Irrigating solutions for intraocular purposes were evaluated for their possible damaging effects on the corneal endothelial cell structure and function. Rabbit corneal endothelium was perfused in vitro with Tis-u-Sol, Travenol Ringer’s Solution, or Travenol Sodium Chloride. The irrigating solutions caused an immediate corneal swelling of 67 μm per hour ± 5 (mean ± standard error), which was not modified by a previous stabilization perfusion with glutathione-bicarbonate Ringer’s (GBR). In contrast, the Plasma-lyte-148 solution which is used in the phacoemulsification procedure, did not cause corneal swelling for more than twenty minutes, and for more than sixty minutes if the cornea was perfused after a GBR stabilization. After more than sixty minutes of corneal swelling, endothelial intercellular junction separations appeared. This breakdown was present with the tested irrigating solutions except for Travenol Ringer’s Solution, which contained calcium. Plasma-lyte was also evaluated in conjunction with the surgical phacoemulsification procedure. The complete procedure or just irrigation with ultrasound did not cause endothelial cell damage similar to a prolonged in vitro irrigation. Instead, endothelial cells were traumatically damaged in varying degrees by the surgical manipulations.

New intraocular surgical procedures such as phacoemulsification require large volumes of balanced salt solutions for irrigation and replacement of intraocular fluids. Short-term exposure of corneal endothelium to these balanced salt solutions may have little effect on the endothelium. However, when a large volume of fluid is used in the anterior chamber for prolonged periods, such as may occur during the phacoemulsification procedure, a potential cell injury mechanism may occur because of ionic shifts between the corneal endothelium and the irrigating solution. As evidence to this possible endothelial damage, corneal edema has been found to be a complication following patients treated with phacoemulsification. It is known, for example, that during phacoemulsification more than 1,000 ml. of a balanced salt solution is circulated through the anterior chamber at a flow rate of 25 ml. per minute during the 30-minute procedure. The irrigating solution is not completely replaced by aqueous until one to two hours after surgery.

Edelhauser and co-workers evaluated
Table I. Compositions of solutions

<table>
<thead>
<tr>
<th></th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>HCO₃⁻</th>
<th>Cl⁻</th>
<th>SO₄²⁻</th>
<th>HPO₄³⁻⁺</th>
<th>Glucose (mg.%)</th>
<th>Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione bicarbonate Ringer's</td>
<td>143.5</td>
<td>5.8</td>
<td>2.5</td>
<td>1.25</td>
<td>25.0</td>
<td>128.2</td>
<td>1.25</td>
<td>1.2</td>
<td>90</td>
<td>—</td>
</tr>
<tr>
<td>Plasma-lyte</td>
<td>140</td>
<td>—</td>
<td>3</td>
<td>—</td>
<td>98</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>27</td>
<td>—</td>
</tr>
<tr>
<td>Tis-u-Sol</td>
<td>137.6</td>
<td>5.8</td>
<td>—</td>
<td>1.6</td>
<td>142.3</td>
<td>1.6</td>
<td>1.1</td>
<td>—</td>
<td>27</td>
<td>—</td>
</tr>
<tr>
<td>Travenol Ringer’s injection</td>
<td>147.5</td>
<td>4</td>
<td>4.5</td>
<td>—</td>
<td>156</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Travenol sodium chloride Rabbit aqueous*</td>
<td>154</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>154</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Rabbit aqueous*</td>
<td>148.3</td>
<td>5.04</td>
<td>1.7</td>
<td>0.78</td>
<td>30.8</td>
<td>103.4</td>
<td>0.64</td>
<td>0.86</td>
<td>89</td>
<td>—</td>
</tr>
</tbody>
</table>

*Reference 11.

cornea endothelial cell function during a three- to six-hour in vitro perfusion of some commonly available intraocular irrigating solutions. Their results showed reduced endothelial function in each of the in vitro corneas. It is yet to be determined if these solutions are inadequate for the endothelium during intraocular surgery.

The purpose of this study was to evaluate the corneal endothelial function and structure after exposure to various intraocular irrigating solutions, in particular Plasma-lyte. The corneal tissue was also evaluated after the surgical procedure of phacoemulsification with Plasma-lyte irrigation. Thus testing the adequacy of Plasma-lyte within its intended application.

Methods

Rabbits weighing 3 to 4 kilograms were killed with an overdose of Nembutal. The paired eyes were excised and mounted on the dual-chambered specular microscope. The isolated cornea was maintained in vitro at 34°C with 15 mm Hg intraocular pressure. The epithelium was left intact and covered with 360 Medical Fluid (Dow-Corning Silicone Oil). In some experiments one of the paired eyes served as the control in which the endothelial surface was perfused with a glutathione-bicarbonate Ringer’s solution (CBR). The other eye of the pair was used according to the experimental protocols I and II as described below. All solutions were perfused at the rate of 60 μl per minute, except when changing from one solution to another, at which time the flow was increased to 120 μl per minute for ten minutes. Corneal thickness measurements were recorded along with specular microphotographs of endothelial cells. At various times, the corneal perfusion was terminated and the tissue was prepared for scanning electron microscopy.

Experiment I. In vitro perfusion.

GROUP A. The corneal endothelium was perfused with Tis-u-Sol, Travenol Ringer’s Injection Solution, or Travenol Sodium Chloride. In a second set of experiments the endothelium was perfused for a period of 60 to 90 minutes (stabilization period) with CBR, followed by perfusion with one of the above mentioned irrigating solutions. Table I lists the chemical composition of the irrigating solutions, their pH, and osmolarity.

GROUP B. Plasma-lyte was perfused to the corneal endothelium immediately after mounting the cornea in the specular microscope or after the stabilization period with CBR.

Experiment II. In vivo experiments.

Rabbits (3 to 4 kilograms) were anesthetized with Nembutal (30 mg. per kilogram), and their pupils dilated with atropine eye drops (4 per cent).

GROUP A. The Cavitron-Kelman Phacoemulsification machine was used to irrigate the anterior chamber of five eyes for five minutes with Plasma-lyte at a rate of 125 ml. per minute, with the ultrasound on setting 9 (40,000 cycles per second). The tip of the instrument was introduced into the anterior chamber at the limbus through a 2.5 mm incision. The lens was not removed, and care was taken to avoid contact with the corneal endothelium. The paired eye served as an unoperated control.

GROUP B. The complete phacoemulsification pro-
procedure was used to remove the lenses in six eyes using the standard procedure (capsulectomy, lens luxation into the anterior chamber, phacoemulsification). The procedure required 3 to 5 minutes of phacoemulsification (ultrasound) time and a total irrigation time of 20 to 30 minutes. The paired eye was an unoperated control.

Immediately after each experimental procedure, the incision was closed with one or two sutures after filling the anterior chamber with PlasmaLyte solution; the animal was killed; the eyes were enucleated and mounted in the specular microscope. Both the operated eye and the non-operated control eye were perfused with GBR. The corneal thickness measurements were recorded and specular microphotographs taken. Upon terminating the perfusion, the excised corneas were prepared for scanning electron microscopy.

Scanning electron microscopy. Excised corneas were fixed in 2.5 per cent cold glutaraldehyde for 4 to 12 hours, rinsed in phosphate buffer, and distilled water, and postfixed in 1 per cent osmium tetroxide for one hour. After rinsing, they were dehydrated in graded concentrations of alcohol and Freon T-F followed by critical point drying with Freon. Samples were coated with gold-palladium and examined in a scanning electron microscope (StereoScan, Cambridge, England) at 10 or 20 KV. Photographs were taken with Polaroid P/N film.

Results

Experiment 1. In vitro perfusion.

GROUP A. Corneal swelling occurred within a few minutes after Tis-u-Sol, Travenol Ringer’s Solution, and Travenol Sodium Chloride had been in contact with the endothelium (Fig. 1). This response was independent of a pretesting GBR stabilization period (Fig. 2). However, when GBR was used, the swelling rate was slightly less [54 ± 4 \( \mu \)m per hour (Mean ± S.E.) with \( n = 4 \)] (Fig. 2), than with immediate perfusion of the irrigating solution, [67 ± 5 \( \mu \)m per hour (Mean ± S.E.) with \( n = 8 \)] (Fig. 1). Endothelial cell damage was observed to occur after one hour of perfusion with Tis-u-Sol or Travenol Sodium Chloride. In contrast to this, even after three or four hours of perfusion with Travenol Ringer’s Injection, endothelial cell changes were not noticed under the specular microscope, although stromal swelling was present. Cell changes and/or corneal swelling induced by perfusion with Tis-u-Sol and Travenol Ringer’s Injection Solution were reversible after perfusion with the GBR solution.

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Fig. 1. Paired rabbit corneas were perfused with Tis-u-Sol (▲, △), Travenol Ringer's Injection Solution (○), and Travenol Sodium Chloride (●). The corneal thickness measurements were adjusted so that the initial experimental measurements were the same, i.e., to reveal the relative change in corneal thickness.

Fig. 2. Four paired rabbit corneas were stabilized with GBR. One of the pair was then used as a control (○) and the other was perfused with Tis-u-Sol (▲). This graph is representative of the corneal thickness changes caused by the endothelial exposure to Tis-u-Sol, Travenol Ringer's Injection Solution and Travenol Sodium Chloride.

took an additional 25 minutes for the cell pattern to deteriorate severely. If the Plasma-lyte solution was changed to GBR, the cell pattern could return to normal in another 15 minutes. An upper time limit for how long the tissue could remain "damaged" and still reverse to normal was not established. The type of cell damage seen appeared to be a breakdown of the junction between endothelial cells.

When Plasma-lyte was perfused with or without a GBR stabilization period, the scanning electron micrographs revealed progressive endothelial damage which was dependent on the duration of the Plasma-lyte exposure to the endothelium. As can be seen in Fig. 7, there was endothelial cell edema with stretching of the cell junctions and ruptures in some areas.

Experiment II. In vivo experiments.

GROUP A. The partial phacoemulsification procedure, i.e., irrigation plus ultrasound for five minutes resulted in considerable variation in response. There was a maximum swelling rate of 58 μm per hour to a minimum of 0 μm per hour. The mean rate was 16 μm per hour ± 22 (S.E.) for n = 5. This correlated with the amount of endothelial cell damage. Some corneas had numerous small areas of cell destruction while others had large areas of destroyed cells, presumably by contact with the phacoemulsification probe. Scanning electron microscopic examination of the endothelium of these corneas showed the endothelial cell layer to be normal with no cell junction disruption except for areas of cell destruction which were apparently caused by the irrigation needle (Fig. 8).

GROUP B. The maximum swelling rate observed after the complete phacoemulsification procedure was 54 μm per hour and the minimum was 6 μm per hour with a mean rate of 26 μm per hour ± 41 (S.E.) for n = 6. The same type of traumatic endothelial cell damage that was observed in Group A existed in these eyes. The areas of endothelial cell destruction were larger than those seen in Group A corneas, and the probe-type trauma appeared more fre-
Fig. 3. The scanning electron micrograph shows the normal pattern of rabbit corneal endothelium after an in vitro perfusion of Travenol Ringer's Solution to the endothelium for three hours.

Fig. 4. Six paired rabbit corneas were perfused with GBR as a control (○) and Plasma-lyte (●) following a GBR stabilization period. This graph is representative of the corneal thickness and endothelial cell changes caused by the exposure to the Plasma-lyte. The arrows indicate: the start of the Plasma-lyte exposure, the corneal swelling, the initial then severe endothelial cell damage, the return to GBR perfusion and, finally, a return to normal endothelial cell pattern.

Fig. 5. Rabbit corneas (n = 4) were perfused with Plasma-lyte from the start of the in vitro perfusion. The corneal thickness measurements were adjusted so that the initial experimental measurements were the same, i.e., to reveal the relative change in corneal thickness.
Immediately. There were lesions varying from one cell size to several millimeters in size. With the specular microscope, these lesions could be seen to be devoid of endothelial cells, yet the surrounding cells appeared normal (Fig. 9). This was confirmed in scanning electron micrographs. A complete study of these alterations is discussed in a separate paper.10

Discussion

The effects of intraocular irrigating solutions on the corneal endothelium relative to the clinical applications have not been critically evaluated in the past. The use of the specular microscope to study eyes perfused in vitro enables us to dynamically profile cornea endothelial function via corneal thickness while observing the endothelial morphology. Unfortunately, it was difficult to precisely separate the endothelial metabolic pump function from its cell barrier function, since an extra rapid corneal swelling was not consistently noticed at the time of endothelial cell junction separation. This problem was apparent for all the irrigating solutions tested (Figs. 1 and 2) except Plasma-lyte. During the Plasma-lyte perfusion (Fig. 4) this inadequate irrigating solution caused corneal swelling while the endothelial cell barrier as seen with the specular microscope appeared to be intact (Fig. 6). The swelling rate increased when the endothelial cell junctions began to appear damaged.

The physiological needs of the endothelium have been outlined in the literature7-9; it has been shown that the cells need an energy source (normally glucose), a list of pertinent ions (particularly so-
Fig. 7. The microphotograph shows a cornea endothelial cell edema caused by exposing the endothelium to Plasma-lyte. The cornea was perfused for 40 minutes past the initial appearance of cell damage.

Fig. 8. The corneal endothelial destruction after partial phacoemulsification (i.e., irrigation and ultrasound) can be seen in this microphotograph.
Fig. 9. The endothelial cell montage was taken with the specular microscope after the rabbit eye had undergone complete phacoemulsification procedure. The normal cell pattern can be seen except for the traumatic removed area of cells.

dium, chloride, calcium, and bicarbonate), a pH of 7.4, and an osmolarity of approximately 306 milliosmoles. Many saline solutions used for intraocular infusion lack these basic needs as can be seen by comparing their compositions (Table I), and results of in vitro perfusions (Figs. 1, 2, 4, and 5). Slight differences in response between irrigation solutions can be explained by their individual compositions. Cell junction rupture (Fig. 6) can be explained by the lack of calcium in the irrigating solution. The immediate corneal swelling upon contact to an irrigating solution (Figs. 1 and 2) could be caused by incorrect pH values, while a delayed swelling (Figs. 4 and 5) could be caused by correct pH values but a solution of inadequate composition. The corneal swelling and endothelial cell changes can be reversed when a physiological maintenance medium (GBR) is supplied to the corneal endothelium (Fig. 4). All of these data indicate that the tested intraocular irrigation solutions are inadequate and damaging to the corneal endothelium.

A realistic evaluation of an irrigating solution should also include its effect on the cornea during and after the solution's normal clinical application. This was done with Plasma-lyte which is the recommended irrigating solution for the phacoemulsification procedure. Since this procedure yields variable results, regardless of the surgeon's feelings of consistency with his technique, it was important to determine the origin of the variations. This study shows that the endothelial damage after phacoemulsification procedure was of a traumatic nature (Figs. 8 and 9) and not caused by irrigating solution composition insufficiencies as in the prolonged in vitro perfusion of Plasma-lyte (Figs. 6 and 4). Thus, the Plasma-lyte solution although it is an inadequate solution when tested in vitro, seemed to be sufficiently adequate for anterior chamber irrigation for a limited period of time as shown in rabbits. A subsequent paper will describe in more detail the endothelial changes which occur during and after the phacoemulsification procedure in which it is confirmed that the irrigating solutions seem to play a minor role.

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REFERENCES
8. Hudson, S.: Evidence for a bicarbonate-