either must be entirely made up fresh or the unstable components (adenosine, glutathione, and bicarbonate) must be individually added to the solution. They contained 4 mg/ml. gentamicin and were refrigerated at 4°C for periods up to 4 days. The unstable components (Nos. 8 to 10, Table I) of the solutions under analysis were checked at this time, and adjustments made if indicated. Every 15 min. for a total of 180 min. corneal thickness was measured three times, and the average value recorded.

**Results.** Perfusion of the corneal endothelium with glutathione-deficient GBR (Fig. 1) resulted in good maintenance of corneal thickness throughout 180 min. with a mean net loss of 4 μm (n=4). After 60 min. of perfusion when the corneal preparations appeared to have equilibrated with the specific temperature and pressure conditions of the perfusion chamber, glutathione-deficient GBR induced a mean swelling of 2 μm/hr. The paired control corneas perfused with GBR containing glutathione underwent a mean net loss of 5 μm over 180 min. and hourly swelling rates of 5 μm/hr. after 60 min.

Corneas perfused with adenosine-deficient GBR (Fig. 2) also appeared to resist swelling, with a mean net loss of 5 μm (n=5) over the 180 min. perfusion. These corneas underwent mean hourly swelling rates of 3 μm/hr. after 60 min. of perfusion. Control corneas perfused simultaneously with adenosine-containing GBR decreased 8 μm in thickness over 180 min. and did not swell at all after 60 min. of perfusion.

GBR deficient in adenosine and glutathione (Fig. 3) demonstrated a mean net gain in corneal thickness of 11 μm (n=5) over the entire basic salt solution at the time of ocular surgery, which is not only inconvenient but allows for the possibility of infectious contamination. It is the attempt of this investigation to analyze what role, if any, adenosine and glutathione play in the effectiveness of GBR to maintain corneal thickness during in vitro perfusion. In addition, the effect of phosphate buffer substitution for bicarbonate buffer is studied in a newly proposed glucose-phosphate-Ringer solution (GPR). No controlled studies have previously been reported which maintained constant pH, osmolarity, temperature, pressure, and electrolyte amounts during simultaneous perfusion with GBR and GBR with labile elements omitted.

**Materials and methods.** Corneas obtained from 5 to 10 lb. albino rabbits were prepared in the manner described by Dikstein and Maurice and mounted in a dual-chambered specular microscope. All infusions were carried out with paired corneas from the same albino rabbit being perfused under identical conditions of temperature (36° to 37°C) and pressure (15 to 20 mm. Hg) for the two solutions under analysis. The basic electrolyte and dextrose components (Nos. 1 to 7, Table I) of the solutions under analysis were either prepared fresh or obtained from stock solutions. They contained 4 μg/ml. gentamicin and were refrigerated at 4°C for periods up to 4 days. The unstable components (Nos. 8 to 10, Table I) were all added fresh at the time of infusion, the pH and osmolarity were also checked at this time, and adjustments made if indicated. Every 15 min. for a total of 180 min. corneal thickness was measured three times, and the average value recorded.

**Simplification of glutathione-bicarbonate-Ringer solution: its effect on corneal thickness.** JAMES K. McENERNEY AND GHOLAM A. PEYMAN.

Glutathione and adenosine can safely be omitted from glutathione-bicarbonate-Ringer solution (GBR) which is used as an irrigating solution during vitrectomy, without significant reduction in the solution’s efficacy or increase in corneal toxicity. Bicarbonate appears to be an essential ingredient and should not be omitted from the Ringer solution.

With the advent of automated instruments to remove and replace vitreous, vitreous surgery has become more prevalent, and with it has come the need for extended intracocular irrigation and vitreous replacement. Currently used intracocular irrigating solutions (normal saline, balanced salt solution, and Ringer-lactate) have been shown to be damaging to the corneal endothelium and result in significant corneal swelling. Carey et al. and others have proposed a glutathione-bicarbonate-Ringer solution containing adenosine (GBR, Table 1), which has demonstrated ability in maintaining the physiologic state of the cornea and lens during perfusion and incubation studies. Currently GBR either must be entirely made up fresh or the unstable components (adenosine, glutathione, and bicarbonate) must be individually added to the solution’s efficacy or increase in corneal toxicity. Carey et al. and others have proposed a glutathione-bicarbonate-Ringer solution containing adenosine, glutathione, and bicarbonate, which is used as an irrigating solution during vitrectomy, without significant reduction in the corneal thickness of albino rabbits prepared in the manner described by Dikstein and Maurice and mounted in a dual-chambered specular microscope.

**References.**


**Key words:** osmotic cataract, boron hydride compounds, lens cation pump, lens Na-K ATPase.
perfusion period and mean hourly swelling rates of 9 μm/hr. after 60 min. Paired controls perfused with GBR containing glutathione and adenosine demonstrated a mean net loss of 6 μm for 180 min. and hourly swelling rates of 2 μm/hr. after 60 min.

During perfusion with GPR, a 60 min. period of equilibration to the environment of the perfusion chamber was not demonstrably evident, due to the dramatic swelling which occurred (Fig. 4). Over the 180 min. of perfusion, GPR resulted in a mean net gain of 106 μm. The hourly swelling rate for the first 60 min. was identical to that of the last 60 min. and equaled 36 μm/hr. Paired corneas perfused with GBR deficient in adenosine and glutathione swelled 22 μm over 180 min. Hourly swelling rates in this group averaged 7 μm/hr. over the 180 min. of perfusion.

Discussion. Edelhauser et al.1 recently correlated hourly swelling rates during specular microscopy with degree of endothelial damage with the
Fig. 3. Mean change in corneal thickness measured during perfusion with GBR lacking adenosine and glutathione (---) and GBR containing adenosine and glutathione (—).  

Fig. 4. GPR (---) induces an increase in mean corneal thickness as compared with GBR lacking adenosine and glutathione (—).
Table I. Chemical composition of intraocular irrigating solutions

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>GBR (gm/liter)</th>
<th>GBR minus glutathione (gm/liter)</th>
<th>GBR minus adenosine (gm/liter)</th>
<th>GBR minus glutathione minus adenosine (gm/liter)</th>
<th>GPR (gm/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Dextrose</td>
<td>0.903</td>
<td>0.903</td>
<td>0.903</td>
<td>0.903</td>
<td>0.404</td>
</tr>
<tr>
<td>3. KCl</td>
<td>0.359</td>
<td>0.359</td>
<td>0.359</td>
<td>0.359</td>
<td>0.410</td>
</tr>
<tr>
<td>4. CaCl₂ (anhyd.)</td>
<td>0.115</td>
<td>0.115</td>
<td>0.115</td>
<td>0.115</td>
<td>0.130</td>
</tr>
<tr>
<td>5. MgCl₂ · 6H₂O</td>
<td>0.159</td>
<td>0.159</td>
<td>0.159</td>
<td>0.159</td>
<td>0.180</td>
</tr>
<tr>
<td>6. NaH₂PO₄ · H₂O</td>
<td>0.119</td>
<td>0.119</td>
<td>0.119</td>
<td>0.119</td>
<td>0.400</td>
</tr>
<tr>
<td>7. NaHO₃</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>1.0079</td>
</tr>
<tr>
<td>8. ACP</td>
<td>0.134</td>
<td>0.134</td>
<td>0.134</td>
<td>0.134</td>
<td>0.400</td>
</tr>
<tr>
<td>9. Glutathione</td>
<td>0.092</td>
<td>0.000</td>
<td>0.092</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>10. NaHCO₃</td>
<td>2.453</td>
<td>2.453</td>
<td>2.453</td>
<td>2.453</td>
<td>0.000</td>
</tr>
<tr>
<td>Osmolarity (mOsm.)</td>
<td>306</td>
<td>306</td>
<td>306</td>
<td>306</td>
<td>303</td>
</tr>
<tr>
<td>pH</td>
<td>7.40</td>
<td>7.40</td>
<td>7.40</td>
<td>7.40</td>
<td>7.20</td>
</tr>
</tbody>
</table>

*Unstable components.

in the glutathione-deficient media under analysis. Glutathione has not been found in aqueous humor in vivo. Glutathione is demonstrated here to aid little to the efficacy of perfusion media and might well be omitted.

Adenosine also was found by Dikstein and Maurice to increase the efficacy of the irrigating solutions in their perfusion study. Anderson et al. found that adenosine omission from a basal salt solution containing glucose had little effect on corneal endothelial (Na⁺, K⁺) ATPase activity during in vitro perfusion. This present experiment offers evidence that although adenosine adds to media's thickness-maintaining ability, its omission does not result in significant difference in efficacy.

Bicarbonate appears in the aqueous of rabbits at 27.7 mmol/liter. Hodson previously demonstrated the detrimental effect of bicarbonate omission in simple media when a toxic Tris buffer was substituted. Further work by Fischbarg has hinted at the importance of sodium bicarbonate during perfusion studies. GPR was an attempt to omit bicarbonate and substitute a stable physiologic buffer. It appears that even with this substitution, corneal thickness could not be maintained without bicarbonate. It seems that a practical alternative to a ready-to-use intraocular solution might include a GBR electrolyte stock to which bicarbonate is added at the time of surgery but in which the glutathione and adenosine are omitted.

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Key words: intraocular irrigating solution, bicarbonate, glutathione, adenosine.

REFERENCES


Acoustic microscopy of the human retina and pigment epithelium. MICHAEL F. MARMOR, H. K. WICKRAMASINGHE, and ROSS A. LEMONS.*

An acoustic microscope uses sound waves rather than light to image a sample, and displays viscoelastic rather than optical properties. The Stanford instrument, operating at frequencies near 1,000 MHz, achieves resolution and magnification that is...