In vivo measurements of oxygen tension in the cornea, aqueous humor, and anterior lens of the open eye

Marcus Kwan,* Juha Niinikoski,** and Thomas K. Hunt

Measurements of oxygen profiles across the cornea, aqueous humor, and anterior lens differed from previously calculated values. The aqueous humor oxygen tension in particular also differed from previously measured values, being approximately 20 mm. Hg higher. Reasons for these differences are discussed. The tissue oxygen gradients measured confirm the high Qo, of lenticular and corneal epithelium and corneal endothelium. The steady state Po, in stroma and anterior nucleus reflects the low Qo, of these tissues. The profiles reaffirm the atmospheric source of oxygen to the corneal epithelium and stroma of the open eye and suggest that the corneal endothelium and anterior lens are completely nourished by the aqueous humor oxygen.

Key words: cornea, aqueous humor, lens, oxygen tension, ocular oxygen gradients, needle oxygen electrodes

The oxygen supply to the cornea and lens of the eye is of much practical interest. The avascular nature of the tissues and media through which light passes to strike the retina allows the application of diffusion theory and circumvents the need for models of capillary blood flow.

Oxygen can be supplied to the eye from the atmosphere, the limbic circulation, the palpebral conjunctiva, and the aqueous humor.

It has been customary to assume that the aqueous humor, limbic vessels, and lacrimal fluid (atmosphere) all contribute to the supply of oxygen to the cornea.1 Recently, however, Fatt and Bieber2 have calculated that all of the oxygen needed for corneal metabolism in the open eye comes directly from the atmosphere and that in the closed eye the palpebral conjunctival capillaries contribute as much as two thirds of the necessary oxygen while the aqueous humor supplies the remainder.

In order to confirm the oxygen profiles calculated by Fatt and Bieber, we used ultramicro oxygen electrodes to make direct measurements of corneal, aqueous, and lenticular oxygen tensions.
Materials and methods

**Ultramicro oxygen electrodes.** Two sizes of needle microelectrodes were used in this study, according to techniques described by Silver. The larger microelectrodes were made of 300 μ platinum-iridium wire (70 per cent platinum, 30 per cent iridium) fused to 30 gauge copper wire with silver solder paint. The platinum-iridium tip was then electropolished in an alkaline solution of sodium cyanide to 1 to 3 μ in diameter with an alternating current of 0.8 to 1.6 amp. at 4 to 9 v. The electrode was then coated with a film of soda lime glass under microscopic vision in a heating coil, leaving the tip exposed. The open-tipped platinum in glass microelectrode was then covered with Epoxylite and hardened at 150° C. for 15 minutes. This hydrophobic resin served as a protective membrane which prevented "poisoning" of the electrode.

The smaller ultramicro electrodes were made by fusing 25 μ platinum wire to 30 gauge copper wire, electropolishing in a saturated solution of sodium nitrite to a tip of 0.5 to 1 μ diameter with an alternating current of 0.1 to 0.5 amp. at 1 to 4 v., sealing with glass as above, and coating with Rhoplex instead of Epoxylite.

The anode in both cases was a thin silver wire coated electrolytically with silver chloride and placed in the conjunctival sac.

**Electrode evaluation.** The electrodes were used only if: (1) there was a linear relationship between current output and oxygen tension; (2) there was a very low current at zero Po2, essentially zero with the spanning available on our picoammeter; (3) the change in current per 1 mm. Hg oxygen was 0.5 x 10^-11 or less; (4) the response time was rapid, virtually 100 per cent in less than half a second; (5) the stirring effect in fluid was nonexistent; and (6) the taper tip was microscopically long and smooth with a point 0.5 to 3 μ in diameter.

Aerated normal saline with 30 per cent glycerol at 33° C. was used for calibration. The temperatures of the rabbit's globe vary from 32.3° C. at the epithelial surface of the central cornea to 33.7° C. at the surface anterior lens. The temperature dependence of the electrodes was only 1.6 to 2.0 per cent per 1° C. Therefore, temperature corrections were not made.

**Experimental procedure.** In addition to the electrodes, the experimental design consisted of a stereobinocular microscope, a micromanipulator to hold and advance the electrode through the cornea, a battery box to supply a polarizing current, a Keithley Model 417 pico-ammeter, and a Leeds & Northrup chart recorder (Fig. 1).

Five New Zealand white rabbits weighing 6 kilograms were anesthetized with sodium pentobarbital (20 to 25 mg. per kilogram) and ether or with ethyl carbamate (Urethan 2 to 2.5 Gm. per kilogram). The eyelids were sewn open and a Flieringa ring was sutured to the perilimbic sclera with 5-0 silk. The rabbit's head was placed in a clamp holder and three guy wires about the Flieringa ring were used to stabilize the globe. This method of restraint and fixation prevented almost all physical movement of the eye except for indentation of the corneal surface with electrode advancement (Fig. 2).

Oxygen tension profiles in rabbits breathing room air were obtained with the electrode piercing the cornea centrally and at right angles to the corneal surface. Measurements were recorded as the needle electrodes were advanced in 5 to 50 μ increments with the micromanipulator. Steady-state values were usually reached prior to each advancement. The 300 μ electrode with the 1 to 3 μ tip was advanced until a steep increase in oxygen tension indicated the tension of the aqueous humor. This occurred after the electrode was advanced approximately 0.5 to 1.2 mm. The variation in distance required for transcorneal penetration reflects the depression deformity of
S-S S'lK SUTURES
ATTACH FLIERINGA RING
EXTRA-LIMBALLY LID SUTURED BACK
WIRE STAYS AROUND FLIERINGA RING

Fig. 2. Preparation of rabbit eye for transcorneal measurement. Close-up view of rabbit globe fixation with the Fleringa ring.

the cornea as the needle electrode penetrated the stroma and was a function of electrode sharpness and corneal rigidity. In three experiments the aqueous humor was traversed and the oxygen tension of the anterior lens was measured. Leakage of aqueous fluid did not occur when the electrode was removed, except after the lenticular measurements when an unsharpened portion of the electrode had traversed the cornea.

Electrodes with thicker shafts resulted in midstromal oxygen tensions of 0 to 15 mm. Hg. We thought this low Po2 was probably due to the large electrode shaft pressing against the stroma, disturbing stromal diffusion of oxygen, and allowing the electrode tip to consume most of the available oxygen. This would not occur in the epithelium and anterior stroma, as the very tip of the electrode was polished to a taper of 1 to 3 μ and the cornea was not deformed. In an attempt to obtain accurate midstromal oxygen tensions, the 25 μ microelectrode with its finer shaft was used. Although the more fragile shaft usually broke before reaching the aqueous humor, steady-state midstromal oxygen tensions from 29 to 30 mm. Hg were obtained in three rabbits.

Results

Both the 300 μ and 25 μ shaft electrodes showed the same oxygen gradients as the epithelium and anterior stroma were traversed. In this area, penetration by the 0.5 to 3 μ tips was easily accomplished without corneal deformity, and thus the distances recorded by the micromanipulator were accurate.

Fig. 3 shows the characteristics of an actual recording. The x axis is in time units rather than distance, which is shown by the arrows on the side of the tracing. The fluctuations in the early part of the tracing corresponded to the slight respiratory movements that were transmitted to the globe, resulting in microscopic movements of the cornea relative to the measuring electrode. As the electrode was advanced, however, it became more firmly embedded in the tissue of the cornea and the small respiratory fluctuations were dampened.

The short plateau areas represent steady-state levels at that particular point in the cornea. The midstromal oxygen tensions are not scaled in distances as the deformity of the cornea prevented accurate measurements of the exact distance advanced into the cornea.

As the endothelium was pierced and the aqueous humor was entered, a consistent and large oxygen gradient was measured. As the electrode traversed the anterior chamber, stable oxygen tensions were observed. However, in a few tracings a step increase of approximately 20 per cent of the current in aqueous humor was seen as the corneal deformity was removed without removing the electrode from the aqueous humor.

Fig. 4 is a composite graph of the transcorneal, anterior chamber, and anterior lens oxygen profile. As the electrode touched the corneal epithelium and was advanced, tissue oxygen tensions dropped precipitously from 123 ± 10 mm. Hg to approximately 65 mm. Hg at the epitheliostromal junction. Stromal tensions with both types of electrodes ranged from 29 to 39 mm. Hg when the size of the shaft did not interfere with the measurements. When the endothelium was pierced and the aqueous humor was entered, a relatively large rise in oxygen tension, averaging 40 mm. Hg, was always recorded.

Sixteen different entrances into the aqueous humor resulted in an average oxygen tension of 72 ± 5 mm. Hg in the
aqueous humor. In experiments in which the anterior chamber was traversed in order to obtain lenticular oxygen tensions, the measured aqueous humor oxygen tensions varied only slightly, the range always remaining within 10 mm Hg (equivalent to plus or minus one standard error of the mean).

Upon contact and entry into the lens, a second rapidly decreasing oxygen tension was recorded. The oxygen tension of 72 mm Hg in the aqueous humor decreased rapidly to 28 mm Hg and then slowly to 21 mm Hg in the anterior portion of the lens where it remained stable as the oxygen electrode was advanced 400 μ.

Discussion

Cornea. The oxygen tensions measured within the cornea were different from those calculated by Fatt and Bieber. They calculated oxygen tension drops of 19 mm Hg, 65 mm Hg, and 0.9 mm Hg across the epithelium, stroma, and endothelium, respectively, for a total oxygen drop of 93 mm Hg which was always decreasing as the cornea was traversed from outside in. In the present study, drops in tension of 55 mm Hg and 35 mm Hg were measured across the epithelium and stroma, respectively. However, across Descemet's membrane and the endothelium, we observed an increase in oxygen tension of 42 mm Hg from the corneal stroma to the aqueous humor rather than the slight decrease calculated by Fatt and Bieber. The larger gradients seen across the epithelium and endothelium suggest that the oxygen consumption is greater in these two cellular layers than the Qo2 = 3.8 × 10^-4 ml O2/ (sec.) (ml.) used by Fatt and Bieber in their calculations or that the oxygen transmissibility (Dk) is smaller. The large endothelial change in oxygen tension suggests that endothelial cells in situ may have a higher Qo2 than the epithelial cells or that Descemet's membrane acts as a marked diffusion barrier for oxygen.

Freeman has measured as QO2 of 14 ×
Fig. 4. Composite transcorneal, aqueous humor, and anterior lens oxygen profile. Corneal and aqueous humor total thickness taken as the average for rabbits from Prince.¹

10⁻¹ ml./(sec.) (ml.) for corneal endothelium and a Qo₂ of 2.65 x 10⁻⁴ ml./(sec.) (ml.) for corneal epithelium. The Dk for endothelium in his study was 0.54 x 10⁻¹⁰ (cm.²) (ml. O₂)/(sec.) (ml.) (mm. Hg). Freeman's experimental findings of a higher Qo₂ and a lower Dk from that assumed by Fatt and Bieber in their calculations for corneal endothelium help to explain some of the qualitative and quantitative difference between our experimental results and their calculated profiles.

The endothelial-aqueous humor gradient direction opposite that of Fatt and Bieber is of particular interest, since it supports the idea that endothelial oxygen is largely obtained from the aqueous humor rather than from the atmosphere via transcorneal diffusion.

The stromal oxygen tensions of 29 to 60 mm. Hg are much lower than the 55 to 120 mm. Hg calculated by Fatt and Bieber. The lower O₂ tension as well as the shallower oxygen tension profile across the stroma reflect the changes in the oxygen tension at both epithelial and endothelial boundaries of the stroma rather than a change in the basic equation and numbers used by Fatt and Bieber for stroma.

Aqueous humor. The average aqueous humor oxygen tension of 72 ± 5 mm. Hg in this report seems high when compared with data from Freidenwald and Pierce,² Heald and Langham,³ and Fatt and Bieber which range from 40 to 55 mm. Hg. On the one hand we might reason that their data represent the upper limit of the aqueous Po₂, since any contamination of their samples or of the traumatically entered anterior chamber by room air would have caused an increase in Po₂.

On the other hand, we could reason that if the cells surrounding the aqueous humor had a high rate of oxygen consumption, then any decrease in oxygen supply to eye caused by trauma might lead to a lower measured oxygen tension. In addi-
tion to our experimental data, a large oxygen requirement for cells lining the anterior and posterior chambers might be deduced from the data of Heald and Langham\textsuperscript{7} who found that the P_{O2} of aqueous humor takes up to 45 minutes to respond maximally to an increase in inspired oxygen from air to pure oxygen but takes only 3 to 5 minutes to return to normal levels after changing from pure oxygen back to room air.

In some of our curves the removal of corneal deformity increased the P_{O2} of the aqueous humor and in others it did not. The sporadic nature of this finding leads us to believe that the change is secondary to some inconsistent factor such as the dislodgement of endothelial cells from the electrode tip rather than a pressure effect on the oxygen supply to the aqueous humor or on the electrode itself.

Our finding of a relatively high aqueous P_{O2} needs confirmation by other methods and investigators, especially in view of previous publications.\textsuperscript{2,6,7} However, tissue or fluid P_{O2} higher than the mean A-V oxygen tension is not inconsistent if the tissue or fluid is a medium of oxygen supply. For example, Kwan and Fatt\textsuperscript{8} have recently measured the palpebral conjunctival P_{O2} in rabbits and found it to be 70 ± 13.3 mm. Hg rather than the previously assumed 55 mm. Hg. Teleologically, this high tissue P_{O2} has "evolved" to supply oxygen to the cornea of the closed eye; perhaps something similar had evolved in the vessels of the iris, ciliary body, and ciliary processes.

Friedenwald and Pierce,\textsuperscript{4} using a nitrogen bubble equilibration and sampling technique, found a posterior chamber P_{O2} of 80 to 90 mm. Hg with the lens removed, as compared to 47 mm. Hg in the anterior chamber. Our measurements with a delicate needle electrode prevented any change in direction once the cornea was penetrated and so we could not differentiate between the anterior and the posterior chamber. Perhaps the pressure of the electrode and its slight movement was enough to increase mixing between anterior and posterior spaces, thereby giving us a higher oxygen tension than previously noted for aqueous humor. At any rate, we were unable to demonstrate an oxygen gradient within aqueous humor and have measured a relatively high aqueous P_{O2}.

**Lens.** The rapidly decreasing oxygen tension recorded as the lens capsule and epithelium are penetrated probably reflects the oxygen consumption of the single anterior layer of lenticular epithelial cells. Both Prince\textsuperscript{1} and Davson,\textsuperscript{9} in reporting the results of Haus, Hockwin, and Kleinfeld, mention the high oxygen uptake of the lenticular epithelium and the very low P_{O2} for the cells in the nucleus of the lens. Howard-Flanders and Pirie\textsuperscript{10} have calculated that the oxygen tension below the lens epithelium was probably less than 20 mm. Hg; however, their calculations were based on an aqueous humor oxygen tension of 50 mm. Hg rather than our measured value of 72 mm. Hg. Thus, our measured value of approximately 28 mm. Hg is not inconsistent with their calculated result given a different initial boundary condition.

The authors are grateful to Professors Irving Fatt, Berkeley, California, and I. A. Silver, Bristol, England, for inspiration and constructive criticism.

**REFERENCES**