The histopathology of experimental dry spots and dellen in the rabbit cornea: a light microscopy and scanning and transmission electron microscopy study

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A large nasal perilimbal elevation was produced in both eyes of 17 rabbits by implantation of Silastic sponges subconjunctivally. Persistent dry spots and/or dellen occurred in 28 eyes, 1 to 8 days after surgery. Small dry spots consisted of as few as five epithelial cells, with plasmalemmal disintegration, separating from the sheet of surface cells. Larger dry spots were characterized by clusters of desquamating cells or irregular cellular defects resulting from loss of two or three cell layers. Dellen developed under deep, partial-thickness or full-thickness epithelial defects. Most commonly, epithelium entered the base of the dellen smoothly, although surface cells showed rounding up, and cell separations were frequently found. An occasional double-layered flap of epithelium at the edge of a defect probably represented an aberrancy of repair, whereas a two- or three-layered tongue of epithelium issuing from under the flap represented epithelial cells moving into the defect. Occasionally, epithelial cell invasion of the anterior stroma was documented by the presence of a rupture of the basal lamina, collagen destruction, and the presence of stromal vesicles. It is concluded that persistent dry spots and dellen represent stages in the progressive development of surface and subsurface epithelial cellular disruption, with secondary anterior stromal alterations.

Key words: dellen, dry spots, cornea, morphology.

These dry and occasionally hazy-appearing dimples were found to appear (1) following a swelling at the limbus caused by episcleritis, scleritis fugax, suffusion, angioma, or a thick pinguecula, after advancement of a rectus muscle, or after a subconjunctival injection; (2) after administration of cocaine; (3) after operation for cataract; (4) with hemeralopia; (5) with paralytic lagophthalmos; and (6) spontaneously in elderly persons. Subsequent reports in the literature confirmed these observations, adding specific aspects of case reports.2-7

Persistent localized holes in the tear
Fig. 1A. Perilimbal elevation is created nasally by surgical implantation of a Silastic retinal sponge subconjunctivally (arrows).

Fig. 1B. Slit-lamp microscopy of the cornea 1 day after sponge implantation shows two dellen (stromal thinning, large arrows) and several dry spots (no stromal thinning, small arrows).

The causes of dellen have been postulated as (1) compression or destruction of limbal vascular supply; (2) interference with local nerve supply; or (3) enhanced localized drying. Although the final pathophysiological picture has not emerged, it seems clear that a paralimbal or corneal elevation, diminished or abnormal blinking, an abnormal or deficient tear film, or corneal hypesthesia, acting together or alone, enhances the development of dry spots and dellen.

Light microscopy of dellen has been performed in only two eyes. E. Fuchs found only thinning of individual epithelial cells in the base of a dellen in an eye enucleated post-mortem for sarcoma. A. Fuchs reported on the histopathology of a
delle noted preoperatively in an eye removed for a sarcoma. Thinned epithelium lined the periphery of the depression, but a full-thickness epithelial defect was present centrally. At the central portion of the lip of the della a proliferation of small epithelial cells thickened the border of the dimple. In both cases the lamellae of Bowman's membrane and anterior stroma were found to be thinned.

Dry spot formation after prolonged air exposure in rabbit corneas has been observed with the scanning electron microscope. The cellular changes involve loss of surface cell microprojections and cell "sloughing."15

The present report constitutes the first study of the clinical and histopathological findings of dry spots and dellen of the cornea in an animal model.

Materials and methods

Seventeen healthy New Zealand albino rabbits, free of ocular disease, were anesthetized with intravenous Nembutal. A drop of tetracaine without preservative was placed into each eye, and the tip of the nictitating membrane excised with heavy curved scissors. A previous study has shown that topical tetracaine does not affect the surface epithelial cell plasmalemma.15 Sharp scissors were used to excise conjunctiva off the inner portion of the nictitating membrane. This dissection was carried down to the limbus nasally to fully expose the nasal quadrant of the sclera. The optimal size implant was found to be Dow-Corning Silastic scleral sponge of dimensions 7.5 mm. by 5.5 mm., cut to a length of 15 mm. and sutured perilimbally and concentric with the limbus under the fashioned conjunctival flap. The sponge was sutured superiorly and inferiorly with 8-0 silk suture. The conjunctiva was pulled up and over the scleral sponge, with reapproximation of the outer margin of the sponge, and sutured with 8-0 silk in a continuous suture (Fig. 1A). The cornea was kept wet with balanced salt solution during this procedure. No antibiotics were instilled in the eye during or after the procedure.

The animals were examined at 24 hr. intervals with a Kowa portable slit lamp. Dry spots and dellen could easily be visualized on the corneal surface by a break-up of the tear film (Fig. 1B). They were present immediately on inspection or developed within seconds. No fluorescein was instilled into the eye. No postoperative care was instituted during this interval.

Tissue preparation. Each animal was sacrificed by an overdose of intravenous pentobarbital. Glutaraldehyde (4 percent) at room temperature was immediately instilled into each eye, and the upper and lower lashes and fur were clamped together to keep the eyes closed prior to tissue excision.

Preparation for scanning electron microscopy. The entire nasal half of the cornea was excised with a razor-blade knife and corneal scissors. The specimen was placed into 4 percent glutaraldehyde and subsequently processed for scanning electron microscopy (SEM) as previously described.17 Specimens were examined in an ETEC autoscan.

Light microscopy and transmission electron microscopy. A razor-blade knife was used to excise small pieces of tissue, including dry spots or dellen. These pieces of tissues were processed for transmission electron microscopy (TEM). Briefly, the tissues were kept in 4 percent glutaraldehyde for approximately 2 hr., followed by a 1 hr. fixation with osmium tetroxide and dehydration in 30 to 100 percent acetone. The specimens were subsequently placed in Spurr's low-viscosity embedding medium and cured for 8 hr. at 70° C. prior to sectioning. Half-micron sections and thin sections were obtained for light microscopy and TEM, respectively. Light microscopy was performed with a Zeiss microscope. TEM was performed with a Philips 300 electron microscope.

Results

Clinical. One to eight dellen and/or dry spots developed in 28 eyes of 17 rabbits between 1 and 8 days after implant surgery. Twenty-three eyes developed one or more dellen or dry spots within 48 hr. after surgery. Of the remaining rabbits, one eye became infected and was discarded. In the initial phase of the study, four of the implants were somewhat small, causing insufficient perilimbal elevation. A high elevation of the implant was critical for dellen and/or dry spot development.

Dry spots were easily identified as small, constant points of discontinuity in the mirrorlike reflection from the tear film (Fig. 1B). They were present immediately on inspection or developed within seconds. Dellen appeared as larger dry holes (up to 0.5 mm.) in the precorneal tear film, with definite thinning of the underlying stroma in the form of a dimple. A complete spectrum of dry areas from small dry spots to large dellen was identified.
Fig. 2. A, Epithelial irregularity of two dry spot areas (arrows) is evident on an otherwise smooth corneal surface. B, this dry spot consisted of a cluster of 5 corneal epithelial cells separating from the contiguous surface. C, Separating epithelial cells in a dry spot show loss of surface microprojections and irregularity and disruption of the plasma membrane. Retraction fibrils (arrowheads) and discrete anterior cellular edges mark the disrupted attachments with adjacent cells. PD, Plasma membrane disruption.
Fig. 3. A, Larger cluster of separating surface and underlying epithelial cells make up a larger dry spot area. B, Higher-power micrograph shows the apparent persistent intracellular adherence within the separating cell cluster. The paucity of cellular microprojections should be noted.
Fig. 4. A, In this dry spot a two- or three-cell thick surface cluster has desquamated from the corneal surface, leaving a jagged edge and base to the defect. B, Within the epithelial defect the anterior membranes of the second or third cell layers are missing, exposing the nucleus, the nuclear envelope, and the intracellular organelles. C, Higher-power micrograph reveals the split enucleated cell and the exposed intracellular organelles. D, TEM micrograph of a third-layer cell showing loss of the anterior plasmalemma, with exposure and degeneration of intracellular organelles. The nucleus is totally exposed. Inset, Light microscopy shows the ragged epithelial defect with two layers of cells remaining. N, Nucleus; NE, outer membrane of nuclear envelope; ER, remnants of endoplasmic reticulum.
Fig. 5. A, Two adjacent depressions in the corneal surface mark the site of a delle. There are many loose surface epithelial cells. B, Although many epithelial cells retain a flat surface, significant numbers of cells are rounded and elevated off the adjacent surface, with diminution or loss of their surface microprojections. Two openings into pits are visible by SEM (arrows). Inset, Light micrograph of an epithelial pit demonstrates the smooth entry of flat surface epithelial cells to the base on one side, full-thickness epithelial involvement, and persistence of the basal lamina.
Fig. 6. Flaps composed of epithelial cells cover variable-sized underlying defects. A, Three epithelial flaps in a row. B, Smooth epithelial flap over a depression and a larger delle present above. D, Delle; F, flap of epithelial cells.
Fig. 7. TEM montage of the epithelial flap showing the double layering of epithelium around a central core of cells. The columnar appearance, apical nuclei, dense staining, and continuity with basal cells in the epithelial sheet suggest origin from the basal layer. Wing and squamous epithelial cells overlie these cells over the tip. A posteriorly. Except for their orientation, these cells are normal. Inset, Epithelial basal cells flap is present at the edge of well-developed delle, with central full thickness epithelial loss. Note the layering of flat epithelial cells over the entirety of the flap extending to the edge of the defect. The tongue of epithelial cells under the flap (arrow) may represent epithelial cellular locomotion into the defect. B, Basal cells; W, wing cells; S, squamous cells.

Dellen and dry spots were evanescent, often present 24 hr. after surgery, sometimes changing in appearance and location, or even disappearing from one observation day to the next.

Mucus threads tended to lie in the crevice created by the perilimbal elevation as it overhangs the adjacent cornea. Dry spots or dellen developed in the cornea within 1 to 4 mm. of the elevation, especially in the absence of a large mucus thread. If the mucus thread was removed atraumatically, dry spots or dellen often subsequently formed.

Light microscopy. Ten corneas in which 20 dry spots or dellen could be identified were examined. Dry spots showed one to three layers of irregular and disrupted superficial and wing epithelial cells. When three or four layers of cells were missing, the resulting defect was a ragged epithelial hole with broken cellular remnants. Underlying basal cells had lost their columnar appearance and polarity. In those cases there was no depression in the underlying stroma (Fig. 4). Leukocytes were occasionally found. No full-thickness epithelial defects were found in dry spots.

The epithelium over dellen was found to be partially or totally missing. Cells at the edges of partial or full thickness epithelial defects were found to be (1) ragged, with broken cellular components or (2) more commonly, smoothly entering the depths of the dellen. Clumps of epithelial cells in the depths of the defect were a common finding. Increased numbers of epithelial cells adjacent to the dellen frequently gave them a thickened appearance.

Less commonly, when epithelial cells entered the base of a delle, there was a thick flap of epithelium pointing into the defect. The flap was composed of a central double-layered core of palisading columnar cells with deep basophilic nuclei, overlying wing cells, and superficial squamous cells.
Fig. 5. For legend see opposite page.
The flap is therefore a double layer of epithelium with basal cells in contact along its central plane. An occasional flap also showed a tonguelike layer of two to three cells protruding into the defect from under the flap. Leukocytes were occasionally associated with dellen.

Narrow, pitlike holes through the full thickness of epithelium were commonly found close to or within dellen (Fig. 5, inset). The interior of the pit was lined by smooth cells of squamous type continuous with the superficial layer or by superficial wing and basal cells in their respective layers.

**SEM.** There were 17 eyes with dry spots or dellen examined by SEM.

Changes in the surface corneal epithelium in areas where small dry spots appeared showed small clumps of as few as five cells lifting up from the flat corneal surface (Fig. 2). Separation of these cells from their neighbors, the presence of retraction fibrils, loss or a significant reduction in the number of microprojections from the surface of the plasma membrane, and disruption of the integrity of the surface plasma membrane were evident. Larger dry spots (0.4 mm.), corresponding to large surface and subsurface clumps of epithelial cells, were found partially separated from the epithelial sheet (Fig. 3). These cells, which were lifting off the surface of the cornea, often remained partially attached to one another.

Other areas where dry spots were visible clinically showed the first and second layer of epithelial cells missing from the corneal surface over an area approximately 0.2 mm. in diameter (Fig. 4). The interior of these cell excavations was very ragged. A curious finding in the floor of these dry spots was the absence of the plasma membrane from the top of the third cell layer (wing cells). This unroofing of the third-layer cell exposed the nucleus in its nuclear envelope and cell organelles. The entire process attracted few or no leukocytes.

Areas identified as dellen, that is, localized depressed areas of the corneal surface, could also be identified by SEM (Figs. 5 and 6). In the center of the depression, as well as around the dellen, some surface epithelial cells showed lifting up of their edges away from the contiguous surface, loss of microprojections on their surface, and destruction of the plasma membrane. Within and around the edges of the dellen there were many epithelial cells showing rounded surfaces that were attached to the underlying cells, retaining many of their microprojections. Inflammatory cells were noted sporadically. SEM verified the openings of pits in and around the dellen as shown by light microscopy. Surface epithelial cells smoothly entered the pit orifice. In the one eye where a dellen was allowed to persist for 7 days, the surface of the area was actually elevated, with mucus accumulation over its surface, many cells showing lifting from their contiguous neighbors, loss of microprojections, and membrane disruption.

**TEM.** Thin sections of isolated desquamating surface epithelial cells showed severe cellular disruption. In those dry spots missing two or three cell layers, loss of the anterior plasmalemma of many of the remaining cells substantiated the SEM findings of exposed nuclei and cytoplasmic organelles (Fig. 4, D).

In those dellen characterized by flaps, a central core of osmiophilic, palisading columnar epithelial cells was found back...
Table I. Diagram of the proposed morphological and clinical development of persistent dry spots and dellen in the rabbit cornea. A lesion can stabilize or repair at any stage. Dellen rarely go on to exhibit collagen loss.

Table I.

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<th>Prolonged Disturbance of the Tear Film</th>
<th>Surface Epithelial Trauma</th>
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<td><strong>Persistent Dry Spot</strong></td>
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<tr>
<td><strong>Suprificial Cellular Disruption</strong></td>
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<td><strong>Partial Thickness Epithelial Loss</strong></td>
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<td><strong>Full Thickness Epithelial Loss</strong></td>
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<td><strong>Anterior Collagen Loss with Epithelial Invasion of Defect</strong></td>
<td>Dellen (Anterior stromal thinning)</td>
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<td>Dellen (Collagen loss)</td>
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The nuclei of these cells were very dense and apically located. The bases of these cells touched one another in a plane through the middle of the flap, and their apices were anterior, posterior, or smoothly curving near the tip of the flap. Succeeding to the surface from this layer, either anteriorly or posteriorly, were two wing cell layers and two or three squamous cell layers. Under some of these flaps a two- or three-layered epithelial sheet was directed toward the defect. The cytological characteristics of each cell layer led to the conclusion that two complete layerings of stratified epithelium were doubled upon one another in the flap.

Attempts to measure the interfibrillar distances of collagen in the bases of dellen failed to show any significant differences from normal values (200 to 500 A). This is thought to be due to the artifact induced by imbibition of fixation fluids.

In two typical dellen showing full-thickness epithelial defects, evidence of superficial collagen destruction and epithelial ingrowth into the stroma was found (Fig. 8). In this area, basal lamina and anterior stroma were interrupted, with the presence of amorphous material, presumably collagen breakdown products, adjacent to the invading epithelial cells. Within some of such amorphous deposits were vesicles clearly defined by a trilamellar membrane. These vesicles contained various amounts of electron-dense substances.

Discussion

The creation of dry spots and dellen in this rabbit model by a surgically induced perilimbal elevation effectively simulates the human disease process. The perilimbal elevation lifts the lid off the peripheral cornea, creating a triangular air space. This prevents resurfacing of peripheral cornea by the lid, by interfering with peripheral lid-globe congruity. It also interrupts the framing of the tear film, creates tear film turbulence, prevents mucin from being rubbed into the surface epithelium, and may interfere with the blink mechanism.

The findings of this study lead to a postulation regarding the development of...
dry spots and dellen in the presence of a perilimbal elevation (Table I). It appears that for a dry spot to consistently appear on the corneal surface, plasmalemmal and intracellular damage to a small cluster of cells are imperative. With continued insult, variable numbers of surface and subsurface cells desquamate separately or as a mass. This leaves a ragged partial-thickness epithelial defect, with intracellular debris exposed on the base. The dry spot development precedes the formation of a dellen as the defect deepens, exposing the basal epithelial cells or the basal lamina. Dellen do not appear prior to this event. Clinical localized thinning of the stroma is reflected in a condensation of the superficial stroma and overlying partial or full-thickness epithelial defects. As dellen form, the ragged epithelial edges and base change to a smoother transition of surface epithelial cells down into the depression. In persistent dellen, with full-thickness epithelial defects, the epithelial edge of the depression rolls upon itself, creating a flap with generative cells (basal) within its core. This flap formation may represent part of the mechanism of repair, but most likely it is an aberrancy of repair. The tongue of flat epithelial cells emerging from under the rolled edge toward the base of the depression most likely represents the first evidence of cellular locomotion into the defect. In rare instances, basal lamina and superficial collagen are destroyed in the base of the dellen, presumably by invading epithelial cells. Although hypothetical, the mechanism of collagen degradation might be by the elaboration of enzymes or precursors from vesicles derived from epithelial cells.

Precorneal tear film dynamics clearly initiate this disease process in rabbit cornea. The fact that persistent dry spots are consistently evidenced by localized cellular damage suggests that this is the common denominator in the formation of dry spots. To support this thesis, localized cellular injury by a cilium, abrasion, prolonged air exposure, or generalized surface cellular injury as by the administration of topical cocaine consistently causes destructive changes in the surface epithelial cell layer. Depending on local conditions, dry spots may or may not go on to dellen formation.

Perilimbal elevations and, directly or indirectly, tear film abnormalities or blinking anomalies may give rise to dry spots and/or dellen by the common avenue of surface and subsurface epithelial destruction. Whether or not these changes occur may depend largely on the interplay of separate but additive factors in a given eye.

REFERENCES