Oxygen Distribution and Consumption in the Cat Retina during Normoxia and Hypoxemia

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ABSTRACT Oxygen tension (PO₂) was measured with microelectrodes within the retina of anesthetized cats during normoxia and hypoxemia (i.e., systemic hypoxia), and photoreceptor oxygen consumption was determined by fitting PO₂ measurements to a model of steady-state oxygen diffusion and consumption. Choroidal PO₂ fell linearly during hypoxemia, about 0.64 mmHg/mmHg decrease in arterial PO₂ (PₐO₂). The choroidal circulation provided ~91% of the photoreceptors' oxygen supply under dark-adapted conditions during both normoxia and hypoxemia. In light adaptation the choroid supplied all of the oxygen during normoxia, but at PₐO₂'s < 60 mmHg the retinal circulation supplied ~10% of the oxygen. In the dark-adapted retina the decrease in choroidal PO₂ caused a large decrease in photoreceptor oxygen consumption, from ~ 5.1 ml O₂/100 g/min during normoxia to 2.6 ml O₂/100 g/min at a PₐO₂ of 50 mmHg. When the retina was adapted to a rod saturating background, normoxic oxygen consumption was ~33% of the dark-adapted value, and hypoxemia caused almost no change in oxygen consumption. This difference in metabolic effects of hypoxemia in light and dark explains why the standing potential of the eye and retinal extracellular potassium concentration were previously found to be more affected by hypoxemia in darkness. Frequency histograms of intraretinal PO₂ were used to characterize the oxygenation of the vascularized inner half of the retina, where the oxygen distribution is heterogeneous and simple diffusion models cannot be used. Inner retinal PO₂ during normoxia was relatively low: 18 ± 12 mmHg (mean and SD; n = 8,328 values from 36 profiles) in dark adaptation, and significantly lower, 13 ± 6 mmHg (n = 4,349 values from 19 profiles) in light adaptation. Even in the dark-adapted retina, 30% of the values were <10 mmHg. The mean PO₂ in the inner (i.e., proximal) half of the retina was well regulated during hypoxemia. In dark adaptation it was significantly reduced only at PₐO₂'s < 45 mmHg, and it was reduced less at these PₐO₂'s in light adaptation.

INTRODUCTION

In cats, hypoxemia (i.e., systemic hypoxia) leads to changes in the standing potential of the eye and in slow components of the electroretinogram (ERG) when arterial...
oxygen tension ($P_{O_2}$) is as high as 60–70 mmHg (Niemeyer et al., 1982; Linsenmeier et al., 1983). Those components that have been measured in humans appear to be similarly sensitive to hypoxemia (e.g., Marmor et al., 1985; Linsenmeier et al., 1987). The components of the ERG that are altered in mild hypoxemia all originate as changes in membrane potential of retinal pigment epithelial (RPE) cells (Linsenmeier and Steinberg, 1986), but RPE cells, which are adjacent to a rich blood supply, are probably not themselves the site of the sensitivity to hypoxemia. Instead, hypoxemia apparently acts on the photoreceptors, altering the ionic environment around both the photoreceptors and the RPE cells, and causing changes in RPE membrane responses (Linsenmeier and Steinberg, 1984, 1986). Measurements of the oxygen distribution in the normoxic cat retina (Linsenmeier, 1986) suggested that the photoreceptors might be very sensitive to hypoxemia, because the $P_{O_2}$ around the metabolically active part of the photoreceptors was normally nearly zero under dark-adapted conditions, leaving no reserve of oxygen that could be used during hypoxemia. The major purposes of this work were to measure the extent of changes in oxygen availability to the photoreceptors during hypoxemia and quantify the consequent changes in photoreceptor oxygen consumption.

The experiments described here are closely related to recent work in which oxygen distribution and consumption have been studied at increased intraocular pressure (Yancey and Linsenmeier, 1989). This study allows a comparison of hypoxemia with pressure elevation, one of which causes tissue hypoxia by a reduction in $P_{O_2}$, and one of which reduces retinal oxygenation by a reduction in blood flow. The retinal effect that is common to these two situations is a reduction in $P_{O_2}$ in the choroidal circulation behind the retina, but they may be different in other respects. Thus, it is useful to compare the two situations. The work presented here also goes beyond the work on elevated pressure in two ways. First, measurements have been made here in the light-adapted as well as in the dark-adapted retina. This is because hypoxemia is known to have smaller electrophysiological effects in light adaptation (Linsenmeier and Steinberg, 1984, 1986). We have predicted that this is due to a greater oxygen reserve during light adaptation, when photoreceptor metabolism is reduced (Linsenmeier, 1986; Haugh et al., 1990), but experimental evidence of this has not previously been available. Second, an attempt was made here to quantify the status of oxygen supply in the vascularized inner half of the retina, a tissue more similar to other parts of the central nervous system than the outer retina, during normoxia and hypoxemia.

**METHODS**

**Animal Preparation and Recording**

The methods used in this work were identical to those described previously (Linsenmeier, 1986; Yancey and Linsenmeier, 1989). Briefly, adult cats were anesthetized with sodium thiamylal during surgery and urethane (200 mg/kg loading dose; 100–200 mg/h maintenance) during recordings. Animals were paralyzed with pancuronium bromide (0.2–0.3 mg/kg/h) and artificially ventilated during recordings to prevent eye movements and to eliminate respiratory compensation during changes of inspired $P_{O_2}$. Stability of heart rate and femoral arterial pressure were monitored continuously as an indication of the level of anesthesia. It should be
noted that in similarly prepared cats, without muscle relaxation, lower doses of urethane have sufficed for light anesthesia (Enroth-Cugell and Pinto, 1970).

Double-barreled microelectrodes were used, in which one barrel contained a recessed oxygen cathode and the other contained saline for voltage recording (e.g., Linsenmeier and Yancey, 1987). Electrodes were polarized at -0.7 V and were calibrated in vitro at 37°C in a saline-filled chamber through which gases containing 4, 8, and 21% O₂ were bubbled alternately. Current was recorded with a picoammeter (model 614; Keithley Instruments, Inc., Cleveland, OH) and stored on FM tape for later analysis. Electrodes were introduced into the intact right eye through a 15-gauge hypodermic needle. The electrode was sealed in with a boot that prevented leakage of vitreous humor but allowed advancement of the electrode with a hydraulic microdrive. All recordings were made in or near the area centralis.

Pao₂, Paco₂, and pHₐ were measured with a blood gas analyzer (model 158; Corning Medical and Scientific, Medfield, MA). During normoxia, the animal breathed air or air supplemented with enough oxygen to keep Pao₂ generally above 90 mmHg. Under these conditions the arterial values were: Pao₂, 95.4 ± 14.4 mmHg (mean ± SD); Paco₂, 29.0 ± 3.3 mmHg; pHₐ, 7.404 ± 0.037 (n = 48 measurements in 14 cats). During hypoxemia, arterial CO₂ and pH were little affected. At Pao₂'s < 50 mmHg the values were: Pao₂, 37.4 ± 9.6 mmHg; Paco₂, 29.1 ± 4.1 mmHg; pHₐ, 7.412 ± 0.055 (n = 19). A side effect of urethane anesthesia is hyperglycemia (Reinert, 1964). Arterial glucose, measured with a Glucometer II (Ames Division, Miles Laboratories Inc., Elkhart, IN), was 235 ± 52 mg/dl (n = 22 samples in 12 cats).

Experiments were done on animals that were initially dark-adapted. Flashes of diffuse white light were presented as the electrode penetrated the retina in order to elicit ERGs that assisted in monitoring the depth of recording. Once the electrode had penetrated the RPE it was withdrawn in steps of 1 μm at 1–2 μm/s all the way to the vitreous humor, generating a profile of Po₂ as a function of depth. Most of the data presented (all of the oxygen consumption data) are from withdrawals of this type. The vitreal surface during withdrawal was taken to be the point at which the b wave of the ERG changed polarity from negative to positive. The electrode was always moved slightly, to a new retinal location, between profiles.

Penetrations were always done in dark adaptation, but in some cases a diffuse white light was turned on ~ 60 s before the electrode withdrawal, so that the withdrawal could be done during light adaptation. This light had an illumination sufficient to saturate the rod photoreceptors (8.6 log [equivalent quanta (555 nm)/deg²·s]) and reduced the photoreceptor oxygen consumption maximally (Linsenmeier, 1986). Light- and dark-adapted series were, in general, performed on different cats. Normoxic dark-adapted profiles were recorded in all animals used for light-adapted series, however, to check that the profiles were similar to those in the animals used for dark-adapted series. These profiles were excluded from the analyses so that the data were not unduly weighted by normoxic profiles in cats where no hypoxemic data were recorded.

Normoxic profiles were recorded first in a typical experiment. The animal was then made hypoxic by replacing some of the inspired air with nitrogen and more profiles were recorded. In some cases the animal was subjected to another degree of hypoxemia before returning to normoxia, and in other cases normoxia was interposed between measurements at each different hypoxemic level. Durations of hypoxemia were generally less than an hour. Choroidal Po₂ changed rapidly at the beginning of hypoxemia, remained stable during hypoxemia, and recovered rapidly after hypoxemia.

Determination of Oxygen Consumption

The oxygen profile in the outer, avascular half of the retina was fitted to a steady-state model of oxygen diffusion (Haugh et al., 1990). This model assumes that oxygen diffuses only in the radial direction (x), and that all oxygen consumption is confined to a single layer of the outer...
retina whose boundaries, $L_1$ and $L_2$, and oxygen consumption, $Q_o$ (in milliliters O$_2$/100 g tissue-min), are determined by fitting oxygen tension data to the model. This layer typically occupies $\sim$10% of the retinal thickness and corresponds to the location of the mitochondria in the photoreceptor inner segments, within the limits of distortion due to the measurement technique (Haugh et al., 1990). The layers on either side of the consuming layer are assumed to have insignificant oxygen consumption compared with the highly consuming layer. These considerations lead to the model equations:

\[
\begin{align*}
\frac{d^2P}{dx^2} &= 0 & 0 < x < L_1 \\
\frac{d^2P}{dx^2} &= \frac{Q_o}{D_k} & L_1 < x < L_2 \\
\frac{d^2P}{dx^2} &= 0 & L_2 < x < L \\
\end{align*}
\]

which are solved subject to the boundary conditions that both $P$ (i.e., $P_0$) and oxygen flux ($D_k \frac{dP}{dx}$) must be equal at the boundary between two adjacent layers. Oxygen diffusion coefficient ($D$) and oxygen solubility ($k$) were assumed to be the same in all layers. The five parameters of the model were then the $P_0$'s at the choroid ($P(0) = P_c$) and at the assumed inner edge of the avascular layer ($P(L) = P_i$), which was taken to be halfway through the retina, the boundaries of the consuming region near the choroid ($L_0$), and near the inner retina ($L_0$), and the oxygen consumption of the consuming layer divided by its oxygen diffusion coefficient and oxygen solubility ($Q_o/D_k$). $P_c$ and $P_i$ were strongly constrained by the data, and only the other three parameters were truly unknown. The fitted and measured values of choroidal $P_0$ were very similar (slope of the relation between fitted and measured = 1.03; $r = 0.993$), but for consistency the $P_i$ is used only to refer to the fitted parameter and the term "choroidal $P_0$" is used otherwise.

Oxygen profiles were measured at an angle of $\sim$45° to the retinal surface, so apparent values of $x$ were multiplied by $\sin 45°$ to give actual radial position. It should be noted that $x = 0$ in the model is at the RPE, while the usual conventions of designating the vitreal border of the retina as 0% retinal depth and the RPE as 100% retinal depth are adopted in discussing the results.

The fitting procedure was slightly different than in previous work (Haugh et al., 1990), since it proved possible to reduce the equations to be solved to only two nonlinear equations in $L_0$ and $L_2$. This allowed us to generate an error surface that could be easily plotted, allowing us to avoid local minima, and giving confidence in the uniqueness of the fits. The fitting minimized the RMS error between the model and the data, where $\text{RMS error} = \left\{ \left( \frac{1}{n} \sum (Y_i - \bar{y}_i)^2 \right)^{1/2} \right\} / n$, and $n$ is the number of data values in a profile, $Y_i$ is a data value (i.e., $P_0$), and $\bar{y}_i$ is the ordinate value of the curve at the same abscissa value.

The figures show values of $Q_o$ and $Q_o/v$ (where $Q_o/v = Q_o (L_2 - L_1)/L$ as discussed in the Results), which are more useful than the parameter derived directly from the modeling, $Q_o/D_k$. To arrive at $Q_o/v$, a value has been assumed for $D$, based on recent measurements in cat retina that indicate a relatively homogeneous value of $\sim$71% of $D$ in saline at 37°C, or $1.97 \times 10^{-5}$ cm$^2$/s (Roh et al., 1990). Oxygen solubility has not been measured for retina, and was assumed to be $2.4 \times 10^{-5}$ ml O$_2$/ml tissue-mmHg. This is the value for whole blood at 37°C (e.g., Goldstick, 1973), and is approximately an average of the values measured for brain by Thews (1960), Ganfield et al. (1970), and Clark et al. (1978). A correction was also applied to $Q_o$ (and $Q_o/v$) to account for the withdrawal distances typically being longer than penetration distances, due to electrode drag. $Q_o$ values were multiplied by $(2L/D_p)^2$, where $D_p$ is the average retinal thickness measured during all penetrations preceding dark-adapted withdrawals ($207 \pm 44$ μm; $n = 64$ profiles) or all penetrations preceding light-adapted withdrawals ($272 \pm 44$ μm; $n = 46$ profiles). The rationale for this correction arises from the solution to the diffusion equations, and is discussed by Yancey and Linsenmeier (1989).
RESULTS

Oxygen Profiles and Oxygen Tension in the Outer Retina

Fig. 1 shows oxygen profiles recorded from one dark-adapted retina during normoxia and at two levels of hypoxemia, illustrating the type of data on which quantitative comparisons are based. The photoreceptors occupy the avascular region, from ~50 to 100% depth from the vitreous. They clearly receive most of their oxygen from the choroid, which is at 100% depth. In all cats the PO$_2$ decreased to very nearly zero even during normoxia as reported previously (Linsenmeier, 1986; Yancey and Linsenmeier, 1989). In dark-adapted profiles the minimum PO$_2$ occurred in the oxygen-consuming layer, which lay between 65 and 80% retinal depth in Fig. 1 as defined by the fits to the diffusion model. In the top two profiles the PO$_2$ remained very low from ~50 to 65% retinal depth, indicating that there was very little diffusion from the retinal circulation to the photoreceptor inner segments, but in the bottom profile and in profiles recorded in other cats there was a more pronounced supply from the retinal side, so that the region having an extremely low PO$_2$ was smaller. During hypoxemia there was a decrease in choroidal PO$_2$. There also tended to be a decrease in the slope of the gradient between the choroid and the point of minimum PO$_2$ that is consistent with the decrease in oxygen consumption discussed below, but
this gradient itself cannot be taken as a measure of consumption, since the other parameters that determine consumption vary between profiles.

In the proximal retina, from 0 to 50% retinal depth, peaks in $P_{O_2}$ were usually observed, presumably reflecting the presence of the capillaries and other small vessels of the retinal circulation. The $P_{O_2}$ distribution in the vascularized part of the retina is discussed separately below.

Examples of oxygen profiles recorded in the light-adapted retina are shown in Fig. 2. Since photoreceptor oxygen consumption is reduced by steady illumination (e.g., Zuckerman and Weiter, 1980; Linsenmeier, 1986), the $P_{O_2}$ decreased more gradually from the choroid to the photoreceptor nuclei. In normoxia all of the oxygen required by the photoreceptors was supplied by the choroid. The oxygen consuming region is particularly evident as the region near the bend in the normoxic profile at the top (74-81% retinal depth according to the fit to this profile). During hypoxemia choroidal $P_{O_2}$ decreased, just as in dark adaptation, but $P_{O_2}$ remained above zero throughout the outer retina until a relatively severe level of hypoxemia was reached, at a $P_{O_2}$ of 44 mmHg in the example shown. During hypoxemia, oxygen was supplied to the photoreceptors by the retinal as well as choroidal circulations.

The relationship between $P_{O_2}$, the fitted value of choroidal $P_{O_2}$, and $P_{aO_2}$ for 60 dark-adapted and 47 light-adapted profiles is shown in Fig. 3. Each animal is represented by a separate symbol. The dashed line represents the situation where choroidal $P_{O_2}$ equals $P_{aO_2}$ and the solid line is a linear regression of all the data.
Retinal PO₂ during Hypoxemia

Line SF and E. Retinal PO₂ during.

- 120
- 100
- 80
- 60
- 40
- 20
- 0

Arterial PO₂ (mmHg)

Choroidal PO₂ (mmHg)

Figure 3. Choroidal PO₂ as a function of arterial PO₂ during normoxia and hypoxemia. The dashed line is the identity line and the solid line is a linear regression of all the data: \( P_c = 0.64 P_{aO_2} + 1.66 \) (\( n = 107 \)). Each cat is represented here and in subsequent figures by a separate symbol. The open symbols (O, □, Δ, V, and ○) are data obtained in light-adapted retinas, and the others are from dark-adapted retinas.

\( r = 0.80 \). Neither the slopes nor intercepts of separate regression lines for dark- and light-adapted data were significantly different. For \( P_{aO_2} > 85 \) mmHg the average value of \( P_c \) was 62.2 ± 14.8 mmHg, and 62 is used later as the standard normoxic value of \( P_c \). As shown already by the examples in Figs. 1 and 2, choroidal PO₂ decreased with \( P_{aO_2} \), showing no obvious region of regulation. Choroidal PO₂ approached \( P_{aO_2} \) more closely as \( P_{aO_2} \) decreased. This is expected from the hemoglobin saturation curve, since equal arteriovenous saturation differences yield smaller arteriovenous PO₂ differences at lower values of \( P_{aO_2} \).

The profiles in Figs. 1 and 2 lead one to expect that the light-evoked increase in PO₂ at any point in the outer half of the retina would be smaller in hypoxemia. The profiles provide only an indirect test of this, however, since dark- and light-adapted PO₂'s were measured at different times and in different animals. In five animals measurements of light-evoked changes in PO₂ were made before and during hypoxemia while an electrode was positioned in the outer retina. Fig. 4 illustrates the result observed in all cases. In this example the electrode was positioned at 84% retinal depth, where the PO₂ in normoxia was nearly zero. During normoxia an increase of

Figure 4. Light-evoked changes in outer retinal PO₂ during normoxia (\( P_{aO_2} = 92 \) mmHg) and hypoxemia (\( P_{aO_2} = 49 \) mmHg). The top traces show PO₂ recorded with an electrode at 84% retinal depth. The initial transient in these records is electrical pickup of the ERG. The middle traces are simultaneously recorded ERGs, showing the b-wave, c-wave, and fast oscillation trough (FOT). The lower trace shows the timing of a 30-s period of illumination at 7.6 log (equivalent quanta [555 nm]/deg²/s) presented to a dark-adapted retina.
20 mmHg was observed when a light with an illumination about one log unit below
rod saturation was turned on for 30 s (top left). 4 min after the onset of hypoxemia
(PcO2 = 49 mmHg) the same illumination elicited almost no increase in PO2 (top
right). The light-evoked increase in PO2 returned to the initial amplitude shortly after
the end of the hypoxicemic episode (not shown). The middle traces are ERGs recorded
simultaneously in the vitreous humor, and show the lack of change in b-wave
amplitude, increase in c-wave amplitude, and deepening of the fast oscillation trough
expected during hypoxemia (Linsenmeier and Steinberg, 1986).

Photoreceptor Oxygen Consumption
As described in Methods, the outer 50% of each profile was digitized and fitted to a
model of oxygen diffusion to determine the oxygen consumption of the photorecep-
tors and the size of the oxygen consuming layer. The model fitted all the data well,
but actually fitted the hypoxemic data slightly better in both light and dark
adaptation. This is important, since it allowed the use of the model originally
developed for the normoxic case with no modifications. The relation between RMS
error (mmHg) and Pc was RMS error = 0.013 (Pc) + 0.53 (n = 112 profiles). The
slope was significantly different from zero (P << 0.001).

Dark adaptation. Values of oxygen consumption (Q2) derived from 64 profiles in
the dark-adapted retina are shown as a function of choroidal PO2 in Fig. 5 a. While
there is a great deal of scatter in the data, Q2 decreased during hypoxemia. The
correlation coefficient was 0.50 and the slope of the regression line was significantly
different from zero (P << 0.001). The size of the consuming layer as a fraction of the
thickness of the whole avascular layer, (L2 - L1)/L, is shown in Fig. 5 b. The thickness
of the consuming layer was 20 ± 11% of the thickness of the whole avascular layer
(10% of retinal thickness), and was not correlated with choroidal PO2 (r = 0.05).
There was no change in the position of the consuming layer (i.e., L1/L) during
hypoxemia (not shown).

At any one choroidal PO2 there was considerable variability among profiles in Q2
and in L2 - L1. At least part of this is probably artifactual and results from variable
amounts of distortion of the profile caused by the electrode, and from the high
sensitivity of the fitted parameters to details of the shape of the profile. One way to
partially deal with the variability, and to obtain more meaningful comparisons of Q
during normoxia and hypoxemia, is to compute an average oxygen consumption of
the avascular layer, Qav = Q2 x (L2 - L1)/L. This is the total oxygen consumption of
the avascular layer divided by its thickness. This measure reduces the variability
because Q2 and L2 - L1 tend to be inversely related to each other (Haugh et al.,
1990). A plot of Qav as a function of Pc for the dark-adapted data is shown in Fig. 6.
The correlation coefficient is somewhat better than for the data in Fig. 5 a (r = 0.67).
The solid regression line has a slope of 0.079 ml O2/100 g tissue·min·mmHg and
indicates that halving Pc roughly halves the oxygen consumption, from 5.1 ml
O2/100 g tissue·min at the "normal" Pc of ~62 mmHg to 2.6 ml O2/100 g tissue·min
at a Pc of 31 mmHg. The conclusion is similar if data from each animal are
considered separately. The regression lines for each cat are shown in the inset. The
slopes of these lines were all positive and had a mean of 0.053 ml O2/100 g
tissue·min·mmHg (±0.034 [SD]; n = 9 cats). The one with the lowest slope is based
on only three points, none of which were at very low $P_c$. Variation of oxygen consumption as a function of time during hypoxemia was not investigated systematically, but no obvious changes were noted between profiles recorded at the same $P_{\mathrm{a}O_2}$ but at different times during a hypoxemic episode. The speed of recovery after hypoxemia was not assessed, but $Q_{\mathrm{m}}$ had usually recovered by the time the next profile was obtained (~15–30 min).

The dashed line in Fig. 6 is a regression line for similar data obtained during elevation of intraocular pressure (from Fig. 8 of Yancey and Linsenmeier, 1989), and

![Figure 5](image)

**FIGURE 5.** Oxygen consumption ($Q_{\mathrm{m}}$) and relative thickness of the consuming layer $(L_2 - L_1)/L$ in the dark-adapted retina during hypoxemia. Each point was derived from a fit of an oxygen profile to the model described in Methods. The regression line in $a$ is: $Q_{\mathrm{m}} = 0.43P_c + 4.19$ ($r = 0.50$; $n = 64$). The regression line in $b$ is: $(L_2 - L_1)/L = -0.00053P_c + 0.23$ ($r = -0.096$). The slope for $(L_2 - L_1)/L$ vs. $P_c$ was not significantly different from zero.

converted to the units used here. The relationship is similar to that found in hypoxemia.

Some of the oxygen used by the photoreceptors diffuses from the retinal circulation, and to evaluate the importance of this source, the diffusion equations were used to compute the oxygen fluxes into the consuming region through the planes at $L_1$ (choroidal supply) and $L_2$ (retinal supply). This requires evaluating the derivative of Eq. 16 of Haugh et al. (1990) at $L_1$ and $L_2$, recognizing that the flux into the consuming region at $L_1$ is positive and at $L_2$ is negative. The percentage of oxygen
supplied by the retinal side (Fr) is 100 times the flux through L₂ divided by the total flux, which can be shown to be:

\[ Fr = 100\left[\frac{(L_1 + L_2)^2}{2L} + \frac{(P_L - P_c)}{((Q_{aw}/Dk)L^2)}\right] \]

This varied from 0 to ~30% among profiles, averaging 9.4 ± 8.1% (n = 65 profiles). Thus, on average, 91% of the oxygen was supplied by the choroid and only 9% by the retinal circulation. The value of Fr did not depend on P_c or P_{Po2}.

**Light adaptation.** Oxygen consumption data for the light-adapted retina are shown in Fig. 7 on the same scale as the data for the dark-adapted retina in Fig. 6. In
light adaptation $Q_2$ and $Q_\text{av}$ during normoxia were lower than their dark-adapted values. The normoxic $Q_\text{av}$ during light adaptation (taken from the regression line at $P_C = 62 \text{ mmHg}$) was $\sim 1.6 \text{ ml O}_2/100 \text{ g tissue-min}$, 33% of the corresponding value in dark adaptation. This is lower than the previously reported value of 60%, which was based on a much smaller sample of light-adapted profiles (Haugh et al., 1990). The average normoxic value for $Q_\text{av}$ is similar to the value that can be calculated by averaging $Q$ over the distal 50% of the retina in Fig. 5 of Alder et al. (1990).

A linear regression of all the data showed some reduction in both $Q_2$ and $Q_\text{av}$ during hypoxemia, but the effect of hypoxemia was much less pronounced than during dark adaptation. The slope of the regression line for $Q_2$ as a function of $P_C$ was not different from zero, and the slope of the regression line for $Q_\text{av}$ as a function of $P_C$ was 28% of the corresponding slope during dark adaptation. On closer inspection it was clear that for four of the five animals in which light-adapted profiles were measured, there was no change in $Q_\text{av}$ (i.e., slope not different from zero) down to $P_{O_2}$'s of 20–30 mmHg. Only the animal with the highest oxygen consumption during normoxia showed a decrease in $Q_\text{av}$ during hypoxemia, and this is the reason that the slope in Fig. 7 is not zero. We could find no reason to exclude these data. The size of the oxygen-consuming region, 18 ± 14% ($n = 46$) of the outer retina, was about the same as in dark adaptation.

During light adaptation the retinal circulation only rarely supplied any oxygen to the photoreceptors when $P_{O_2}$ was $> 60 \text{ mmHg}$ (3 of 29 profiles). At lower $P_{O_2}$, however, the retinal circulation supplied some of the oxygen in 14 of 18 profiles. For these 14 the percentage ($F_r$) supplied by the retinal circulation was 10.6 ± 9.7%.

**Inner Retina**

The profiles cannot be used to derive the oxygen consumption of the vascularized part of the retina, because the exact geometry of the vasculature is unknown, and because three-dimensional gradients of $P_{O_2}$ must exist, but cannot be measured. The extent to which inner retinal $P_{O_2}$ is regulated during hypoxemia can be determined, however, by making frequency histograms of $P_{O_2}$ for the inner 50% of the profiles. This technique has been used in many other tissues (e.g., Lübbers, 1977) in which no single value of $P_{O_2}$ characterizes tissue oxygenation. Histograms of tissue $P_{O_2}$ in different ranges of $P_{O_2}$ are shown in Fig. 8 for the dark-adapted retina. Each profile contributed $\sim 230$ values to these "grand" histograms. A small number of points (0.3%) were excluded because they appeared to represent measurement noise.

A wide range of retinal $P_{O_2}$ was measured at every $P_{O_2}$, but only part of the range was typically seen in an individual profile. The standard deviation of the normoxic ($P_{O_2} > 85 \text{ mmHg}$) histogram compiled from all profiles was 12.6 mmHg, whereas the average standard deviation in 36 histograms prepared from individual profiles was 5.7 mmHg, and only 4 profiles had standard deviations of $P_{O_2} > 10 \text{ mmHg}$. Histograms from two representative individual profiles are shown in Fig. 9. Clearly, a penetration through most retinal locations would not yield a range of $P_{O_2}$'s as large as seen in Fig. 8, but the grand histograms represent the overall likelihood of encountering particular values of $P_{O_2}$ in the inner half of the retina. Corresponding histograms for the light-adapted retina are shown in Fig. 10. In both light and darkness the distributions were positively skewed, as is typical in other tissues.
The amount of skewness was not large, however, and the median Po$_2$ was never more than 2.5 mmHg below the mean. Strikingly, even in normoxia in the dark-adapted retina, ~30% of the values were <10 mmHg.

Statistical analysis of inner retinal Po$_2$ was not performed on the means of the grand histograms, since the number of points was so large that even trivial differences would have appeared statistically different. Instead, the mean Po$_2$ was computed for each profile and then the mean and SEM of the profile means were computed. These are shown graphically in Fig. 11. Table I gives the statistical analysis of these means, which was done with the nonparametric Mann-Whitney test, since at some ranges of P$_{O_2}$ the profile mean Po$_2$'s had a nonnormal distribution (based on normal probability plots). In the dark, the hypoxemic mean Po$_2$ was different from the normoxic mean Po$_2$ only when P$_{O_2}$ was <45 mmHg. The
FIGURE 9. Examples of histograms obtained from individual dark-adapted profiles of inner retinal PO₂ during normoxia.

FIGURE 10. Frequency distribution of inner retinal PO₂ in light adaptation at various ranges of PO₂. The means and standard deviations are: for PO₂ > 85 mmHg, 12.6 ± 5.8 mmHg (n = 4,349 values); for 60 < PO₂ < 85 mmHg, 10.7 ± 8.9 mmHg (n = 2,282 values); for 45 < PO₂ < 60 mmHg, 7.9 ± 5.7 mmHg (n = 2,310 values); for PO₂ < 45 mmHg, 9.3 ± 6.8 mmHg (n = 1,838 values).
maintenance of inner retinal $P_{O_2}$ is in striking contrast to the linear reduction in choroidal $P_{O_2}$ during hypoxemia (Fig. 3).

During light adaptation the absolute change in inner retinal $P_{O_2}$ was smaller under hypoxic conditions, and the mean was significantly different from normoxic $P_{O_2}$ only for $P_{O_2}$ in the 45-60 mmHg range. Comparison of dark- and light-adapted $P_{O_2}$ for each range of $P_{O_2}$ (last row of Table I) showed that the means were significantly lower in the light than in the dark during both normoxia and mild hypoxemia.

Since the peaks in $P_{O_2}$ typically appeared to be in the more proximal part of the inner retina, we also examined whether the mean $P_{O_2}$ was higher in the layer from 0 to 25% retinal depth than in the layer from 25 to 50%. This was done with paired $t$ tests comparing the mean $P_{O_2}$’s in each of these layers within each profile. In the dark-adapted retina the $P_{O_2}$ was higher in the more proximal layer for the normoxic profiles (by 4.0 ± 7.5 mmHg; $P = 0.003$) and for the whole sample of profiles (by

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>$P_{O_2}$ in Inner Retina</th>
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<tr>
<td></td>
<td>$P_{O_2}$ (mmHg)</td>
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<tr>
<td></td>
<td>&lt;45</td>
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<tr>
<td>Dark</td>
<td></td>
</tr>
<tr>
<td>Mean of profile</td>
<td></td>
</tr>
<tr>
<td>Means ± SEM</td>
<td>8.5 ± 2.0</td>
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<tr>
<td>$n$ (profiles)</td>
<td>8</td>
</tr>
<tr>
<td>$P$ value for hypoxic vs. $P_{O_2}$ &gt; 85</td>
<td>0.005</td>
</tr>
<tr>
<td>Light</td>
<td></td>
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<tr>
<td>Mean of profile</td>
<td></td>
</tr>
<tr>
<td>Means ± SEM</td>
<td>9.3 ± 2.1</td>
</tr>
<tr>
<td>$n$ (profiles)</td>
<td>8</td>
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<tr>
<td>$P$ value for hypoxic vs. $P_{O_2}$ &gt; 85</td>
<td>NS</td>
</tr>
<tr>
<td>$P$ value for light vs. dark</td>
<td>NS</td>
</tr>
</tbody>
</table>
3.0 ± 6.8 mmHg; P < 0.001). This is consistent with the previously reported difference in Po2 in the two peaks that are usually observed in the inner retina (Yancey and Linsenmeier, 1989). There was no difference in mean Po2 in the two sublayers of the inner retina for hypoxemic profiles (P<sub>02</sub> < 85 mmHg) alone. During light adaptation, no significant differences were found, although the more distal layer tended to have a slightly higher Po2 during normoxia (by 2.0 ± 4.7 mmHg; P = 0.08).

**DISCUSSION**

The data presented here provide a relatively complete description of intraretinal Po2 in the cat during hypoxemia, and they are the only such data for any species. Many of the conclusions to be drawn support hypotheses that arose from electrophysiological results and from earlier work on retinal blood flow and vitreal oxygen tension.

**Oxygen Consumption**

Choroidal Po2 decreases during hypoxemia because compensatory increases in choroidal blood flow apparently do not occur (Bill, 1962; Friedman and Chandra, 1972). An increase in flow would have a limited effect in any case, since P<sub>02</sub> during relatively mild hypoxemia (60–70 mmHg) falls below the normoxic choroidal venous Po2, forcing choroidal venous Po2 to fall. In dark adaptation the decreased choroidal Po2 decreases the flux of oxygen to the inner segments, since the Po2 at some point in the inner segment layer was already nearly zero during normoxia. This deficit on the choroidal side is not made up by increased supply to photoreceptors from the retinal circulation (i.e., Fr does not increase during hypoxemia), so the large oxygen demand of the photoreceptors cannot be sustained. In the dark, therefore, Q<sub>av</sub> decreases by 0.79 ml O2/100 g tissue-min for a 10 mmHg decrease in choroidal Po2. Light decreases Q<sub>av</sub>, which increases outer retinal Po2. This buffers the photoreceptors against the hypoxic decrease in choroidal Po2, and Q<sub>av</sub> decreases by only 0.23 ml O2/100 g tissue-min (considerably less in most animals) for the same 10 mmHg change in choroidal Po2.

Since only one level of illumination was used here, it is reasonable to ask how much light is necessary to protect photoreceptor oxygen consumption from hypoxemia. Certainly the dependence on light is graded, but based on the size of light-evoked changes in Po2 in the cat retina (Linsenmeier, 1986), it appears that only in the two or three log units below rod saturation would light have a protective effect. At lower illuminations, the Po2 in the outer retina reaches almost zero during normoxia, and oxygen consumption should therefore be sensitive to hypoxemia not only in dark adaptation, but also over much of the scotopic range.

The decrease in dark-adapted oxygen consumption could conceivably affect many processes in the photoreceptor. Earlier evidence suggested that hypoxemia reduced the activity of the photoreceptor Na<sup>+</sup>/K<sup>+</sup> pump, leading to an increase in extracellular [K<sup>+</sup>] in the dark and a slowing of the time course of light-evoked changes in [K<sup>+</sup>] (Linsenmeier and Steinberg, 1984; Shimazaki and Oakley, 1984). The present results support this, since the reduction in oxygen consumption should lead to a deficit in
ATP production that would be expected to reduce the ion flux through the pump. Whether other cellular processes are also affected is not known. In the light, much smaller increases in extracellular [K+] were found during hypoxemia (Linsenmeier and Steinberg, 1984). This is consistent with the present finding that much smaller changes in consumption occur, so that ATP production and sodium pumping are less affected.

One objective of this work was to find the relationship of photoreceptor oxygen consumption to PaC in dark adaptation (Fig. 6). We would like to assert that the regression line has physiological significance, that is, that oxygen consumption falls approximately linearly as soon as PaC is reduced. This is consistent with electrophysiological data, particularly the lack of a threshold for the onset of hypoxemic changes in the light peak (Linsenmeier et al., 1983), and with the prediction that when choroidal Po2 falls there is no way to prevent a change in oxygen consumption. A linear relationship of Qav to PaC is not as clear in the data as one might have hoped, however, and it could be argued that over a range of PaC, from ~40 to 70 mmHg, Qav is nearly independent of PaC. Such a plateau may exist, and the relationship may be more complex than expected, but it is at least equally likely that a truly linear relationship between PaC and Qav is somewhat obscured by a combination of measurement error and physiological variation in Q. In this in vivo analysis, oxygen consumption is a highly derived quantity that is subject to error from at least two sources: (a) distortion of the profile due to the presence of the relatively large oxygen microelectrode, and (b) errors in the calibration of the oxygen electrode. We attempted to deal with the first of these by making the best correction possible for distortion in the apparent length of the profile during withdrawal (see Methods), but this correction is not perfect. The regression line relating Qav to PaC was also recomputed for several types of length correction and after excluding profiles in which L was >25% different from the average L for all profiles in one cat. These made little difference to the slope of the regression line or to the correlation coefficient. Small errors in estimating Po2 from the measured currents are always possible, because even perfectly reproducible in vitro calibrations may not apply exactly in vivo, due to possible changes in temperature or to the diffusion coefficient of the material in the electrode recess. Such errors might add some scatter to the data, but should be in a consistent direction. Another possible source of error is that the model itself is inappropriate. This is unlikely, because (a) the model fit well during both normoxia and hypoxemia, (b) the model is consistent with the anatomy of the photoreceptors, and (c) relative measurements of Q in the retina do not depend very strongly on the choice of the model (Haugh et al., 1990).

While some variation in Q is due to measurement error, there is probably also real physiological variation at a particular value of PaC. Rod density varies by a factor of 1.7 between the center of the area centralis and an eccentricity of 10–15° (Steinberg et al., 1973). This would be expected to influence Qav, but it was not possible to know the rod density at each recording site. It is also possible that in certain regions a greater oxygen supply from the retinal circulation allows a higher Q than would be predicted based on choroidal Po2 alone.
Glycolysis

The retina is known to have a very high glycolytic capacity, which increases under anaerobic or hypoxic conditions (e.g., Graymore, 1959; Winkler, 1986; Yamamoto and Steinberg, 1989), and one can ask whether glycolysis is able to compensate completely for the loss of respiration during hypoxemia. The 36 mol of ATP obtained from 1 mol of glucose during respiration require 6 mol of O₂, so the loss of each mole of O₂ requires that six more moles of ATP be generated glycolytically. The production of these ATP requires the utilization of 3 mol of glucose, replacing the 1 mol of O₂ and 1/6 mol of glucose that had been used in respiration. Therefore, the loss of each oxygen molecule requires three more glucose molecules to be used for glycolysis. As an example, consider the decrease in dark-adapted oxygen consumption (Qₒ₂) from 5.1 ml O₂/100 g·min at a normoxic Pₒ₂ of 62 mmHg to 2.6 ml O₂/100 g·min at a Pₒ₂ of 30 mmHg (Pₒ₂ ~ 50 mmHg). This is a difference of 0.11 mmol O₂/100 g·min, so 0.33 mmol/100 g·min more glucose would be required to keep ATP production constant. While there have been many measurements of lactate production in the retina, only Törnquist (1979) has measured it in the outer retina of the cat in vivo. From the choroidal arteriovenous difference and choroidal blood flow, the lactate production was found to be 1.91 × 10⁻⁴ mmol/min. Assuming that this lactate was generated by glycolysis in the outer half of the retina (wet weight ~ 65 mg), the normoxic utilization of glucose for glycolysis would be ~ 0.15 mmol/100 g·min. A similar value was obtained in pig (Törnquist and Alm, 1979). Thus, to completely compensate for the loss of ATP from oxidative metabolism, glycolysis would have to be about three times as great at Pₒ₂ = 30 mmHg as at Pₒ₂ = 62 mmHg, utilizing 0.48 vs. 0.15 mmol glucose/100 g·min. Törnquist’s value is probably for light adaptation, which is at least somewhat lower than what is expected in dark adaptation (Winkler, 1986; Yamamoto and Steinberg, 1989), so at Pₒ₂ = 30 mmHg the value might need to be somewhat greater than 0.5 mmol glucose/100 g·min. These values, derived from Qₒ₂, assume that glycolysis is spread out over the whole photoreceptor, but to the extent that glycolysis is compartmentalized, the local glycolytic rate would have to be even higher. Such an increase in glycolysis is not out of the question, since anaerobic glucose utilization rates of 0.5 to > 0.6 mmol glucose/100 g·min have been reported for whole albino rat retina (Graymore, 1959; Winkler, 1986). The fact that there are effects of mild retinal hypoxemia on electrical signals from the outer retina suggests, however, that glycolytic compensation is not complete, or perhaps that a high rate of glycolysis carries its own detrimental consequences.

Comparison with Intraocular Pressure Elevation

When intraocular pressure was elevated, choroidal Po₂ and oxygen consumption were reduced in the dark-adapted retina (Yancey and Linsenmeier, 1989). Despite other changes, such as acidosis, that probably accompanied the reduction in choroidal blood flow during intraocular pressure elevation, and the blood pressure elevation that sometimes occurs in hypoxemia, the relationship of Qₒ₂ and Pₒ₂ was very similar in both cases. Since there was a strong relationship between perfusion pressure (mean arterial pressure minus intraocular pressure) and Pₒ₂ in the earlier study, and between
\( P_{aO_2} \) and \( P_c \) here, we can give a relationship between the perfusion pressure (PP) and the \( P_{aO_2} \) that would have equal effects on \( P_c \) and therefore on \( Q \). This relationship is:

\[
PP = 1.39 P_{aO_2} - 26.3
\]

As an example, an arterial \( P_{O_2} \) of 50 mmHg would have the same effect as a PP of 43 mmHg, or for an animal with a mean arterial pressure of 110 mmHg, an intraocular pressure of 67 mmHg.

**Inner Retinal \( P_{O_2} \)**

The average inner retinal \( P_{O_2} \) in dark adaptation is close to 20 mmHg. This value has been based on many measurements and it is similar to previous estimates of inner retinal \( P_{O_2} \) obtained from vitreal and inner retinal measurements in cats and pigs (Alm and Bill, 1972; Tsacopoulos, 1979; Enroth-Cugell et al., 1980; Alder et al., 1983; Linsenmeier, 1986). The wide range of \( P_{O_2} \) observed here was not apparent from the earlier measurements. Mean inner retinal \( P_{O_2} \) decreased during illumination, consistent with changes that sometimes had been observed when \( P_{O_2} \) was recorded at a point in the inner retina during 10–60 s of illumination (Linsenmeier, 1986). It is not known whether the decrease during illumination represents a decrease in blood flow or an increase in oxygen consumption, but the former seems more likely. A decrease in retinal blood flow during illumination has been found in some experiments (e.g., Feke et al., 1983; Bill and Sperber, 1990). The only data directly pertinent to light-induced changes in inner retinal oxygen consumption are deoxyglucose measurements showing no difference in accumulation between darkness and steady illumination (Bill and Sperber, 1990).

Even during dark adaptation inner retinal \( P_{O_2} \) is low compared with tissue \( P_{O_2} \) in most other organs. A survey of \( P_{O_2} \) histograms from various tissues (Kessler, 1974; Leniger-Follert, 1985; Lund, 1985; Schuchhardt, 1985) shows universal agreement that the myocardium has mean \( P_{O_2} \)'s as low as the inner retina. These are both metabolically active tissues with relatively low venous \( P_{O_2} \)'s (human retinal venous saturation = 46% [Sebag et al., 1989], corresponding to a venous \( P_{O_2} \) of ~24 mmHg; coronary sinus \( P_{O_2} = 20 \) mmHg [Lund, 1985]). Whether the retina is similar to any other, possibly more heterogeneous, tissues is less clear. Some microelectrode studies in skeletal muscle show low \( P_{O_2} \)'s (e.g., Heinrich et al., 1985). Mean \( P_{O_2} \)'s in the range of 15–25 mmHg have also been found in the cerebrum of guinea pig (e.g., Lübbers, 1977) and rat (Feng et al., 1988), but only rarely in cat (Gronczewski and Leniger-Follert, 1984), where the mean is instead generally >30 mmHg (Leniger-Follert, 1985) and there are few values <10 mmHg. Most of the recordings in cat brain have been made with surface electrodes, however, and the \( P_{O_2} \) distribution might be different deeper in the tissue. In cardiac and skeletal muscle, surface electrodes generally give higher values for \( P_{O_2} \) than microelectrodes within the tissue (e.g., Harrison et al., 1990).

Inner retinal \( P_{O_2} \) is well regulated during hypoxemia in both dark and light adaptation, which may be especially important since the values are already low in normoxia. The results reported here support measurements made in the vitreous close to the retina during hypoxemia (e.g., Alm and Bill, 1972; Tsacopoulos, 1979; Enroth-Cugell et al., 1980) in showing that retinal \( P_{O_2} \) is regulated at least as well as
tissue PO2 in brain, heart, or skeletal muscle (Leniger-Follert et al., 1975; Schuchhart, 1985; Harrison et al., 1990). This regulation is the result of a well-known vasodilation and increase in retinal blood flow during hypoxemia (e.g., Eperon et al., 1975). Regulation of retinal PO2 is important for maintaining function, since the failure of the ERG b-wave and ganglion cell sensitivity begins when this regulation breaks down at Pao2 < 40 mmHg (e.g., Linsenmeier, 1990). As noted earlier, photoreceptor metabolism and some ERG components are affected by hypoxemia at considerably higher Pao2. This raises the question of how the inner retina manages to preserve function under these conditions, and this requires further study.

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