Oxygen levels beneath the closed eyelid.

NATHAN EFRON and LEO G. CARNEY.

The level of oxygen at the anterior corneal surface beneath the closed eyelid is shown to be equivalent to an atmosphere of 7.7% oxygen. This finding is in good agreement with assumptions which have been based on the oxygen level at the palpebral conjunctiva. However, in some instances a significant amount of oxygen is derived not only from the palpebral conjunctiva but also from the atmosphere as a result of an imperfect palpebral aperture seal.

The oxygen tension at the anterior corneal surface during prolonged lid closure, such as during sleep, has been of interest for some time. It is now of increased significance because of the present popularity of extended-wear contact lenses.

The oxygen tension at the palpebral conjunctiva was found by Fatt and Bieber1 by direct measurement to be 55 ± 5 mm Hg (equivalent to 7.4% oxygen). They assumed this to be the level of oxygen available to the cornea when the eyelids are closed, and they based subsequent oxygen profile calculations, with and without contact lenses of varying oxygen transmissibilities, on this closed-eye boundary condition.2 Grote and Zander,2 on the other hand, assumed in their oxygen profile calculations that the level of oxygen at the cornea of the closed eye corresponded to that of arterial blood and was 90 mm Hg (equivalent to 12.1% oxygen).

In this study, we have sought to determine, by corneal oxygen uptake measurements in the in vivo human eye, the level of oxygen available to the cornea during eyelid closure.

Methods. The technique of determining the equivalent level of oxygen at the corneal surface was based on that of Hill.3 Corneal oxygen uptake recordings were made on the unanesthetized corneas of 12 young adults. None of these subjects had any significant past ocular history. A fast response-time Clark-type polarographic oxygen electrode (with a 12.7 μm Teflon membrane and 2 mm cathode) was used. This electrode had the advantage over those electrodes used previously4 for human in vivo corneal oxygen uptake measurements of reducing the length of time of the electrode on the subject’s eye.

The depletion of oxygen from the electrode was recorded on a Hitachi QPD 54 pen recorder (Hitachi, Ltd., Tokyo, Japan), and the depletion rate was then calculated according to the formula:

$$R = \frac{\Delta P_{O_2}}{t - t_0}$$

where $\Delta P_{O_2}$ is the arbitrarily chosen depletion range of 150 to 40 mm Hg, $t$ is the depletion time (sec), and $t_0$ the electrode response time over the above range (usually 0.5 to 1.0 sec). The experiment was essentially a two-stage process.

Stage 1. Calibration of corneal response. Air, nitrogen, and gases of known oxygen content were each passed over the eye through an airtight gas mask for a 5 min interval. Immediately following exposure of the corneal surface to each gas, the electrode was removed from an air-equilibrated saline bath at 37° C and placed upon the cornea, and the depletion rate was recorded. Thus the effect of the gas of known oxygen content could be expressed by the response ratio, $q$, which is defined as:

$$q = \frac{R_{O_2} - R_k}{R_{N_2} - R_{AIR}}$$

where $R_k$ is the depletion rate obtained after passing the gas of known oxygen content over the eye and $R_{AIR}$ and $R_{N_2}$ are the depletion rates obtained after exposure of the cornea to air and nitrogen, respectively. This procedure was carried out on each subject with gases of 5%, 10%, and 15% $O_2$ (all balance nitrogen), each gas being used six times. A linear relationship could then be derived which could predict from the electrode response the equivalent level of oxygen present at the anterior corneal surface.

Stage 2. Test procedure. The cornea was exposed sequentially to air and nitrogen for 5 min intervals, and the electrode responses were recorded after exposure to each gas. Two experiments were then conducted. In the first experiment, the subjects closed their eyes for 5 min, and the oxygen depletion rate was recorded immediately upon opening the eyelid. The response ratio was then expressed as:

$$q = \frac{R_{N_2} - R_c}{R_{N_2} - R_{AIR}}$$

where $R_c$ is the depletion rate obtained following the test condition. The equivalent oxygen level at the anterior surface of the cornea was then de-
Table I. Corneal response calibration data

<table>
<thead>
<tr>
<th>Subject</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>19</td>
<td>33</td>
<td>19</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>Equation predicting oxygen levels ($\delta$) from response ratio $q$</td>
<td>$\delta = 16q-0.71$</td>
<td>$\delta = 19q-3.21$</td>
<td>$\delta = 19q-1.29$</td>
<td>$\delta = 12q+3.83$</td>
<td>$\delta = 11q+3.77$</td>
</tr>
<tr>
<td>Correlation coefficient*</td>
<td>0.89</td>
<td>0.91</td>
<td>0.89</td>
<td>0.74</td>
<td>0.77</td>
</tr>
</tbody>
</table>

*Statistically significant in all cases ($p < 0.001$, except for subject 10 where $p < 0.01$).

Table II. Level of oxygen at corneal surface beneath closed lid in air and in nitrogen

<table>
<thead>
<tr>
<th>Subject</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of oxygen beneath closed lid in air (%) (Mean ± S.D.)</td>
<td>7.0 ± 1.9</td>
<td>8.1 ± 4.2</td>
<td>2.2 ± 1.9</td>
<td>7.0 ± 6.0</td>
<td>8.0 ± 1.9</td>
</tr>
<tr>
<td>Level of oxygen beneath closed lid in nitrogen (%) (Mean ± S.D.)</td>
<td>2.7 ± 1.4</td>
<td>8.7 ± 3.7</td>
<td>2.8 ± 2.7</td>
<td>3.8 ± 7.3</td>
<td>8.9 ± 0.8</td>
</tr>
<tr>
<td>Significance (p value) of difference between the two test conditions (t-test)</td>
<td>&lt;0.002</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not significant.

terminated from the calibration data. This was repeated six times on each of the 12 subjects. In the second experiment, nitrogen was passed over the closed eye for the entire 5 min interval to ensure that the oxygen reaching the cornea came only from the palpebral conjunctiva. This second experiment was also carried out six times on each of the 12 subjects.

Results. The calibration data of Table I show that a linear relationship exists between the response ratio values and known oxygen levels. In Table II, results are given for the levels of oxygen beneath the closed lid in air and beneath the closed lid in a nitrogen (anoxic) environment. The mean level of oxygen at the corneal surface beneath the closed lid in air was 7.7% ± 3.8 (56.7 mm Hg). This was not significantly different from the level found beneath the closed lid in an anoxic environment. However, in three of the 12 subjects used in this study, there was significantly less oxygen available to the cornea when nitrogen was passed over the closed eye, suggesting that in these subjects there was a reduced effectiveness of the palpebral aperture as a seal to oxygen from the atmosphere. Indeed, such a result is not unexpected, since Howitt and Goldstein7 have shown that in 23% of 145 subjects a physiologic lagophthalmos was evident. This would inevitably contribute to the variations in the results under the two closed-eye conditions used in the present study.

Discussion. It was found in this study that the mean level of oxygen at the corneal surface beneath the closed lid in air is 7.7% oxygen. It would seem, then, that oxygen profile calculations based on an assumed closed-lid boundary condition of 7.4% oxygen are well-founded. Corneal oxygen profile calculations using this boundary condition assumed that the only supply of oxygen to the cornea with lid closure was from the palpebral conjunctiva. To test this assumption, we replaced the ambient air during lid closure with an anoxic environment and found that there was no statistically significant change in the mean level of oxygen at the cornea. However, in three of the 12 subjects used in this study, there was significantly less oxygen available to the cornea when nitrogen was passed over the closed eye, suggesting that in these subjects there was a reduced effectiveness of the palpebral aperture as a seal to oxygen from the atmosphere. Indeed, such a result is not unexpected, since Howitt and Goldstein7 have shown that in 23% of 145 subjects a physiologic lagophthalmos was evident. This would inevitably contribute to the variations in the results under the two closed-eye conditions used in the present study.

We thank H. Barry Collin for his valuable suggestions on the preparation of this paper.

From the Corneal Biophysics Laboratory, Department of Optometry, University of Melbourne, Parkville, Victoria, Australia. Submitted for publication Dec. 30, 1977. Reprint requests: Dr. L. G. Carney, Department of Optometry, University of Melbourne, Parkville, Victoria, Australia 3052.

Key words: corneal oxygen uptake, oxygen electrode, closed eye, palpebral aperture seal, palpebral conjunctiva.
Rhodopsin determinations in C57BL/6J-pallid strain mice. M. G. LEWIS AND D. T. ORGANISCIAK.

The influence of light environment on rhodopsin concentration per eye was determined in littermate pigmented and nonpigmented C57BL/6J-pallid gene mice reared under cyclic light or continuous dark environments. Attempts to exacerbate a congenital manganese deficiency in pallid strain mice included dietary deprivation and supplementation with manganese and exposure to intense light followed by the determination of rhodopsin recovery rates in darkness. Homozygous pallid mice (pa/pa) reared in cyclic light had rhodopsin levels which were significantly lower than heterozygous (+/pa) or homozygous (+/+ ) black control mice. Dark-rearing resulted in a significant increase in rhodopsin per eye in pallid strain mice and equivalent levels in adult mice, but young pallid strain mice did not achieve the same rhodopsin concentration as young +/+ mice. Although dietary manganese deprivation or supplementation did not significantly alter rhodopsin levels among pallid mice, the deficient diet resulted in lower rhodopsin per eye in the young +/+ control animals. The recovery of rhodopsin in darkness following intense light exposure was equal and complete within 24 hr for most genotypes. However, recovery by pallid mice after 24 hr was significantly lower than by pigmented or albino genotypes.

In studies of the retina, environmental light is a variable shown to have effects on rhodopsin content and rod outer segment (ROS) length, ROS phospholipid/opsin ratios, disc shedding, and the rate of phagocytosis. Eye pigmentation is an additional factor which may mediate the effects of light environment on rhodopsin concentrations. We recently obtained a strain of C57BL/6J mice which carry the mutant gene pallid and display a dilution of pigmentation enabling the homozygous

REFERENCES