Ultrastructural Effects of Sodium Chloride on the Corneal Epithelium

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Ten excised rabbit corneas were bathed posteriorly with glutathione bicarbonate Ringer's solution (GBR), while anteriorly the bathing solution was either GBR or sodium chloride solution (NaCl). All solutions had an osmolarity of 305 ± 2 mOsm/kg. The corneas were fixed after 150 min exposure to the solutions, and prepared for electron microscopy. Morphometric analysis of the electron micrographs was conducted by an observer unaware of the anterior bathing solution. In each case, the epithelium was examined along a 1500 μm stretch of the basement membrane. Cells were categorized as normal, abnormal, and sloughing. Abnormal cells showed cytoplasmic and nuclear pallor, and disrupted cell membranes. Sloughing cells showed partial separation from the underlying epithelium. Corneas exposed to NaCl showed statistically significant differences from those exposed to GBR; the differences occurred in both the number of abnormal cells, and the number of sloughing cells. All observed ultrastructural changes were limited to the surface region of the epithelium. It is concluded that sodium chloride solution is inadequate at maintaining the epithelial surface. Invest Ophthalmol Vis Sci 30:116-121, 1989

The normal epithelial surface appearance has been described in a number of histologic studies.¹⁻³ The effects of preservatives⁴ and contact lenses⁵⁻⁶ on the surface of the cornea have been studied by examining ultrastructural changes. As there is now evidence from the laboratory specular microscope that the ionic composition of the precorneal bathing solution can influence ocular surface structure,⁷⁻⁸ it is important to verify these changes at the ultrastructural level. This study compares the effects of topical sodium chloride solution and Ringer's solution on the structure of the rabbit corneal epithelium.

Materials and Methods

The animals used in the current study were treated in accordance with the ARVO Resolution on the Use of Animals in Research. Corneas from New Zealand White rabbits were mounted in a laboratory specular microscope at a temperature of 35°C. The endothelial surface was perfused with glutathione bicarbonate Ringer's (GBR) solution with the following composition: NaCl (111.5 mM), KCl (4.8 mM), CaCl₂·2H₂O (0.8 mM), MgCl₂·6H₂O (0.9 mM), NaHCO₃ (29.3 mM), NaH₂PO₄ (0.9 mM), glucose (5.0 mM), adenosine (0.5 mM), glutathione (0.3 mM). The osmolarity of the solution was 305 ± 2 mOsm/kg checked by a freezing point depression osmometer (Model 2007, Precision Systems, Sudbury, MA), and the pH adjusted to 7.5 by bubbling with a mixture of 95% air/5% CO₂. The GBR solution was perfused over the endothelium at a rate of 0.3 ml/hr at a pressure of 20 mmHg. Each cornea was perfused for 150 min. Periodic measurements of the entire corneal thickness were made with the specular microscope in order to check hydration changes. Corneal swelling was less than 6% for the duration of all experiments.

The epithelial surface was superfused with either GBR or isosmolar NaCl solution at a rate of 6 ml/hr. The NaCl had a concentration of 165.8 mM, giving an osmolarity of 305 ± 2 mOsm/kg. The two corneas of the rabbit were perfused consecutively, the epithelium of one cornea with NaCl, and the other with GBR. However, the order was varied so that three of the first corneas were bathed with NaCl and two bathed with GBR. A total of five corneas were bathed with NaCl and five with GBR.

Following the 150 min perfusion regime, the corneas were placed in fixative (3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2). The vials containing the corneas were coded so that the electron micro-
A ~ricle in the plane of the cut. The code revealing which corneas received so-
dium chloride solution was disclosed only after the cells had been counted from all preparations, as de-
scribed below. Specimens dissected from the central cornea were first post-stained in 1% osmium tetrox-
ide for 3 hr and thereafter dehydrated through an alcohol series. Subsequently the tissue was cleared in propylene oxide for 30 min followed by 4–10 hr in a 1:1 mixture of propylene oxide and Spurr’s epoxy. Infiltration of the cornea by pure Spurr’s epoxy was
accomplished overnight. The pieces of tissue were then transferred to block molds for 8 hr at 70°C to
polymerize the plastic.
Semithin (1 µm) toluidine blue-stained sections were obtained for light microscopic observations, and
were also used to determine the ideal block orientation for thin transverse sections. The unfilmed, 75 × 300 mesh copper grids with thin sections mounted with the epithelium perpendicular to the short side of the mesh apertures were double stained in 3.5% uranyl acetate for 20 min and with Reynold’s lead citrate for 10 min. The ultrastructural observations were made with a Jeol 100C transmission electron micro-
scope calibrated with a carbon grating.
Cells of the epithelium were counted from electron micrographs of standardized magnification (×2132)
in sections that together covered 1500 µm as measured along the basement membrane. The sections used to cover the required area were all taken from different blocks of tissue and, therefore, the same area could not be analyzed twice. For reasons of repeata-
bility only epithelial cells which contained a nucleus, or a portion of a nucleus, were included in the count. All cells were placed into either the normal or the abnormal category. If two or more of the criteria
listed in Table 1 were manifest, a cell was classified as abnormal, otherwise the cell was considered normal. Detached cells within 10 µm of the intact epithelial surface were included in the count, since they were assumed to be adherent to the cornea at some point beyond the plane of the cut.
The total number of sloughing cells was also counted. This third category was not required to show a nucleus in the plane of the section. As before, the cells had to be within 10 µm of the epithelial surface. Loss of desmosomal junctions was considered the minimum criterion for sloughing, and inclu-
sion in this category. Feasibly, both normal and abnormal cells could be considered in this count but no normal cells were found detached from the underlying cornea. Therefore, the sloughing cell count contained only abnormal cells, with or without the nu-
cleus in the plane of the cut.

### Results

The epithelium of the corneas bathed with GBR solution contained tightly packed, electron-dense cells (Fig. 1), although along the epithelial surface these cells frequently lost some of their electron den-
sity. Occasionally a surface cell with more pronounced pallor was encountered. Usually such cells had a reduced number of desmosomal junctions with the internally adjacent cells, and appeared to be in a
stage of sloughing.

The epithelium exposed to NaCl solution showed several changes in the surface layers, while the wing and basal cell layers remained normal (Figs. 2–4). The abnormal cells had two or more of the degenera-
tive characteristics listed in Table 1. Larger areas in the corneas subjected to NaCl solution appeared to suffer exfoliation of squamous cells, which detached in sheets (Fig. 2). Cells appearing to be in the process of exfoliation were frequently bloated (Fig. 3).

Quantitative results are shown in Table 2. For ab-
normal cells, the median number appearing in 1500 µm of epithelium was three cells for GBR solution, compared with 26 cells for NaCl. These two catego-
ries were significantly different (P < 0.01, two-tailed Mann-Whitney U Test). For sloughing cells, the me-
edian number was six cells for GBR and 69 cells for NaCl. Again, these two categories were significantly different (P < 0.01, two-tailed Mann-Whitney U Test). For normal cells, the median was 479 cells for GBR, and 464 cells for NaCl. The number of normal cells in the corneas treated with NaCl did not differ significantly from the number of normal cells in the corneas treated with GBR.

### Discussion

The ultrastructural appearance of the rabbit cor-
neal epithelium bathed in GBR resembled closely

| Characteristics of epithelial cell abnormalities |
|-----------------|-----------------|
| **Cytoplasm**    | Loss of normal texture |
|                   | Cytoplasmic pallor   |
|                   | Loss of organelles   |
|                   | Empty areas         |
|                   | Loss of microvilli   |
| **Nucleus**      | Loss of heterochromatin |
|                   | Loss of normal texture |
|                   | Nuclear pallor       |
|                   | Nuclear membrane disruption |
| **Plasmalemma**  | Disruptions of the plasmalemma |
| **Relation to adjacent cells** | Loss of desmosomal junctions |
that of the normal primate and rabbit corneas as described in earlier studies.\(^1,2\) However, corneas bathed in NaCl had significantly more abnormal epithelial cells than the group exposed to GBR. Since the protocol for both groups, including the osmolarity of the bathing solutions, was identical, it is concluded that the difference in the content of the solution provoked the increased incidence of abnormal cells in the NaCl-exposed epithelium.

Cells with a reduced number of desmosomal junctions with their internal neighbors were interpreted as being in the process of sloughing. This process was significantly accelerated by exposing the epithelium to NaCl solution. In addition, sloughing cells showed degenerative changes in the plasmalemma, cytoplasm, and nucleus, which were induced or accelerated by NaCl solution. The NaCl solution frequently caused a swelling of the cell, but this may have been secondary to disruption of the cell membrane.

The EM results presented here, and results from the laboratory specular microscope,\(^7\) show that the surface cells of the corneal epithelium are dependent on the composition of the epithelial bathing solution. Thus an additional role is perceived for the precorneal film. It is known as the medium which provides the environmental osmolarity and pH, and gives the epithelium access to atmospheric oxygen, as well as being the principal refracting surface of the eye. In addition, it can now be viewed as supplying several cations and anions to the cells of the anterior epithelium. These include potassium in addition to sodium and chloride, and possibly phosphate, bicarbonate, magnesium and calcium.\(^7\)

It has been suggested\(^7\) that the NaCl-induced appearance of the epithelium, as observed with the laboratory specular microscope, might be due to an increase in sloughing rate. That is, the increased light scatter of the superficial cells when bathed in NaCl is due to accelerated cellular senescence and desquamation. This interpretation is confirmed by the morphometric data presented here. The median number of sloughing cells is ten times greater in corneas bathed with NaCl solution compared with corneas bathed in GBR. The implication is that the overall sloughing rate of the corneal epithelium is ten times higher in NaCl solution than in GBR. However, these measurements show the process of desquamation at one instant in time, and do not indicate the pace of this process, that is, the rate at which cells are actually liberated from the surface. NaCl solution could be interfering with the normal exfoliative mechanism, opening up intercellular spaces and inhibiting the rate at which cells leave the epithelial surface. That is, the absence of essential ions extends the time required for the process of exfoliation. Thus there are two possible interpretations of the data. First, NaCl causes the sloughing rate to be ten times faster than normal. Second, NaCl slows the process of desqua-
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mation so that ten times as many desquamating cells adhere to the epithelial surface. The question cannot be resolved with certainty at this stage. However, reported NaCl-induced appearance of sheets of cells leaving the corneal surface has been interpreted as supporting the accelerated sloughing hypothesis. Also, there is initial evidence from the human eye showing an increase in epithelial squamous cell sloughing when the eye is irrigated with NaCl. By counting cells sampled from the precorneal film, a solution containing additional ions such as potassium and bicarbonate showed fewer epithelial cells. Thus there is at least preliminary evidence that the short-term constituents of tear solutions affect the sloughing rate in the human eye.

How far into the epithelium does the influence of NaCl extend? In this study, cells which were structurally abnormal, but were not yet sloughing, were encountered near the epithelial surface. Therefore, pre-exfoliative changes can be detected before cells actually desquamate. The number of abnormal cells was small in relation to the number of normal cells. Hence the ultrastructural changes induced by NaCl occur close to the surface of the epithelium. The fact that no ultrastructural changes were detectable deep in the epithelium does not necessarily mean that the effect is limited to the epithelial surface. Little is known of the homeostatic mechanisms maintaining epithelial thickness. Does increased sloughing act as a stimulus to mitosis? As there is little understanding of

Fig. 2. Induced epithelial changes. Abnormal cells (A) have remained adherent to the surface but were not included in the count due to the lack of a nucleus in the plane of the section. The exfoliating abnormal cells (S) containing a nucleus were included in both the abnormal and sloughing cell groups, while those sloughing cells without a manifest nucleus (star) were considered only for the latter group. All internal cells are normal (N). ×3600 (NaCl-bathed cornea).

Fig. 3. Surface cells with changes. Along the surface the cells are swollen and their cytoplasm pale. One cell has a disintegrating nucleus (star). Internal cells are normal (N). ×3600 (NaCl-bathed cornea).

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cell-to-cell communication or of the feedback mechanisms controlling the rate of epithelial turnover, it is not possible to determine whether metabolic changes in the basal or wing cells occur without being manifest ultrastructurally. The susceptibility of mitosis to exogenous influence is known, and recently the influence of oxygen deprivation on cell replication has been shown.  

Table 2. Corneal epithelial cell count from ten eyes  

<table>
<thead>
<tr>
<th>Cornea</th>
<th>NaCl</th>
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<th>GBR</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>A</td>
<td>S</td>
<td>N</td>
<td>A</td>
<td>S</td>
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<td>7</td>
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<tr>
<td>3</td>
<td>464</td>
<td>26</td>
<td>69</td>
<td>8</td>
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<tr>
<td>4</td>
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<td>81</td>
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<td>Median</td>
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<td>26</td>
<td>69</td>
<td>479</td>
<td>3</td>
<td>6</td>
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</tbody>
</table>

Each cornea was bathed anteriorly in NaCl or GBR, while posteriorly they were all bathed in GBR. The criteria for classification of cells into the various categories are described in Material and Methods.  

- N = number of normal cells; A = number of abnormal cells; S = number of sloughing cells.

The principal barrier to the diffusion of substances from the precorneal film into the anterior chamber is constructed from the intercellular junctions of the superficial layers of the cornea. Any bathing solution which interferes with the stability of the superficial cells can be expected to increase the permeability of the epithelium. This has been shown in a number of ways. For instance, it has been demonstrated that a decrease in short-circuit current and an increase in the rate of diffusion occurs when the epithelial barrier is compromised. Under more traumatic circumstances it has been shown that preservatives such as benzalkonium chloride and chlorobutanol can increase the permeability of the epithelium. Exposing the cornea to a hypotonic solution causes opening of the intercellular spaces.

Do the effects of exposing the in vitro rabbit cornea to NaCl have a correlate in the human tear system? In the human eye there is a continuous turnover of the precorneal tear film, which is also reconstituted with every blink. Thus it is unlikely that the corneal environment remains constant for extended periods of time. Perhaps only during sleep, or in the extended wear of contact lenses, is the epithelium isolated from the normal perturbations which occur in the tear film. Although there are no eye conditions whose pathogenesis can be ascribed to ionic deficiencies, there are two situations where the sustaining role of tear ions must be examined. First of all in the dry eye, where tear supplements are designed to replace deficient naturally produced tears, and second in contact lens wear where the precorneal film is of reduced thickness and often altered composition. It is important to examine the need for tear supplements to contain all the necessary ingredients to maintain the corneal surface. Is a sterile isotonic solution of sodium chloride adequate, or is it necessary to include other ions such as potassium? These issues have been raised before but have not been addressed, perhaps because of difficulty in identifying the correct test whose results are pertinent to the human eye.

Key words: corneal epithelium, sodium chloride, Ringer's, sloughing, exfoliation, tear composition

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References