Measuring Oxygen Tension in the Anterior Chamber of Rabbits

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PURPOSE. Measuring the concentration of oxygen in the aqueous humor without penetrating the eye would provide a new dimension in understanding aqueous humor and corneal dynamics. In this study a preinvasive method was developed for determining the cameral oxygen concentration in anesthetized rabbits by measuring the excited-state lifetime of a phosphorescent dye.

METHODS. A scanning ocular fluorometer was designed to excite phosphorescence with a brief flash of light and to measure the decay of luminescence for as long as 1000 μsec after excitation. The measurement window was scanned through the depth of the anterior chamber or fixed at the mid-anterior chamber. A depot of the phosphorescent dye Pd-urophorphyrin was injected into the vitreous of eight pigmented rabbits, and within a few days the dye was measurable in the anterior chamber. The excited-state lifetime of this dye is inversely correlated to oxygen concentration and was calibrated by measuring the lifetime of dye in cuvettes equilibrated with oxygen-nitrogen mixtures. Oxygen tensions were determined from lifetimes measured in the open eye, under a polymethylmethacrylate (PMMA) contact lens, under two oxygen-permeable contact lenses, and immediately after lid closure.

RESULTS. Oxygen tension in the mid-anterior chamber before placing a PMMA contact lens was 23 ± 3 mm Hg (mean ± SD; n = 6). After 20 minutes of PMMA lens wear, oxygen tension decreased to 4 ± 2 mm Hg. When the focal diamond was scanned through the anterior chamber, oxygen tension was 24 ± 5 mm Hg near the corneal endothelium and decreased to 17 ± 8 mm Hg near the crystalline lens. Under the PMMA contact lens this gradient reversed: Oxygen tensions near the endothelium and lens were 3 ± 2 mm Hg and 6 ± 2 mm Hg, respectively. Lid closure for 10 minutes or longer decreased the mid-anterior chamber oxygen tension from 21 ± 2 mm Hg (n = 19 measurements from seven animals) to 10 ± 3 mm Hg (n = 15 measurements from five animals).

CONCLUSIONS. Measuring excited-state lifetime of phosphorescent dyes in the anterior chamber provides a useful method for determining oxygen concentration in vivo, without penetrating the eye. Cameral oxygen tension under PMMA contact lenses are significantly lower than in the uncovered eye. The profile of oxygen tension through the anterior chamber suggests that oxygen is supplied transcorneally to the aqueous humor. (Invest Ophthalmol Vis Sci. 1998;39:1899-1909)

The circulation of aqueous humor is critical to the health of the cornea. This fluid movement supplies nutrients and removes metabolites from the cornea, a process that in vascularized tissues is supported by blood circulation. In contrast, oxygen must be supplied by other sources because the oxygen content of the aqueous humor does not seem to be high enough to supply the cornea with all the oxygen it uses.1 Most measurements of oxygen tension and mathematical modeling of oxygen movement suggest that in the open eye, oxygen is supplied to the cornea by direct transfer from the atmosphere, and if access of the corneal epithelium to the atmospheric oxygen is restricted, oxygen concentration in the anterior chamber and in the cornea decreases.2-5 When the cornea is covered, other sources of oxygen, such as the iris vessels, the ciliary body, and the limbic tissues, are unable to maintain cameral oxygen concentrations in the uncovered eye. Although oxygenation of the aqueous humor is influenced by oxygen saturation of the blood,6,7 it is unclear how oxygen concentrations are affected by medications that constrict blood vessels or change aqueous humor flow rate.

Most of our knowledge about oxygen kinetics in the anterior segment have come from direct measurements in the aqueous humor, although some have inferred cameral oxygen concentration from measurements at the bare corneal stroma.4 In early studies, oxygen was assayed from samples of aqueous humor obtained by paracentesis.7 Friedenwald and Pierce6 introduced a bubble of nitrogen or air into the anterior cham-
ber, and after period of equilibration with the aqueous humor, they removed it and assayed it for oxygen. Others have measured oxygen directly in the anterior chamber and cornea through custom-made polarographic microelectrodes that were introduced through a corneal or scleral opening in experimental animals \(^5\) or at surgery in humans \(^11\) to 14. All these methods require disruption of the eye by penetration with a needle or electrode or by scraping the corneal epithelium and leave open to question the effect of this intervention on the concentration of oxygen, the object of the measurement.

The ability to measure oxygen in the aqueous humor without touching the eye would provide a new dimension in understanding physiologic processes of the undisturbed anterior segment. In this study we describe a technique to determine cameral oxygen concentration in experimental animals by measuring the phosphorescence lifetime of an exogenous dye. The technique requires the injection of a depot of dye into the vitreous several days before oxygen measurements, and can be considered minimally preinvasive.

The method is based on the quenching of phosphorescence by oxygen. When a phosphorescent molecule absorbs a photon, it can remain in an excited state for several microseconds to several seconds before releasing its energy as a second photon, in contrast to most fluorescence molecules, which decay from the excited state in less than a microsecond. The lifetime of this excited state depends not only on the characteristics of the dye molecule, but also on its interaction with other molecules in the environment. If the excited molecule interacts with molecular oxygen, for example, it can be induced to release its energy by means other than emitting a photon. This process, called quenching, diminishes the intensity of phosphorescence and shortens its decay by an amount that is closely related to the concentration of the quenching molecule. Quenching can be used to study the molecular environment of the phosphor; by measuring the decrease in phosphorescence intensity or the change in phosphorescence lifetime, the concentration of the quencher can be determined. This principle is well suited for measuring oxygen in solutions, because oxygen is an efficient quencher of most phosphorescent dyes. Quenching has been used to measure oxygen concentration in cell culture \(^15\), the cardiovascular system \(^16\) to 19, the cerebral cortex \(^20\) to 22 and other systems in vitro \(^23\) to 25. Harvitt and Bonanno \(^26\) recently measured oxygen tension in the tear film of rabbits, when corneas were exposed to air and under contact lenses, by measuring quenching of Pd-mesotetra (4-carboxyphenyl) porphine in a 1% albumin solution.

The use of phosphorescence quenching to measure oxygen concentration requires a suitable phosphorescent dye, a means of exciting the dye with either a flash or a modulated light source, and a method of measuring the change in phosphorescence intensity or the change in lifetime of luminescence. In a dynamic system such as the aqueous humor where dye concentration can change throughout the experiment, measuring lifetime is more reliable, because lifetime is independent of dye concentration.

In this study we designed an ocular phosphorimeter capable of measuring the excited-state lifetime of phosphorescent dyes in the anterior chamber. It is similar to the instrument used by Harvitt and Bonanno \(^26\) in that it measures the decay of luminescence after a brief excitation flash. The device has a small measurement window that can be scanned through the anterior chamber, and was used to measure phosphorescence lifetime and oxygen concentration in the anterior chambers of anesthetized pigmented rabbits when their corneas were uncovered or covered with contact lenses and after lid closure.

**METHODS**

**Theory**

Detailed descriptions of the physical process of phosphorescence, its dependence on oxygen, and principles of its use in measuring oxygen concentration have been provided elsewhere \(^27\) to 31 and will not be repeated in detail here. Briefly, when a phosphorescent dye is excited by a flash, luminescence decreases exponentially:

\[
I = I_0 e^{-t/\tau}
\]

where \(I\) is luminescence at time \(t\), \(I_0\) is the luminescence immediately after the excitation flash, and \(\tau\) is the excited-state lifetime. As concentration of a quencher (molecular oxygen in this system) increases, \(\tau\) decreases according to the Stern-Volmer correlation \(^27\):

\[
\frac{1}{\tau} = \frac{1}{\tau_0} + k_q [O_2]
\]

where \(k_q\) is a quenching constant, \([O_2]\) is the concentration of oxygen, and \(\tau_0\) is the phosphorescence lifetime in the absence of oxygen. This correlation provides a means of determining concentration of oxygen. If \(k_q\) and \(\tau_0\) are known for a particular dye, then oxygen concentration can be determined by measuring \(\tau\). The instrument that we describe excites phosphorescence in the anterior chamber with a brief flash and records luminescence for 1000 \(\mu\)sec after each flash. The excited-state lifetime \(\tau\) is calculated from the decay of luminescence, and oxygen concentration is determined by using Eq. 2.

**Instrument Design**

A custom-designed scanning ocular fluorophotometer described elsewhere \(^32\) was fitted with a light source and photometric detection system suitable for exciting and measuring phosphorescence (Fig. 1). The light source, a xenon flashlamp (Model FX-800u; EG\&G Electro-optics, Salem, MA), was mounted with its arc at one focus of an ellipsoidal reflector. The image of the arc at the second focus was filtered and relayed by two planoconvex lenses to an optical fiber (0.6 mm diameter) that was routed to the excitation lens assembly. A pair of planoconvex lenses focused light from the optical fiber to the anterior chamber (1:1 conjugate ratio). Each flash had a duration of less than 2 \(\mu\)sec and a maximum energy of 360 \(nJ\) at the eye. This energy could be diminished as needed to accommodate a range of dye concentrations.

Luminescence was collected by a second pair of planoconvex lenses and focused on the second optical fiber. The optical axis of this emission path and the optical axis of the excitation lenses intersected at their focal points at an angle of 90°. The focal diamond was approximately cylindrical and coaxial with the optical axis of the excitation lens and was
Scanning Ocular Phosphorimeter

FIGURE 1. Schematic of ocular phosphorimeter. Excitation light from a Xenon flash lamp was filtered and routed to the excitation lens assembly through an optical fiber. The excitation lens focused the image of the fiber in the anterior chamber. Luminescence was collected by an emission lens and transferred through a second optical fiber to an emission filter and photomultiplier tube. A discriminator and photon counter measured luminescence for 1000 μsec after each flash. The sequence of events was coordinated and recorded by a computer. The lens assembly could be translated in three dimensions, and the focal diamond could be scanned through the anterior chamber during measurement.

approximately 1 mm in diameter, 0.5 mm long, and 1.1 mm in its anteroposterior depth.

The other end of the emission fiber was routed to a photometric measurement assembly. Luminescence was filtered by a long-pass absorption filter that blocked all wavelengths shorter than 600 nm. A photomultiplier tube (model R928; Hamamatsu, Bridgewater, NJ) with a multialkali photocathode and extended red response to 930 nm measured luminescence after each flash. Its anode was connected to a preamplifier and amplifier-discriminator (model C604A; Thorn EMI Electron Tubes, Fairfield, NJ) that generated square-wave pulses with a maximum width (dead time) of 25 nsec from each photoelectron detected.

A custom-designed circuit and dedicated microcomputer (based on an M68332 processor; Motorola, Schaumburg, IL) recorded luminescence after each flash by counting pulses from the discriminator in 20 consecutive intervals, each 50 μsec long (Fig. 2). At the end of this sequence (trial) the circuit stored the number of photons detected in each interval, paused for 20 msec to ensure that residual phosphorescence had vanished, and repeated the flash and recording trial. After each repeated trial, the device added the number of photons detected in each interval to the number in the corresponding interval on previous trials. After 32 consecutive trials, data in the 20 registers represented the average luminescence at 50-μsec intervals beginning 25 μsec after the flash (midpoint of first interval). The program stored these data and reset all 20 registers to zero. It then repeated the recording sequence of 32 trials with a 12-μsec delay imposed between the flash and the beginning of the first 50-μsec interval. Two more sequences were recorded with delays of 23 μsec and 34 μsec. At the end of these four groups of measurements, the luminescence decay was represented by the total number of photons detected in 80 overlapping intervals, each 50 μsec wide and centered at approximately 12-μsec steps. In some animals six groups of measurements (six delays) were recorded.

The dedicated microcomputer timed and coordinated the flash and the photon counting circuit and stored data. Parameters of the scan (number of trials, delays, and count time) were downloaded from, and luminescence data were uploaded to, a laboratory computer. After each sequence, custom programs graphed luminescence and calculated first-order decay constants.

In many measurements luminescence clearly did not decrease as a single exponential curve (Eq. 1), and a biexponential curve provided a better fit to the data:

\[ I = I_1 e^{-t/\tau_1} + I_2 e^{-t/\tau_2} \]

where \( I_1, I_2, \tau_1, \) and \( \tau_2 \) are constants. Equation 3 was fitted by the least-squares method and a curve-stripping technique to obtain a long and a short time constant (\( \tau_1 \) and \( \tau_2 \)). Luminescence during the first 30 μsec after each flash was sometimes erratic, possibly because of the slow recovery of the photomultiplier tube from the bright fluorescence signal during the
FIGURE 2. Method of recording luminescence after excitation flash. Vertical lines on top represent photoelectron pulses after the excitation flash at time 0. Pulses were counted during consecutive 50-μsec intervals \((n_{0,1}, n_{0,2}, \ldots)\). The beginning of the first interval was delayed \((d_{0}, d_{1}, d_{2}, d_{3}, \ldots)\) to offset time of each interval. The minimum delay, \(d_{0}\), was 6 μsec to ensure that luminescence from the flash had disappeared. Centers of intervals are indicated by asterisks above the time scale.

flash. For this reason, data from the first 60 μsec were not used to calculate decay constants. \(\tau_1\) and \(\tau_2\) were dependent on oxygen concentration, although the long time constant was somewhat more sensitive than the short constant to changes in oxygen concentration. In this study we used and calibrated the long decay constant as a measure of oxygen concentration. When luminescence decreased as a single exponential, the curve-fitting technique provided a very small short time constant, and the long time constant was approximately equal to the lifetime determined by fitting the data to a single exponential.

The operator aligned the instrument with the anterior chamber by positioning on the cornea the images of a pair of linear-filament lamps. Both lamps were mounted just lateral to the excitation and emission lens assemblies and were focused at the focal diamond through simple biconvex lenses. When the instrument was properly aligned, the images of the filaments were in focus and overlaid each other. The operator then pressed a button to initiate measurement, and the instrument turned the alignment lights and room lights off and measured phosphorescence in a static mode or a scanning mode.

In the static mode, the computer initiated the measurement sequence without repositioning the focal diamond. Measurement of phosphorescence in this mode required approximately 4 seconds. In the scanning mode, a servomotor advanced the lens assembly between groups of 32 trials, and phosphorescence was measured at 15 discrete positions centered at 0.3-mm intervals on a line through the anterior chamber. A complete set of measurements in this mode required approximately 15 seconds. Phosphorescence lifetime was calculated from the luminescence decay curve at each position.

Phosphorescent Dye

The phosphorescent dye Pd-uroporphyrin,

33 supplied as a crystalline powder, was dissolved in a 100 mM phosphate buffer (pH 7.4) and filtered (0.22-μm pore size; Millipore, Bedford, MA) to make a 1-mM stock solution. A portion of this solution was diluted 1000:1 for calibration, and the remainder was saved for injection into the vitreous. The optimum wavelengths of excitation, fluorescence, and phosphorescence were 405 nm, 620 nm, and 670 nm, respectively.

Calibration

Oxygen concentration was determined from phosphorescence lifetime by solving Eq. 2 for \([O_2]\). The Stern-Volmer coefficients \((k_q\) and \(\tau_q)\) were first determined by fitting Eq. 2 to lifetimes measured at several known oxygen concentrations. Gas mixtures at 5%, 2%, 1.18%, and 0.68% oxygen balanced with nitrogen were obtained from a local vendor. These mixtures were bubbled for a minimum of 45 minutes through a distilled water bath (to humidify and reduce evaporation of the test solution), and then through approximately 1 ml of the 1
The equilibrated partial pressures of dissolved oxygen correspond to 38, 15, 9, and 5 mm Hg, respectively. Oxygen concentrations were reduced to zero by adding 75 μg/ml glucose oxidase (Sigma, St. Louis, MO), 12.5 μg/ml catalase (Sigma), and 0.3% glucose,28 and bubbling with nitrogen. This sample was then sealed for at least 1 hour. Phosphorescence was measured by operating the instrument in the static mode and the lifetime (long lifetime) was calculated by fitting Eq. 3 to luminescence.

Phosphorescence lifetime of the dye was measured in solutions that simulated some of the conditions that may be encountered in the anterior chamber. Lifetime was measured in solutions that contained rabbit albumin (Fraction V; Sigma) at concentrations equal to 10, 25, 50, and 100 mg/dl. It was also measured in solutions of differing ionic strength (110 mM, 150 mM, and 200 mM) and pH (7.2, 7.4, and 7.6), and in solutions that contained amino acids and other substances found in aqueous humor (Dulbecco’s modified Eagle’s medium 13000, recommended concentration and 4:1 dilution; Life Technologies, Grand Island, NY). In some mixtures an anti-foaming agent (Antifoam 289; Sigma) was added at the rate of 1 nl/ml. This did not affect lifetime, but it allowed bubbling without loss of solution caused by foaming. All solutions were measured between 33°C and 34°C, the approximate temperature of aqueous humor. Temperature dependence of dye in phosphate buffer was also measured at between 20°C and 38°C. Except when examining the effect of albumin, all solutions contained rabbit albumin at a concentration of 50 mg/dl.

Lifetime in the absence of oxygen, τ₀ in Eq. 2, was assumed to be equal to the lifetime in the solutions that contained the glucose oxidase and catalase. The quenching constant k.q was assumed to be equal to the slope of the line that passed through τᵢ⁻¹ and was fitted to the inverse of lifetimes in solutions equilibrated with the gas mixtures.

Animals

Phosphorescence lifetime of Pd-uropporphyrin was measured in the anterior chambers of eight pigmented rabbits. All procedures and experiments conformed to the ARVO Statement the use of Animals in Ophthalmic and Vision Research.

The dye diffused into the aqueous humor from a depot that was injected into the mid vitreous 3 to 14 days before phosphorescence measurements. Each animal was anesthetized intramuscularly by 50 mg/kg ketamine and 5 mg/kg xylazine. A 30-gauge needle was inserted into the globe through the superior rectus at the pars plana, the tip was advanced to mid vitreous, and 50 μl or 100 μl of the filtered stock solution was injected. The needle was then quickly withdrawn.

Within 3 days of injection, concentrations of dye in the anterior chamber were sufficient to measure. During each measurement session, animals were anesthetized with ketamine and xylazine and were placed in an animal holder in front of the optical assembly. Corneas were kept moist by rinsing with an ophthalmic irrigating solution (Dacrose; Iolab, Claremont, CA).

We measured phosphorescence lifetime in the anterior chambers of eyes without contact lenses and in eyes with one of three types of contact lenses placed on the cornea to manipulate oxygen concentration: polymethylmethacrylate (PMMA), Equalens II (Polymer Technology, Boston, MA), and Fluorex 700 (GT Laboratories, Glenview, IL). All lenses were 11.5 mm in diameter, had a base curve of 46.50 D, and a power of −3.0 D. Properties related to oxygen transmissivity of the lenses are shown in Table 1. Phosphorescence was measured through the lens, and although intensity of phosphorescence was somewhat reduced, the lenses had no effect on phosphorescence lifetime.

We also examined the effect of lid closure on anterior chamber oxygen concentration. Phosphorescence lifetime was first measured for several minutes. The eyelids were then closed and secured with tape for 3 to 20 minutes. Phosphorescence in the mid-anterior chamber was remeasured for several minutes immediately after reopening the eyes.

Results

Inverse phosphorescence lifetime increased linearly with oxygen concentration in the solutions that were equilibrated with gas mixtures (Fig. 3). When 10 mg/dl rabbit albumin was included in the solution, these data shifted downward, but when higher concentrations typically found in the rabbit anterior chamber were included, they shifted no farther than they did with 10 mg/dl albumin. The Stern-Volmer coefficients, kₜ = 2.4 × 10⁷(SM) (350/(S mm Hg)) and τ₀ = 847 μsec, derived from measurements of dye in the 50 mg/dl albumin solution at 33°C to 34°C, were used to determine oxygen concentration from phosphorescence lifetime in the anterior chamber.

None of the variations in ionic strength, pH, or amino acids and other components of culture medium systematically affected this correlation. However, phosphorescence lifetime shortened as temperature increased. In solutions equilibrated with 2% oxygen (15 mm Hg) lifetime decreased by 6.7 μs/°C. If temperature in the anterior chamber were unknown, the temperature dependence would have induced an error of approximately 1.4 mm Hg/°C when the cornea was exposed to air.

Phosphorescence intensity of most measurements in the mid-anterior chamber decayed biexponentially after excitation.

### Table 1. Oxygen Properties of Contact Lenses

<table>
<thead>
<tr>
<th>Lens Type</th>
<th>Dk*</th>
<th>Central Thickness (mm)</th>
<th>Dk/L†</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMMA</td>
<td>0.2</td>
<td>0.49</td>
<td>0.041</td>
</tr>
<tr>
<td>Fluorex 700</td>
<td>70</td>
<td>0.47</td>
<td>14.9</td>
</tr>
<tr>
<td>Equalens II</td>
<td>125</td>
<td>0.46</td>
<td>27.2</td>
</tr>
</tbody>
</table>

PMMA, polymethylmethacrylate.

* Dk, oxygen permeability coefficient. Units are ×10⁻¹¹ (cm²/sec) (ml O₂)/(ml mm Hg).
† Dk/L, oxygen transmissibility (Dk divided by lens central thickness). Units are ×10⁻⁹ (cm/sec) (ml O₂)/(ml mm Hg).
The solid lines in Figure 4 represent Eq. 3 fitted to these data. When the eye was opened and the cornea uncovered in this example, \( \tau_1 \) and \( \tau_2 \) were 35 \( \mu \)sec and 111 \( \mu \)sec respectively. When a PMMA contact lens was placed on the cornea, \( \tau_1 \) and \( \tau_2 \) increased during the next 10 minutes to 79 \( \mu \)sec and 320 \( \mu \)sec, respectively. The long lifetime was more sensitive than the short lifetime was to changes in oxygen concentration and was used to calibrate the measurement. The curves illustrated in Figure 4 correspond to partial pressures of oxygen equal to 22 mm Hg and 6 mm Hg, without and with the contact lens, respectively.

Mean oxygen concentration during the 5 minutes before placement of the PMMA lens was 23 \( \pm \) 3 mm Hg (mean \( \pm \) SD; \( n = 6 \) animals). Before placing contact lenses, oxygen tension typically ranged from 20 mm Hg to 29 mm Hg, although in one animal it was only 11 mm Hg (Table 2). It is unclear why oxygen was lower in this animal, although lifetime was measured after the rabbit had been anesthetized for approximately 40 minutes. In other animals the duration of anesthesia did not seem to reduce oxygen concentration.

When the focal diamond was scanned through the anterior chamber, oxygen was clearly distributed nonuniformly between the cornea and crystalline lens (Fig. 6). Mean oxygen concentration was 24 \( \pm \) 5 mm Hg (mean \( \pm \) SD; \( n = 6 \) animals) just inside the cornea and decreased to 17 \( \pm \) 8 mm Hg approximately 2 mm behind the cornea, just in front of the crystalline lens. When a PMMA contact lens was in place for 15 to 20 minutes, oxygen concentration throughout the anterior chamber decreased, and this gradient reversed; mean oxygen concentration just inside the cornea decreased to 3 \( \pm \) 2 mm Hg, whereas concentrations near the lens decreased to only 6 \( \pm \) 2 mm Hg. After placement of the Fluorex 700 and Equalens II, oxygen concentration near the cornea decreased to 5 \( \pm \) 3 mm Hg (\( n = 4 \) animals) and 12 \( \pm \) 11 mm Hg, respectively, but near the crystalline lens, it decreased to approximately 6 \( \pm \) 1 mm Hg and 8 \( \pm \) 4 mm Hg.

When a contact lens was placed on the cornea, oxygen tension in the mid-anterior chamber rapidly decreased by an amount that depended on the oxygen permeability of the lens (Fig. 6). Oxygen decreased the fastest under the PMMA lens and the slowest under the Equalens II. After 20 minutes of PMMA, Fluorex 700, and Equalens II wear, the mean oxygen concentration in the mid-anterior chamber was reduced to 4 mm Hg, 6 mm Hg, and 8 mm Hg, respectively (Table 2). When lenses were removed, oxygen returned to pre-lens placement concentrations within 5 to 10 minutes.

During eyelid closure, oxygen concentration in the mid-anterior chamber diminished, although not as much as it did under the contact lenses. In seven animals, mean oxygen concentration 2 minutes before lids were taped closed was 21 \( \pm \) 2 mm Hg (\( n = 19 \) measurements from seven animals; Fig. 7). On opening the eyelids, oxygen concentration was lower than it
Table 2. Oxygen Tension (mm Hg) Measured in Mid-Anterior Chamber before and 20 Minutes after Contact Lens Placement

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>PMMA Before</th>
<th>PMMA 20–22 min</th>
<th>Fluorex 700 Before</th>
<th>Fluorex 700 20–22 min</th>
<th>Equalens II Before</th>
<th>Equalens II 20–22 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>4.2</td>
<td>14</td>
<td>6.9</td>
<td>11</td>
<td>9.0</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>7.2</td>
<td>24</td>
<td>9.7</td>
<td>25</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>6.9</td>
<td>27</td>
<td>8.5</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>3.2</td>
<td>20</td>
<td>4.1</td>
<td>24</td>
<td>4.6</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>2.5</td>
<td>26</td>
<td>4.2</td>
<td>23</td>
<td>6.0</td>
</tr>
<tr>
<td>6</td>
<td>23</td>
<td>2.2</td>
<td>22</td>
<td>3.8</td>
<td>20</td>
<td>4.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>23 ± 3.2</td>
<td>4.4 ± 2.2</td>
<td>22 ± 4.6</td>
<td>6.2 ± 2.6</td>
<td>21 ± 5.4</td>
<td>7.9 ± 3.6</td>
</tr>
</tbody>
</table>

PMMA, polymethylmethacrylate.
FIGURE 5. Oxygen tension through the depth of the anterior chamber before (●) and during (■) polymethylmethacrylate contact lens wear. Each curve represents a separate scan. Scans were aligned in an anteroposterior direction on the basis of the fluorescence signal during the flash, which was greatest when the focal diamond was in the cornea and least when it was in the anterior chamber. Oxygen tension was highest near the cornea and decreased through the depth of the anterior chamber in the uncovered eye. During contact lens wear, oxygen tension just inside the cornea decreased to less than 4 mm Hg, and the gradient reversed; oxygen tension increased slightly through the depth of the anterior chamber.

and unbound fluorescein, whereas polarization of fluorescence has been used to measure bound fluorescein. The quenching of phosphorescence by molecular oxygen makes this property useful for measuring oxygen near the optic nerve head, in cultured cell systems, in the cardiovascular system, in the cerebral cortex, and in other systems in vitro. This experiment we measured quenching by exciting the dye with a pulsed light source and measuring the transient luminescence for several hundred microseconds after the flash. The technique was sensitive and worked best at low oxygen concentrations, it did not consume oxygen, and it did not disrupt the tissue after the initial injection of the dye. We showed that this technique could be used to measure oxygen in the anterior chamber and to measure changes in oxygen in response to simple manipulations such as contact lens placement and lid closure. The ability to measure phosphorescence quickly and to scan the measurement window through the anterior chamber has the advantage of determining gradients of oxygen in the anterior segment.

The average oxygen concentrations of 20 mm Hg to 29 mm Hg in eyes exposed to room air are consistent with, although slightly lower than, most measurements made by others, which have ranged from 23 mm Hg to 72 mm Hg. Our measurements at various depths in the anterior chamber suggested that oxygen concentration is somewhat higher just inside the cornea than it is just in front of the lens. If the atmosphere is the primary source of oxygen to the aqueous humor and oxygen is used by deep tissues, then oxygen concentration should be higher near the cornea, as we measured. Kwan et al. did not find this difference when they advanced an oxygen-sensitive probe through the anterior chamber, although the aqueous humor may have been stirred in their experiment when the probe penetrated the anterior chamber.

When the PMMA contact lens was placed on the cornea to reduce transfer from the atmosphere, anterior chamber oxygen tension quickly decreased to near zero just behind the endothelium, although it remained somewhat higher near the iris and lens. This reversed gradient suggests that in the absence of an influx from the atmosphere, some oxygen in the aqueous humor can be supplied by the iris or by aqueous from the posterior chamber. It must be remembered that animals in these experiments were anesthetized and made no eye movements. In alert subjects that make frequent eye movements that mix aqueous humor, differences in oxygen concentration...
The oxygen-permeable lenses also reduced cameral oxygen tension, but not as quickly or to as low a point as it reached under the PMMA lens. This response is consistent with the graded reduction of oxygen in the pre-corneal tear film under oxygen-permeable lenses, shown by Harvitt and Bonanno. If the pre-corneal tear film is the source of anterior chamber oxygen, then the changes in anterior chamber oxygenation should follow a pattern similar to changes in the tear film.

Oxygen tension clearly decreased during the first 10 minutes of eyelid closure, but stabilized after longer periods. The final concentration under closed lids, a reduction of 52%, was considerably less than the reduction by 73% measured by Barr and Silver with an electrode during lid closure. It is unclear why oxygen concentration remained higher in this study, although it may have been related to unknown variables such as levels of anesthesia or perfusion of surrounding tissues. The delay between opening the eye and measurement was less than 10 seconds and was short enough that concentrations should not have changed significantly. These measurements suggest that when the lids are closed and in the absence of eye movements, as during non-rapid-eye-movement sleep, oxygenation of the cornea and anterior chamber is maintained at approximately half of its open-eye concentration by blood flow to the conjunctiva and lids, although what happens after several hours of lid closure is unknown. Maurice recently suggested that eye movements during rapid-eye-movement sleep serve in part to mix aqueous humor through the anterior chamber to eliminate oxygen gradients and replenish oxygen consumed by the cornea during long periods of lid closure.

The greatest disadvantage of this technique is the need to inject the dye intravitreally before measuring it in the anterior chamber. We also attempted to introduce dye into the anterior chamber topically and by iontophoresis, but these methods did not yield a sufficient cameral concentration to make meaningful measurements, and dye concentrations were detectable for only a few hours. If the needle used for intravitreal injection is small (27- to 30-gauge) and sharp and enters the eye well away from the anterior segment, trauma and its effects on the anterior chamber should be minimal, particularly after a recovery of several days. The intravitreal depot provides stable concentrations of dye in the anterior chamber for several days to a few weeks.

The temperature dependence of the dye is a potential source of error. Standard solutions should be measured at the temperature of the tissue of interest. We assumed that the aqueous humor was approximately 33°C in these animals, the same temperature that has been measured in aqueous humor of anesthetized cats. We measured corneal surface temperatures between 33°C and 34°C with a noncontact infrared thermometer.

Figure 6. Oxygen tension in mid-anterior chamber during contact lens wear. One of three lenses was placed on the cornea at time 0 and was removed at times indicated by E, P, and F (Equalens, polymethylmethacrylate [PMMA], and Fluorex 700 [FLX], respectively). Oxygen decreased the fastest and to the lowest concentrations under the PMMA lens.
FIGURE 7. Oxygen tension in mid-anterior chamber immediately after lid closure. Each symbol represents one rabbit (seven rabbits total). Solid and dotted horizontal lines represent mean and SD of oxygen tension before closing the lid. Oxygen tension was measured within 10 seconds of reopening eyes that were closed for the time indicated on the abscissa.

temperature probe in two anesthetized rabbits, and the same probe was used to verify temperature of the tube that contained the calibration solutions. If the deep aqueous humor had been warmer than the solutions, we could have overestimated oxygen concentration by approximately 1.4 mm Hg/°C.

Several other water-soluble derivatives of fluorescein and palladium porphines are phosphorescent and sensitive to oxygen and have been used or proposed for use as molecular probes in cultured tissue and in perfused organ systems. Many of these compounds interact strongly with protein, and a small amount of albumin decreases sensitivity by partially shielding the phosphor from oxygen. In some studies, this principle has been used to the investigators' advantage; an abundance of albumin shifts the calibration curve to include physiologic oxygen concentrations and renders calibration insensitive to small changes in albumin concentration. Unfortunately, the introduction of excess albumin into the anterior chamber could induce an inflammatory response that would disturb the blood-aqueous barrier and oxygen kinetics.

Dyes that interact strongly with albumin can become concentrated in the cornea and crystalline lens and would have a different calibration in these tissues than in the anterior chamber. If these dyes were administered to aqueous humor without additional albumin, the correlation between oxygen and phosphorescence lifetime would be sensitive to variations in the low native albumin concentration. Any inflammation or other disruption of the blood-aqueous barrier would introduce an uncertainty in the measurement unless albumin concentration could be determined independently.

The dye used in the present study, Pd-uroporphyrin, is ideal for measuring oxygen in the living eye. It is not sensitive to small variations in the ionic environment of the aqueous humor that occur between subjects or with placement of a contact lens. Similar to most phosphorescent dyes, a small amount of albumin (10 mg/dl) decreases the sensitivity of lifetime to a range of oxygen concentrations that are normally found in the anterior chamber. This dye has a limited interaction with proteins and unlike most other phosphors, the phosphorescence lifetime of Pd-uroporphyrin does not shift when albumin concentrations increase from 10 mg/dl to 50 mg/dl (Fig. 3). This range includes mean aqueous humor protein concentrations that have been determined by others. Oxygen measurement is therefore insensitive to small differences in albumin concentration within an eye or between eyes.

This method provides an opportunity to study oxygen kinetics in the living eye after minimal disturbance. It is unnecessary to penetrate the cornea or sclera during the measurement, and because the luminescence lifetime is measured after exciting the dye with a flash, transparent barriers such as contact lenses that decrease measured intensity do not affect the measurement. This technique will provide a useful method for studying oxygen kinetics in eyes exposed to a variety of atmospheres, eyes under contact lenses, and eyes that have
been treated with medications that may change blood flow and possibly disrupt the normal oxygenation.

References


Effect of Nitroprusside on Arteriolar Constriction after Retinal Branch Vein Occlusion

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PURPOSE. The development of extended areas of nonperfused capillaries after branch vein occlusion (BVO) is correlated to the secondary constriction of the arteriole crossing the occluded area. The decrease in nitric oxide (NO) in tissue that occurs early after BVO accounts for the secondary arteriolar constriction. The present study shows that the administration of an NO donor can reverse the secondary arteriolar vasoconstriction observed after BVO.

METHODS. Simultaneous preretinal NO profiles and arteriolar diameter measurements were performed in miniature pigs after experimental BVO. The effect of preretinal microinjections of the NO donor sodium nitroprusside (SNP) on the arteriolar diameter was studied.

RESULTS. Significant arteriolar vasoconstriction (mean arteriolar diameter, 92.1% ± 3.3% of control; n = 7, P = 7.4 × 10^-5) and a simultaneous decrease in the preretinal NO concentration ([NO]) (preretinal [NO], 20% ± 15.6% of control; n = 5, P = 0.0005) were observed 4 hours after BVO. Microinjection of the NO donor SNP (1 mM applied by puffer) near the constricted retinal arteriole caused a segmental, reversible arteriolar dilation that reached its maximum 20 minutes after the injection (mean arteriolar diameter; 110.8% ± 7.5% of control; n = 6, P = 0.02) and was completely reversed 60 minutes later (n = 6).

CONCLUSIONS. Local administration of NO donors may contribute to the restoration of the retinal arteriolar blood flow after BVO and thus may improve the supply of oxygen and nutrients to the injured tissue. (Invest Ophthalmol Vis Sci. 1998;39:1910–1917)

The development of extended areas of nonperfused capillaries after branch vein occlusion (BVO) in humans is correlated to the secondary constriction of the arteriole that crosses the occluded area. This constriction occurs within hours of BVO and can persist for months. Arteriolar constriction occurs after BVO. The continuous production and release of nitric oxide (NO) by the retinal tissue controls the arteriolar tone in the dilating retina in the miniature pig. Local NO release becomes impaired soon after BVO and accounts for the secondary arteriolar constriction. The present study shows that the administration of an NO donor sodium nitroprusside (SNP) can reverse the secondary arteriolar vasoconstriction that occurs after BVO.

MATERIALS AND METHODS

Animal Preparation

All experiments were conducted in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Miniature pigs (10-12 kg; Arare, Geneva, Switzerland) were prepared for experiments, as described. Briefly, anesthesia was maintained during the experiment by continuous perfusion of 100 mg/h metomidate (Hypnodil, Jansen Pharmaceutica, Beerse, Belgium) and 0.1 mg/h tubocurarine. The animal was ventilated at approximately 18 strokes/min, with a continuous flow of 20% O₂ and 80% NO₂. Blood pressure was monitored in the femoral artery. Paco₂, Pao₂, and pH were measured intermittently.

The animal was prepared for surgery as follows: The head was fixed to a head holder to avoid respiratory movements, the upper and lower eyelids and a rectangular area of the skin around the eye were removed, the bulbar conjunctiva was detached, the sclera was carefully cleaned to 5 mm from the limbus, the superficial scleral vessels were thermocauterized, and an incision at the pars plana was performed to allow introduction of the microprobes and micropipettes. The cornea was protected with diluted sodium hyaluronate and fitted with a fundus contact lens on which a fiberoptic cable was placed to illuminate the fundus. The NO microprobe and puffer micropipettes filled with the chemicals to be tested were introduced into the eye through a scleral hole and then advanced with a micromanipulator, through the vitreous, until positioned close to a retinal arteriole.

Electrochemical NO Microprobe

The NO microprobe consisted of a platinum microelectrode inserted into a borosilicate glass micropipette that was filled with 30 mM NaCl solution (pH 3.5) and had its tip covered by a Nafion membrane. Nitric oxide was oxidized at the surface of the Pt-microelectrode, which was polarized at +0.9 V, and the resultant current was measured (see Ref. 8 for further details). The NO microprobe was inserted into the eye, as described.