

# Original Articles

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## The effects of ophthalmic drugs, vehicles, and preservatives on corneal epithelium: a scanning electron microscope study

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*Scanning electron microscopy was found to be of considerable use in evaluating the effects of various commonly used drugs, vehicles, and preservatives on the corneal surface. Of the preparations tested 0.3 per cent gentamicin caused many central cellular microvilli to stand up prominently. Moderate losses of peripheral microvilli, with mild superficial cellular desquamation was noted with 0.25 per cent phospholine iodide, 2 per cent pilocarpine, 2 per cent fluorescein, and Fluor-I-Strip. The top layer of epithelial cells desquamated with 4 per cent cocaine or neopolycin treatment. The top two layers of cells were lost when 0.01 per cent benzalkonium chloride was instilled. When cell death occurred severe membrane disruption was accompanied by loss of microvilli and rupture of intercellular tight junctions. These studies show that the cytotoxicity of topical ocular preparations can be tested in an in vivo model and evaluated by scanning electron microscopy.*

Ophthalmic solutions are formulated to achieve long shelf-life, effective antimicrobial action, comfort to the patient, penetration and action of the active agent(s), and minimal side effects. Tissue reactions from these preparations are often tolerated

to gain one or more specific benefits. However, there are frequent situations when specific drug components may induce serious iatrogenic diseases, possibly vitiating any beneficial effects on the primary disease process.

Clinicians first became aware of some of the dangers of topical ocular medications with the frequent use of cocaine.<sup>1</sup> Other local anesthetics, including those presently used such as proparacaine and tetracaine, were also found to create diseases by their continual use; epithelial defects followed by sterile corneal infiltrates, neovascularization, and scarring have been well documented.<sup>2-4</sup>

Gassett and co-workers<sup>5</sup> using 10× or 100× the concentrations of benzalkonium chloride (BAK) employed in ophthalmic preparations, demonstrated conjunctival necrosis, corneal ulcers, and massive iritis

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Supported in part by National Institutes of Health Grant No. 1-RO1-EY01342-01 and by a Fight For Sight Grant, Fight For Sight, Inc., New York City.

Submitted for publication July 29, 1975.

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**Table I.** Complete list of drugs, vehicles, and preservatives used in the animal portion of the study with their respective SEM corneal effects. Each preparation was tested in 4 to 6 rabbit eyes

<i>Topical preparation</i>		<i>SEM surface effect</i>	
<i>Preparations causing no plasma membrane effects:</i>			
Drugs:	Atropine 1% Chloromycetin 0.5% Epinephryl borate 1% Gentamicin 0.3% Proparacaine 0.5% Tetracaine 0.5%	Surface epithelial microvilli normal in size, shape, and distribution.  No denuded cells. Cell junctions intact.  Plasma membranes not wrinkled.  Usual number of epithelial "holes."	
Vehicles:	Boric acid 5% in petrolatum mineral oil Methylcellulose 0.5% Polyvinyl alcohol 1.6% Saline 0.9%		
Preservatives:	Chlorbutanol 0.5% Disodium edatate 0.1% Thimerosal 0.01%		
<i>Preparations causing moderate plasma membrane effects:</i>			
Drugs:	Phospholine iodide 0.25% Pilocarpine 2% Fluorescein 2% Fluor-I-Strip (wet with one drop 0.9% saline)		Most cells normal.  Some cells show loss of microvilli and wrinkling of plasma membranes.  A small number of cells showed disruption of plasma membrane with premature cellular desquamation.
<i>Preparations causing significant plasma membrane injury and cell death:</i>			
Drugs:	Cocaine 4% Neopolycin (No BAK)		Complete loss of microvilli. Wrinkling of plasma membranes. Premature desquamation of top layer of cells.
Preservatives:	Benzalkonium chloride 0.01%		Severe epithelial microvillous loss.
Drug + Preservative:	Pilocarpine 2% or Gentamicin 0.3% + Benzalkonium chloride 0.01%		Severe membrane disruption. Death and desquamation of 2 superficial layers of cells over 3-hour period.

followed by corneal scarring and vascularization in the rabbit eye. Similar concentrations of thimerosal and chlorbutanol produced mild or no conjunctival or corneal reactions.

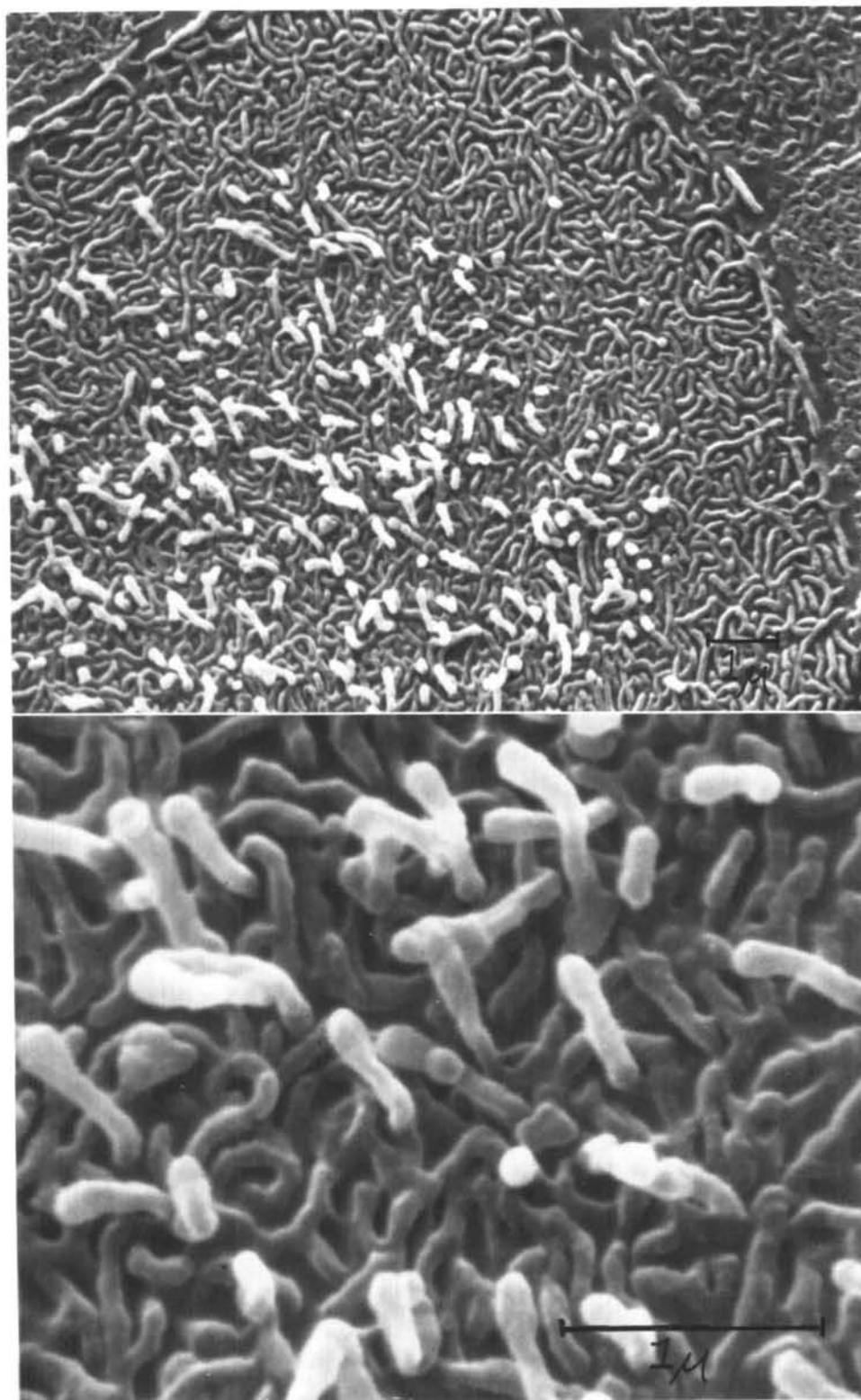
Drug effects on the cornea have been investigated by several other techniques including cultures of epithelial explants,<sup>6, 7</sup> measurement of epithelial regrowth after abrasion or keratectomy,<sup>8</sup> and injection of the solutions directly into the stroma or intracamerally.<sup>9, 10</sup>

When tetracaine, and a number of other ophthalmic preparations, was applied to the corneal surface scanning electron microscopy (SEM) revealed loss of surface microvilli,<sup>11</sup> interruption of cell membranes, and clumping of tonofilaments.<sup>12</sup>

In the present SEM study we attempted to separate and evaluate the component effect of some commonly used topical drugs, vehicles, and preservatives on the surface corneal epithelium. These preparations were tested in vivo in their clinical concentrations and volumes.

#### Materials and methods

To separate the effects of drug components the active drug, preservative, or vehicle was dissolved in 0.9 per cent saline at neutral pH (Table I: solutions, concentrations, and results). These separate solutions were instilled individually in both eyes of New Zealand albino rabbits, 2 to 3 kilograms in size. With the upper lid held up drops were applied to the upper corneoscleral limbus allowing the fluid to flow down evenly onto the cornea. At preselected time intervals the animals were anesthetized with intravenous



**Fig. 1.** Thirty minutes after 0.3 per cent gentamicin application many microvilli appear erected in the central portions of significant numbers of cells.

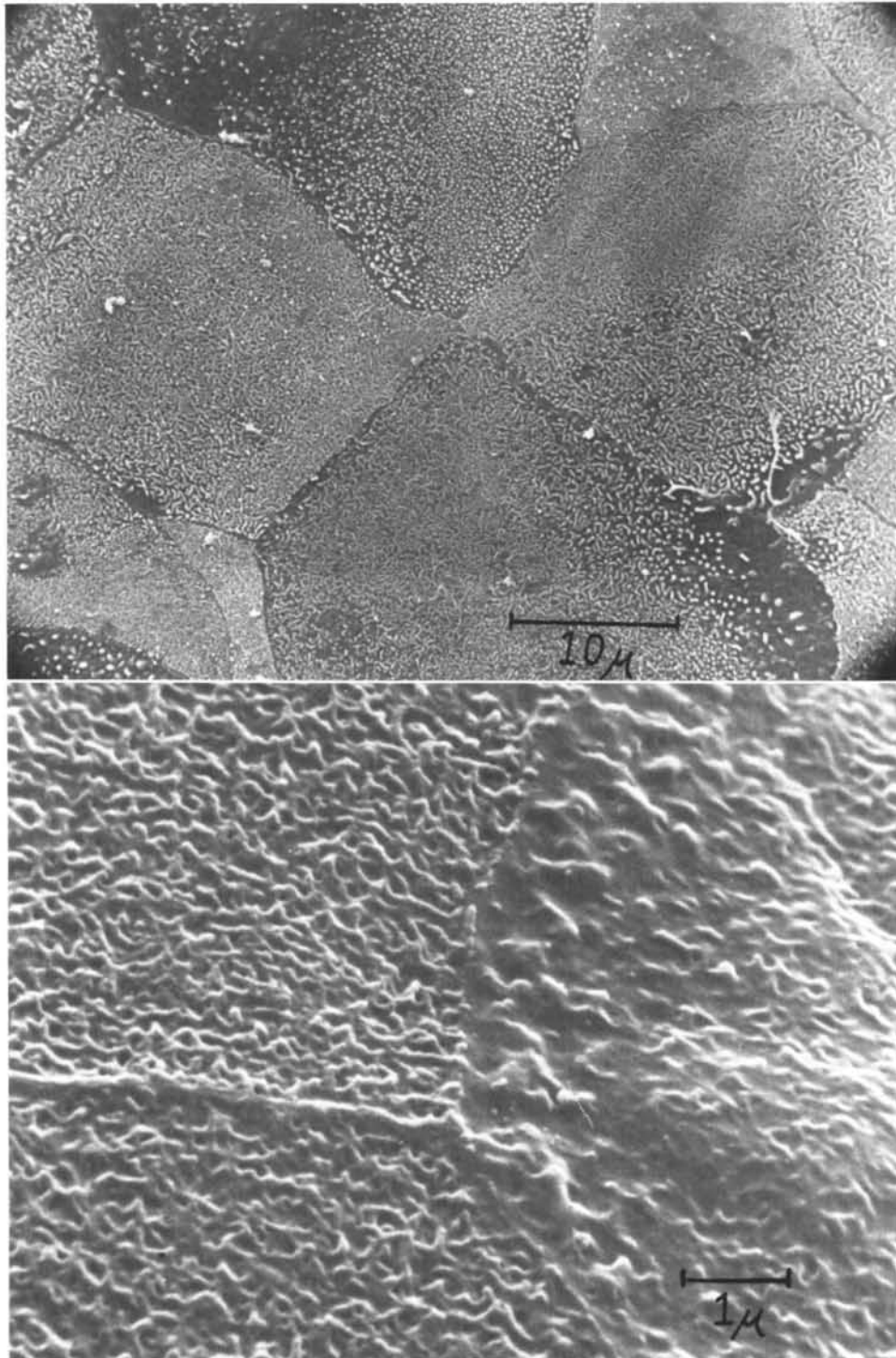


Fig. 2. Thirty minutes after 2 per cent pilocarpine applied to corneal surface. Significant decreases in microvilli, especially at the cell periphery, are noted primarily on dark cells. Lower photo-higher power view.



**Fig. 3.** Fifteen minutes after 0.01 per cent benzalkonium chloride application large numbers of surface cells retract from their cell contacts leaving retraction fibrils. Many cell edges are peeling up. Note the loss of microvilli from the edges of cells retracting or peeling. Inset: high power of retraction fibrils. RF = retraction fibrils.

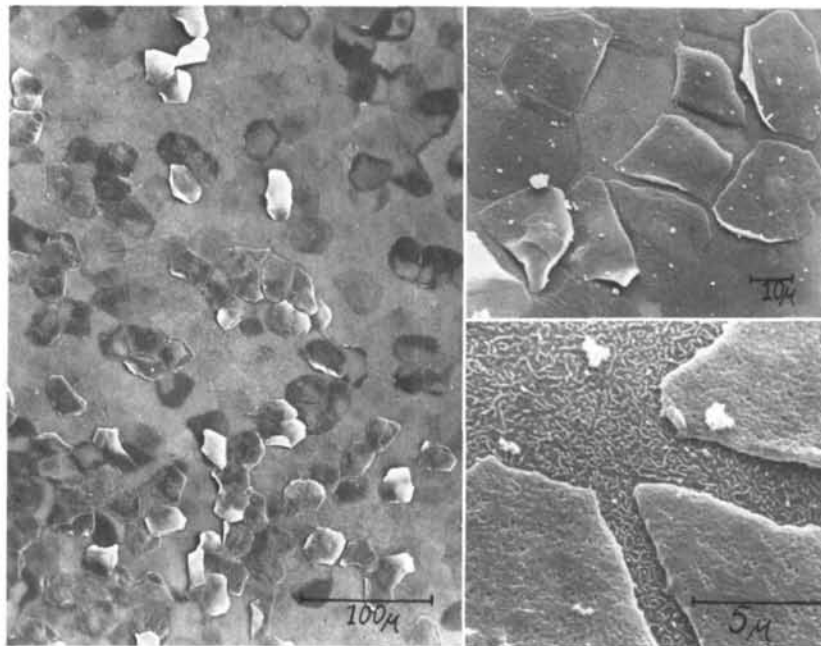
sodium pentobarbital, the eyes flooded with warm 4 per cent glutaraldehyde, and corneas removed as previously described.<sup>13</sup> The preparation of specimens for SEM has been described elsewhere.<sup>13</sup> Specimens were examined in a Cambridge S4 stereoscanner.

In preliminary experiments, 0.01 per cent BAK was found to be very injurious to surface corneal epithelium. It was therefore studied at 5, 15, and 30 minutes, 1, 3, and 6 hours after application. From this data the maximal damaging effects of BAK were found to be at 30 minutes and 1

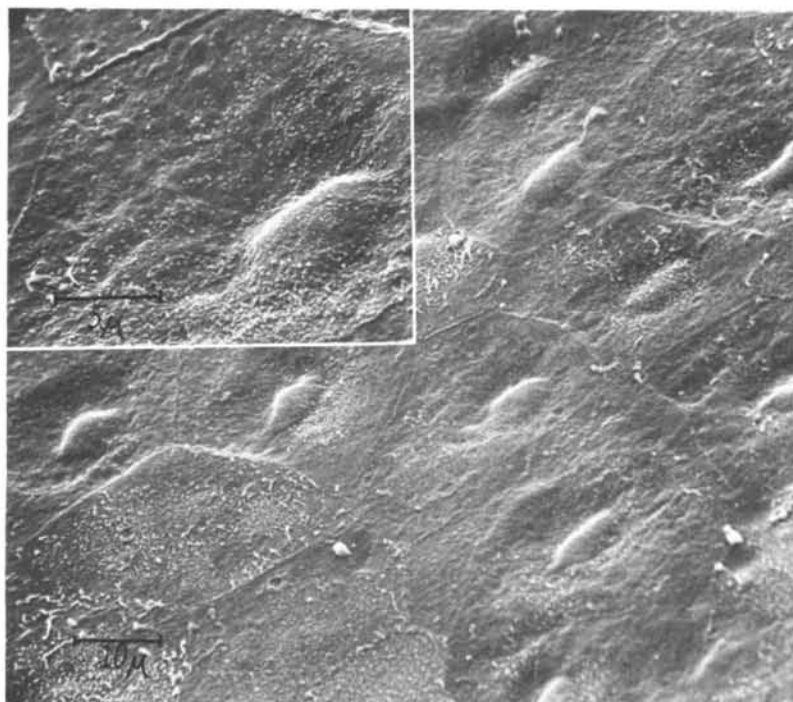
hour. Since a complete time course could not be carried out for each preparation tested, all subsequent corneas were removed at 30 minutes or 1 hour after instillation of the preparation.

Another five rabbits were anesthetized and a 12 mm. diameter circular area of epithelium scraped off the cornea with a No. 15 Bard-Parker blade leaving basal lamina intact.<sup>14</sup> Twenty-four hours later 0.01 per cent BAK was applied to the corneas as previously described and 30 minutes later the corneas were removed.

Two corneal buttons from humans with kerato-



**Fig. 4.** Thirty minutes after 0.01 per cent benzalkonium chloride application. Most of the top layer of cells are desquamating. Insets: severe degenerative membrane changes are notable in these dying cells.



**Fig. 5.** One hour after 0.01 per cent benzalkonium chloride. First layer of cells has desquamated exposing second cell layer with nuclear bulge. Inset: adjacent to the nuclear bulge a depression marks the indent of the nucleus from the top cell. Note the sparse and small microvilli on the recently exposed cells.



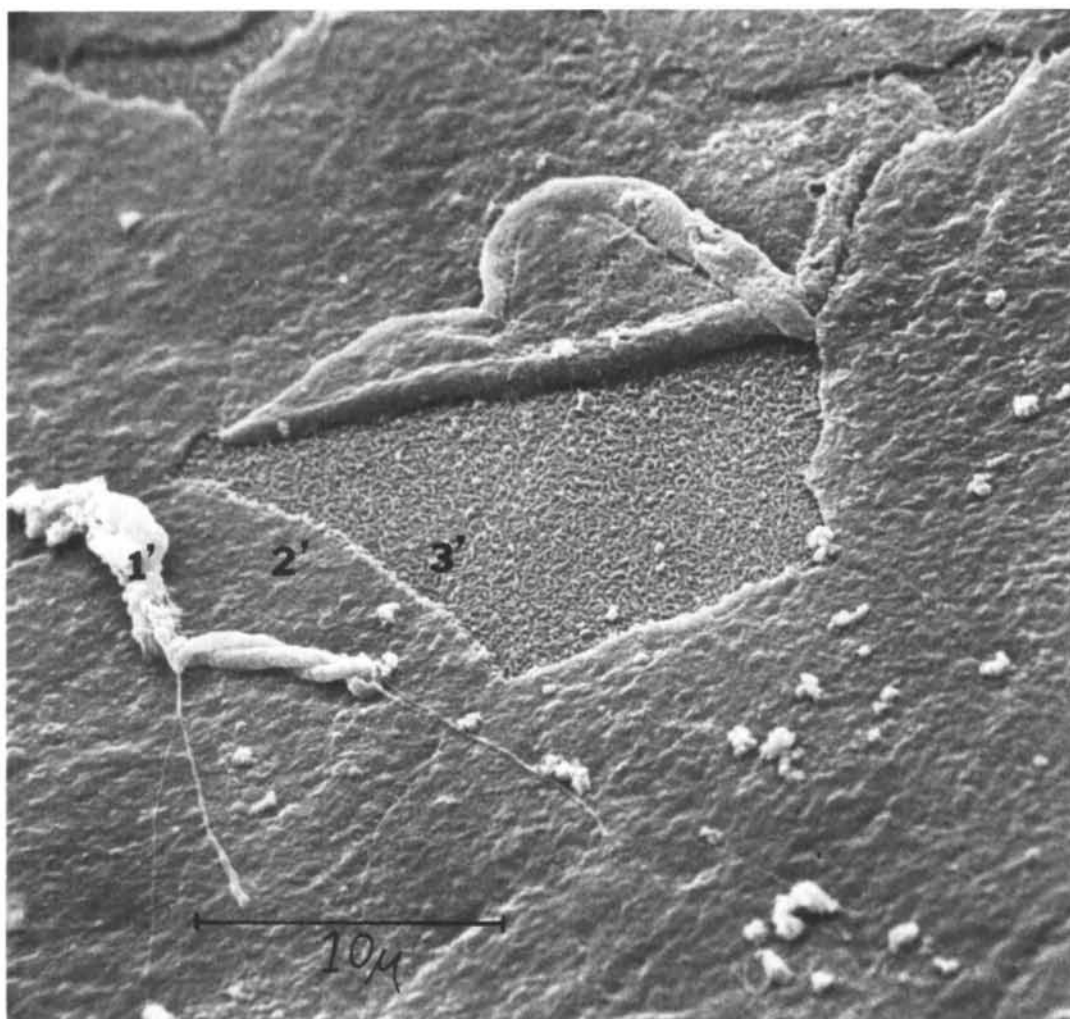


Fig. 6. Three hours after application of 0.01 per cent benzalkonium chloride. The second layer cells have also undergone extensive degenerative membrane changes. In this case the third layer cell is now exposed with a normal microvillous appearance. A first layer cell appears to be rolled up on the surface. Note remaining retraction fibrils. 1' = first or top layer cell, 2' = second layer cell, 3' = third layer cell.

conus were obtained one hour after application of 2 per cent pilocarpine alone or 2 per cent pilocarpine with 0.01 per cent BAK. Corneas were kept wet with 0.9 per cent saline solution, removed by trephination, and placed in warm 4 per cent glutaraldehyde for further preparation.

### Results

Table I summarizes the results of the animal tissue studies. The findings are separated into preparations having (1) no demonstrable surface effects, (2) moderate plasma membrane effects with moderate

cell desquamation, or (3) severe plasma membrane effects with significant cell desquamation.

*Preparations causing no plasma membrane effect.* Noteworthy in this group are the pure forms of the topical anesthetics, 0.5 per cent tetracaine and 0.5 per cent proparacaine. Significant numbers of central cellular microvilli were erected when treated with 0.3 per cent gentamicin (Fig. 1).

*Preparations causing moderate plasma*

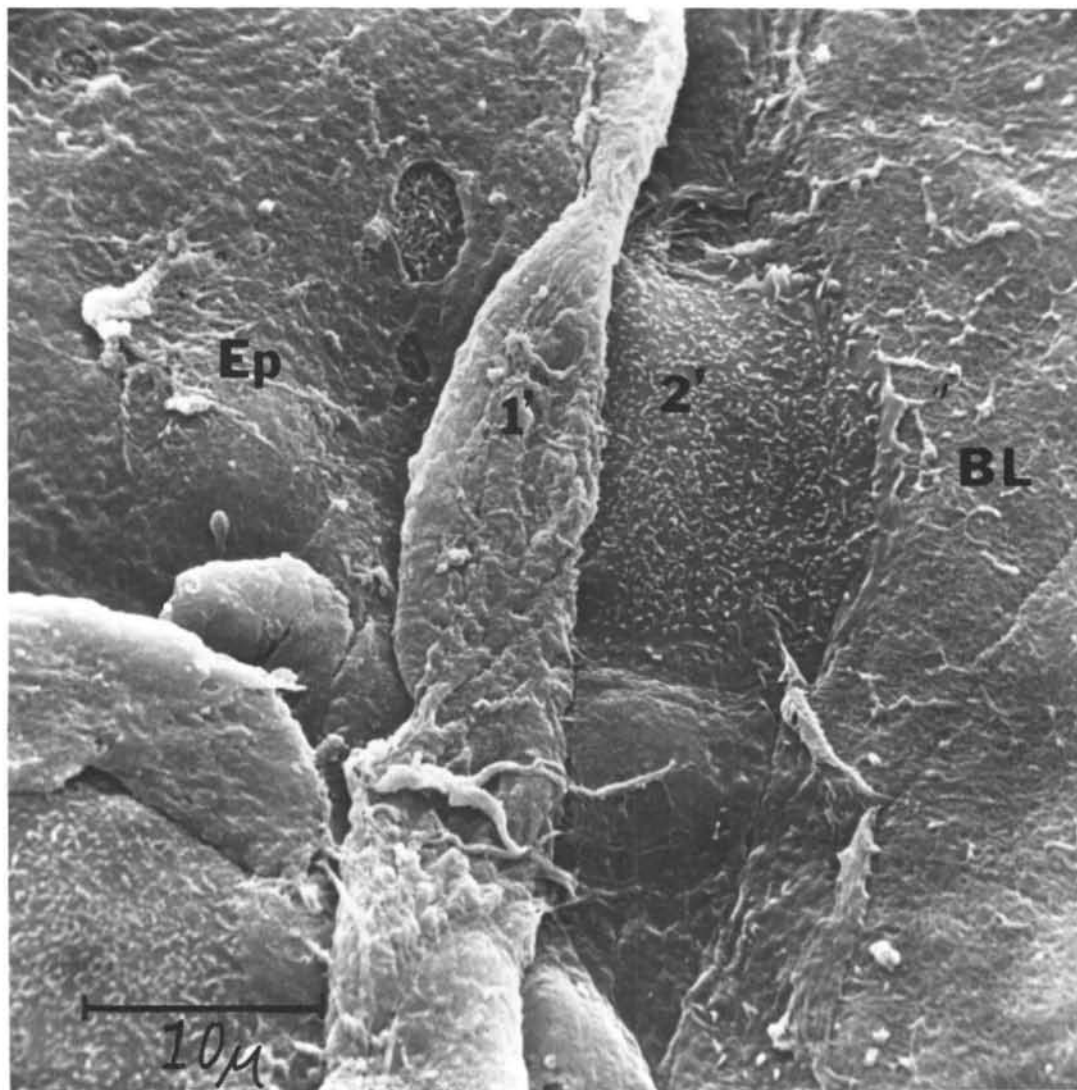


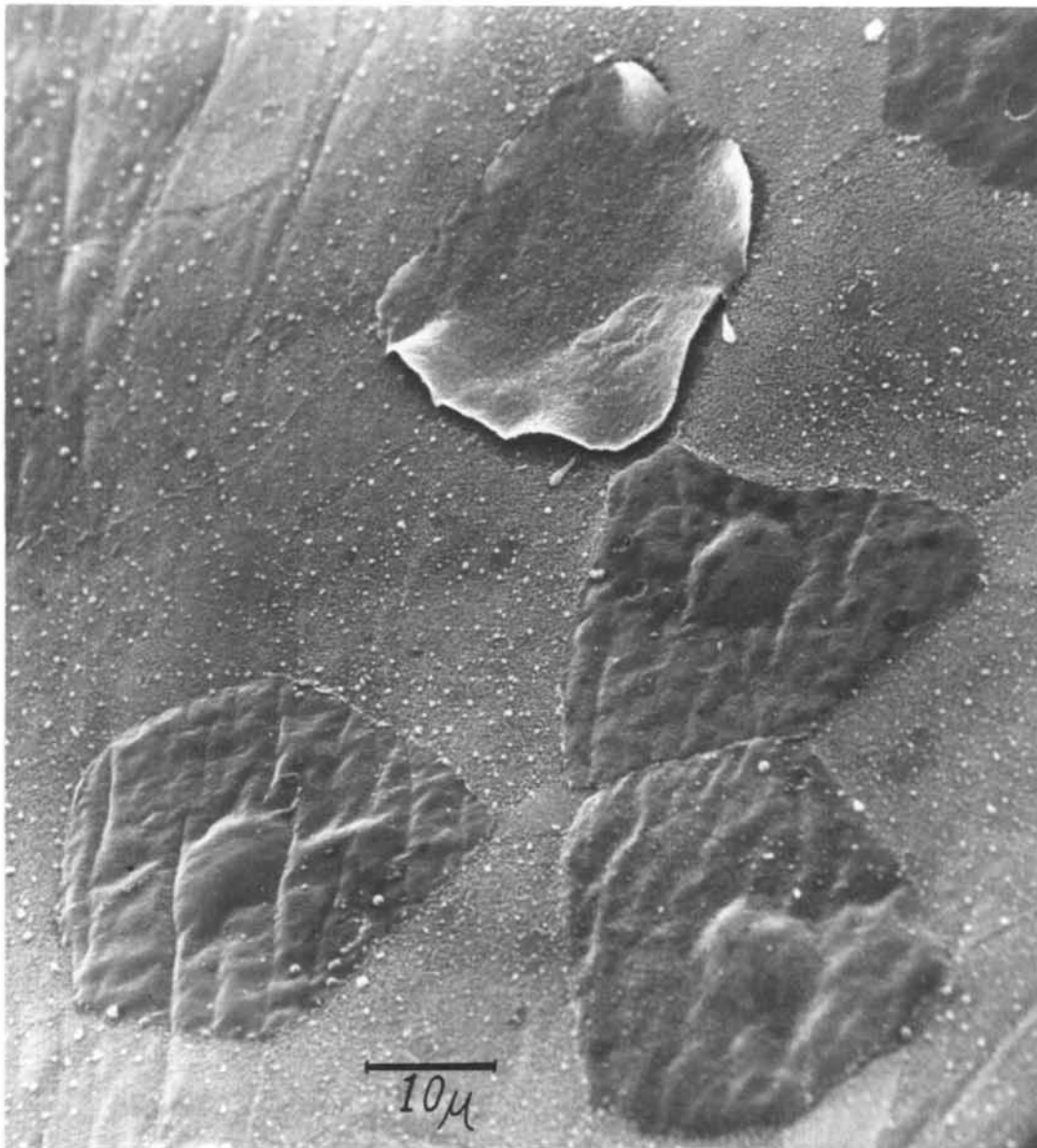
Fig. 7. Thirty minutes after 0.01 per cent benzalkonium chloride was applied to actively migrating corneal epithelial cells. The advancing layer of cells lose their locomotor activity (ruffles and filopodia) showing extensive membrane degeneration. The advancing cell peeled back exposing the underlying cell showing cellular adherence to the basal lamina. BL = basal lamina, Ep = epithelium, 1' = 1st cell layer, 2' = 2nd layer.

*membrane effects.* The membrane effects of these drugs (Table I) were particularly noticeable in the upper half of the cornea near the site of drug application (Fig. 2).

*Drugs causing significant plasma membrane injury and cell death.* At 5 minutes 0.01 per cent BAK caused some cells to peel up at their borders, exposing cells

beneath. At 15 minutes many adjacent cells separated from one another leaving plasma membrane strands as evidence of tight junction (Fig. 3). Thirty minutes after application, most of the top epithelial cells were loosely lying on the corneal surface (Fig. 4). These peeling cells showed extensive disruption of their plasma membranes. By 1 hour, most of these



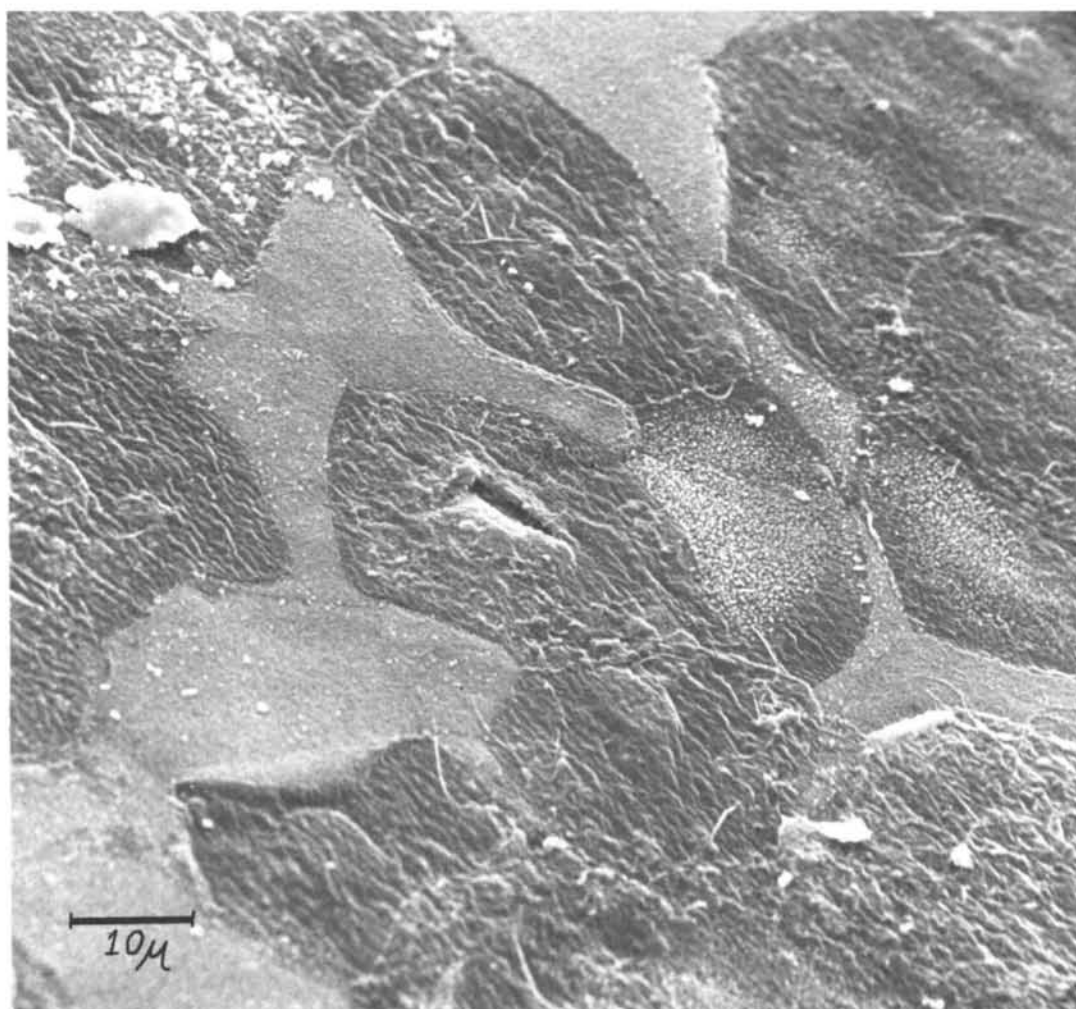


**Fig. 8.** Thirty minutes after 4 per cent cocaine applied to the cornea resulted in loss of microvilli, wrinkling of plasma membrane, and premature desquamation of most surface cells.

surface cells had prematurely desquamated, exposing prominent nuclear bulges in the second cell layer. Adjacent indentations in this second cell layer represent posterior nuclear bulges from the desquamated top layer of cells (Fig. 5). Three hours after BAK application the second layer of cells showed extensive plasma

membrane damage and was desquamating (Fig. 6). After six hours the newly exposed third cell layer had a nearly normal epithelial appearance.

Thirty minutes after 0.01 per cent BAK solution was applied to migrating corneal epithelium, 24 hours after abrasion, the leading edge had lost its adhesion to the



**Fig. 9.** Thirty minutes after neopolycin instillation. Surface cells have lost their microvilli and show membrane wrinkling. Most of the top layer of cells will desquamate.

basal lamina and was peeled back (Fig. 7).

Thirty minutes after topical 4 per cent cocaine or neopolycin (without BAK) application, most surface epithelial cells showed complete loss of microvilli, wrinkling of plasma membranes, and premature desquamation (Figs. 8 and 9). Most of the top layer of cells were lost in the ensuing hour.

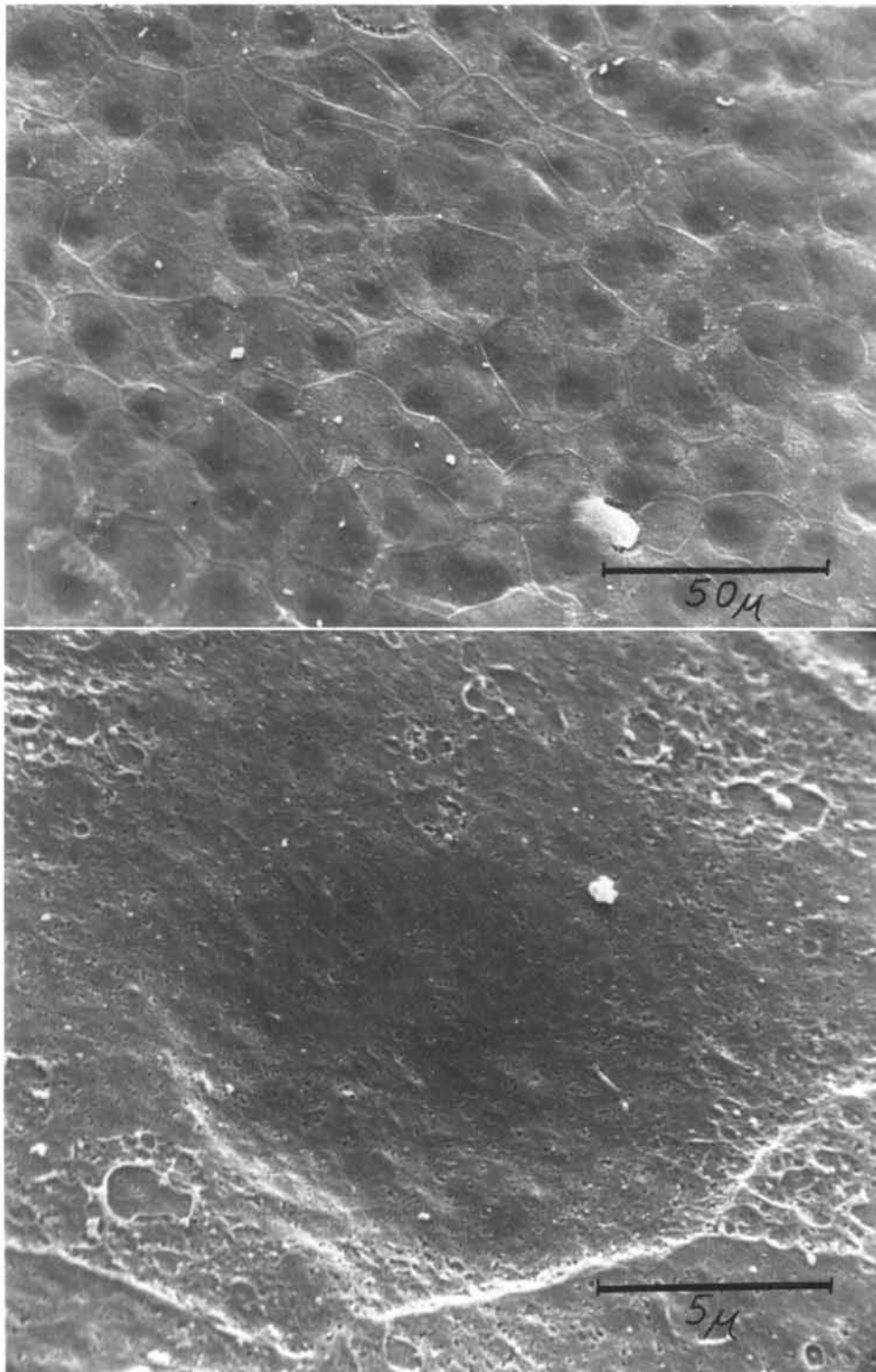
**Drug effects on human cornea.** When 2 per cent pilocarpine with 0.01 per cent BAK was applied to the human cornea, about one hour prior to excision, the top layer of cells were found to be missing.

Prominent nuclear indentations were noted from the desquamated surface layer. The second layer cell showed severe plasma membrane disruption and destruction (Fig. 10).

One hour after application of 2 per cent pilocarpine alone loss of microvilli and plasma membrane destruction were noted in the surface layer of cells (Fig. 11).

#### Discussion

The variable magnification of SEM, from 20× to 100,000× allows for sampling and evaluation of large areas of the corneal surface as well as minute cellular attach-



**Fig. 10.** Keratoconus patient treated one hour before with two drops of 2 per cent pilocarpine containing 0.01 per cent benzalkonium chloride. Note the indents in the second layer cell surface from posterior bulges of destroyed top layer cells. Lower photo shows severe membrane destruction in second layer cell.

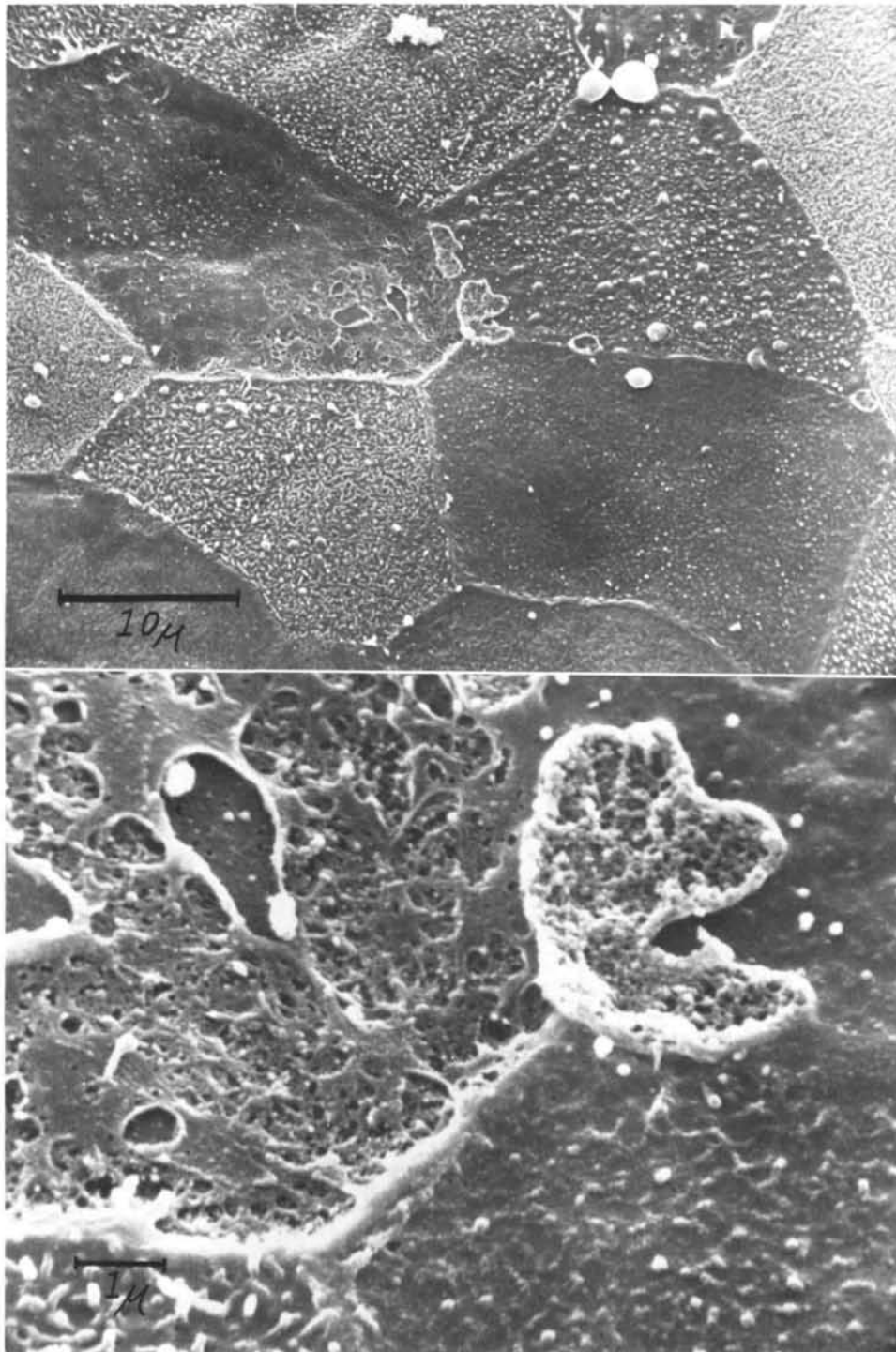


Fig. 11. Keratoconus patient treated one hour before with two drops of 2 per cent pilocarpine alone. Loss of microvilli with some plasma membrane destruction is evident.

ments or plasma membrane changes not easily detected by any other means. The results of this study strongly suggest that the cytotoxicity and membrane disruption effects of ocular topical preparations may be predicted on the basis of SEM observations.

A large number of frequently used topical preparations caused little or no cellular damaging effects. Although antiglaucoma drugs, such as 2 per cent pilocarpine and 0.25 per cent phospholine iodine, caused moderate plasma membrane disruption and cell death they are probably harmful only to an occasional patient, particularly one with pre-existing epithelial or tear film abnormalities. The mechanism of this cellular injury is unknown.

When preparations causing severe plasma membrane disruptions and cell death (e.g., neopolycin, cocaine, or BAK-containing drugs) are used frequently, iatrogenic impediment of the epithelial healing process and shortening of the tear film break-up time can be expected.<sup>15</sup> Rucker and co-workers<sup>16</sup> has shown that the slowest rate of epithelial regrowth occurred in eyes receiving a solution containing 0.01 per cent BAK. The loss of membrane activity and peeling back of the leading epithelial cells, noted in this SEM study, 30 minutes after BAK application, strongly suggests adverse effects of BAK on cell locomotion and adhesion.

BAK is a powerful cationic detergent which destroys bacteria after ionic attraction. A detergent effect on plasma membranes accounts for its epithelial toxicity when used frequently, even in the normal eye. To avoid these damaging effects in eyes with epithelial disease, topical medications could be packaged in sterile, small volume drug containers.<sup>18</sup>

From this study, and those in the literature, the extensive use of BAK in ophthalmic preparations must be critically reviewed and its use, if indicated, curtailed. Although this study was not intended to be encyclopedic, use can be made of the

data in drug formulations, especially by the increased incorporation of preservatives shown to be relatively harmless to surface epithelium.

M. Tarkanian is thanked for his contributions in the initial phases of this work. Dr. Keith Porter is thanked for his encouragement and gracious permission to use the scanning electron microscope at MCD Biology, Boulder Campus, University of Colorado. C. Paterson is thanked for critical manuscript review.

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