The Retinal Oxygen Profile in Cats

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This study records for the first time the retinal tissue oxygen partial pressure as a function of location within the retina of the domestic cat. Tissue pO$_2$ was recorded with oxygen sensitive microelectrodes that use the polarographic principle. The mean vitreal pO$_2$ close to the internal limiting membrane was 20.2 ± 2.3 mmHg. The internal limiting membrane does not act as a diffusion barrier for oxygen. As the electrode was advanced into the inner retina, the tissue pO$_2$ rose gradually to a value of 24.6 ± 2.3 mmHg and then fell to a minimum of 12.0 ± 5.5 mmHg before rising again to a value of 29.2 ± 2.5 mmHg. Further insertion resulted in a sudden steep rise of tissue pO$_2$ values to 72.0 ± 5.1 mmHg, after which there was no further alteration in measured values. Although the exact location within the retina of the recording electrode was not known, it is probable that the tissue pO$_2$ minimum occurs at about the level of the inner nuclear layer. Therefore, it is probable that the retinal avascular layers receive their oxygen supply primarily from the choroidal circulation in the cat. Invest Ophthal-mol Vis Sci 24:30–36, 1983

In cats, as in primates, the retina is nourished by both retinal and choroidal circulations. The retinal blood supply forms two capillary beds in the inner retina, one in the nerve fiber layer and the other in the inner nuclear layer. Intermediate between the retinal and choroidal vasculature lies the avascular outer retina that comprises the outer plexiform layer, the photoreceptors, the pigment epithelium, and Bruch's membrane. The extent to which these layers derive their oxygen supply from the choroidal or retinal circulation has been a matter for speculation. Certainly a considerable gradient in the tissue partial pressure of oxygen (p$_T$O$_2$) would be expected in the avascular retinal region with a minimum p$_T$O$_2$ occurring at the diffusion balance point between the two circulations. Apart from one study in the pig eye in which measurements of retinal p$_T$O$_2$ were made every 50 microns through the retina, there have been no published values for the retinal p$_T$O$_2$ profile. A knowledge of the retinal p$_T$O$_2$ profile both in the normal condition and with experimentally induced vascular disorders would provide important information about the relative roles of the two circulations. In addition, the role of alterations of p$_T$O$_2$ in human retinal and choroidal diseases ranging from tissue death to chronic edema to neovascularization is not understood. To date, the retinal p$_T$O$_2$ has been inferred from vitreal pO$_2$ measurements made close to the internal limiting membrane. The validity of the interpretation of these measurements as reflecting retinal p$_T$O$_2$ relies on the assumption that there is no major diffusion barrier to oxygen in the inner retina.

This paper compares vitreal pO$_2$ values and retinal tissue partial pressure of oxygen profiles in the cat eye for the first time (preliminary results have already been presented). The results demonstrate that vitreal pO$_2$ values recorded close to the internal limiting membrane do faithfully reflect inner retinal p$_T$O$_2$ values in the nerve fiber layer but that there does exist a substantial p$_T$O$_2$ gradient within the retina.

Materials and Methods

General Surgery

Adult domestic cats of weight 2.5 to 5.5 kg were used. Anesthesia was induced by an initial intravenous injection of 1–2 ml Saffan (alphaxalone 9–18 mg, alphadalone acetate 3–6 mg). After tracheal cannulation, the sympathetic trunk was severed bilaterally. The cats were ventilated at 33 strokes/min, with 20% O$_2$, 80% N$_2$. After an initial dose of 80 mg Flaxedil (gallamine triethiodide) to achieve paralysis, a constant intravenous infusion of Flaxedil (5 mg/kg/hr) and Saffan (2 ml/hr) produced stable anesthesia and paralysis. Systolic and diastolic blood pressures were monitored continuously via the femoral artery.
using a Hewlett Packard transducer and monitor (78205B) (mean values: 149 ± 7 mmHg systolic, 112 ± 4 mmHg diastolic; errors represent the standard error estimate of the mean). Arterial blood gases and pH were measured at hourly intervals using a Corning blood gas analyzer 161 (mean arterial paO2 = 102 ± 5 mmHg, paCO2 = 35 ± 2 mmHg and pH = 7.403 ± 0.018). Rectal temperature was maintained at 37.5 C, and the ECG and the pupil size of the unoperated eye were monitored continuously. Experiments usually lasted 10 hours and successful results were obtained from five cats. On average, two to three oxygen microelectrodes were used for each successful experiment.

Ocular Surgery

Topical Neosynephrine (phenylephrine HCl 10%) was used to retract the nictitating membrane. A metal eye ring was sutured to the limbus and attached to the electrode micromanipulator system to minimize eye movements. The pupil was dilated with mydriacyl (Tropicamide 1%) eye drops, and the cornea was protected with a gas permeable contact lens of zero power. The temporal sclera was punctured 5-6 mm posterior to the limbus with a hypodermic needle, and the oxygen sensitive microelectrode was inserted through the needle into the puncture hole and tracked across the vitreous under direct ophthalmoscopic control until within a few hundred microns of the retinal surface.

Manufacture of Oxygen Microelectrodes

Oxygen-sensitive microelectrodes that use the polarographic principle were constructed by a modification of the technique described by Silver.17 Twenty-five-micron platinum iridium (Pt/Ir) wire was etched in sodium nitrite to achieve a tip size of about 1 micron. This was connected to 50-micron copper wire that had been threaded through borosilicate capillary glass of inside diameter 280 microns. The glass was then melted down, under gravitational pull using a heating coil so that the Pt/Ir was insulated to the tip. The tip was coated with a Rhoplex® membrane (Rohme Haas) to set up a constant diffusion barrier for O2 that minimizes artifacts due to local variations in the oxygen diffusion coefficient in tissue and also biologic contamination of the Pt/Ir surface. The final electrode length was 11 cm and outside shank diameter was 420 microns.

Electrode Response and Calibration

The polarogram (current/voltage characteristic) was measured for each electrode placed in air-equilibrated buffered saline with a silver/silver chloride gross reference electrode. This ensured that the polarogram contained a plateau region so that a suitable operating bias voltage could be chosen (usually −0.7 to −0.8 volts). The oxygen generated current was measured with a Keithley picoammeter and was monitored continuously on a chart recorder. In Figure 1, three polarograms are plotted for a typical microelectrode. The continuous line is for the bare electrode. The reduction in current caused by two successive coatings of the platinum tip with Rhoplex is shown by the short and long dashed lines respectively. The bias voltage independent plateau is satisfactory so that biologically induced voltage variation commonly encountered in the eye of up to 10 mV would produce less than 1% change in inferred pO2 values. The electrodes were then calibrated in buffered saline solutions that were equilibrated to different partial pressures of oxygen. The pO2 values of these solutions were checked with a Corning blood gas analyzer 161. Electrodes had a response time of less than 1 second, a temperature coefficient of no more than 5%/centigrade degree, and a negligible stirring effect. Only pO2 results obtained in which the before and after calibration curves agreed within 10% are presented. Electrodes were linear in their pO2 response from 0 mmHg to 400 mmHg.

Experimental Regime

After placement of the oxygen electrode into the vitreous (reference in the connective tissue of the
Fig. 2. The vitreal pO\textsubscript{2} in mmHg as a function of electrode position as the electrode is advanced from the entry point into the eye to the internal limiting membrane of the retina.


t head), its integrity was checked both by the magnitude of the initial vitreal pO\textsubscript{2} values, and also by the electrode’s response to changes in pao\textsubscript{2}. Only electrodes giving initial vitreal values of pO\textsubscript{2} of between 15 mmHg and 25 mmHg were assumed to be functioning correctly,\textsuperscript{14} and were therefore used for the experiments. Electrodes that measured vitreal pO\textsubscript{2} values greatly in excess of 25 mmHg were usually found to have a damaged tip or cracked glass insulation, whereas electrodes measuring very low values of vitreal pO\textsubscript{2} had often acquired an adherent tissue layer from scleral or ciliary body contamination to the Rhoplex membrane so that the effective oxygen diffusion barrier to the Pt/Ir surface was increased. Satisfactory electrodes were tested for oxygen sensitivity by ventilating the animal on 100% O\textsubscript{2} for 5 min. After the vitreal pO\textsubscript{2} had returned to its original value, the microelectrode was advanced across the vitreous, and the vitreal pO\textsubscript{2} was measured every 1 mm. When the electrode was judged by direct ophthalmoscopy to be within a few hundred microns of the internal limiting membrane, the electrode was advanced slowly into the retina using the fine control of the micromanipulator drive. Entry into the retina was usually signalled by an increase in the noise level of the current recording, a major component of which was due to the occurrence of small oscillations that were in synchrony with the forced ventilation of the cat. At present, the cause of these oscillations is unknown. In preliminary experiments with electrodes having larger tip diameters, this increased noise level occurred simultaneously with the visually observed retinal dimpling as the electrode first pushed the retina rather than penetrated. Dimpling and hence tissue damage was minimized in later experiments by constructing electrodes with small tip diameters and sharper profiles.

The electrode was advanced obliquely through the retina and readings of p\textsubscript{T}O\textsubscript{2} were made every 10 microns. A 1-micron tip electrode can be assumed to be measuring the average p\textsubscript{T}O\textsubscript{2} of a spherical tissue volume of radius 5 microns to 10 microns,\textsuperscript{18,19} so that there is little advantage to be gained by improving the spatial resolution of the readings. The electrode was advanced until p\textsubscript{T}O\textsubscript{2} values similar to those of arterial blood were measured. Here it was assumed that the electrode had entered the choroid. Frequently the electrode tip would break prior to reaching this point at a location we inferred to be Bruch’s membrane. After successful penetration to the choroid the electrode was withdrawn, and the p\textsubscript{T}O\textsubscript{2} readings were repeated during withdrawal to check for electrode induced tissue damage. The retinal entry point was located within an area bounded by the area centralis on the temporal side, a superior inferior line at 20° nasal, and nasotemporal lines at 20° superior and 10° inferior to the area centralis.

Results

Vitreal Profile

In Figure 2, a typical vitreal pO\textsubscript{2} profile is plotted as a function of location from the point of entry of the electrode into the vitreous where it lies close to the ciliary body, to the point at which it enters the retina. The curve shows a minimum where the electrode passes closest to the lens. Such curves gave values of between 0.4 mmHg/mm and 7 mmHg/mm as the oblique, pO\textsubscript{2} gradient in vitreous, with the gradient becoming larger as the electrode approaches the internal limiting membrane. These oblique gradients were not converted to gradients perpendicular to the retina as a sufficiently comprehensive data set of vitreal pO\textsubscript{2} values was never measured in any one eye.

Retinal Profile

In Figure 3, the retinal p\textsubscript{T}O\textsubscript{2} is plotted as a function of oblique distance into the retina measured in microns. At location −40 microns the electrode is in the vitreous, and it can be seen that there is no step change in the measured p\textsubscript{T}O\textsubscript{2} as the electrode enters the retina at 0 microns. However, the p\textsubscript{T}O\textsubscript{2} in the retina rises to 27 mmHg as the electrode moves to a position 40 microns within the retina. Further advancement is followed by a p\textsubscript{T}O\textsubscript{2} fall to a minimum of 9 mmHg at 60 microns, and then after a gradual
rise to 30 mmHg at a penetration distance of 140 microns, the pO₂ value rises sharply to 72 mmHg at 180 microns where the electrode is presumed to have entered the choriocapillaris.

This profile was similar in all successful recordings. In three cases, the tissue profile could not be replicated during withdrawal of the electrode. In such cases, there was little variation in pO₂ as a function of retinal location during withdrawal, and it must be assumed that the electrode had caused tissue damage. Indeed, in some instances, the withdrawal was followed by a small amount of bleeding from the entry point. Qualitatively similar retinal profiles were recorded from the retinas of another eight cats in which the electrode calibration was unknown, therefore, these results have not been presented.

In two serendipitous cases, the retinal profile was maintained on withdrawal of the microelectrode from the choroid. Such a situation is shown in Figure 4 where the full and dashed lines are for electrode insertion and withdrawal respectively. The withdrawal profile is displaced 20 microns from the insertion one and this is presumed to be due to a DC movement of the retina rather than to backlash in the micro-
A summary of the retinal $p_TO_2$ profile results from the five successful animals is shown in Figure 5 in which the average vitreal $pO_2$ just prior to retinal entry ($20.2 \pm 2.3$ mmHg), the inner retinal $p_TO_2$ maximum ($24.6 \pm 2.3$ mmHg), the retinal $p_TO_2$ minimum, ($12.0 \pm 5.5$ mmHg), the $p_TO_2$ of the inner side of Bruch's membrane $29.2 \pm 2.5$ mmHg, and the choroidal $pO_2$ values ($72 \pm 1$ mmHg) are displayed. Exact retinal location is not stated as the position at which these values occurred, depended on the entry point of the electrode into the retina and the obliquity of its path through the retina.

**Discussion**

Vitreal $pO_2$ values have been reported by previous workers for both conditions of oxygen equilibrium and for conditions of oxygen diffusion transients caused by hypoxia, ischemia, and altered intraocular pressure. The results presented here are in accord with these measurements and serve to validate the present measuring techniques. The observed reduction of vitreal $pO_2$ with distance from the retina in steady state conditions implies that the posterior lens is a consumer of vitreal oxygen.

The absence of a discrete change in measured $pO_2$ values as the oxygen-sensitive microelectrode is moved from the vitreous into the inner retina implies that the inner retina offers no diffusion barrier to the movement of oxygen. The consequence of this is that the vitreal $pO_2$ values will mimic those of the inner retina fairly closely. The absolute relationship between $p_TO_2$ values in the inner retina and some location in the vitreous will depend on the magnitude of the oxygen diffusion coefficient, the ocular geometry, the distance of the vitreal location with respect to the retina, and the magnitude of the oxygen consumption by the posterior lens and vitreous. Previously, vitreal $pO_2$ values, usually measured within a few hundred microns of the internal limiting membrane, have been used to estimate retinal tissue $pO_2$ qualitatively. A more recent attempt to relate vitreal and retinal $p_TO_2$ values quantitatively has been made by modelling the diffusion of oxygen in a simplified version of the cat eye.\(^1\)

The advantages of inferring retinal $p_TO_2$ values from vitreal values are that all tissue damage is avoided. However, the ability to measure retinal $p_TO_2$ directly avoids making assumptions about the constancy of diffusion coefficients during altering states of tissue oxygenation and, of course, extends the measurement of retinal $p_TO_2$ to include the whole retina, not just that section close to the internal limiting membrane. There is no doubt that for this series of experiments in a large number of cases the microelectrode caused tissue damage, but by careful selection of microelectrodes to accord with a suitable tip geometry, such tissue damage could be minimized.

From the data presented here on the retinal $p_TO_2$ profile, it is clear that there exists a substantial tissue oxygen gradient within the cat retina. Mathematical analysis has been performed on the expected tissue oxygen gradient within the retina where it has been assumed that there are two sources of oxygen: the choroidal and retinal supplies, as discrete boundary layers on either side of the avascular retinal layer. In this analysis the simplifying assumption was made that the solubility coefficient $\alpha$, the oxygen diffusion coefficient $D$, and tissue oxygen consumption $Q$, are all maintained constant across the retina. The resulting predicted retinal $p_TO_2$ profile consists of two paraboli meeting at the balance point between the two circulations where $p_TO_2$ is a minimum.

From the form of the measured $p_TO_2$ profile (Figs. 3, 4), it is clear that this homogeneous assumption is not valid in the cat retina and that one or more of $\alpha$, $D$, and $Q$ must vary as a function of retinal location. Moreover, although the choroidal supply can be considered to be a discrete boundary source of oxygen in the outer retina, the retinal supply has two capillary beds: the outer one being more venous in nature than the inner retinal capillary bed. This means that the retinal blood supply, considered as an oxygen source is distributed throughout a considerable retinal region, and therefore cannot be considiered...
erred as a discrete source for mathematical analysis. In the absence of measurements of retinal $\alpha$, $D$, and $Q$, then a detailed consideration of the reasons for the exact form of the retinal $p_{T}O_2$ profile is not warranted. However, there are two important conclusions to be drawn from the measured results.

The steep rise in $p_{T}O_2$ values in the outer retina from $29.2 \pm 2.5$ mmHg to $72.0 \pm 5.1$ mmHg, which is close to arterial $pO_2$ values, is assumed to occur across Bruch's membrane. This is certainly a high electrical resistance membrane, and it would appear that it has a lower oxygen diffusion coefficient than the surrounding retinal tissue. A similar diffusion barrier to oxygen has also been shown to exist in the pig eye.\(^5\) It is possible that the clinical procedure of photocoagulation causes an alteration in this diffusion barrier with as yet unknown consequences for retinal oxygenation.

The existence of a $p_{T}O_2$ minimum value in the cat retina demonstrates that there is indeed a balance point between the retinal and choroidal circulations. The low $p_{T}O_2$ value of $12 \pm 5.5$ mmHg at the minimum position would place this location most at risk for any impairment to either retinal or choroidal circulation. The exact location of this minimum $p_{T}O_2$ point is as yet unknown and awaits the perfection of techniques that enable the recording location to be specified more precisely. In future work it is proposed to combine both electrophysiologic and histologic techniques to estimate optimal recording locations. The oxygen-sensitive microelectrode can be used to record both the induced oxygen currents and the local electroretinogram,\(^20,21\) the changing form of which allows retinal location to be partially specified.\(^22\) The variation in thickness of the cat retina and its component layers as a function of retinal location has not been well documented. Detwiler\(^23\) states that the retinal thickness in the cat varies from 259 microns at its thickest to 182 microns in the midperiphery. The problem of locating electrode recording location is confounded by the oblique entry passage of the electrode through the retina. In our recordings, the position of the retinal $p_{T}O_2$ minimum occurred 60 to 80 microns from the internal limiting membrane. For an electrode entering the retina normally this would place the minimum position just at the outer surface of the inner nuclear layer.\(^1\)

Any error introduced by oblique entry would bring this position into the inner nuclear layer. Thus, these preliminary results point to the balance point between the retinal and choroidal circulations being close to the inner nuclear layer. If this is so, then the avascular layers of the retina must receive all their oxygen by diffusion from the choroidal circulation, and the retinal circulation must only supply those tissues that it passes through together with the posterior lens and vitreous. The presence of the $p_{T}O_2$ minimum in this location implies that neither circulation is capable of adequately compensating for an impairment to the other, without the use of artificial means such as a hyperoxic breathing mixture or an increased oxygen diffusion through Bruch's membrane.

The further development of this recording technique for use in retinal tissue will allow considerable advances to be made in our understanding of vascular-based retinal pathology.

**Key words:** oxygen, microelectrodes, retina, cat, polarography, vitreous.

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**References**


