Essential Ions for Maintenance of the Corneal Epithelial Surface

William G. Bachman* and Graeme Wilson

It is generally believed that tears are required to furnish only oxygen to the corneal epithelium. However, as tears are a very complicated solution, it is likely that other factors are essential to the cells of the corneal surface. The amount of light scattered from the epithelial surface of the excised rabbit cornea was examined with the in vitro specular microscope while the epithelium was bathed in different solutions. It was shown that the epithelial surface was maintained best with a buffered solution containing potassium, calcium, magnesium, phosphate and bicarbonate, in addition to sodium chloride. The solution was named Basic Tear Solution (BTS). The effect was not due to osmolarity. Potassium was particularly important, as corneas bathed with sodium chloride and potassium chloride were maintained better than corneas bathed with sodium chloride only. The appearance of the epithelial surface was different in these bathing solutions. In sodium chloride the surface scattered more light and more cells were sloughed. Least light was scattered in BTS, and cell-sloughing was at a minimum. Thus, the rate at which cells were sloughed from the epithelial surface and the quality of the surface were dependent on the bathing solution. Invest Ophthalmol Vis Sci 26:1484-1488, 1985

It is generally accepted that oxygen is the only metabolic requirement offered to the cornea from the tears,1,2 and other nutrients are supplied by the aqueous humor and limbal blood vessels. Yet, in a preliminary report,3 the sloughing rate of epithelial cells was shown to be influenced by the composition of the bathing fluid. Excised rabbit corneas observed with the Maurice in vitro specular microscope4 scattered more light from the surface epithelium when the sloughing rate of cells increased. The sloughing rate increased in sodium chloride solution relative to isosmotic Glutathione Bicarbonate Ringer’s solution (GBR). These findings have important implications for the composition of epithelial bathing solutions.

Maurice in vitro specular microscopy is used here to quantify the influence of several tear solutions on the rabbit corneal epithelium. A bathing solution is considered inadequate when its presence leads to increased surface epithelial light scattering, as this is associated with an increased rate of desquamation. A tear substitute is suggested whose composition satisfies the short-term maintenance requirements of the epithelial surface.

Materials and Methods

The in vitro specular microscope used in these experiments was modified by connection to a small computer and digital timer. In this way, both thickness measurements and their time sequence could be recorded automatically as the operator adjusted the fine focus of the microscope. The specular microscope was equipped with a 5X eyepiece and a 40X water-immersion objective (Zeiss, West Germany). The eyepiece could be connected to a photomultiplier (Gamma Scientific; San Diego, CA) with a fiber optic to allow measurement of relative light scatter.

Eyes from New Zealand white rabbits were enucleated with the conjunctival sac and eyelids intact, as described by Dikstein and Maurice,4 and the corneas were mounted in a temperature-controlled environment (35°C).

A preliminary series of experiments was carried out to determine if it was possible to isolate one constituent which, when added to sodium chloride, would maintain the surface epithelium as well as GBR. Potassium chloride was the only single additional constituent which reduced the amount of light scattered by the anterior epithelium. The addition of any other single salt or component gave results very similar to sodium chloride alone. Conversely, GBR minus glutathione, GBR minus adenosine, GBR minus glucose, or GBR

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From the Department of Physiological Optics, School of Optometry, University of Alabama at Birmingham.

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* Formerly from the Department of Physiological Optics, School of Optometry, University of Alabama at Birmingham, currently at U.S. Army Aeromedical Research Laboratory, Ft. Rucker, Alabama.

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Reprint requests: Graeme Wilson, PhD, Department of Physiological Optics, School of Optometry, University of Alabama at Birmingham, AL 35294

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minus all of these, appeared to maintain the superficial epithelium as well as GBR itself.

On the basis of these preliminary experiments, four solutions were tested: GBR, NaCl, NaCl plus KCl, and a Basic Tear Solution (BTS). BTS was formulated as a tear substitute, and contained sodium chloride (680 mg/dl), potassium chloride (140 mg/dl), calcium chloride (6.4 mg/dl), magnesium chloride (12.1 mg/dl), sodium bicarbonate (218 mg/dl), sodium phosphate (9.3 mg/dl). Calcium\(^6\) and magnesium\(^7\) were included in BTS because both have been shown to be important in maintaining cell adhesion. The concentration of magnesium in human tears has not been measured, so its concentration in BTS was made equal to that of GBR which is comparable to aqueous humor. The potassium concentration was adjusted to make it similar to that in tears.\(^8\)

The endothelial perfusion solution was always GBR, whose composition was the same as the epithelial GBR shown in Table 1. The four perfusion solutions were prepared in the laboratory immediately before use, and the osmolarity checked with an osmometer (Model 2007, Precision Systems, Sudbury, Mass.). The osmolarity was 305 ± 2 mOsm/kg. The pH of the buffered solutions was adjusted to 7.5 by bubbling with 95% air/5% CO\(_2\). Both the epithelial and endothelial surfaces were perfused at a rate of 6 ml/hr. The pressure at the endothelial surface was 20 mmHg.

Beginning 10 min after mounting, and at each 10-min interval thereafter, a minimum of 5 measurements of the thickness of the anterior bright band of the epithelium were performed. Periodic measurements of the entire corneal thickness were made in order to monitor the swelling or deswelling of the cornea. Corneas which swelled more than 6% were rejected. Each experiment ran for a total of 150 min.

The rabbit experiments were run in 2 series. In both series, the solution bathing the epithelial surface was varied, but the endothelial perfusate was always GBR. In the first series, NaCl, NaCl + KCl, and GBR were compared. The two eyes of the rabbit were perfused consecutively, and the epithelial solutions were run in a random order. Each solution was run 5 times to make a total of 15 runs. In the second series of experiments, the solutions compared were NaCl, BTS, and GBR. As in the first series, each solution was run 5 times.

A companion experiment using the corneas of one cynomolgus monkey (Macaca fascicularis) was conducted to check the validity of the results on a primate. The corneas were mounted and perfused in the same manner as the rabbit corneas. The epithelial surface of 1 eye was bathed with NaCl solution and the epithelial surface of the other with GBR. The endothelial surface was always perfused with GBR.

The investigations described in this paper conform to the ARVO Resolution on the Use of Animals in Research.

### The Anterior Bright Band

The interface between the superficial cells of the corneal epithelium and the bathing fluid often scatters more light than any other region of the cornea, as is shown in Fig. 1. In the normal rabbit cornea, the amount of light scattered in this region depends on the age of the superficial cells. Cells which are about to slough scatter more light than cells which have only recently reached the epithelial surface. Thus, when the cornea is rapidly sloughing epithelial cells, more light is scattered from the cornea/tear interface than in stable conditions. Cells can slough singly or in aggregates, or sometimes in whole sheets of contiguous cells,\(^3\) as has been observed in humans.\(^9\) In all cases, the thickness of the zone of scattered light at the epithelial interface increases when cells are undergoing exfoliation. This zone, as it appears in the Maurice specular microscope,

### Table 1. Composition of epithelial bathing solutions

<table>
<thead>
<tr>
<th></th>
<th>GBR</th>
<th>BTS</th>
<th>NaCl + KCl</th>
<th>NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>652.0</td>
<td>680.0</td>
<td>930.0</td>
<td>970.0</td>
</tr>
<tr>
<td>KCl</td>
<td>36.0</td>
<td>140.0</td>
<td>36.0</td>
<td></td>
</tr>
<tr>
<td>CaCl(_2) (2)H(_2)O</td>
<td>11.6</td>
<td>6.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MgCl(_2) (6)H(_2)O</td>
<td>12.1</td>
<td>12.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaHCO(_3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaH(_2)PO(_4)</td>
<td>10.3</td>
<td>9.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>90.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenosine</td>
<td>13.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione</td>
<td>9.2</td>
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All concentrations expressed in mg/dl.

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Fig. 1. A scan of the rabbit cornea taken with a photomultiplier attached to the eyepiece of the in vitro specular microscope. The corneal epithelium was bathed in NaCl. In this scan the anterior bright band is about 20 \(\mu\)m thick and scatters more light than any other layer of the cornea.
Fig. 2. In vitro specular microscope view of the anterior bright band (ABB) of the rabbit corneal epithelium and stroma following 100 min in NaCl. Intercellular spaces of epithelial cells are visible. The anterior stroma scatters more light than the highly transparent cells of the basal epithelium (bar = 10 μm).

is referred to here as the anterior bright band (ABB). The ABB cannot easily be observed in conventional slit-lamp biomicroscopy, as it is obscured by the proximity and intense scatter of the tear/air interface.

Measurement of the thickness of the ABB was obtained by first adjusting the fine focus of the microscope so that a hairline in the eyepiece appeared to touch the anterior surface of the ABB. The fine focus was then adjusted so that the hairline touched the posterior surface of the ABB. The difference between these two positions gave the thickness of the ABB and was recorded automatically.

Results

The appearance of the corneal epithelium after 100 min in NaCl is shown in Figure 2. The ABB had in-

creased to almost half the thickness of the epithelium. Intercellular spaces were visible at a level corresponding to wing cells. The deep epithelium scattered very little light. After 200 min in NaCl, pre-exfoliative sheets could sometimes be observed separating from the underlying epithelium (Fig. 3).

In the first series, shown in Figure 4, corneas perfused with GBR showed no change in ABB thickness after 150 min of perfusion. Corneas perfused with NaCl underwent a mean net gain in ABB thickness of 15.2 μm in 150 min. Corneas perfused with NaCl plus KCl underwent a mean net gain of 5.2 μm in 150 min. Data collected from 100 min to 150 min for each of the test conditions is significantly different from the other two (P < 0.05, Studentized Range Test).

In the second series, shown in Figure 5, corneas perfused with GBR showed a mean net gain of 0.6 μm in ABB thickness after 150 min of perfusion. Corneas perfused with NaCl underwent a mean net gain in superficial thickness of 15.2 μm after 150 min. Corneas

Fig. 3. In vitro specular microscope view of a sheet of cells sloughing from the corneal epithelium following 200 min in NaCl. A bright layer of cells is shown desquamating from the epithelial surface. The underlying surface scatters less light. Towards the right of the photomicrograph, the desquamating cells are still attached, and the anterior bright band (ABB) thickness includes the old surface and the new surface. Towards the left, beyond the edge of the desquamating sheet, the ABB thickness is considerably reduced (bar = 10 μm).
perfused with BTS showed a mean net loss of 0.2 μm. The difference between GBR and BTS is not statistically significant ($P > .05$, Studentized Range Test).

In the monkey, 150 min of perfusion with NaCl solution resulted in a net gain of 11.3 μm in the thickness of the ABB. With GBR the net gain was 0.3 μm. This result is similar to individual runs on a rabbit cornea. The data is shown in Figure 6.

**Discussion**

The superficial corneal epithelium is currently seen to be important from several viewpoints. It serves as the substratum of the precorneal film which is the principle refracting surface of the eye. It provides the principle barrier to diffusion of substances from the tears into the corneal stroma and anterior chamber. It is a region in which local events such as trauma and the composition of the bathing medium determine the longevity and turnover rate of epithelial cells.

These multiple roles, and the need for transparency, impose restrictions on the life history of the corneal epithelium over and above the maintenance of vital functions. Furthermore, events on the surface influence the life history of these cells. Pfister and Burstein showed with scanning electron microscopy that benzalkonium chloride caused epithelial cells to desquamate prematurely. In general, however, the environmental factors influencing sloughing have not been studied.

This current study demonstrates our ability to manipulate the condition of the surface epithelium and to facilitate, or possibly inhibit, sloughing rate by the composition of the bathing medium. As the condition of the surface epithelium is critical for the stability of the precorneal film, there is a strong implication that pathological states of the corneal surface/precorneal film could be bettered by attention to the composition of the bathing medium.

Potassium has a high concentration in tears. Its complementary role with sodium in maintaining the internal environment of the cell is established. The demonstration here that its addition to the bathing medium reduces the amount of surface light scatter,
and hence the sloughing rate, shows that it should be included in tear substitutes.

There is a strong suggestion that calcium, magnesium, bicarbonate, and phosphate should also be included. Calcium and magnesium exert a stabilizing role in cell adhesion. Dohlman\textsuperscript{15} believed that a tear substitute should include potassium, calcium, and magnesium. Calcium might not be acceptable where calcification of the cornea and conjunctiva is a problem, or in soft contact lens wear, but its demonstrated contribution to glycogen metabolism, endocytosis, and cell motility\textsuperscript{6} argue for its inclusion. The concentration of magnesium in tears is not known, but it has been implicated in membrane permeability\textsuperscript{8} and cell aggregation,\textsuperscript{7} and is present in GBR and many cell culture media.

These experiments do not demonstrate that calcium, magnesium, bicarbonate, and phosphate are each essential to the corneal surface. They do demonstrate that, together with sodium, potassium and chloride, these ions in a buffered solution maintain the epithelium as well as a more complex solution containing glucose and large molecules. This supports the idea that tears do not supply glucose to the corneal epithelium, or at least precorneal glucose is not essential. To demonstrate an essential role for all of these ions within the constraints of pH and osmolarity, and with freedom to manipulate concentration, is a large task beyond the scope of this study.

The results in the rabbit, and the indication that a similar result might be expected in monkey, suggest that this approach to tear substitutes should be explored in humans. Work on the physico-chemical properties of tears\textsuperscript{14} and the surface activity of tear substitutes\textsuperscript{16} can be complemented by addressing the nutrients required by the corneal epithelium.

**Key words**: epithelium, corneal epithelium, dry-eye, keratoconjunctivitis sicca, tears

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**References**