Prevention of stromal ulceration in the alkali-burned rabbit cornea by glued-on contact lens. Evidence for the role of polymorphonuclear leukocytes in collagen degradation.

K. R. Kenyon, M. Berman, J. Rose, and J. Gage

Stromal ulceration of the alkali-burned rabbit cornea was found to be associated invariably with phagocytically active polymorphonuclear leukocytes (PMNs). A glued-on methylmethacrylate lens applied to corneas soon after burning, however, prevented re-epithelialization and also prevented PMN infiltration of the stroma and stromal ulceration. Subsequent partial detachment or complete removal of the lens resulted in epithelial resurfacing of the stroma, PMN infiltration, and stromal ulceration. Glued-on lenses applied to already ulcerating corneas arrested further ulceration by prohibiting additional PMN infiltration. Either surface debridement or glued-on methylmethacrylate rings also prevented re-epithelialization and ulceration in stromas not infiltrated by PMNs, but neither treatment was sufficient to prevent ulceration in corneas already containing numerous PMNs. The data suggest the possibility that the epithelium stimulates infiltration of the stroma by PMNs which then participate in stromal matrix degradation. Although no claim is made that only PMNs mediate matrix destruction in corneal ulceration, the efficacy of the lens would seem to be due to exclusion of the epithelium and the consequent prevention of stromal infiltration by PMNs.

Key words: cornea, ulceration, alkali, polymorphonuclear leukocyte, contact lens, collagenolysis, cyanoacrylate tissue adhesive, electron microscopy

Although extensive evidence documents the actions of collagenase and other proteases in corneal ulceration,1-4 the specific roles of various cell types are not well understood. The ability of epithelium from ulcerating corneas to lyse collagen gels5-9 suggests those cells as one source of collagenase. In addition, keratocytes in tissue culture have also been shown to produce collagenase.10 Moreover, the occurrence of polymorphonuclear leukocytes (PMNs) in actively ulcerating corneas,9, 11-13 and the well-known presence of collagenases14, 15 and other hydrolases16, 17 within the cytoplasmic granules of such cells implicate PMNs also as effectors of stromal matrix degradation.
Earlier glued-on lens experiments suggested that preventing re-epithelialization of the cornea and protecting the stroma from the tear film prevented stromal ulceration after alkali burns. The efficacy of the glued-on lens has been attributed to its ability to prevent collagenase of epithelial origin from reaching the stroma. It has become clear, however, that various other cell types might also participate in ulceration and that the efficacy of the lens might be due also to its effect on those cell types. The possibility that matrix degradation might involve interactions among different cells and the fact that regulation of collagenase activity can occur at different levels have prompted us to re-examine the effect of the glued-on lens at the light and electron microscopic levels in an attempt to understand the role of the various cell types in ulceration.

Materials and methods

Sixty-five albino rabbits (2 to 4 kg) were utilized in these experiments in five experimental groups as follows (summarized in Table I).

**Group 1. Alkali burn only (30 eyes).** Rabbits were anesthetized with intravenous pentobarbital and topical Proparacaine. A filter paper disc, 7 mm in diameter, was wetted with 4N sodium hydroxide (NaOH) solution and applied to the central cornea, for 2 min to produce a severe alkali burn. Precautions were taken to avoid involvement of peripheral cornea, conjunctiva, and lids. Immediately after burning, the cornea was rinsed with approximately 5 cc of normal saline solution, loosely adherent epithelium was gently debrided with a cotton applicator, and erythromycin ointment was instilled.

**Group 2. Contact lens (50 eyes).**

A. **Continuous application (30 eyes).** At intervals varying from 2 to 10 days after the standard alkali burn, loosely adherent corneal epithelium was removed with a cotton applicator, and polymethylmethacrylate contact lenses (approximately 9 mm in diameter) were bonded centrally to the stromal surface by isobutyl 2-cyanoacrylate tissue adhesive (Braun Melsungen) applied evenly to the periphery of the lens to form a complete adhesive ring. Erythromycin ointment was then applied.

B. **Lens applied on day 2, epithelial ingrowth permitted after day 14 (14 eyes).** Contact lenses were glued in place, as previously described, on day 2 following the alkali burn and allowed to remain in position until day 14. At that time the adhesive seal was broken for approximately 180 degrees of arc to permit epithelial ingrowth beneath the lens without removal of the lens.

C. **Lens applied on days 2 to 7, removed on day 14 (10 eyes).** Contact lenses were glued in position at various times from day 2 to day 7 following the alkali burn, maintained in position until day 14, and then removed entirely.

**Group 3. Rings (15 eyes).** Plastic rings (9 mm in outside diameter), made by removing from contact lenses of the central area 5 mm. in diameter (provided by D. R. Korb, O.D.), were glued by the previously described technique on day 2 after alkali burn, and left in position until day 21 (Fig. 1, G).

**Group 4. Epithelial debridement (19 eyes).**

Subgroup A. Beginning at day 2 after alkali burn and repeating on alternate days, all loosely adherent regenerating corneal epithelium was gently debrided with a cotton applicator (10 eyes) for a total of 21 days.

Subgroup B. Beginning on day 2 and continuing on alternate days until day 14, epithelial debridement was performed, after which time the epithelium was allowed to regenerate undisturbed (9 eyes).

**Group 5. Controls (16 eyes).**

A. **Contact lens or ring only (12 eyes).** Epithelium was removed from normal (non-alkali-burned) corneas by scraping with a Bard-Parker blade, and either a lens or ring was glued on centrally by the previous technique.

B. **Epithelial debridement every other day only (14 eyes).** Normal corneas were initially scraped with a Bard-Parker blade, and then the regenerating epithelium was debrided on alternate days with a cotton applicator.

Eyes of all treatment groups were examined daily, and slit-lamp biomicroscopy was performed when appropriate. Erythromycin ointment was instilled in the cul-de-sac following each examination. The extent of epithelial defects was determined with fluorescein or methylene blue–Azure II staining. The extents of stromal ulceration and neovascularization were recorded at each examination. Except in group 1 (alkali burn only) whose animals were sacrificed at intervals from day 6 to day 21 after injury, all other experimental groups were maintained until day 21, unless corneal perforation necessitated earlier termination. Any
Table I. Summary of histopathologic observations in alkali-burned corneas

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No. of eyes</th>
<th>Histopathology (at day 21)*</th>
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<tr>
<td></td>
<td></td>
<td>Epithelium</td>
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<tr>
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<td>-</td>
</tr>
<tr>
<td>1. Alkali burn only</td>
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<td>4</td>
</tr>
<tr>
<td>Sacrificed &lt;day 10*</td>
<td>26</td>
<td>17</td>
</tr>
<tr>
<td>Sacrificed day 10-21*</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>2. Alkali + lens</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Continuous from Day 2</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Nonulcerating when lens applied (2-10 days)</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Ulcerating when lens applied (7-10 days)</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>B. Apply lens day 2, permit epithelium under lens after day 14</td>
<td>15</td>
<td>14</td>
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<tr>
<td>C. Apply lens day 2-7, remove day 14</td>
<td>12</td>
<td>9</td>
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<tr>
<td>3. Alkali + ring (continuous from day 2)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>4. Alkali + epithelial debridement</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>A. QOD until day 21</td>
<td>10</td>
<td>10</td>
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<tr>
<td>B. QOD until day 14 only</td>
<td>9</td>
<td>7</td>
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<tr>
<td>5. Control</td>
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<td>12</td>
</tr>
<tr>
<td>A. Contact lens or ring</td>
<td>4</td>
<td>4</td>
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<tr>
<td>B. Epithelial debridement QOD</td>
<td>4</td>
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QOD = Every other day.

*All experimental groups terminated at day 21 unless otherwise specified. – = absent, + = mild, ++ = moderate, +++ = marked.

animal with clinically suspected ocular infection was immediately excluded from the study.

Eyes were enucleated immediately after animals were sacrificed by overdose of intravenous pentobarbital. Eighty-five eyes were prepared for light microscopic examination by fixation of the intact globes in 10% neutral formaldehyde, excision of the corneal segment, standard dehydration, paraffin embedding, serial sectioning, and staining with hematoxylin and eosin.

Forty-five corneas were prepared for transmission electron microscopy by fixation with 1.5% buffered glutaraldehyde followed by postfixation with 1% buffered osmium tetroxide. After fixation, the corneas were dehydrated in graded alcohols and embedded in epoxy resin. Serial sections were taken at various levels, stained with para-phenylenediamine, and examined by phase-contrast microscopy. Representative areas were ultrathin-sectioned, double stained with uranyl acetate–lead citrate, and examined with a JEOL 100-B transmission electron microscope.

Serial or semithin sections were examined by light microscopy for fibroblasts (keratocytes) and inflammatory cells (PMNs and monocytes), and the degree of stromal ulceration within the central 5 mm of the cornea was estimated.

Results

Group 1. Alkali burn only. The severe corneal alkali burn in these experiments provided a highly reproducible end-point of stromal ulceration within the 21-day term of the experiments. In the positive control group of 30 eyes observed to determine the natural clinical course following this standard injury, dense, opalescent opacification of the central cornea was instantaneous and persisted throughout the period of observation (Fig. 1, A). Edema of the uninvolved peripheral cornea cleared completely by day 3. Epithelium from the corneal periphery began to grow in about day 3 and usually resurfaced the cornea completely with a thin, irregular (hence fluorescein staining), and loosely adherent epithelial sheet by day 7. In some instances, the resurfacing by the epithelium was incomplete or epithelium was nonadhe-
Prevention of stromal ulceration

Inflammatory cells | Ulceration
---|---
- | + | ++ | +++
2 | 2 | - | + | ++ | +++
1 | 4 | 7 | 14 | 4 | 2 | 10 | 10
16 | 1 | 3 | 18 | 1 | 1
6 | 5 | 1 | 2 | 11 | 2 | 1
1 | 2 | 7 | 2 | 2 | 2 | 6
4 | 6 | 5 | 4 | 2 | 7 | 2
5 | 4 | 1 | 7 | 2 | 1
1 | 6 | 2 | 1 | 5 | 3
1 | 7 | 1 | 3 | 12
2 | 2 | 4

rent, so that epithelial defects were observed. Superficial and deep stromal neovascularization from the limbus began about day 5 and progressed steadily to reach the central cornea within 15 days. Stromal ulceration was only rarely evident before day 9. With the slit-lamp, however, development of clear lacunar spaces in the central stroma could often be observed on day 7, even in the presence of an intact epithelium. Such stromal lacunae would progressively enlarge, so that between days 9 and 14, epithelial breakdown and grossly apparent stromal ulceration would progress rapidly to descemetocele formation and perforation (Fig. 1, B). In this manner, 22 of 26 eyes (85%) developed unequivocal ulcers within 2 weeks. Although a direct relationship cannot be appreciated from the tabulated observations (Table I), there appeared to be a positive correspondence between the numbers of acute inflammatory cells (both adhering to the corneal surface and infiltrating the anterior stroma) and the extent of ulceration. The presence or absence of epithelium and/or fibroblasts did not seem to relate to ulceration. In the four nonulcerating corneas, the relative absence of PMNs and/or the presence of extensive vascularization in the central cornea were noteworthy.

More detailed histopathologic examination of the control ulcerating corneas revealed the epithelium, when present, to be one to two attenuated cell layers (Fig. 3 and Fig. 4, inset). Ultrastructurally, these epithelial cells appeared rather quiescent and unspecialized with respect to synthetic and secretory activity, not having extensive endoplasmic reticulum, Golgi apparatus, lysosomes, or other secretory organelles (Fig. 4). Fibroblast-appearing cells, though numerous in the peripheral stroma, were altogether absent from the central ulcerating zone for at least 2 weeks. Those fibroblastic cells at the periphery contained abundant rough-surfaced endoplasmic reticulum as their major cytoplasmic organelle as would seem consistent with their usual reparative function of collagen synthesis and secretion (Fig. 11). Most striking was the intense infiltrate of acute inflammatory cells, particularly PMNs, within the anterior stroma. With phase-contrast microscopy, extensive vacuolization of innumerable PMNs and numerous mononuclear macrophages was evident.
Fig. 1. A, Immediately after sodium hydroxide application to the central cornea, there is dense opacification of the burned area. B, Sixteen days after alkali burn, the stroma has melted to produce a large central descemetocele. C, With a contact lens (edge indicated by arrowheads) glued on at day 2 following alkali burn, ulceration is prevented through day 21. D, If the glued-on lens is removed at day 14 (before central cornea has completely vascularized), then ulceration to descemetocele occurs before day 21. E, By day 9, after a deep ulcer had developed, a lens was glued on. F, Twelve days later (day 21), this same ulcer had not progressed, but instead showed clinical and histologic evidence of healing (see Fig. 9) (edge of lens at arrowheads). G, This cornea, with ring (between arrowheads) glued on at day 2, vascularized and did not ulcerate by day 21. H, Epithelial debridement on alternate days can also result in nonulcerated, vascularized cornea on day 21.
Fig. 2. Alkali burn only. By day 18, an extensive descemetocele has perforated (asterisk). There is a large central epithelial defect. PMNs are innumerable throughout the remaining stroma. A fibrocellular retrocorneal membrane has developed. (Hematoxylin and eosin; ×90.)

Inset: At area indicated by asterisk in main figure, break in Descemet's membrane is filled by fibrin in which PMNs are enmeshed. (Hematoxylin and eosin; ×300.)

Phagocytes could be resolved (Figs. 4 and 5, insets). These cells appeared to be highly active with respect to the formation of large phagosomes (Figs. 4 to 6) and the discharge (degranulation) of their enzyme-containing lysosomes (Fig. 7). Occasionally, the apparent coalescence of the lysosomal granules with large phagocytic vacuoles could be discerned (Figs. 6 and 7). The PMNs therefore appeared to be stimulated with respect to their usual activities of phagocytosis and enzymatic digestion. The accompanying mononuclear cells also appeared to be engaged in the phagocytosis of extracellular material as well as expended PMNs (Fig. 8). Fibrillar degeneration of collagen or the presence of recognizable collagenous fragments within phagosomes could not be identified directly; however, the fine, amorphous, granular composition of the degraded extracellular matrix did appear to be like the material within phagosomes. We conclude therefore that these cells are involved with the degradation of the extracellular matrix of the corneal stroma.

**Group 2. Contact lens.** Lenses applied (usually at day 2) following the alkali burn were well tolerated without additional lens-induced inflammation, infection, or neovascularization (Fig. 1, C). Within the group of 20 corneas receiving lenses (days 2 to 10), only two relatively minor stromal ulcers developed by day 21. In both of these ulcerating corneas, only acute inflammatory cells with neither epithelial nor fibroblastic cells were present in the central cornea. Among the clinically nonulcerating corneas (18 of 20), we observed histologically that the epithelium had been completely excluded from the corneal surface under the lens. Most surprising was the complete acellularity of the otherwise intact central stroma, as was confirmed by phase-contrast and transmission electron microscopy even at day 21 (Fig. 9). In the subgroup of six eyes which had been actively ulcerating when the lens was applied (at day 7 to 10, Fig. 1, E), clinical observation at day 21 indicated that no ulcer had worsened and that three of the six ulcers had improved significantly (Fig. 1, F). Histologic examination of these corneas disclosed that, in fact, no intact PMNs were present in the
central cornea (Fig. 9), although they must have been there when the lens was applied. Instead, extensive cellular debris, often recognizable as PMN remnants, was apparent in the anterior stroma (Fig. 9). Thus the glued-on contact lens appeared capable of inhibiting continued PMN infiltration. The contact lens also appeared to retard both vascular ingrowth and fibroblastic infiltration of the central cornea. Vascularization did, however, eventually extend under the lens to the central corneal region.

In nine of the 14 eyes whose glued-on lenses were lifted at day 14, considerable rep epithelialization had occurred under the lens by day 21. Ulcers, however, developed in only three of these nine eyes, and in each of the three, large numbers of acute inflammatory cells also existed centrally. Of particular interest was the third subgroup of 10 eyes in which contact lenses had been applied at days 2 to 7 and then removed at day 14. Although 85% of positive control eyes had ulcerated by this time (day 14), no ulcers were observed at day 14 in the glued-lens eyes. Eight of the 10 corneas, however, underwent rapid ulceration within 7 days after removal of the lens (Fig. 1, D). The histopathology of these ulcerating corneas was identical to that of positive controls, particularly with respect to innumerable PMNs. The two nonulcerating corneas also exhibited acute inflammatory infiltrates but were also totally vascularized.

To determine whether the ability of the glued-on lens to prevent ulceration was perhaps attributable to mechanical or hypoxic effects of the contact lens or due to the exclusion of epithelial and inflammatory cell types, two additional experiments were done: (1) glued-on rings and (2) epithelial debridement.

**Group 3. Rings.** In 14 of the 15 eyes having rings from day 2 to day 21, the epithelium was completely excluded from the central cornea (Fig. 1, C). Nonetheless, by day 21, 11 eyes evidenced significant ulceration, thereby indicating that the simple exclusion of epithelium does not prevent ulcers. Complete correspondence was, however, apparent between the development of ulceration and the presence of PMNs adherent to and within the anterior stroma (Fig. 10). In both ulcerating and nonulcerating corneas, there was a significant fibroblastic proliferation into the central cornea, and in several nonulcerating eyes, extensive vascularization was present. Thus the epithelium is also apparently not obligatory for inflammatory, fibroblastic, or neovascular responses.

**Group 4. Epithelial debridement.** Simple mechanical removal of the loosely adherent epithelium was accomplished by gentle de-
Fig. 4. Alkali burn only. Inset: Phase-contrast photomicrograph of ulcerating cornea shows continuous bilayer of epithelial cells and intense acute inflammatory-cell infiltration of superficial stroma. (Paraphenylenediamine; ×1100.) Survey electron micrograph demonstrates epithelial cells to lack secretory specializations, melting stromal matrix to have undergone dissolution of fibrillar collagen, and several PMNs to have engaged in extensive phagocytic activity. (×7000.)

bridement every 2 days (Fig. 1, H). Among these 10 eyes, only three developed stromal ulcers. In all three, only acute inflammatory cells were present in the central cornea. Moreover, the results of contact lens removal could be reproduced by simply discontinuing debridement at day 14. In such eyes, ulcers developed in eight of nine corneas within the next 7 days. Again the histopathology demonstrated the presence of large numbers of PMNs in the ulcerating areas.

Group 5. Controls. Significant acute in-
Fig. 5. Alkali burn only. Inset: During early ulceration, deeper stroma is also intensely infiltrated with acute inflammatory cells, particularly vacuolated PMNs. (Phase-contrast microscopy, paraphenylenediamine; ×750.) Main figure shows electron micrograph of this area with intensely active PMNs and disorganized stromal collagen. (×7500.)

Discussion

Collagenases are thought to initiate degradation of fibrillar, helical collagen in corneal ulceration, as in other situations where collagen is degraded. Previously a primary role of the corneal epithelium in stromal collagen degradation was suggested by the cor-
Fig. 6. Alkali burn only. An active PMN displays several phagocytic vacuoles plus enzyme-containing granules (which appear to fuse with phagosomes). The cell may be attempting continued phagocytosis (asterisk). The surrounding extracellular collagen appears extensively degraded. (×19,000.)

Relation between epithelial defects and stromal ulceration. Indeed, the findings that epithelium lyses collagen gels\(^1\), \(^2\), \(^7\), \(^8\) had prompted the initial attempts to prevent ulceration by gluing on a lens to keep the epithelium from the corneal stroma.\(^18\) The additional observation that ulcerations in both humans and rabbits occurred in cases where epithelium had insinuated beneath the glue bond and migrated over the stroma reinforced the view of the importance of the epithelium.

It has become increasingly apparent that collagenase production and collagen degradation in various systems are complex processes involving interactions between different cell types,\(^{10}\), \(^{24}\)–\(^{26}\) involving also the regulation of collagenase at several different levels.\(^{21}\) In the alkali-burned cornea, however, although epithelium has long been thought to participate in stromal destruction, neither its exact role(s) nor that of the PMN, the predominant inflammatory cell type present,\(^{8}\), \(^{11}\)–\(^{13}\) has been established. These realizations led us to re-evaluate the histopathology of cell populations involved in the ulcerating cornea following alkali burns.

The alkali burn used in the present study destroyed all the cellular elements of the central cornea. Subsequently, epithelium began to resurface the cornea, and as described by Hughes,\(^11\) PMNs began to infiltrate the stroma. They entered from the limbus and moved centrally in the superficial stroma.
somewhat behind the regenerating epithelium. As ulceration developed, the ulcer region contained innumerable PMNs. At the fine-structure level these PMNs contained many phagolysosomes, sometimes in continuity with degraded extracellular matrix, and appeared to be playing an active role in the stromal ulceration. Wound fibroblasts from the peripheral stroma, at least early after the burn, were not observed in the ulcer region, and the epithelium in this area did not show ultrastructural evidence of secretory activity.

In contrast to ulcerating corneas, alkali-burned corneas to which lenses had been glued did not ulcerate and showed a very different histologic picture. In such corneas, where epithelium had been successfully kept back by the lens, the central stromas were essentially completely cell-free. The PMN infiltrate that was so marked in the ulcerating corneas was absent in the central stroma, as the PMNs remained at the periphery, about coextensive with the corneal epithelium. At the electron microscopic level, these PMNs gave no indication of participation in matrix destruction. Thus the experiments with the glued-on contact lens clearly indicate that viable cells are necessary for stromal ulceration following alkali burns and that ulceration is not simply the passive breakdown of alkali-denatured collagen and proteoglycans. The glued-on lens has the ability therefore to prevent ulceration by maintaining the acellularity of the stroma. That the prevention of ulceration is not just a hypoxic effect of the lens...
is suggested by the observation that simple mechanical epithelial debridement similarly retards cellular repopulation and ulceration of the central stroma. Moreover, ulceration can, indeed, occur under the glued-on lens as was demonstrated by lifting up one sector of the lens. In such cases, epithelium migrated under the lens, PMNs infiltrated the stroma, and ulceration resulted. At the electron microscopic level, the PMN appeared to be active in matrix degradation. Corneas from which lenses were completely removed also ulcerated rapidly, demonstrating again that the potential for ulceration was still present.

The observations that PMNs appear active in the degradation of stromal matrix and that the glued-on lens prevented both re-epithelialization and PMN infiltration suggest that epithelium might produce a signal which attracts PMNs into the stroma. In this view, blocking epithelial resurfacing should also prevent the stromal infiltrate. To test the possibility that it is the absence of the epithelium and not the presence of lens which results in the lack of the PMN infiltrate, control experiments were done in which rings of the same outer dimension as the lens were glued onto alkali-burned corneas. Ulceration

Fig. 8. Alkali burn only. Inset: Phase-contrast microscopy reveals clear demarcation between degraded stroma above and intact fibrillar collagen below. Other than epithelial cells above, only PMN and mononuclear cells are evident in the stroma. (Paraphenylenediamine; ×11,000.) In the zone of stromal liquefaction (note granular amorphous quality of degraded stroma), a macrophage contains expended PMN remnants. (×9000.)
Fig. 9. Lens glued on at day 2. A, At day 21, the central stroma remains nearly acellular and has not ulcerated. A fibrous membrane has formed posterior to Descemet's membrane (circled). (Hematoxylin and eosin; ×200.) B, Phase-contrast microscopy of anterior stroma shows the acellular collagenous lamellae to be intact. (Paraphenylenediamine; ×1200.) C, Phase-contrast photomicrograph of area circled in top left figure resolves loose fibrocellular layer posterior to Descemet's membrane. (Paraphenylenediamine; ×600.) Lens glued on at day 9 (cf. Fig. 1, E and F). D, At day 21, the stroma is moderately edematous but not ulcerated. No intact cells are evident. (Hematoxylin and eosin; ×175.) E, At higher magnification, the cellular material present in the anterior stroma is identifiable as remnant debris of acute inflammatory cells. (Hematoxylin and eosin; ×600.)
Fig. 10. Ring glued on at day 2. Left, On day 21, the central stroma of this clinically nonulcerating cornea is intact and acellular. (Hematoxylin and eosin; x115.) Right, On day 18, this ulcerating cornea has large numbers of PMNs layered at the anterior surface and throughout the stroma. Keratocytes are also present. (Hematoxylin and eosin; x370.)

still occurred in 10 of the 14 glued-ring eyes where epithelium had been excluded successfully. Histologic examination revealed a dense infiltrate of PMNs in the 10 ulceratingstromas as well as in the mucous coagulum trapped between the ring and the anterior corneal surface. It was not possible to decide therefore whether the PMNs in the ulceratingstromas came in part from the PMN at the periphery or wholly from the tear fluid. Possibly PMNs entering the stroma from the tears themselves released chemotactic factors that attracted the peripheral PMNs into the stroma. In the four eyes that did not ulcerate, the central stromas were almost completely acellular, like those of nonulcerating eyes with glued-on lenses. Thus the glued-ring experiments do not permit an unequivocal statement that PMNs are attracted into the stroma by the epithelium; it is clear, however, that stromal ulceration can occur in the absence of the corneal epithelium and can be mediated by PMNs alone.

PMNs contain hydrolases, including collagenase, that can contribute to matrix degradation. Although the roles of such hydrolases in corneal ulceration have not yet been determined, elastase might also be expected to play an important role by cleaving through the terminal crosslink-containing regions of collagen to facilitate solubilization of the fibril after additional, intrahelical cleavage by collagenase. PMNs have been implicated on morphologic grounds as effectors in various pathologic situations. Indeed, there is evidence of the participation of PMNs in degradation of the stromal matrix after thermal burns, in Mooren's ulcer, and in retinol-deficient rats. It is also known that PMNs must receive some kind of stimulus to become phagocytically active and to secrete their destructive hydrolases. Our demonstration that PMNs infiltrate non-alkali-burned corneas with glued-on lenses but show no sign of phagocytic activity suggests that the stimulus for phagocytosis is present in the alkali-burned cornea but not in the normal cornea. Although immune com-
Fig. 11. For legend, see opposite page.
plexes and anaphylotoxins elicit phagocytosis in other PMN systems, the nature of the stimulus in the alkali-burned cornea is completely unknown. It is known that membrane-enclosed hydrolases are released extracellularly in the process of phagolysosome formation when the phagocytic vacuole is still open to the extracellular space. As in the case of PMNs presented with immune complexes embedded in Millipore filters or in collagen membranes, the difficulty in phagocytosing large fibrils of stromal collagen could result in the extracellular release of high levels of hydrolases.

Although our results suggest strongly that the PMN has an important role in corneal ulceration, we do not suggest that the PMN alone is responsible for stromal destruction. Corneal epithelium from the ulcerating rabbit cornea does exhibit a low level of collagenolytic activity in vitro, and normal epithelium appears to stimulate corneal fibroblasts to secrete latent collagenase in cell culture. As already indicated, epithelium might stimulate the infiltration of PMNs into the corneal stroma after alkali burns. Of possible relevance is the report of Weimar that linear injuries of the rat cornea cause stromal infiltration by PMNs, presumably due to liberation of a serine protease that generates chemotactic peptides. Human skin and rabbit skin have recently been reported to produce proteases that are very active in stimulating PMN infiltration, and the cornea might also produce such a protease. Although the mechanism by which PMNs are attracted into the alkali-burned cornea is not yet understood, the interaction between corneal cells and inflammatory cells is clearly an important area for future study. In this regard, it should also be noted that lysates of PMNs (probably cathepsin B) are reported to activate latent collagenase from corneal fibroblasts. Moreover, plasmin activates latent corneal collagenase; plasminogen activator from corneal cells and from PMNs might generate plasmin (from plasminogen in the stroma) to generate chemotactic fragments for PMNs as well as to activate collagenase.

As therapy for preventing ulceration after alkali burns in both rabbits and humans, the glued-on lens was successful so long as the glue bond held secure and the epithelium did not get under the lens. The results of the present work additionally indicate that the glued-on lens can not only prevent ulceration when applied soon after the burn but can also arrest ulceration that has progressed even to descemetocele. Thus, in cases where central stromal ulceration is progressive and does not respond to other therapies, as sometimes occurs in stromal herpetic disease and in keratomalacia, we suggest that the glued-on lens might be used successfully to prevent further ulceration.

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