Corneal Epithelial and Aqueous Humor Acidification During In Vivo Contact Lens Wear in Rabbits

Claude Giasson*† and Joseph A. Bonanno*

**Purpose.** Based on contact lens-induced stromal acidification of the cornea, it has been suggested that the corneal epithelial and endothelial cells also become acidotic during contact lens wear. This alleged acidification may have a role in altered cell appearance and metabolism during contact lens wear. This study investigated the effects of anoxia, carbon dioxide retention, and contact lens gas transmissibility on the epithelial and aqueous humor pH in living rabbits.

**Methods.** Epithelial intracellular pH (pHi) and aqueous humor pH were fluorophotometrically measured with a pH sensitive-dye (BCECF) during contact lens wear or exposure to various gas mixtures.

**Results.** Polymethylmethacrylate (PMMA) lens wear acidified epithelial cells by preventing CO2 efflux and by inducing hypoxia. Increasing lens oxygen transmissibility decreased epithelial acidification. After initiation of rigid, gas-permeable (RGP) lens wear or CO2-air exposure, pHi dropped transiently and then recovered partially. This recovery of pHi was not observed during anoxia, whether induced by PMMA lens wear or exposure to 100% N2. The aqueous humor also acidified during PMMA lens wear, a phenomenon not observed during RGP lens wear. Changes in aqueous pH were smaller, slower, and delayed when compared to their epithelial counterparts.

**Conclusions.** Hypoxic contact lens wear acidifies the corneal epithelium and aqueous humor. The aqueous humor pH change indicates a probable endothelial acidification during hypoxic contact lens wear; the pH changes are caused by two separate and additive effects, CO2 retention and hypoxic acidosis. Increases in the oxygen transmissibility of the lens decrease the cellular acidosis, which might minimize cellular complications arising from contact lens wear. We estimate that a lens with an oxygen transmissibility (Dk/L) of 300 X 10^-11 (cm/sec)(ml O2/ml X mm Hg) is needed to prevent epithelial pHi changes in the open eye. In contrast, lenses with $Dk/L$ as low as $18 X 10^{-9}$ (cm/sec)(ml O2/ml X mm Hg) can prevent aqueous humor pH changes. Invest Ophthalmol Vis Sci. 1994;35:851-861.

Contact lens wear can induce many corneal alterations, including the formation of epithelial edema and microysts, stromal edema and folds, and endothelial edema (ie, blebs) and polymegathism.$^{1,2}$ These changes in corneal morphology are accompanied by cellular changes, including increased glycolytic activity (ie, increased lactate and proton production, and diminished adenosine triphosphate levels), reduction in mitotic activity, thinning of the epithelium, slowing of wound healing rates, and a small loss of endothelial functional capacity.$^1$ Furthermore, studies indicate that changes in pH can influence corneal hydration control.$^{3,4}$ Increased lactate levels are responsible for stromal and epithelial edema,$^5$ and corneal acidosis alone can lead to the formation of endothelial blebs.$^6$ The precise cause of the other effects is unknown, however. Cellular acidosis has been implicated in these other changes, because it is known that intracellular...
pH (pHi) is a key regulator of enzymatic activity, and cellular processes such as mitotic activity, cell migration, and ion and fluid transport mechanisms; however, no direct measurements of in vivo epithelial or endothelial pH have been made during contact lens wear.

It is likely that lens wear alters epithelial and possibly endothelial pH in vivo, because it has been shown that the hypoxia and CO2 retention caused by lens wear both contribute to stromal acidosis. A preliminary report has indicated epithelial acidosis due to contact lens wear in vitro. Another study using perfused corneal epithelial cells, however, has shown an alkalization in response to hypoxia. Although acute exposure to increased CO2 levels will in general initially acidify cells, it has been shown that cells can respond by regulating their pHi to levels below, equal to, or above the resting cell pHi, depending on the type of pHi regulatory mechanisms present. Thus it is possible that corneal cell acidosis due to lens wear could be transient. To answer these questions, a direct measurement of pHi under lens-wearing conditions in the living eye is needed.

In this study, we used the pH-sensitive fluorescent probe BCECF to measure surface epithelial and aqueous humor pH in living rabbits. We attempted to determine if epithelial and endothelial pHi are affected by contact lens wear; observe whether or not cells in vivo can regulate their pHi in response to lens wear, hypoxia, and increased pCO2; and estimate the minimum lens oxygen transmissibility (Dk/L) needed to prevent pHi changes in the cornea. This is the first report of the effects of contact lenses on cellular pH in living animals.

MATERIALS AND METHODS

All experiments used Dutch-belted rabbits except for the oxygen transmissibility study, which was done on 24 New Zealand White rabbits. The animals were treated according to the ARVO Resolution on the Use of Animals in Research.

BCECF Loading

BCECF-AM [2',7'-bis(2-carboxyethyl)-5-(and-6)carboxyfluorescein, acetoxymethyl ester] (Molecular Probes, Eugene, OR) was used to measure epithelial pHi. Stock solutions (10 mM in DMSO) were stored desiccated at -20°C. For epithelial loading, the dye was diluted to 280 μM with saline solution. This mixture was poured into the central hollow reservoir of a Lucite cylinder positioned on the central cornea. At its periphery, the ocular side of this device provided a concave radius matching the corneal surface. A circular groove connected by tubes to a syringe allowed for stable positioning on the cornea by suction. This device was placed on the cornea of the rabbit for 20 to 30 minutes. To determine the extent of epithelial cell loading provided after a typical period of dye exposure, one cornea was dissected, placed on a coverslip, and observed with epifluorescent illumination at X400 magnification. The superficial epithelial cells were intensely fluorescent. When focusing into deeper epithelial layers, however, the fluorescence intensity decreased dramatically, indicating that only superficial cells incorporated the dye. If the corneal surface was lightly rubbed with a cotton swab to remove superficial cells, fluorescent cells were not seen. Thus, all references to epithelial pHi are from the most superficial layers.

We attempted to measure endothelial pHi by intracamerall injection of BCECF-AM or 5-(and-6)-carboxy-4',5'-dimethylfluorescein diacetate. Although this procedure successfully loaded endothelial cells, the dye also hydrolyzed within the anterior chamber, leading to very large anterior chamber fluorescence signals, thus making the distinction between pHi and extracellular pH (pHo) impossible. Because pHi has been shown to correlate directly with pHo, we decided to measure aqueous humor pH, which would reflect trends in endothelial pHi. For aqueous humor pH experiments, BCECF free acid (22 mM) was loaded into the anterior chamber by iontophoresis across the cornea. After corneal anesthesia, filter paper (Whatman No. 2 cut to a diameter of 3.5 mm), saturated with the dye, was placed on the superior limbus of the cornea. An iontophoretic probe, a 3-mm diameter solid copper wire, was applied directly on the filter paper and current was applied for 5 to 10 minutes (9 V, 200 μA). The filter paper was removed from the tear film and the cornea was rinsed thoroughly. Data collection began 45 to 60 minutes later, a delay necessary for the aqueous humor to incorporate the dye. Aqueous fluorescence was measured through the central inferior cornea to avoid dye in the superior corneal area.

The Slit-Lamp Fluorophotometer

The fluorophotometer has been described previously. Briefly, the illumination system of a Nikon (Garden City, NY) biomicroscope has been replaced by a mercury arc lamp. The dye was excited by 440- and 490-nm interference filters (half bandwidth 7.5 and 7.3 nm, respectively; Ealing Electro-Optics, Hol- liston, MA) mounted on a spinning wheel alternated at 40 Hz. Circular and slit apertures were used, which, when projected by the illumination system, were 2.4 mm in diameter and 6.0 X 0.3 mm (length X width), respectively. The large circular aperture could be used for epithelial measurement because the dye was trapped in the cellular layer. This maximized the signal-to-noise ratio. The slit aperture was used for ante-
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The eyes were promptly removed and were placed in phosphate-buffered solutions at different pH. These solutions had a concentration of BCECF of 2.5 μM. About 125 μl of calibrating solution was carefully poured between two microscope slides, separated by 90 μm. Separations up to 400 μm (completely filling the measurement window) gave similar fluorescence ratio findings. Glass plates and solutions were suspended in front of the microscope, and fluorescence was measured for each solution. The data generated by this method were consistent and repeatable. A nonlinear regression of the following equation

\[
\text{Fluorescence ratio} = a + b \times \frac{10^{(pH-pK)}}{1 + 10^{(pH-pK)}}
\]

was highly significant, and yielded values of 0.17, 1.03, and 7.29 for a, b, and pK, respectively ($r^2 = 0.99$). Ratios obtained from aqueous experiments were transformed to absolute pH using this function.

Experimental Procedures

Before each experiment, the rabbit was sedated and anesthetized with an intramuscular injection of a mixture of ketamine hydrochloride (120 mg/kg) and xylazine (6 mg/kg). Then it was placed on a stand in front of the fluorophotometer, and background fluorescence was measured. Depending on the experiment, background was also measured with contact lenses or goggles in place. Background intensity typically represented 2.5% of the total signal at 440 nm, and less than 0.5% at 490 nm. After topical anesthesia with proparacaine hydrochloride, dye was applied either to the superficial epithelium or into the aqueous humor.

Gas Exposure Experiments. The cornea was exposed to gases through a single eye cup goggle that fit snugly around the orbit. The free edge of a short Lucite tube glued to a flat front window of Lucite was contoured to fit the orbitofacial morphology of the rabbit, and was covered with a lining of modeling clay to improve the seal. It enclosed a volume of 33 ml to and from which gases were delivered by inflow and outflow ports. Gases used (100% air, 5% CO2-95% air, 100% N2, or 4.8% CO2-95.2% N2) were humidified by passage through a gas-washing bottle placed in a water bath set at 37°C. Depending on the quality of the goggle seal on the rabbit, the flow of gas was adjusted until a small positive pressure inside the goggle was achieved. Gas exposure experiments started and ended by delivering air (control gas) to the cornea of the animal. Analyzed experiments included only those in which introduction and removal of the experimental gas mixture brought about equal and opposite changes in pH.

Contact Lens Experiments. We used contact lenses of similar design but of different gas permeability (Dk): polymethylmethacrylate (PMMA), Oxyflow f30,
TABLE 1. Parameters of the Contact Lenses Used in This Study

<table>
<thead>
<tr>
<th>Materials</th>
<th>Dk</th>
<th>L (mm)</th>
<th>Dk/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymethylmethacrylate (PMMA)</td>
<td>0</td>
<td>0.17</td>
<td>0</td>
</tr>
<tr>
<td>Oxyflow 130</td>
<td>30*</td>
<td>0.17</td>
<td>18†</td>
</tr>
<tr>
<td>Fluorex 700</td>
<td>70</td>
<td>0.18</td>
<td>39</td>
</tr>
<tr>
<td>Oxyflow 151†</td>
<td>150</td>
<td>0.16</td>
<td>94</td>
</tr>
<tr>
<td>Quantum 2</td>
<td>145</td>
<td>0.17</td>
<td>85</td>
</tr>
<tr>
<td>Hi-Dk</td>
<td>200</td>
<td>0.17</td>
<td>121</td>
</tr>
</tbody>
</table>

All lenses were made according to the following parameters: base curve: 7.00 mm, Power: —3.00 D, Diameter = 10.0 mm, central thickness (L) = 0.16–0.18 mm.

* Units of permeability are \( \times 10^{-11} \) (cm²/sec)(ml O₂/ml*mm Hg).
† The list of Dk refers to the oxygen permeability coefficients as measured by the manufacturer.
‡ Oxygen transmissibility is the oxygen permeability coefficient divided by lens thickness (L) in cm and is therefore expressed as \( \times 10^{-10} \) (cm/sec)(ml O₂/ml*mm Hg).

Oxyflow 151 was rejected from the study because of differential absorbance at the excitation wavelengths.

Fluorex 700, Oxyflow 151 (Concise, San Leandro, CA); and Quantum 2, Hi-Dk (Polymer Technology, Wilmington, MA). Table 1 lists the material characteristics and contact lens parameters. One of the lenses (Oxyflow 151) was rejected from the study because of differential absorbance at the two excitation wavelengths.

In all contact lens experiments, lenses were inserted without interrupting data collection (except for aqueous experiments). This typically blocked the light path, producing a dramatic drop in the signal for a few seconds. This drop served as a reference for initiation of lens wear.

**Statistics**

Unless otherwise mentioned, the data were analyzed with paired, unilateral t tests. Mean values ± standard error of the estimate (n = sample size) are presented. Rates of acidification were calculated by linearly regressing data points 1 minute before and 1 minute after the indicated time.

**RESULTS**

**PMMA Contact Lens Experiments**

Figure 1 shows that PMMA lens wear acidified both the aqueous and the superficial corneal epithelium. Ten minutes of lens wear acidified epithelial cells by 1.32 ± 0.04 pH units, which represents 93% of the pH change observed at steady state (20 minutes; Table 2). By comparison, 10 minutes of lens wear acidified aqueous pH by 0.12 ± 0.02 units, a value representing only 59% of the pH change observed at 30 minutes. There was no pH recovery during PMMA lens wear, indicating the cells’ inability to produce a net extrusion of protons. On lens removal, epithelial pH briefly overshot, then returned to the baseline value.

Lens wear increases CO₂ levels and decreases O₂ levels in the cornea. To determine the relative contribution of each to changes in epithelial and aqueous pH, we exposed corneas of living rabbits to various gas mixtures. Figure 2 shows that changing the gas in contact with the cornea from 100% air to 5% CO₂ (balance air), which simulates a lens with \( \text{Dk}_{\text{CO}_2} = 0 \), that is, a PMMA lens (without hypoxia), caused epithelial pH to drop rapidly by 1.25 units (mean = 1.00 ± 0.11

![FIGURE 1. Effect of PMMA lens wear on epithelial (O) and aqueous (x) pH. The two traces are from separate experiments. The gap in the epithelial trace was included to make the removal of the lens coincident with the aqueous trace.](http://iovs.arvojournals.org/pdfaccess.ashx?url=data/journals/iovs/933180/)
TABLE 2. Epithelial and Aqueous pH Change (ΔpH) and Rates of pH Change (dpH/min) During PMMA Contact Lens Wear and Exposure to Gas Mixtures

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>PMMA</th>
<th>CO₂-Air</th>
<th>N₂</th>
<th>CO₂-N₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change (ΔpH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelium Maximal values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>-1.32 ± 0.04 (9, 7)</td>
<td>-1.00 ± 0.11 (8, 4)</td>
<td>-1.11 ± 0.13 (4, 3)</td>
<td>-1.24 ± 0.18 (3, 2)</td>
</tr>
<tr>
<td>15</td>
<td>-0.78 ± 0.09 (8, 4)</td>
<td>-0.78 ± 0.11 (3, 5)</td>
<td>-0.91 ± 0.15 (4, 2)</td>
<td>-1.14 ± 0.13 (3, 2)</td>
</tr>
<tr>
<td>20</td>
<td>-0.77 ± 0.18 (4, 2)</td>
<td>-0.92 ± 0.12 (4, 3)</td>
<td>-1.03 ± 0.13 (4, 3)</td>
<td>-1.22 ± 0.17 (3, 2)</td>
</tr>
<tr>
<td>30</td>
<td>-0.76 ± 0.23 (2, 2)</td>
<td>-1.10 ± 0.19 (3, 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqueous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>-0.12 ± 0.02 (3, 3)</td>
<td>-0.05 ± 0.05 (3, 2)</td>
<td>-0.01 ± 0.01 (3, 2)</td>
<td>-0.05 (2, 2)</td>
</tr>
<tr>
<td>20</td>
<td>-0.17 ± 0.03 (3, 3)</td>
<td>-0.08 ± 0.01 (3, 2)</td>
<td>-0.02 ± 0.02 (3, 2)</td>
<td>-0.07 (2, 2)</td>
</tr>
<tr>
<td>30</td>
<td>-0.20 ± 0.04 (3, 3)</td>
<td>-0.09 ± 0.01 (3, 2)</td>
<td>-0.03 ± 0.03 (2, 2)</td>
<td>-0.09 ± 0.01 (2, 2)</td>
</tr>
<tr>
<td>40</td>
<td>-0.20 ± 0.04 (3, 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rates of pH change (dpH/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-0.35 ± 0.04 (9, 7)</td>
<td>-0.43 ± 0.03 (9, 4)</td>
<td>-0.11 ± 0.03 (5, 3)</td>
<td>-0.40 ± 0.04 (4, 2)</td>
</tr>
<tr>
<td>2</td>
<td>-0.18 ± 0.02 (9, 7)</td>
<td>-0.05 ± 0.02 (9, 4)</td>
<td>-0.09 ± 0.02 (5, 3)</td>
<td>-0.05 ± 0.02 (4, 2)</td>
</tr>
<tr>
<td>3</td>
<td>-0.13 ± 0.02 (9, 7)</td>
<td>0.03 ± 0.01 (9, 4)</td>
<td>-0.10 ± 0.02 (5, 3)</td>
<td>-0.04 ± 0.02 (4, 2)</td>
</tr>
<tr>
<td>10</td>
<td>-0.02 ± 0.005 (9, 7)</td>
<td>0.00 ± 0.02 (6, 3)</td>
<td>-0.03 ± 0.01 (5, 3)</td>
<td>-0.02 ± 0.01 (5, 2)</td>
</tr>
<tr>
<td>20</td>
<td>-0.01 ± 0.01 (8, 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers in parentheses are presented as (n₁, n₂) where n₁ indicates the number of experiments and n₂ refers to the number of different rabbits used. Means ± SEM are presented.

units, n = 8). The greatest acidification was reached at 2.6 ± 0.2 minutes of CO₂ exposure, and was followed by a partial recovery of 0.24 units. By contrast, the aqueous response to CO₂-air was monotonic, and pH was still decreasing after 10 minutes. Thirty minutes of tear film CO₂-air exposure decreased aqueous pH from 7.63 ± 0.02 to 7.54 ± 0.03 (n = 3). On CO₂ removal, epithelial pHovershot baseline by a value approximately equal to the CO₂-induced recovery. These data are summarized in Table 2.

To test the effect of hypoxia alone, we exposed the cornea to 100% N₂. As shown in Figure 3, epithelial pH drops smoothly, reaching a steady state within 50 minutes. The maximal acidification was 1.10 ± 0.11 pH unit (Table 2). The same length of N₂ exposure acidified aqueous pH by only 0.03 ± 0.03 unit, a change that was not statistically significant (Table 2; P = 0.25).

Because PMMA lens wear causes both tear anoxia and CO₂ retention to 5%, we should be able to simu-

FIGURE 2. Effect of 5% CO₂-95% air on epithelial and aqueous pH. At first arrow, the gas flowing in the goggle was switched from air to CO₂-air. The gap in the epithelial trace was included to make CO₂ removal coincident with the aqueous trace.
late lens wear by exposure to 5% CO₂–95% N₂. The precise specialty mixture obtained from the supplier was 4.8% CO₂–95.2% N₂. Table 2 shows that this mixture caused the largest epithelial acidifications observed in the goggle experiments (1.24 ± 0.18), very close to the one resulting from wearing PMMA contact lens (1.42 ± 0.05; see Table 2). Furthermore, the overall shape of the acidification with CO₂–N₂ is similar to that for the PMMA lens (compare Figs. 1 and 4), and in both cases, no pH recovery was observed. On the other hand, a 30-minute exposure to this mixture acidified the aqueous by 0.09 ± 0.01 pH unit. Because this pH change is not different from that caused by CO₂–air exposure alone, it is concluded that hypoxia has little if any effect on aqueous pH.

To learn which process (O₂ decrease or CO₂ increase) is responsible for a given phase of lens-induced acidification, we calculated and compared the average rates of acidification at similar times of lens wear, or gas exposure (Table 2). The average rates of pH change after 1 minute of either PMMA lens wear or CO₂ exposure were found not to be significantly dif-

![Figure 3](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933180/)

**FIGURE 3.** Effect of 100% N₂ on epithelial and aqueous pH. At first arrow, the gas flowing in the goggle was switched from air to 100% N₂. The gap in the epithelial trace was included to make N₂ removal coincident with the aqueous trace.

![Figure 4](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933180/)

**FIGURE 4.** Effect of 4.8% CO₂–95.2% N₂ on epithelial (inferior trace) and aqueous pH (superior trace). The epithelial trace includes exposure to 5% CO₂–air as well.
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ferent (bilateral t test, \( P = 0.16, n = 9 \)). The PMMA- and 100% N\(_2\)-induced rates of pHi changes at 3 and 10 minutes also were found not to be significantly different by an independent bilateral t test (at 3 minutes: \( t = -1.021, P = 0.37 \); at 10 minutes: \( t = 1.474, P = 0.17 \)). These findings suggest that CO\(_2\) is responsible for the initial fast drop in pHi, and hypoxia is responsible for the later sustained pHi drop.

Gas-Permeable Contact Lens Experiments

It has been shown that the drop in stromal pH caused by a contact lens is inversely proportional to its gas transmissibility. We investigated if this relationship held for epithelial pHi. In these experiments, rigid gas-permeable (RGP) contact lenses were tested relative to PMMA controls. Thus, the acidification produced by each RGP lens could be compared within a paired PMMA experiment. To avoid distortion of the data by the temporal sequence of contact lens insertion, half the experiments started with the RGP contact lens while the other half started with the PMMA lens. Figure 5 shows the effects of an RGP lens on epithelial pHi. The maximal pHi change was at about 5 minutes. At this point, pHi began a partial recovery and approached a new steady state after only 10 minutes. Conversely, PMMA lens wear induced continual acidification over this time period. Removal of the RGP lens generally brought pHi transiently higher than baseline.

Table 3 shows the maximal pHi change (\( \Delta \text{pHi} \)), the pHi change at 10 minutes (\( \Delta \text{pHi}_{10} \)) of lens wear for RGP and paired PMMA lenses, and the ratio of paired changes expressed as

\[
\left( \frac{\Delta \text{pH}_{\text{RGP}}}{\Delta \text{pH}_{\text{PMMA}}} \right)
\]

The mean drop in pHi after 10 minutes of RGP contact lens wear was significantly smaller for all permeable materials than corresponding acidification from PMMA lens (\( P < 0.05 \) for all). The initial rates of pHi drop between PMMA and RGP lenses, however, were similar. Yet, at 2 minutes and later, all the RGP lenses caused slower acidification than the paired PMMA lens. At 10 minutes of wear, PMMA-induced rates of pHi change were still decreasing, whereas those induced by RGP lenses were increasing. These data are summarized in Table 4.

To determine whether there were differences in the lens-induced acidification among RGP lenses, we compared the ratios of pHi change at 10 minutes of RGP lens wear to pHi change at 10 minutes of PMMA lens wear,

\[
\left( \frac{\Delta \text{pH}_{\text{RGP}}}{\Delta \text{pH}_{\text{PMMA}}} \right)
\]

and, with a one-way analysis of variance, found significant differences in this ratio among RGP lenses (\( F_{3,45} = 4.08, P < 0.05 \)). Using a pairwise comparison between the ratios obtained by lenses from the lower

![Figure 5. Paired experiment showing epithelial pH during RGP (Oxyflow f30) and PMMA contact lens (control) wear. Inset shows a linear regression of the ratio of pH drop after 10 minutes expressed as (ΔpH\(_{\text{RGP}}\))/(ΔpH\(_{\text{PMMA}}\)) as a function of nominal Dk/L\(_{O_{2}}\).](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933180/)
### TABLE 3. Contact Lens-Induced Decrease in Epithelial pH

<table>
<thead>
<tr>
<th>Material</th>
<th>RGP</th>
<th>PMMA</th>
<th>RGP/PMMA†</th>
<th>n₁, n₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorex 700</td>
<td>0.93 ± 0.07*</td>
<td>1.39 ± 0.07*</td>
<td>0.67 ± 0.04</td>
<td>13, 10</td>
</tr>
<tr>
<td>ΔpHi</td>
<td>0.83 ± 0.07*</td>
<td>1.34 ± 0.06*</td>
<td>0.62 ± 0.05¹-²</td>
<td>13, 10</td>
</tr>
<tr>
<td>Quantum2</td>
<td>0.95 ± 0.05*</td>
<td>1.46 ± 0.05*</td>
<td>0.65 ± 0.03</td>
<td>12, 10</td>
</tr>
<tr>
<td>ΔpHi</td>
<td>0.87 ± 0.05*</td>
<td>1.40 ± 0.05*</td>
<td>0.62 ± 0.03³-⁴</td>
<td>12, 10</td>
</tr>
<tr>
<td>Hi-Dk</td>
<td>0.74 ± 0.03*</td>
<td>1.58 ± 0.08*</td>
<td>0.48 ± 0.03</td>
<td>12, 5</td>
</tr>
<tr>
<td>ΔpHi₉₀</td>
<td>0.67 ± 0.04*</td>
<td>1.44 ± 0.08*</td>
<td>0.48 ± 0.04³-⁴</td>
<td>12, 5</td>
</tr>
<tr>
<td>ΔpHi₉₀</td>
<td>0.87 ± 0.04*</td>
<td>1.58 ± 0.08*</td>
<td>0.57 ± 0.04</td>
<td>12, 6</td>
</tr>
<tr>
<td>ΔpHi₉₀</td>
<td>0.70 ± 0.05*</td>
<td>1.52 ± 0.08*</td>
<td>0.48 ± 0.04³-⁴</td>
<td>12, 6</td>
</tr>
</tbody>
</table>

n₁ = number of experiments; n₂ = number of different rabbits used; Max ΔpHi = difference between pH just before lens insertion and lowest pH value during lens wear; usually Max ΔpHi occurs either at about 5 min of RGP lens wear or at lens removal for PMMA lenses; ΔpHi₉₀ = pH change 10 min after lens insertion. Means ± SEM are presented.

* Significant at P < 0.05 as evaluated with a unilateral paired t-test.
† The numerals in superscript in the RGP/PMMA column represent the 4 pairwise comparisons tested: 1, Fluorex 700-Quantum2 (t = 2.46); 2, Fluorex 700-Hi-Dk (t = 2.50); 3, Oxyflow 30-Quantum2 (t = 2.45); 4, Oxyflow 30-Hi-Dk (t = 2.49). Lenses from the higher Dk/L group produced significantly smaller ratios than the ones from the lower Dk/L group (t₄,₀₅,₅ = 2.32, as corrected by Bonferroni multiple comparison method).

Dk/L group (Oxyflow 30, Fluorex 700) with the ones from the higher Dk/L group (Quantum 2, Hi-Dk), we found four significantly smaller pairwise ratios for the lens from the higher Dk/L group (P < 0.05). Therefore, increasing contact lens transmissibility significantly decreased the lens-induced epithelial acidification among RGP contact lenses. The inset in Figure 5 illustrates the linear regression of

\[
\frac{\Delta pH_{RGP}}{\Delta pH_{PMMA}}
\]

as a function of the nominal Dk/L of the contact lens. Using this regression, we would predict that a lens with a Dk/L of more than 300 × 10⁻⁹ (cm/sec)(ml O₂/ml X mm Hg) would be needed to prevent any epithelial pH change.

No change in aqueous humor pH could be demonstrated during wear of any of the RGP lenses (n = 6) over 20 to 30 minutes. Thus, a Dk/L of only 18 × 10⁻⁹ (cm/sec)(ml O₂/ml X mm Hg) is sufficient to prevent aqueous pH changes in the open eye.

### DISCUSSION

We investigated the effects of contact lens wear on corneal epithelial and aqueous pH in living rabbits.

### TABLE 4. Rates of Epithelial pH Change During Contact Lens Wear

<table>
<thead>
<tr>
<th>RGP Material</th>
<th>Time (min)</th>
<th>dpH/dt (RGP)</th>
<th>dpH/dt (PMMA)</th>
<th>n₁, n₂</th>
</tr>
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<tbody>
<tr>
<td>Fluorex 700</td>
<td>1</td>
<td>-0.37 ± 0.03</td>
<td>-0.40 ± 0.02</td>
<td>13, 10</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.11 ± 0.02*</td>
<td>-0.22 ± 0.02*</td>
<td>13, 10</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-0.05 ± 0.01*</td>
<td>-0.14 ± 0.01*</td>
<td>13, 10</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>+0.02 ± 0.005*</td>
<td>-0.01 ± 0.005*</td>
<td>13, 10</td>
</tr>
<tr>
<td>Oxyflow 30</td>
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<td>-0.39 ± 0.02</td>
<td>11, 9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-0.16 ± 0.03*</td>
<td>-0.22 ± 0.02*</td>
<td>11, 9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-0.09 ± 0.03*</td>
<td>-0.14 ± 0.01*</td>
<td>11, 9</td>
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<tr>
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<td>-0.02 ± 0.01*</td>
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<tr>
<td>Quantum2</td>
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<td>12, 5</td>
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<tr>
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<td>-0.21 ± 0.03*</td>
<td>12, 5</td>
</tr>
<tr>
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<td>-0.15 ± 0.01*</td>
<td>12, 5</td>
</tr>
<tr>
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<td>10</td>
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<td>-0.03 ± 0.01*</td>
<td>12, 5</td>
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<tr>
<td>Hi-Dk</td>
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<td>-0.39 ± 0.03</td>
<td>-0.48 ± 0.03</td>
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<td>12, 6</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>10</td>
<td>+0.04 ± 0.01*</td>
<td>-0.02 ± 0.01*</td>
<td>12, 6</td>
</tr>
</tbody>
</table>

Rates (in dpH/min) are presented 1, 2, 3, and 10 min after lens insertion. Rates during PMMA and RGP lens wear were tested for significant differences with a unilateral paired t-test (dpH/dt (RGP) > dpH/dt (PMMA)). n₁ = number of experiments; n₂ = number of different rabbits used.

* Significant differences (P < 0.05).
† Significantly larger than 0 (P < 0.05) as evaluated with a unilateral t-test; only the rates at 10 min were tested.
‡ Significantly smaller than 0 (P < 0.05) as evaluated with a unilateral t-test; only the rates at 10 min were tested.
Twenty minutes of PMMA lens wear caused a decrease in epithelial pH of 1.42 units and a decrease in aqueous pH of 0.20 units. There was no recovery in pH, indicating the inability of epithelial cells to produce a net extrusion of protons. The aqueous humor acidification reported here was smaller than that previously reported under similar conditions (0.30 pH units). Considering both the aqueous humor acidification and the drop in stromal pH known to accompany hypoxic contact lens wear in humans, it is likely that PMMA lens wear acidifies the corneal endothelium because pH will tend to vary directly with pH0.

The effect of PMMA lens wear could be roughly simulated by exposure to 4.8% CO2-95.2% N2. Furthermore, the analysis of dpHi/dt suggests that the fast epithelial acidification during hypoxic lens wear is due to CO2 retention, whereas the slower component is hypoxic in origin. Theoretical work by Fatt and Bieber, and Lin showed that the time needed for CO2 to reach diffusional equilibrium is less than 90 seconds in the closed-eye condition or under an RGP contact lens. Second, a steady state stromal pH was reached after only 5 to 7 minutes of corneal exposure to 5% CO2. In addition, during PMMA contact lens wear, oxygen is depleted rapidly from the tear reservoir, and oxygen consumption decreases, a phenomenon that can lead only to a decrease in CO2 production. Thus, it seems unlikely that CO2 would significantly affect pH after 5 minutes of lens wear, and, therefore, the continued acidification is due primarily to hypoxia.

Although the goggle simulated the steady-state condition, the initial changes were dissimilar. When a PMMA lens is placed on the eye, CO2 will build up by diffusion from the anterior chamber, which will take at most a few minutes. In contrast, when the goggle is used, superficial epithelial pCO2 will essentially equilibrate with the gas instantaneously. Therefore it is not surprising that the initial pH drop during CO2-N2 exposure tends to be more rapid than when PMMA lenses are used. The overall quality of the change was the same, however: a fast initial drop, a secondary slow drop in pH, and the absence of acid recovery. The absolute changes were not as large during CO2-N2 exposure (1.24 vs. 1.42) as during PMMA lens wear. Several factors could contribute to this discrepancy: that the gas was 4.8% CO2-95.2% N2 rather than 5% would account for only about 0.02 pH units; a small temperature decrease due to the gas flow, slowing the temperature-sensitive metabolic reactions; and the difficulty in maintaining a completely sealed goggle on the rabbit. Interestingly, this smaller pH drop with gas exposure than with a PMMA lens is paralleled by the smaller corneal edema reported during 100% N2 exposure compared to PMMA lens wear.

In the case of the aqueous humor, hypoxia (N2 goggle) caused minimal and statistically insignificant changes in pH. Interestingly, 30 minutes of 5% CO2-air application at the tears drops aqueous pH consistently from 7.63 to 7.54. Given that this environment simulates closed-eye conditions, this result is close to the 0.13 drop in aqueous pH after 9 hours of lid closure maintained by sutures, but smaller than the one reported by Thomas et al after 1 hour of eye closure in rabbits. These reports of aqueous humor acidification after exposure of the cornea to 5% CO2 or during eye closure suggest that in the rabbit, open-eye, steady-state aqueous humor pCO2 is less than 38 mm Hg (5%).

We can estimate the open-eye, steady-state aqueous pCO2 in the following manner. When the eye is exposed to 5% CO2-air from the goggle, we can assume that aqueous pCO2 is very close to 38 mm Hg (5%). The measured pH is 7.54, so from the Hender-Hasselbalch equation, the calculated [HCO3-] = 31.4 mM. Assuming that the aqueous [HCO3-] changes very little when the eye is exposed to air, then

\[
[CO2] = \frac{31.4}{10^{(pH-pK_a)}}
\]

The open-eye pH was 7.63; hence, pCO2 is calculated to be 31 mm Hg or 4.06%, close to previous estimates of aqueous CO2 content, 4.25% and 4.38%, in the rabbit. Given that plasma pCO2 and posterior chamber pCO2 are 40 mm Hg, there must be a small decreasing gradient of aqueous CO2 in the posterior-to-anterior direction when the eye is open. Thus, it is not surprising that when a contact lens that is essentially impermeable to CO2 is placed on the cornea, CO2 efflux is blocked, aqueous pCO2 increases, and pH drops.

Is the observed pH change in the epithelium during contact lens wear consistent with the change in CO2 tension that occurs under these conditions? The mean maximal drop in epithelial pH after CO2 exposure averaged 1.00 pH unit. Such a pH drop would be expected if epithelial pCO2 increased ten times after exposure to CO2, assuming that [HCO3-] was the same in the open- and closed-eye situations. And, because cells from the superficial epithelium are exposed to air in the open-eye condition, they will experience the largest relative increase in CO2 tension during PMMA lens wear, and thus will acidify the most. A tenfold change in CO2 tension indicates that the intracellular pCO2 in the open eye is approximately 3.8 mm Hg (0.5%). The corneal stroma will acidify less because mid-stromal CO2 content will rise from about 2%, its approximate level during the open-eye condition, to 5% (for a decrease in stromal pH by 0.40 pH units). This prediction agrees well with the empirically determined 0.37 drop in human stromal pH after exposure...
to CO₂-air. In light of the pCO₂ gradient across the cornea, these pH changes seem reasonable.

In the current in vivo study, nitrogen-induced hypoxia acidified epithelial cells of the rabbit cornea. This finding should be contrasted with the alkalization after inhibition of respiration (by nitrogen exposure or cyanide application) in epithelial explants of rabbit cornea. This apparent contradiction can be explained by examination of the experimental conditions. First, the in vitro epithelial preparation was rubbed to expose basal cells. Second, this preparation was perfused, and rapidly enough (20 changes of volume/minute) to consider the extracellular pH to be constant. Thus, cellular acids were rapidly washed from the cells and from the extracellular space. In contrast, in the in vivo model presented here, any lactate and protons extruded from the epithelial cell diffuse from the extracellular space toward the stroma, thereby causing stromal acidosis and stromal lactate accumulation. In turn, the lingering of lactate and protons in the extracellular space and stroma are expected to decrease the electrochemical gradient for lactate and proton efflux from the cell, resulting in epithelial acidosis.

A lens that would not induce any pH change is highly desirable. The RGP contact lenses tested here induced smaller pH drops in the epithelium and in the aqueous humor than PMMA contact lenses. This finding is not surprising because tear film oxygen and carbon dioxide tensions are directly and inversely related to lens gas transmissibility, respectively. In addition, RGP lenses allowed epithelial pH to recover partially from acidification, as observed during the CO₂-air exposure experiments. The Dk/L required to avoid any epithelial pH change can be estimated by the x-intercept of the regression line displayed in the inset of Figure 5. The estimated Dk/L is over 300 X 10⁻⁹ (cm/sec)(ml O₂/ml X mm Hg), which represents, for the thicknesses used in this study, a DkO₂ of more than 500 X 10⁻¹¹ (cm/sec)(ml O₂/ml X mm Hg). Although rough, this estimate indicates that a very large permeability coefficient is required to prevent any epithelial pH change. Because DkO₂ and DkCO₂ are linearly related, either one can be used to express relative permeability to O₂ or to CO₂. Therefore, the estimated Dk/L is large because baseline epithelial pCO₂ is low, and the lens must be highly transmissible to prevent any epithelial CO₂ accumulation. Clinical evidence suggests that the epithelium can tolerate small amounts of acidosis as encountered during simple eye closure, or during daily wear of gas-permeable contact lenses. Because we could not demonstrate any change in aqueous humor pH with RGP lens wear, these lenses are not expected to alter much, if at all, the corneal endothelial pH in the open-eye condition. Our attempt to measure directly endothelial pH in vivo was not successful owing to hydrolysis of the AM ester within the anterior chamber. Using an in vitro isolated perfused cornea, we have found that these RGP lenses will cause a small (~ 0.1 units), measurable endothelial pH drop (unpublished observations).

In summary, changes in epithelial and aqueous pH were measured during lens wear and exposure to various gas mixtures. The fast component of epithelial acidification observed after the insertion of a PMMA or RGP contact lens is caused by CO₂ retention; the lens-induced cellular acidification is large because epithelial CO₂ tension during lens wear reaches a level many times its initial baseline level. Hypoxia also acidifies the corneal epithelium, and is responsible for the slow phase of the pH drop observed during PMMA lens wear. During RGP lens wear, epithelial cells partially recover from a contact lens-induced acid load, and thus face a higher steady state pH than during PMMA lens wear. pH measurement in the aqueous humor indicates that endothelial cells experience an acidification during PMMA lens wear; however, this acidification was not observed during the wearing of RGP contact lenses. Thus, RGP lenses alter the corneal environment less and are less likely to exert adverse effects on the physiology and metabolism of corneal cells.

Key Words
aqueous humor, contact lens, corneal epithelium, hypoxia, pH

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References
Corneal Epithelial and Aqueous Humor pH


