Corneal hydration studied in stromal segments separated by interlamellar discs

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Interlamellar plastic discs inserted into cat corneas provide a means by which segments of the cornea can be studied with regard to swelling and water movement in vivo. The corneas are physiologically normal as demonstrated by their transparency, swelling properties, and histologic structure. The observation that one disc causes no change in the hydration of the stroma anterior or posterior to the disc is consistent with the hypothesis that active movement of water occurs across both the epithelium and the endothelium. The middle stromal segment which is created by inserting two interlamellar discs also remains transparent and physiologically normal. This raises the possibility that the stroma itself is a site of metabolic activity which is sufficient to sustain a normal state of stromal hydration. It further indicates that metabolic interaction between the stroma and the overlying epithelium or the underlying endothelium is not essential to the normal state of corneal tissue.

The insertion of one and two interlamellar plastic discs within the corneal stroma was first described in rabbits by Bock and Maumenee. It was found that within 48 hours the stroma anterior to the disc became edematous and eventually eroded away. Similar results were obtained by Knowles; however, he did observe that monkeys tolerated one interlamellar disc well.

The possibility of inserting well-tolerated interlamellar discs into the cornea of experimental animals is interesting. It would permit one to study a segment of the cornea which either is separated from the epithelium or endothelium by a single disc or is isolated from both limiting membranes between 2 discs. The present investigation provides evidence that 1 or 2 interlamellar discs can be successfully inserted and well tolerated by the cat cornea with little or no alteration of the histologic structure. In addition, the separation of the stroma into 2 segments by a disc has provided an opportunity to study corneal swelling and water movement in vivo.

Methods

Male cats weighing 5 to 10 pounds were anesthetized with Pentothal sodium, 30 mg. per kilogram body weight intraperitoneally. The skin about the eye was shaved and prepared for operation in the usual manner; operative sterility was maintained. Exposure and fixation were obtained by a lateral canthotomy and 4 limbal stay sutures. A vertical incision 5 to 6 mm. long and about 2 mm. inside the lateral limbus was made with a Beaver knife. Through this approach a Gill knife was introduced along the corneal plane splitting the lamella ahead of it to receive a 5 or 6 mm. polypropylene disc.* The latter was then slipped into the stromal cleft, thus dividing the cornea into an anterior stromal segment covered by

*Supplied by Dow Chemical Company. It is 9/16 thick and is considered to be impermeable to water vapor but permeable to CO₂ and O₂.
Table I. Summary of corneal thickness measurements after insertion of discs

<table>
<thead>
<tr>
<th>Before operation</th>
<th>Days after operation</th>
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<tbody>
<tr>
<td>Number of eyes</td>
<td>1 to 4</td>
</tr>
<tr>
<td>Thickness (mm.)*</td>
<td>0.57 ± 0.03</td>
</tr>
<tr>
<td>% increase (versus pre-operative)</td>
<td>26.3</td>
</tr>
<tr>
<td>Probability (versus pre-operative)</td>
<td>&lt; 0.005</td>
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*Mean ± S.D.

epithelium and a posterior one covered by endothelium. In experiments in which another disc was similarly placed it was introduced through an incision near the medial limbus. The depth of the incisions varied, one shallow and one deep, in an effort to place one just posterior to the epithelium and the other just anterior to the endothelium. In this way the cornea was divided into 3 stromal segments with the middle one isolated by the discs from both the epithelium and endothelium.

An area of overlying epithelium, approximately 6 mm. in diameter, was removed from 2 corneas in which single interlamellar discs had been placed 4 weeks earlier. With the aid of biomicroscopy the endothelium and Descemet's membrane were removed from the corneas of 2 eyes with the blunt curve of a bent needle. The extent of this procedure was later confirmed by histologic examination. The eyes were observed for periods of 1 to 28 days with periodic measurements of the corneal thickness.

Measurements of central corneal thickness were made pre- and postoperatively with a corneal pachymeter mounted on the illuminating arm of the Haag-Streit biomicroscope. Corneas for histologic study were fixed in 10 per cent formalin and stained with hematoxylin and eosin, periodic acid-Schiff, colloidal iron, toluidine blue, and Alcian blue. The latter proved helpful as a stain for acid mucopolysaccharides and provided a histologic method for recognizing corneal swelling.

Results

The central corneal thickness of 64 normal cat eyes was 0.57 ± 0.03 mm. (mean ± S.D.). One or 2 interlamellar discs were inserted and successfully maintained in 36 eyes for periods lasting up to 15 weeks at which time the eyes were used for other studies. There was always some corneal swelling during the postoperative period which was probably the result of surgical trauma. Although it appeared to be greater after insertion of 2 discs than after one, this could not be demonstrated statistically. During the first 4 days following operation the corneal thickness increased 26 per cent to a mean of 0.72 mm. (p &lt; 0.005, Table). Deturgescence began during the latter half of the first week with gradual decrease in corneal thickness. Although the cornea usually regained its transparency and appeared perfectly clear by the end of the second week, it did not attain its normal thickness until the third to fourth week. All corneas that were followed after this time remained deturgesced and transparent.

Fig. 1. Cornea of a cat eye, containing one interlamellar disc, 8 weeks after its insertion. The cornea was transparent and clear. The temporal margin of the disc is revealed by the reflections of the photographic flash. The nasal margin which is just inside the pupillary border is not evident in the photograph.
The stromal segments which resulted from inserting the discs were individually observed by biomicroscopy. During the initial period of swelling there was an increase in stromal density, but this gradually disappeared so that each segment was usually clear and transparent by the second week (Fig. 1).

Histologic study of eyes 6 weeks after insertion of 1 or 2 discs revealed the architecture and stromal pattern to be normal (Figs. 2 and 3). The keratocytes in each of the stromal segments were normal in appearance and number with an occasional polymorphonuclear cell interspersed (Fig. 4). Sections stained for acid mucopolysaccharide showed normal periodic acid-Schiff, colloidal iron, and Alcian blue staining throughout, and no loss of metachromasia with toluidine blue.

Removal of corneal epithelium was followed within 4 to 6 hours by swelling of the underlying cornea except in the area posterior to the disc. The posterior segment remained deturgesced and perfectly clear even though the anterior segment clouded and swelled to almost twice its original thickness during this time. When epithelium was removed from an eye with two discs, both the middle and posterior segments remained clear and deturgesced during the same time interval (Fig. 5).

Removal of the endothelium and Descemet's membrane revealed a similar pattern of swelling. During the first 6 hours there was marked swelling of the overlying stroma except in the area anterior to the disc. Despite a threefold increase in thickness of the posterior segment, the anterior segment remained clear and did not swell during this time.

Discussion

Evidence that the stroma remains physiologically normal when separated from either or both of the limiting membranes is based on its transparency, swelling properties, and histologic structure. An evaluation of the transparency has been made by gross examination and by biomicroscopy. It is well known that the slightest changes in the structure of the corneal collagen will cause marked dispersion of the light as seen in slit-lamp examination. It is no surprise that there is a transient period of corneal edema with diminished transparency following insertion of either 1 or 2

Fig. 2. Cornea 6 weeks after insertion of 2 interlamellar discs. (The discs were removed during sectioning.) The 3 stromal segments were separated by clefts which were potential spaces in vivo, but became artificially widened during fixation. The histology of all 3 segments appeared normal. (Hematoxylin and eosin. ×25; reduced 1/2.)

Fig. 3. Cornea 4 weeks after insertion of one interlamellar disc. The architecture and histologic picture were normal. (Hematoxylin and eosin. ×25; reduced 1/2.)

Fig. 4. Middle stromal segment of cornea shown in Fig. 2. The keratocytes were normal in appearance and number. The stromal pattern was also normal. (Hematoxylin and eosin. ×100; reduced 1/2.)

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interlamellar discs. That this is a reversible phenomenon, however, is a good indication that the fibrillar structure and metabolic activity in the cornea returns to its normal state.

Further convincing evidence that the corneal stroma behind or in front of the disc remains normal is seen in measurements of corneal thickness and observations of the swelling properties following removal of either the epithelium or the endothelium. It is readily appreciated that any disturbance to the limiting layers results in swelling of the cornea. To be sure, this is partly responsible for the increased corneal thickness immediately after insertion of a disc. However, by the third to fourth week the tissue regains its normal thickness and remains unchanged throughout the entire period of the experimental observations. The method used in this study for corneal thickness is adequate to determine changes of less than 2 per cent. After removal of the epithelial and endothelial layers, the stroma anterior and posterior to the disc behaves in an exactly analogous way to normal stromal tissues.

Histologic examination supports the conclusion that the tissue anterior and posterior to the disc remains normal in two ways: first, the tissue in the disc area stains in a manner comparable with that beyond the margins of the disc; and, second, the specific histochemical stains suggest that there has occurred no alteration of the mucopolysaccharide content. Hematoxylin and eosin stain reveals that the histologic structure of the corneal segments created by 1 or 2 discs remains normal. Staining of the mucopolysaccharides with P.A.S., Alcian blue, colloidal iron, and toluidine blue reveals no alteration in the staining properties of the experimental compared with control corneas.

By no means was insertion of interlamellar discs 100 per cent successful. In approximately 2 out of every 10 eyes in which one disc was placed, there developed stromal degeneration anterior to the disc—similar to that described when discs were placed in rabbit eyes.² The morbidity almost doubled when 2 discs were inserted. However, every cornea that survived the first 2 week postoperative period remained transparent and histologically normal thereafter.

The physiologic significance of the results lies in their relationship to the current concepts of the mechanism controlling deturgescence and to the recent studies of metabolic interaction. In steady-state conditions the tendency for water or solute to move into the cornea is balanced by an equivalent movement in the outward direction.₂, ⁷ The observation that isolation of an area of stroma from the overlying limiting membranes does not cause it to swell indicates that if active transport is involved
in the maintenance of normal deturgescence, it can occur over a distance of at least 3 mm. in a lateral direction. This is of special interest in view of the finding that removal of a limited area of epithelium or endothelium results in swelling which is restricted to the column of stroma bordered by the denuded area. Thus, whereas intact epithelium immediately adjacent to a denuded area is unable to maintain deturgescence beneath that area, the tissue between two interlamellar discs does remain deturgesced. Furthermore, the observation that one disc causes no change in swelling anterior or posterior to the disc is consistent with the hypothesis that active transport must occur across both the epithelium and the endothelium.

That there is a metabolic interaction between the component layers of the cornea has recently received strong support by the observations of Herrmann8 on the uptake of radioactive glycine by the stroma and the findings of Smelser9 on the uptake of radioactive sulfate. Herrmann has suggested that corneal stroma is dependent upon the epithelium directly over it for its metabolic energy. Yet, in the present experiments the middle and posterior stromal segments appear normal as long as 15 weeks (the duration of the experiments) after their separation from the overlying epithelium by the plastic discs. It appears from these experiments that if a linear structural metabolic chain connecting the stromal components with the epithelial cell does exist, it is not essential to the normal state of the tissue. Although the middle segment has access to freely diffusing metabolites and to the epithelium not directly over it, one must consider the possibility that the stroma itself is the site of metabolic activity10 of the type that might sustain mucopolysaccharide synthesis and maintain normal corneal hydration. Studies of the uptake of radioactive sulfur by the middle stromal segment also suggest that mucopolysaccharide synthesis occurs here.11

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REFERENCES