Adults of this study, the mean keratometric reading was 39.35 D (8.58 mm). Standard error values ranged from 0.10 to 0.47 D, with a mean of 0.235 D. For kittens weighing less than 1000 gm, the range of standard errors was 0.17 to 0.86 D, with a mean of 0.391 D. Although the measurements from young kittens were slightly more variable, the apparent prevalence of higher corneal astigmatism probably reflects the complex, coordinated growth process that must occur during early postnatal weeks between the various optical elements of the eye.

I am grateful to Ann Emerson, Barbara Lee, Susan Hartley, and A. B. Bonds for help with this study.

From the School of Optometry, University of California, Berkeley. Support was provided by research grant EY01175 and Research Career Development Award EY00092 from the National Eye Institute, Bethesda, Md. Submitted for publication Aug. 27, 1979. Reprint requests: Dr. R. D. Freeman, University of California School of Optometry, Minor Hall, Berkeley, Calif. 94720.

Key words: cornea, radius of curvature, cats, kittens

REFERENCES

Preservative cytotoxic threshold for benzalkonium chloride and chlorhexidine digluconate in cat and rabbit corneas. Neal L. Burstein.

Benzalkonium chloride (BAC) and chlorhexidine digluconate (CDG) were applied to rabbit and cat corneal epithelium in clinically used concentrations. Corneas were fixed ½ hr later and examined by scanning electron microscopy (SEM). BAC was found to produce a progressive increase in damage at concentrations between 0.001% and 0.01% as determined by SEM. CDG pro-
duced less damage than BAC at any concentration. Cats lacrimated copiously and blinked frequently after instillation of drops; rabbits did not. No significant difference was found between the two species, however, in their response to the preservative agents tested. It is presumed that binding of these surface active agents occurs almost immediately and is unaffected by tear film dilution. Studies measuring permeability increase in the human eye after preservative use are required to allow clinical interpretation of the data presented here.

Previous papers detailing the cytotoxic effects of drug and preservative agents by SEM have used rabbits as test animals. Since rabbits seldom blink and do not lacrimate heavily even after irritation, results of these tests have been questioned. Concentrations of agents used experimentally have usually been high and/or varied over 10-fold increments. Thus evaluation of actual damage which may result clinically from the range of preservative concentrations commercially available has been difficult.

In this paper, two surface-active preservatives currently in use are tested in cat and rabbit eyes in vivo to determine the effect of tears in diluting or binding the agents and preventing damage to corneal epithelium. A range of concentrations of both preservatives is used to determine the threshold of detection of surface change as well as the minimum concentration causing epithelial exfoliation.

**Materials and methods.** Solutions containing benzalkonium chloride (BAC) or chlorhexidine digluconate (CDG) were prepared in concentrations of 0.01%, 0.0075%, 0.005%, 0.0025%, and 0.001% in 0.86% NaCl with 0.09% phosphate. Solutions were pH 7.3 to 7.4, and osmolarity was 297 to 301 mOsm. Solutions were assayed and found to be within 5% of stated preservative concentration.

Unbuffered 0.1% solutions of BAC and CDG were used on some corneas to determine the effects of higher than clinical doses. Control solution consisted of buffer and saline alone.

New Zealand white rabbits and cats quarantined 3 weeks had 0.1 ml of test solution instilled at the apical margin of the cornea. Contra lateral eyes received different concentrations or control solutions. Six eyes of each species were tested for each preservative at each concentration, and buffer solution alone was used as control.

Thirty to 40 min after instillation of solutions, animals were sacrificed by sodium pentobarbital overdose. Corneas were kept moist with warm 4% glutaraldehyde in 0.1M phosphate buffer before and during trephine removal of 6 mm buttons. Specimens were fixed overnight, rinsed 20 min in 20% acetic acid (Mucomyst; Meade-Johnson) to remove surface mucins, and postfixed 2 hr in buffered osmium tetroxide. After quartering to prevent excessive shrinkage and wrinkling, tissues were dehydrated 20 min each in 30%, 50%, 70%, 90%, 95% and 100% acetone, critical point-dried with liquid carbon dioxide, shadowed with 150A gold, and viewed by SEM examination.

Photographs were taken of each specimen at low, intermediate, and high magnifications to evaluate the frequency and degree of cell surface disruption and the appearance of cellular microvilli, microvilli, and borders. A numerical rating system was developed to aid in the categorization of relative damage to corneas (Table I). High-magnification photographs presented herein were selected to be as representative as possible of the overall appearance of corneas at low magnification.

**Results.** The epithelial surfaces of both species were relatively smooth in untreated or buffer-treated control corneas, with less than 2% of cells uplifting. However, some control corneas exhibited wrinkling or smoothing of individual cell surfaces (a numerical rating of 1), so that the averaged control group rating was 0.3 for rabbits and 0.5 for cats, with cats showing greater variability in surface. No effects were detected from buffering solution alone. As noted by Pfister, cat corneas contained a number of dark cells, with no cellular "holes" as in rabbits.

For both cats and rabbits, all concentrations of preservatives tested produced some variability in numerical ratings. One animal from each group

<table>
<thead>
<tr>
<th>Table I. Numerical evaluation of damage to corneal surface</th>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No visible damage seen by SEM; less than 2% of cells peeling, no wrinkling or smooth membranes; normal microvilli</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Some wrinkling or smoothing of cell surface; reduced numbers of microvilli on cells seen as &quot;dark&quot; at low magnification</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Prominent wrinkling and flattening of most cell surfaces; little peeling of cell borders observed</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Cell peeling and lifting of older, dark cells apparent, top cell layer without major disruption</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Top cell layer exfoliating, second layer intact beneath, preventing long-term compromise of barrier properties</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Second cell layer peeling beneath first, revealing membranes of third layer; physiological resistance of corneal epithelium severely compromised</td>
<td></td>
</tr>
</tbody>
</table>
generally ranked one numerical grade higher or lower than the average for the group as a whole. This may be partially attributed to variation in preservative application, lacrimation, or biological variability. Since cat and rabbit corneal epithelial changes were similar after identical treatment as reflected in Fig. 1, only cat corneal epithelial photographs are presented here. Rabbit epithelial changes have been reviewed and representative photographs published previously.3

**Benzalkonium chloride.** At 0.001%, BAC did not produce major change to corneal surface cells, although marginal microvilli were sometimes lost in both cat and rabbit. The small number of cells which were seen uplifting are believed to be due to natural exfoliation.1 At concentrations of 0.005%, BAC produced some cell wrinkling (Fig. 2, b). Margins of cells occasionally lifted, and surface microvilli were often lost at the edges of cells. This effect is also seen on some corneas exposed to 0.0025% BAC (Fig. 2, a) and may be the first sign of binding of surfactants to the plasma membrane.

As previously reported for rabbits,1 at 0.01% and 0.0075% BAC, concentrations produced lifting of cells, peeling, and exposure of underlying cell layers in both species (Fig. 2, c and d). The entire apical layer was exfoliating or in the process of exfoliation, with the second cell layer apparently intact beneath. Some corneas of both species exposed to this level of preservative showed only wrinkling of cell surfaces.

**Chlorhexidine digluconate.** Results have not been previously reported for chlorhexidine toxicity as seen by SEM examination of the epithelial surface, and so 0.1% was used to indicate the types of cell damage caused by an overdose of the preservative. This produced uplifting of large areas of cells.

No damage was detected at concentrations of 0.001% or 0.0025%. These corneas resembled controls (Fig. 3, a). At 0.005% CDG, some cell borders were denuded of microvilli, leading to a darkened appearance (Fig. 3, b). At 0.075%, older cells were completely denuded of peripheral microvilli (Fig. 3, c). At 0.01% CDG caused occasional cell margin ruffling and uplifting (Fig. 3, d).

**Discussion.** There is little difference in apparent sensitivity between cat and rabbit corneas when exposed to the same topical concentration of a given preservative, although statistical tests were not applied to these data since the damage scale is subjective and nonlinear. Blinking and lacrimation might occur too late after instillation of preservative agents to prevent damage to superficial corneal cells. The present evidence supports the hypothesis that binding of surface-active agents to the cellular epithelium and perhaps intercalation into the bilamellar membrane occur rapidly. Such may not be the case with mercurial preservatives, which show less damage in vivo than in vitro,1 perhaps due to tear film dilution and binding. Chlorhexidine is used in concentrations between 0.0025% and 0.005% in this country. In this range, damage from single topical application is minimal. This observation cannot be extended, however, to its use in soft contact lenses which may bind the preservative or act as a reservoir for
Fig. 2. Cat corneas ½ hr after BAC instillation. a, 0.0025% BAC. Margins are smooth on some older cells; rating = 1. b, 0.005% BAC. Microplicae are nearly lost from surface on upper cell; rating = 2. c, 0.0075% BAC. Cells are peeling at margins, revealing intact cells underneath; rating = 3. d, 0.01% BAC. Entire top surface is exfoliating. Note cracks and holes in top layer cells, probably caused by detergent disruption of membranes; rating = 4. (a and b, bar = 1 μm; c and d, bar = 10 μm.)
Fig. 3. Scanning electron micrographs of cat corneas ½ hr after CDG. a. Control, buffer only applied. Note microplicae on surface, with some blebs, particularly at cell margins; rating = 0. b. 0.005% CDG. Borders of cells have lost some microplicae; rating = 1. c. 0.0075% CDG. Older, dark cells have lost peripheral microplicae and microvilli; rating = 1. d. 0.01% CDG, clinically used concentration. Some ruffling, lifting edges are seen on cells; rating = 1 to 2. (Bars = 1 μm.)
its slow release, possibly increasing exposure due to length of contact time.

It is noteworthy that BAC is used in concentrations between 0.004% and 0.02% commercially. The lower concentrations used show far less damage to the epithelial surface than the higher concentrations still present in many ophthalmic solutions. Where possible, the formulation of these solutions should be limited to the minimum compatible with sterility.

Extrapolation of these data to the human eye must await a nondestructive test of damage in response to preservative agents, such as the use of fluorescein to measure changed corneal permeability.9

Specific proprietary formulations should be evaluated by the increasingly sensitive tests of corneal side effects now available, and consideration must be given to the addition of the minimal concentration of preservatives compatible with bacterial prophylaxis.

From the Division of Ophthalmology, Stanford University Medical Center, Stanford, Calif. Supported by NIH grant ET 07053. Submitted for publication May 21, 1979. Reprint requests: Dr. Neal L. Burstein, Division of Ophthalmology, Stanford University Medical Center, Stanford, Calif. 94305.

Key words: toxicity, cytotoxicity, benzalkonium chloride, chlorhexidine digluconate, preservatives, corneal epithelium, scanning electron microscopy, membrane effects

REFERENCES


Altering the course of cataracts in diabetic rats. S. FUKUSHI, L. O. MEROLA, AND JIN H. KINOSHITA.

A potent new aldose reductase (AR) inhibitor was effective in preventing cataractous changes in diabetic rats. Untreated diabetic rats developed early lens changes by 3 weeks and dense nuclear opacities by 6 to 9 weeks. In contrast, diabetic rats treated with the AR inhibitor showed less lens changes in 5 months of the experiment.

Aldose reductase (AR) has been implicated in the initiation of sugar cataracts.1 The most convincing support for this thesis has come from the use of AR inhibitors which were shown to delay the onset of lens changes in this type of cataracts in animals.2 3 In the case of galactose cataract, the evidence is substantial in that several AR inhibitors, structurally different, were found to retard cataracts in rats fed galactose.4

Galactose feeding in young rats leads to dense nuclear opacities in 2 to 3 weeks. In contrast, a much longer and variable period of 6 to 9 weeks is required for the frank opacity to appear in diabetic rats. Thus, in diabetic rats, it has been difficult to assess the effectiveness of AR inhibitors which are only marginally active and capable of delaying the onset of the nuclear opacity by only a few weeks. For this reason, the South American rodent degu has been used, which when made diabetic, develops cataracts by 2 weeks. With these diabetic degus it was possible to show that treatment with the AR inhibitor quercitrin could delay the formation of nuclear opacity. However, since so much information about diabetic cataracts was previously derived from studies with rats, it was considered important to demonstrate that an AR inhibitor could alter lens opacification in diabetic rats. This has now been accomplished with the use of the very potent AR inhibitor CP-45,634 (d-6-fluoro-spiro(chroman-4,4'-imidazolidine-2',5' dione) developed by the Pfizer, Inc. Previously, Peterson et al.5 have demonstrated this inhibitor to be effective in retarding cataracts in galactosemic rats.

Methods. Rats of the Charles River strain weighing approximately 80 gm were made diabetic by an intravenous injection of streptozotocin (100 gm/kg body weight). Lens polyols were analyzed by the gas-liquid chromatographic method as previously...