagnosis: Bu + 3.

- **Extreme.** Pressure to palpation was firm, the cornea had marked clouding, pits, bedewing, healed old ulcers, frequent pannus, and circum-corneal injection. Fundi were not seen due to corneal clouding. Epithelial smears: both Pap and Giemsa staining methods showed many advanced cornified cells; fragmented, pyknotic nuclei and vacuolated, non-nucleated cells. Diagnosis: Bu + 4.

**Experimental procedure.** Aseptic procedures were followed, yet two eyes became infected during the course of the study. These eyes were not included in the analytical data.

Eyes were rinsed with sterile physiological saline and anesthetized with two drops of 3 per cent cocaine in Ringer’s solution. The cornea was scraped with the sharp edge of a scalpel blade until the stroma was exposed to the limbic boundary. Efficiency of the procedure was determined by staining each scraped eye with a 2 per cent solution of sodium fluorescein. This immediately revealed any areas not completely denuded of epithelium. Such areas were rescraped and reexamined. When the epithelium had been completely removed, the eyes were rinsed with sterile saline and restained with fluorescein for photographic recording. Each eye received twice daily irrigation followed with topical applications of 1 per cent atropine sulfate and chloromycetin-polymyxin until regeneration was complete.

Photographs were made with a 35 mm. Exa camera (f3.5 Tessar lens) using a combination of visible and ultraviolet light. The long wavelength U-V light elicited a brilliant primary fluorescence from the fluorescein-stained tissues. The light was obtained by covering a Honeywell Model 65A Strobomar flash unit with a non-specific “black-light” filter. Visible illumination was provided by overhead fluorescent room lights. A No. Y-2 (Med.) yellow filter provided desirable contrast and a No. +3 copy lens provided an initially enlarged image. Each eye was photographed immediately after scraping and at daily intervals thereafter (Figs. 1 to 8). Exposures were made on Kodak Tri-X panchromatic film at 1/50 second exposure at an f-4 stop. The subject-to-lens and subject-to-light distances were 9" and 11" respectively.

Images from the negatives were magnified with a photographic enlarger 3x as determined by measurements of the image reference scale with a No. 12 Vernier Caliper.
Fig. 1. Local—normal No. 790; right eye immediately after scraping.

Fig. 2. Local—normal No. 790; right eye 24 hours (day 1) after scraping; 22.9 per cent regenerated.

Fig. 3. Local—normal No. 790; right eye 48 hours (day 2) after scraping; 55.2 per cent regenerated.

Fig. 4. Local—normal No. 790; right eye 96 hours (day 4) after scraping; 98.5 per cent regenerated.

Fig. 5. Extreme buphthalmic No. 789; left eye immediately after scraping.

Fig. 6. Extreme buphthalmic No. 789; left eye 24 hours (day 1) after scraping; 42.9 per cent regenerated.

Fig. 7. Extreme buphthalmic No. 789; left eye 48 hours (day 2) after scraping; 58.7 per cent regenerated.

Fig. 8. Extreme buphthalmic No. 789; left eye 96 hours (day 4) after scraping; 89.2 per cent regenerated.
The enlarged images of the limbic boundary and the fluorescent denuded surface were traced on bond paper. The dimension of the denuded surface was calculated by averaging the results of three planimeter tracings of each image. The range of the extreme planimeter readings did not exceed 13.5 mm.2 on the enlarged image, so that when the data were reduced to the normal equivalent, the readings were accurate to within ±1.5 mm.2 of corneal area.

The small numbers of animals involved in the individual groups and the wide variability of performance by individual animals within groups severely limited the amount of pertinent statistical interpretation that could be developed from our data. However, all the statistics in this report and the major conclusion drawn from them were reviewed by our Biometry Department using their computer facilities. They subjected the data to an Analysis of Covariance test that adjusts for the initial difference in eye size and permits a meaningful comparison between groups of eyes.

**Observations**

The following data were extracted from Tables I and II. They are presented here in summary form grouped according to the types of animals used.

**Normal—local, 9 eyes.** The mean corneal area was 125.0 mm.2 (range 113.0 to 132.7 mm.2). The average cumulative regeneration rate was 29.3 per cent by the end of the first day and 90+ per cent by the end of the fourth day. All eyes had completed regeneration by the end of the fifth day.

**Normal JAX, 6 eyes.** The mean corneal area was 165.6 mm.2 (range 143.1 to 188.6 mm.2). The average cumulative regeneration rate was 31.8 per cent by the end of the first day and 93.2 per cent by the end of the fourth day. All eyes had completed regeneration by the end of the fifth day.

**Buphthalmic—predisposed, 6 eyes.** The mean corneal area was 181.2 mm.2 (range 165.0 to 213.5 mm.2). The average cumulative regeneration rate was 23.3 per cent by the end of the first day and 89.9 by the end of the fourth day. None of the eyes completed regeneration by the end of day 5, although 2 eyes completed on day 6. Three eyes (Nos. 798L and 799R & L) showed periods of regression. Regeneration was complete in all eyes by the end of day 9.

**Buphthalmic—mild, 5 eyes.** The mean corneal area was 219.4 mm.2 (range 188.6 to 240.4 mm.2). The average cumulative regeneration rate was 36.7 per cent by the end of the first day and 87.3 per cent by the end of the fourth day. One eye completed regeneration on day 4. Four eyes (Nos. 781R & L and 787R & L) showed periods of regression. Regeneration was complete in all eyes by the end of day 14.

**Buphthalmic—extreme, 8 eyes.** The mean corneal area was 260.3 mm.2 (range 226.9 to 298.5 mm.2). Average cumulative regeneration rate was 45.8 per cent by the end of the first day and 89.7 per cent by the end of the fourth day. None of the eyes were complete before the end of day 7. Seven of the eight eyes showed some degree of regression. Eye No. 786L developed an ulcer after day 9 and did not complete regeneration. All other eyes were complete by the end of day 21.

**General review of statistical data.** The amount of corneal epithelium regenerated daily was compared for individual eyes and by designated groups by means of the Analysis of Covariance Test. It was found that there was no significant difference among the five groups of eyes for day 1, day 2, day 3, or day 4. On day 5, however, there is a significant difference between the local—normal animals and all four groups of JAX animals. Without exception, in all the local—normals regeneration reached 100 per cent during day 5. Among all the JAX animals, only 3 of the 25 eyes reached 100 per cent by day 5. The cumulative average for all the JAX eyes at the end of day 5 was 95 per cent.

Of primary importance is the finding that among the JAX animals there was no significant difference in the performance of the JAX normal group and any of the buphthalmic groups. Nor was there any statistical difference among the buphthal-
Table I. Area of corneal epithelium (in square millimeters) regenerated per day.

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Actual Corneal Area (mm²)</th>
<th>RIGHT CORNEA</th>
<th>LEFT CORNEA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DAYS AFTER SCRAPING</td>
<td>DAYS AFTER SCRAPING</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 11</td>
<td>1 2 3 4 5 6 7 8 9 10 11</td>
</tr>
<tr>
<td>790</td>
<td>R - 132.7 L - 132.7</td>
<td>35.8 10.0 38.0 46.5 2.5</td>
<td>35.8 10.0 38.0 46.5 2.5</td>
</tr>
<tr>
<td>791</td>
<td>R - 122.7 L - 122.7</td>
<td>35.8 10.0 38.0 46.5 2.5</td>
<td>35.8 10.0 38.0 46.5 2.5</td>
</tr>
<tr>
<td>792</td>
<td>R - 122.7 L - 122.7</td>
<td>22.8 50.5 49.5 10.0</td>
<td>22.8 50.5 49.5 10.0</td>
</tr>
<tr>
<td>793</td>
<td>R - 113.0 L - 113.0</td>
<td>10.6 28.5 48.5 23.0 2.5</td>
<td>10.6 28.5 48.5 23.0 2.5</td>
</tr>
<tr>
<td>795</td>
<td>R - 132.7 L - 1</td>
<td>27.8 33.5 21.0 42.0 8.5</td>
<td>27.8 33.5 21.0 42.0 8.5</td>
</tr>
<tr>
<td>788</td>
<td>R - 165.0 L - 166.8</td>
<td>65.4 24.0 27.4 15.0 8.5</td>
<td>15.0 0.5 0.5 0.5</td>
</tr>
<tr>
<td>802</td>
<td>R - 188.6 L - 176.6</td>
<td>54.3 46.0 27.0 31.5 3.0</td>
<td>1.5 2.5 11.0</td>
</tr>
<tr>
<td>803</td>
<td>R - 153.9 L - 143.1</td>
<td>15.7 70.4 44.0 12.0 1.0</td>
<td>15.7 70.4 44.0 12.0 1.0</td>
</tr>
<tr>
<td>797</td>
<td>R - 165.0 L - 165.0</td>
<td>16.1 46.5 31.5 43.5 21.5 6.0</td>
<td>16.1 46.5 31.5 43.5 21.5 6.0</td>
</tr>
<tr>
<td>798</td>
<td>R - 165.0 L - 165.0</td>
<td>32.7 47.5 37.0 42.0 6.0</td>
<td>-3.0 3.0</td>
</tr>
<tr>
<td>799</td>
<td>R - 213.5 L - 213.5</td>
<td>69.2 47.0 49.0 28.0 5.0</td>
<td>-5.0 20.5</td>
</tr>
<tr>
<td>781</td>
<td>R - 224.9 L - 240.4</td>
<td>37.6 92.9 26.0 13.0 42.0</td>
<td>-14.0 2.4 4.0</td>
</tr>
<tr>
<td>782</td>
<td>R - 240.4 L - 240.4</td>
<td>147.8 14.7 68.9 9.0</td>
<td>147.8 14.7 68.9 9.0</td>
</tr>
<tr>
<td>787</td>
<td>R - 201.0 L - 188.6</td>
<td>77.7 43.5 30.0 21.0 14.5</td>
<td>1.0 -3.5 1.5</td>
</tr>
<tr>
<td>786</td>
<td>R - 292.5 L - 292.5</td>
<td>105.1 69.4 25.0 8.0 6.0</td>
<td>0.4 -13.9 6.5</td>
</tr>
<tr>
<td>799</td>
<td>R - 254.3 L - 240.4</td>
<td>103.0 38.0 47.0 26.5 7.5</td>
<td>-8.0 19.5 7.0</td>
</tr>
<tr>
<td>796</td>
<td>R - 240.4 L - 226.9</td>
<td>61.6 67.9 28.5 20.0 0.5</td>
<td>1.5 8.0 (Regeneration Complete on Day 21)</td>
</tr>
<tr>
<td>801</td>
<td>R - 254.3 L - 268.7</td>
<td>131.3 82.9 34.0 -26.0 29.5 -20.5 23.0 14.5</td>
<td>131.3 82.9 34.0 -26.0 29.5 -20.5 23.0 14.5</td>
</tr>
</tbody>
</table>
mic groups that would relate to the severity of the buphthalmia. This strongly suggests that the condition responsible for the delayed completion of the final 5 per cent of epithelial regeneration in all the JAX groups was some factor common to all these animals—possibly associated with their larger eye size but not a function of the bu gene. It further indicates that buphthalmia, even in its extreme form, does not adversely affect corneal epithelium regeneration.

Multiple scrapings—preliminary observations. In anticipation of a follow-up study to the current one, a series of experiments was run to determine if there was an appreciable difference in the regeneration rate of second- and third-set regenerations. The results are not completely conclusive, but sufficient information is available to be of interest in this context.

All the rabbits involved in this present study were rescraped at various intervals after their initial regeneration was complete. In all cases the second-set regeneration rate approximated their original rate. The different intervals between scrapings did not appear to influence the rates.

One local—normal animal (not included in the present study) was scraped three consecutive times: the second scraping was 7 days after the first (1 day after completion of regeneration); the third scraping was 27 days after the second (21 days following completion of the second set regeneration). The results of the third set regeneration were: 122 mm.² (74 per cent) regenerated by end of 24 hours; 13 mm.² (8 per cent) regenerated during the second day; and 30.0 mm.² (18 per cent) regenerated during the third day to complete the regeneration. This was the shortest time we have recorded for complete regeneration.

Epithelial looseness and fragility. During the initial scraping procedure it was noted that the corneal epithelium of the buphthalmic rabbits was frequently very loose and fragile. The degree of looseness was generally correlated with the severity of the buphthalmia so that in the extreme —buphthalmic animals the epithelium could easily be removed in large sheets by lightly wiping the cornea with a moist gauze sponge. This same pattern of looseness was also observed for the regenerated epithelium when all of the eyes were rescraped. Unfortunately these observations did not lend themselves to quantitative expression but were merely subjective evaluations that were made in conjunction with the rescraping procedures.

The extent of the looseness was related to the length of time between scrapings. If the eyes were rescaped within a week of the completion of initial regeneration then the epithelium was only slightly adherent and could be easily removed in all five groups of animals. If ten days to two weeks elapsed before the second scraping, the epithelium of the local—normals, the JAX normals and the predisposed—buphthalmics all appeared tightly adherent to the underlying stroma, approximately equivalent to the original epithelium. Among the mild and extreme buphthalmics, however, the regenerated epithelium remained loose two to three weeks after the initial completion of regeneration.

Discussion

The well-documented phenomenon of rapid corneal epithelial regeneration was again demonstrated in this study. However, as far as we can determine, this is the first time that the rate of comeal epithelial regeneration has been determined quantitatively and the comparative rates used as an experimental parameter.

In view of the involvement of the corneal epithelium in advanced glaucoma it was expected that the regeneration efficiency of buphthalmic eyes would be affected by the disease and that the affect would probably result in an increase in the time required for regeneration. As shown in Tables I and II, this was not the case. Not only does the regeneration efficiency not decrease in the more extreme buph-
Table II. Percentage of corneal epithelium regenerated per day.

Thalmics, it actually appears to be more efficient at least in the early stages of regeneration. It is particularly interesting that the average regeneration rate for the extreme bu group shown in Table II for day 1 was 46 per cent whereas the comparable figure for the local—normal group was only 23 per cent. Thus during the first day following scraping the extreme bu eyes regenerated twice as rapidly, on the average, as did the local—normals. Of equal interest, however, is the speed with which this and the other differences in rate were compensated for so that by day 3 the cumulative average rate for both the local—normal and extreme bu groups was the same at 83 per cent.

Another apparent trend that deserves comment concerns the tendency among the JAX animals as a group to exhibit periods of regression during the regeneration of the final 5 per cent of the epithelium. These periods are indicated in Table I by the negative values. When the incidence of these periods is compared for the different groups it appears that there is a
A comparison of corneal epithelium regeneration

A direct relation between the severity of the buphthalmia and the occurrence of the regression; however, the small number of samples in each group and the variability among groups prevent us from knowing if this is actually a meaningful difference. When analyzed statistically the differences were found not to be significant.

That both the delay in completion of the regeneration and the periods of regression are found in all groups of JAX animals, whether normal or buphthalmic, strongly suggests that this is a genetic trait or some other factor unrelated to the bu gene that is shared by all JAX animals used in this study. Based on the foregoing we concluded that there is no relationship between buphthalmia and the ability of the corneal epithelium to regenerate.

It must be pointed out, however, that in this study the criterion for epithelial regeneration is merely the epithelial recovery of denuded stroma as demonstrated by fluorescein staining. Khodadoust and his associates6 have recently shown that mere epithelial recovery is not a definitive criterion for regeneration, but that the re-establishment of the normal tight adhesion of the epithelial layer to the underlying stroma is an integral second phase of regeneration. They showed that when the normal corneal epithelium was removed by light scraping (as was done in our study), the epithelium was completely regenerated by the end of 5 days and tight adhesion of the new epithelium was re-established by the end of 6 to 7 days. If, instead of scraping, the epithelium was removed by keratectomy so that a portion of the stroma was removed with it, the epithelium still covered the wound as quickly as before, but the new epithelium remained loose and “unadhesive” for periods of up to six weeks. Khodadoust concluded from preliminary electron microscopy studies of his material that, when the epithelium was removed by scraping, the basement membrane remained essentially intact and the re-establishment of adhesion in these cases involved an attachment of the regenerated epithelial cells to the old basement membrane. In the keratectomized animals, the basement membrane was removed with the epithelium. Thus, although epithelial migration occurred just as readily over the denuded stroma, tight adhesion, the second component of regeneration, could not be established until a new basement membrane was synthesized.

This similarity between the above description and our observations on the regenerated epithelium is quite interesting. The normal animals of both groups and the predisposed buphthalmics behaved as did the animals in the scraping experiments of Khodadoust. The epithelium covered the stroma in approximately a week but was only loosely attached at that stage. It then became tightly adherent during the second week after regeneration. The manifest buphthalmics, on the other hand, resembled the animals whose basement membranes had been removed by keratectomy even though their epithelium had been removed by only light scraping. This similarity suggests that the basement membrane of the buphthalmic rabbits may differ from the normals and could be either initially deficient or of such a nature that it is removed by the light scraping. The loose condition of the original epithelium at the time of initial scraping would seem to favor the former hypothesis.

Khodadoust suggests that the pathological condition “recurrent corneal erosion” may result from a deficiency in the basement membrane. We would like to propose that the epithelial fragility and looseness associated with human glaucoma and demonstrated by our buphthalmic rabbits may be related to a similar failure in the adhesive relationship between the epithelium and the basement membrane. Histological and electron microscopic studies are planned to examine this relationship more closely.

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REFERENCES


