The Etiology of Transient Endothelial Changes in the Human Cornea

Brien A. Holden, Lewis Williams, and Steve G. Zantos

To investigate the etiology of contact lens–induced transient endothelial changes (blebs) in the human cornea, the effects of five different stimuli on corneal thickness and the appearance of the corneal endothelium were assessed. The stimuli included: (1) a silicone contact lens; (2) a silicone contact lens in combination with anoxia; (3) anoxia alone; (4) a thick hydroxyethyl methacrylate (HEMA) contact lens; and (5) a gas mixture of 9.8% carbon dioxide, 20.5% oxygen, and the balance nitrogen. The silicone lens alone produced no significant alteration in endothelial appearance and little change in corneal thickness. However, when nitrogen gas was passed in front of the lens, a typical bleb response was observed. This indicates that the physical presence of a contact lens is insufficient by itself to produce transient endothelial changes. Anoxia alone induced corneal swelling and endothelial bleb formation, indicating a metabolic component in the bleb response. The gas mixture containing 9.8% carbon dioxide also altered the endothelial appearance but had no significant effect on corneal thickness. The thick HEMA lens produced changes in both the appearance of the endothelium and corneal thickness. The only factor common to the stimuli which induced blebs would appear to be their ability to change the pH in or near the corneal endothelial layer. Invest Ophthalmol Vis Sci 26:1354–1359, 1985

The initial observation by Zantos and Holden1 that contact lenses produce changes in the appearance of the corneal endothelium (blebs) has been substantiated by a number of authors.2–4 Although subsequent studies have shown that blebs can be induced independent of contact lens wear by manipulating precorneal oxygen levels,5–6 the etiology of the contact lens induced response remains a matter for conjecture. In particular, it is not clear whether blebs are produced by a single factor such as precorneal hypoxia, or whether a combination of factors associated with contact lens wear is implicated in the response. In addition, controversy still exists in the literature as to whether alterations in the oxygen tension of the precorneal environment can affect endothelial oxygen levels.7–9

In the experiments reported in this article, we isolated factors associated with contact lens wear which induced blebs from those which did not. The etiology is more complicated than previously suspected,5–6 since blebs can be produced independent of precorneal hypoxia. We also found that blebs could be induced without apparently increasing corneal hydration, suggesting that this response does not compromise endothelial pump or barrier function at least in the short term.

Materials and Methods

Subjects

Twenty young adults, from whom informed consent had been obtained, participated in these experiments. None of the subjects were contact lens wearers and all were free from any apparent ocular disease.

Environments

On separate visits at least 24 hr apart, one eye of each of 10 subjects was exposed to the following environments for 1 hr: (1) a tight-fitting, thick silicone contact lens of high oxygen transmissibility (Titmus Eurocon; Aschafenburg, Germany 0.32 mm center thickness). The estimated oxygen availability under this lens was equivalent to an atmosphere of 18.1% oxygen10; (2) the same silicone contact lens in conjunction with atmospheric anoxia; (3) atmospheric anoxia produced by humidified nitrogen gas; (3) atmospheric anoxia produced by humidified nitrogen gas; (4) a tight-fitting, thick hydrogel contact lens of low oxygen transmissibility (Hydron; Hydron Australia; Sydney, Australia, 0.31 mm center thickness, 38.6% water content HEMA). The estimated oxygen availability under this lens was equivalent to an atmosphere of approximately 1% oxygen.11 (5) A humidified gas mixture nominally containing 9.8%
Fig. 1. A typical endothelial bleb response. The stimulus in this case was a thick HEMA contact lens. A, Prior to lens insertion; endothelial bleb response, expressed as percentage area covered by blebs, is 0.18% (×130). B, Peak response to stimulus (5.40% area under blebs).

carbon dioxide, 20.5% oxygen, and the balance nitrogen. Corrected for temperature (24°C) and altitude (10 m above sea level), the gas mixture actually contained 9.5% carbon dioxide after humidification.

The contralateral eye, which remained exposed to the air without a stimulus, served as a control in all experiments.12

Procedure

The gases were presented to the eye in sealed goggles after being bubbled through water at 37°C. The composition of the gases was verified by a Radiometer PHM 73 Blood Gas Analyser (Radiometer; Copenhagen, Denmark).

Central corneal thickness was measured before and after exposure to each test environment using an electronic pachometer. A Diagnostic Concepts Model 6090 pachometer (Diagnostic Concepts; San Diego, CA) was used for all experiments except the carbon dioxide study (5) for which the Payor-Holden micro-pachometer (Optometric Vision Research Foundation; Sydney, Australia) was used.

The corneal endothelium was photographed before, after, and at approximately 5-min intervals during exposure to each test environment using a noncontact specular microscope. The same endothelial area was relocated by fixing the angles of observation and illumination and the direction of subject fixation. The latency of the endothelial response was determined by continuously monitoring the endothelium over the first few minutes of exposure to each test environment until a bleb response was first detected. The change in appearance of the endothelium for each measurement occasion was quantified by comparing the endothelial appearance with a graded series of photographic slides in which the percentage area of the endothelium covered by dark zones (blebs) had been previously determined using projection planimetry. Figure 1 shows an...
Table 1. Endothelial bleb response to five different stimuli

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Latency of response (min)</th>
<th>Time to maximum response (min)</th>
<th>Endothelial response (% area)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Maximum response</td>
</tr>
<tr>
<td>a. Silicone lens</td>
<td>10.4 ± 6.4</td>
<td>23.8 ± 24.6</td>
<td>0.23 ± 0.18</td>
</tr>
<tr>
<td>b. Silicone lens + N₂</td>
<td>5.2 ± 2.6</td>
<td>23.2 ± 5.7</td>
<td>2.80 ± 2.85</td>
</tr>
<tr>
<td>c. Anoxia (N₂)</td>
<td>5.9 ± 3.6</td>
<td>32.4 ± 12.9</td>
<td>2.68 ± 2.03</td>
</tr>
<tr>
<td>d. HEMA lens</td>
<td>4.0 ± 2.3</td>
<td>28.6 ± 7.1</td>
<td>3.83 ± 3.44</td>
</tr>
<tr>
<td>e. CO₂ gas mixture†</td>
<td>4.9 ± 2.2</td>
<td>22.8 ± 2.9</td>
<td>3.43 ± 1.98</td>
</tr>
</tbody>
</table>

All results are presented as mean ± standard deviation.

* Student’s t-test: α = 0.05, df = 9, t₀ = 2.26.
† Subjects 11–20 were used in this study. All other studies involved subjects 1–10.

example of a typical endothelial bleb response, in this case to a thick hydroxyethyl methacrylate (HEMA) contact lens.

Results

The average endothelial bleb responses to the five different stimuli are summarized in Table 1. The endothelial response is expressed in terms of percentage area covered by the endothelial blebs. The latency of the bleb response and the time to reach maximum response (in minutes) are also presented.

Table 2 summarizes the average corneal swelling (percent) induced by each of the five stimuli.

In all studies the change in endothelial appearance followed the pattern described by Zantos and Holden. The response reached a maximum between 20 and 35 min after the stimulus was applied and then subsided at a slower rate than it had developed. After 1 hr of exposure to the stimulus, the endothelial response observed had decreased, on average, to approximately 50% of the maximum response.

Table 2. Corneal swelling response to five different stimuli

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Test eye</th>
<th>Control eye</th>
<th>Difference (test-cont)</th>
<th>Significance level*</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Silicone lens</td>
<td>1.14 ± 1.24</td>
<td>0.03 ± 1.47</td>
<td>1.10 ± 1.10</td>
<td>t = 3.00</td>
</tr>
<tr>
<td>b. Silicone lens + N₂</td>
<td>2.72 ± 1.97</td>
<td>0.96 ± 0.99</td>
<td>1.76 ± 1.73</td>
<td>t = 3.05</td>
</tr>
<tr>
<td>c. Anoxia (N₂)</td>
<td>3.00 ± 1.32</td>
<td>0.55 ± 0.91</td>
<td>2.46 ± 1.40</td>
<td>t = 5.27</td>
</tr>
<tr>
<td>d. HEMA lens</td>
<td>3.58 ± 0.97</td>
<td>-0.25 ± 1.21</td>
<td>3.83 ± 0.84</td>
<td>t = 13.68</td>
</tr>
<tr>
<td>e. CO₂ gas mixture†</td>
<td>0.21 ± 1.39</td>
<td>0.52 ± 1.34</td>
<td>-0.32 ± 1.89</td>
<td>t = 0.51 ns</td>
</tr>
</tbody>
</table>

All results are presented as mean ± standard deviation. ns: not significant.

* Student’s t-test; α = 0.05, df = 9, t₀ = 2.26.
† Subjects 11–20 were used in this study. All other studies involved subjects 1–10.

Contact Lenses

The silicone contact lens produced a slight increase in corneal thickness (1.1 ± 1.2%) and a slight alteration in the appearance of the endothelium (0.2 ± 0.2%).

When an anoxic environment was presented in conjunction with the silicone lens, significant alterations in both corneal thickness and endothelial appearance resulted (2.7 ± 2.0% and 2.8 ± 2.9%, respectively). The endothelial response peaked at 23 ± 6 min.

The thick hydrogel lens, on the other hand, produced a 3.6 ± 1.0% increase in corneal thickness and an obvious endothelial response of 3.8 ± 3.4%. Within 7 min all subjects exhibited an endothelial response, which peaked at 29 ± 7 min.

Anoxia

Anoxia alone resulted in alterations to both corneal thickness and endothelial appearance. These changes were similar in magnitude to those produced in the anoxia/silicone lens experiment (3.0 ± 1.3% and 2.7
Fig. 2. Endothelial bleb response (% area) vs time (minutes). The stimulus in this case was a humidified gas mixture nominally containing 9.8% carbon dioxide, 20.5% oxygen, and the balance nitrogen. Data for each subject is presented to illustrate the large variation in individual responses.

± 2.0%, respectively). The endothelial response peaked at 32 ± 13 min.

**Carbon Dioxide**

The gas mixture containing carbon dioxide produced no significant corneal thickness increase (0.2 ± 1.4%) but resulted in a significant endothelial response (3.4 ± 2.0%) which peaked at 23 ± 3 min.

In all experiments, the endothelial response was subject to very large individual variation. Figure 2, which presents individual data of the bleb response to the CO2 gas mixture, shows this clearly. In all cases where an endothelial response occurred the response subsided within 5 min of removal of the stimulus. The rate of decay of the bleb response was rapid and was complete in most cases within 2–3 min.

No changes in corneal thickness or endothelial appearance in the control eyes were detected in any of the experiments performed. This implies no interaction between the test and control eyes, as predicted by Efron et al.12

**Statistical Analysis**

All five stimuli produced a statistically significant maximal endothelial response (Student's t-test, $P < 0.05$). However, it is relevant to point out that the endothelial mosaic is seldom featureless; a baseline endothelial appearance is generally rated in the range of 0.1% to 0.4%. The endothelial response to the silicone lens (0.23%), therefore, can be regarded as insignificant.

Analysis of variance (ANOVA), combined with Fisher's protected t-test (LSD) procedure, confirms that the endothelial response to the silicone lens was significantly less than the endothelial response to the other four stimuli ($F_0 = 3.38, P < 0.05$). In addition, ANOVA indicates that these latter four stimuli produced endothelial responses which were not significantly different from one another.

Statistical analysis of the 1-hr corneal swelling responses shows that a statistically significant level of swelling was produced by all stimuli except the gas mixture containing carbon dioxide (Student's t-test, $P < 0.05$). Analysis of variance, combined with Fisher's LSD test, indicates that the HEMA lens produced significantly more corneal swelling over the 1-hr period than the other stimuli ($F_0 = 11.57, P < 0.05$).

**Discussion**

**Exogenous Factors Contributing to Endothelial Changes**

Endothelial changes in response to contact lens wear might be ascribed to several different factors, including the mechanical effects of the lens, reduced corneal oxygenation, interference with carbon dioxide (CO2) ef- flux or changes in corneal pH.

In these experiments, we have shown that the presence of a lens (silicone) by itself does not induce or enhance endothelial changes, suggesting that mechanical effects do not contribute to the endothelial response to lens wear.
The other four stimuli that we investigated all induced a significant endothelial bleb response (Table 1). Two points are worthy of attention: (1) the time course of both the onset of the endothelial response and the recovery were virtually identical for these four stimuli; and (2) all four stimuli produced endothelial responses of about the same magnitude.

It is possible, therefore, that a common factor underlies the bleb response induced by these four effective stimuli.

An increased precorneal CO₂ level, which was an effective stimulus in producing blebs, must result in an increase in the CO₂ dissolved in the corneal tissue. The first possibility, then, is that an increase in corneal carbonic acid in its dissociated form (H⁺ + HCO₃⁻) is responsible for bleb formation.

A relatively oxygen-impermeable HEMA lens also stimulates bleb formation. The major effect of this stimulus on corneal CO₂ levels is to impede the escape of CO₂ from the anterior surface of the cornea; consequently, CO₂ levels will equilibrate with those of the aqueous and uveal vasculature. This would induce a rise in H₂CO₃ concentration in the region of the endothelium. In addition, the low oxygen transmissibility of the contact lens will cause epithelial hypoxia and a consequent increase in the lactic acid concentration in the cornea.

Passing nitrogen gas across the cornea (with or without an oxygen-permeable contact lens) also induces bleb formation. Since the tricarboxylic acid cycle is inoperative in the corneal epithelium under this condition, CO₂ production by the epithelium (and possibly by the stroma) must be diminished. It is difficult to see how the H₂CO₃ concentration near the endothelium could rise in these circumstances; it seems, rather, that it should fall. For this reason, an increase in corneal CO₂ concentration cannot be a necessary factor in bleb formation.

In the nitrogen experiments, as with the HEMA lens, hypoxia probably caused the lactic acid concentration in the corneal stroma to increase. However, lactic acid production would not be significantly affected in the CO₂ experiment; therefore, an increase in lactic acid production is also not a necessary factor in bleb formation.

It appears, therefore, that although increases in either CO₂ or lactic acid concentration in the corneal stroma always seem to accompany the appearance of blebs, neither factor by itself is essential for bleb formation. The most obvious change in the corneal environment which may be produced equally well by increases in either lactic acid or CO₂ concentration is a lowering of pH. Therefore, we tentatively suggest that transient endothelial changes are produced by reductions in corneal stromal pH.

It would be of interest to evaluate the effects of the endothelium of manipulation of endothelial pH levels independent of other factors. However, it is difficult to conceive of a suitable approach using human subjects, as ethical considerations preclude the use of invasive techniques. Attempts by one of the authors (LW) to produce an endothelial bleb response by topical instillation of aerated ocular buffer solutions of varying pH have been unsuccessful; however, the authors feel that it is doubtful whether endothelial pH levels are significantly affected by this procedure, given the barrier properties of the various corneal layers.

Although animal studies provide an alternative approach, an animal model for transient endothelial changes has yet to be identified; no bleb response to lens wear or anoxia has been observed in monkeys, cats, or rabbits. However, unpublished experiments by one of the authors (LW) indicate that pH levels in the anterior aqueous in rabbits are affected by precorneal conditions such as anoxia, thick HEMA contact lenses and exposure to a gas mixture containing 16% CO₂. This supports the work of Gamm and Lapina, who report a decrease in aqueous pH with hydrogel lens wear.

Corneal Thickness

Carbon dioxide gas passed in front of the cornea produced a significant endothelial response, but resulted in no significant change in corneal thickness. This important result indicates that transient endothelial changes can occur without significantly altering corneal hydration. In addition, it suggests that corneal swelling with contact lens wear is unlikely to be attributable to an increase in corneal CO₂ concentration.

Corneal thickness changes in anoxic environments (contact lens–induced or gas–induced) were all statistically significant. However, the corneal swelling response to the HEMA contact lens was significantly greater than to the other anoxic stimuli. This interesting result suggests that factors other than hypoxia may contribute to the edema response during contact lens wear.

We found no significant correlation between the magnitude of swelling and the magnitude of the endothelial response. This supports the conclusion that the transient endothelial response does not in itself affect the endothelial pump or barrier function, at least in the short term.

In summary, our results show that the presence of a contact lens is not a sufficient stimulus by itself for endothelial bleb formation. Various other changes in the precorneal environment can produce endothelial blebs irrespective of whether corneal swelling occurs or not. We suggest that pH changes in the corneal...
stroma may be responsible for producing the endothelial bleb response.

**Key words:** cornea, endothelial blebs, contact lenses, hypoxia, carbon dioxide, pH

**Acknowledgments**

The authors gratefully acknowledge the contributions of Ms. Helen Swarbrick and Dr. Daniel O'Leary in the preparation of this paper, and the assistance of Professor Richard Hill and Dr. John Schoessler of Ohio State University in the conduct of a number of these studies.

**References**