

# Effects of Light and Darkness on Oxygen Distribution and Consumption in the Cat Retina

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**ABSTRACT** These experiments were done to investigate the effects of light and darkness on the oxygenation of the retina in anesthetized cats. Measurements were made with double-barreled oxygen microelectrodes capable of recording both oxygen tension ( $PO_2$ ) and local voltages. Diffuse white illumination presented to a dark-adapted retina led to an increase in  $PO_2$  of up to 30 mmHg in the outer half of the retina. Changes were maximal at ~75% depth, corresponding to the outer nuclear layer. No change or decrease in  $PO_2$  was observed in the inner retina. Light-evoked increases in outer retinal  $PO_2$  were graded with the duration and strength of illumination, and were maximal in response to 60 s of illumination at rod saturation. For these stimuli, the increase at the onset of illumination was slower (average half-time, 12.2 s) than the recovery at the end of illumination (average half-time, 5.9 s), but for stimuli above rod saturation,  $PO_2$  recovered much more slowly. The profile of  $PO_2$  was measured during electrode penetration and withdrawal and during light and dark adaptation. Dark-adapted profiles were characterized by a minimum  $PO_2$  of nearly 0 mmHg at depths of 65–85%, and a steep gradient from the minimum to the choroid. During light adaptation at rod saturation,  $PO_2$  was elevated in the outer half of the retina and the minimum was eliminated. Fits of the profiles to a one-dimensional model of oxygen diffusion indicated that light reduced the oxygen consumption of the outer retina to ~50% of its dark-adapted value.

## INTRODUCTION

The nutrient supply to the retina of higher mammals is complex. The inner retina is supplied directly by one vascular bed, the retinal circulation, while the outer retina, comprising about half of the retinal thickness, is supplied by diffusion from the retinal circulation and from the choroidal vessels that lie behind the retina. Oxygen gradients within the avascular outer region are determined by the unique features of oxygen supply and consumption of this tissue. First, the choroidal and retinal circulations have very different flow rates and control mechanisms (e.g., Bill, 1984) and provide different boundary conditions on the two sides of the avascular layer. Second, photoreceptor oxygen

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consumption is considered to be high (e.g., Cohen and Noell, 1965), and should be localized primarily to the inner segments of the photoreceptors, where mitochondria are numerous.

Previous measurements of intraretinal oxygen tension in mammals have shown that the oxygen tension is relatively uniform in the inner retina, reaches a minimum at some point in the outer retina, and rises to a maximum value at the tips of the outer segments, near the choroid (Tsacopoulos et al., 1976; Alder et al., 1983). Although these studies have supported our basic expectations about oxygen distribution, they have not considered that retinal illumination may be an important variable. Retinal oxygen consumption is reduced by light in frog (Sickel, 1972; Kimble et al., 1980; Zuckerman and Weiter, 1980) and monkey (Stefansson et al., 1983). In frog, this change in consumption has a large effect on the intraretinal oxygen distribution *in vitro* (Zuckerman and Weiter, 1980), but in the intact mammalian retina, the magnitude and time course of light-evoked changes in consumption, and their effect on oxygen distribution, have not been measured previously.

A further limitation of previous studies in mammals is that they have been purely descriptive, although it has been realized for some time that a rather simple diffusion analysis is possible for part of the retina (Dollery et al., 1969; Linsenmeier, 1978). The analysis is simple because diffusion of oxygen through the outer retina should occur in only the radial direction, and because the oxygen supply of this region is confined to its boundaries, so that supply and consumption are spatially separate. This type of modeling can allow the determination of values of oxygen consumption that would otherwise be impossible to obtain *in vivo*.

This study had three primary goals. The first was to determine the magnitude and time course of changes in intraretinal oxygen tension ( $PO_2$ ) in the cat retina during illumination. The second was to measure the profile of  $PO_2$  as a function of intraretinal depth under carefully controlled conditions of illumination. The final goal was to use these measurements in a diffusion analysis to obtain estimates of the oxygen consumption of the outer retina, and to determine how this varies with illumination.

## METHODS

### *Preparation*

Conditioned adult cats were initially anesthetized with sodium thiamylal injected intravenously (20 mg/kg). This anesthetic was used during the insertion of two venous cannulae for drug infusion, a femoral artery cannula for monitoring pressure and sampling blood, and a tracheal cannula. The animal was mounted in a head-holder of the type described by Brown (1964), and the temporal side of the right eye was surgically exposed. During these procedures, the thiamylal was gradually replaced by urethane (200 mg/kg), which was the anesthetic during recording (100–200 mg/h). Atropine (0.3 mg) was given subcutaneously to minimize secretions and 1 ml of Combiotic (a mixture of penicillin [ $2 \times 10^5$  U/ml] and streptomycin [250 mg/ml]) was given intramuscularly. During experiments, animals were paralyzed with pancuronium bromide (Pavulon, 0.1 mg/kg·h) and artificially ventilated at a rate and tidal volume suitable for maintaining arterial  $PO_2$

above 90 mmHg and arterial pH in the range of 7.35–7.45. Room air was sometimes supplemented with oxygen to keep the arterial  $PO_2$  normal. Measurements of arterial values were made intermittently with a PHM 71 blood gas analyzer (Radiometer America, Westlake, OH). The animal's temperature was controlled with a water-filled heating pad connected to a water bath and temperature controller (model 73A, Yellow Springs Instruments, Yellow Springs, OH). Blood pressure was monitored with a pressure transducer (model 377, Harvard Apparatus, South Natick, MA) connected to a chart recorder and mercury manometer. The pupil was dilated with atropine sulfate (1%) and neosynephrine (1%) and the cornea was protected by a contact lens.

### *Electrodes*

Oxygen microelectrodes were introduced into the optically intact right eye through a 15-gauge hypodermic needle inserted ~10 mm posterior to the limbus. The needle was held in a positioner similar to that described by Steinberg et al. (1968) and was advanced with a hydraulic microdrive (model 670WPC, David Kopf Instruments, Tujunga, CA).

Double-barreled oxygen microelectrodes were used. One barrel measured oxygen tension polarographically and the other measured voltage. Electrodes were made of double-barreled glass in which each barrel had an outer diameter of 0.7 mm and an inner diameter of 0.35 mm. The finished electrodes were similar to those described by Whalen et al. (1967) in having a recessed oxygen cathode, but the construction method was different. Briefly, the glass was pulled to an appropriate shape (Brown-Flaming P-77 micropipette puller, Sutter Instrument Co., San Francisco, CA) and the tip was broken to a size of 1–2  $\mu\text{m}$ . A Wood's metal wire was inserted into one barrel and pushed toward the tip while the tapered region of the glass was heated to just above the melting point of the Wood's metal. The metal was not pushed all the way to the tip, but instead a recess of 15–75  $\mu\text{m}$  was left. The voltage reference barrel, which contained a glass fiber, was filled with an electrolyte solution (generally 0.5 M potassium acetate) and the electrode was beveled (model BV-10 micropipette beveler, Sutter Instrument Co.). The Wood's metal was electroplated with gold (Dalic 3020 solution, Sifco Metachemical, Independence, OH) at a current of ~0.1 nA, so that the final recess was 5–15  $\mu\text{m}$  in length. The final size of the tip was ~5  $\mu\text{m}$ , measured perpendicular to the shank of the electrode.

During calibration and experiments, the gold cathode was polarized at  $-0.7$  V with respect to an Ag/AgCl reference electrode. In vitro calibrations were done in a temperature-controlled tonometer similar to that described by Proctor and Bohlen (1979), with 4, 8, and 21% oxygen alternately bubbled through saline. The sensitivity of 14 electrodes employed in the experiments was  $0.05 \pm 0.036$  pA/mmHg. (All statistics are given as means  $\pm$  standard deviation.) The minimum resolvable change in  $PO_2$  was 2–5 mmHg, depending on the sensitivity of the electrode. The calibration was checked in vivo by recording the vitreal oxygen current during a period of  $N_2$  breathing at the end of the experiment, and by recognizing that the choroidal  $PO_2$  should be near the arterial  $PO_2$ , since only ~1 vol%  $O_2$  is removed from choroidal blood (e.g., Alm and Bill, 1972). The time constant of these electrodes could not be assessed in the tonometer, but theoretically they should respond in  $\leq 200$  ms (Schneiderman and Goldstick, 1978). This was verified in some experiments by withdrawing the electrode rapidly from the subretinal space, where the retinal  $PO_2$  is high, to the vitreous, where the  $PO_2$  is much lower.

The reference electrode for the oxygen barrel was an Ag/AgCl wire in the posterior chamber. In addition to the oxygen signal, two voltages were recorded routinely. First, changes in intraretinal DC potential and local responses to light (local electroretinograms [LERGs]) were recorded between the reference barrel of the microelectrode and an Ag/AgCl reference electrode behind the eye. Second, the vitreal ERG was recorded

between a second Ag/AgCl wire in the vitreous and the same retrobulbar reference electrode. Further details of these techniques were as reported previously (Linsenmeier and Steinberg, 1982).

### *Recording*

Oxygen currents were measured with a picoammeter (model 614, Keithley Instruments, Cleveland, OH). The intraretinal voltage signal was recorded through a unity gain amplifier (M4A or M-707A, W-P Instruments, Inc., New Haven, CT) and amplified with a 5A18N module of a storage oscilloscope (5111A, Tektronix Instruments, Beaverton, OR). The vitreal signal was recorded through a unity gain amplifier (FD-223, W-P Instruments, Inc.) and amplified by the 5A22N module of the oscilloscope. All signals and a voice/stimulus channel were recorded on an FM tape recorder (model Store 4DS, Racal Recorders, Inc., Sarasota, FL) and chart recorder (model 440, Gould, Inc., Cleveland, OH).

### *Visual Stimulation*

The animal was enclosed in an electrically shielded, light-proof cage that allowed dark adaptation to be maintained. Stimuli were generated by a two-channel optical bench (of which only one channel was used in the present study) outside the cage, and light was conducted from the optical bench to a mirror above the cat's eye by a fiber optic bundle. The source was a tungsten iodide H1 automobile headlamp bulb. Timing of stimuli was controlled by an electronic shutter (Uniblitz, Vincent Associates, Rochester, NY) and timer (model S-100, Winston Electronics, San Francisco, CA). Illumination was controlled with a neutral density wedge filter. The maximum illumination was 4.2 photopic  $\mu\text{W}/\text{cm}^2$  at the cornea, measured by placing the detector head of a photometer in the position of the cornea. This is  $\sim 9.6$  log equivalent quanta (555 nm)/ $\text{deg}^2 \cdot \text{s}$  (hereafter abbreviated as  $q/\text{deg}^2 \cdot \text{s}$ ). Empirically this was found to be  $\sim 1$  log unit above rod saturation, judged from the b- and c-waves of the ERG. Enroth-Cugell et al. (1977) found that this type of bulb produced an equivalent illumination at 500 nm that was  $\sim 0.2$  log units lower than at 560 nm. White light was used in all experiments. To ensure that the retina was illuminated diffusely, an opal glass or paper screen was inserted between the mirror and the cat's eye. (This has been taken into account in the calibration.) For simplicity, the misnomer "flash" will sometimes be used to refer to periods of illumination  $\leq 300$  s in duration.

### *Retinal Penetrations*

Microelectrode penetrations of the retina were made in or near the area centralis, away from visible vessels. Successive penetrations were made close to each other, in general, but never in exactly the same spot. Penetrations were done under dark-adapted conditions. At selected depths, flashes of light were given, eliciting LERGs and changes in oxygen tension. Distance through the retina is given in terms of percent, where zero is the vitreal-retinal interface and 100 is the basal side of the retinal pigment epithelium (RPE) or the choriocapillaris. Zero was determined from the transient negative deflection observed in the voltage recording as the electrode hit the inner limiting membrane, and successful penetration of this membrane was indicated when a negative-going light response replaced the vitreal-positive b-wave. Penetration of the RPE and entry into the choroid was signaled by the voltage drop across the RPE (the transepithelial potential [TEP]) (e.g., Steinberg et al., 1985). 100% is often taken to be the apical surface of the RPE, but adoption of this convention would make very little difference. The electrode track length between the vitreous and RPE for 46 penetrations in 11 cats was  $352 \pm 104 \mu\text{m}$ , which is similar to that observed when recording with a very small single-barreled microelectrode (personal

observations). Assuming that the electrode hit the retina at an angle of  $45^\circ$  (based on the entry point and the size of the cat eye), this would imply an actual retinal thickness of  $249\ \mu\text{m}$ . The thickness of the cat retina in this region measured histologically is  $150\text{--}220\ \mu\text{m}$  (Prince et al., 1960; Landers, 1978; Vogel, 1978).

The LERGs recorded during acceptable penetrations (Fig. 1) changed as expected with depth (e.g., Brown and Wiesel, 1961), and the time course and maximum amplitudes of the intraretinal b- and c-waves were the same as those observed in recordings made with very small single-barreled electrodes (Brown and Wiesel, 1961; Linsenmeier and Steinberg, 1983). This, in addition to the normal total depth of the penetrations, provides some evidence that the relatively large oxygen electrode did not cause excessive local damage to the retina. (In some cases, it was impossible to obtain normal LERGs, and these penetrations were aborted after  $\sim 50\ \mu\text{m}$ .) Also, as expected, transient shifts in voltage accompanied the stepwise penetrations, but there was essentially no DC potential anywhere between the vitreous and subretinal space that might have affected the polarization of the oxygen electrode.

The major difference between voltage recordings obtained with these electrodes and with single-barreled electrodes was the difficulty of penetrating the RPE cleanly. Generally a TEP of  $10\text{--}15\ \text{mV}$  is observed in a sudden step, and the ERG suddenly reverses polarity. In the present experiments, the TEP was often smaller and the LERG sometimes retained its subretinal polarity while becoming smaller. These features probably resulted from a larger decrease in RPE shunt resistance in these experiments than in ones with smaller microelectrodes, but in some cases (Fig. 8), optimal penetrations of the RPE were achieved.

## RESULTS

### *Light-evoked Oxygen Responses*

*Outer retinal oxygen responses.* Fig. 1 shows a series of oxygen responses and LERGs evoked by 15-s flashes of light at increasing depths within the retina. The stimuli were just adequate to produce saturating b- and c-wave responses (and oxygen responses) presented to an initially dark-adapted retina. The main effect illustrated here is the substantial increase in oxygen tension ( $PO_2$ ) that occurred over approximately the outer half of the retina. No increase in  $PO_2$  was observed in the inner half of the retina or in the choroid. The light-evoked  $PO_2$  increase was maximal, on the average, at  $75 \pm 8\%$  retinal depth ( $n = 13$  penetrations). This was not a sharply defined point, however. In some penetrations, similar light-evoked changes in  $PO_2$  were observed over a range of depths, from  $\sim 65$  to  $\sim 85\%$ . These depths all correspond to the outer nuclear layer (Vogel, 1978). The maximum amplitude of the  $PO_2$  increase depended on stimulus duration and illumination, as discussed below, but an indication of the amplitude is given by the average maximum increase of  $22.7 \pm 11.3\ \text{mmHg}$  ( $n = 17$  penetrations in 10 cats) observed in flashes ranging from 10 to 120 s in duration.

The oxygen responses of Fig. 1 also show the apparent oscillation of  $PO_2$  in the outer retina and choroid that was observed to some extent in almost every penetration. This oscillation occurred in synchrony with respiration. It was observed only at depths beyond the position of the minimum dark-adapted  $PO_2$  (see below), although irregular fluctuations were often observed in the inner retina. The oscillation was not observed in the voltage recording, except when

the electrode was actually touching the RPE or was in the choroid. This suggests that the  $PO_2$  oscillation was not due to electrode bending, but instead to a real oscillation of  $PO_2$ . This will be considered further in the Discussion.

*Time course of oxygen responses.* While the oxygen responses in Fig. 1 may appear to have reached a plateau within 15 s, the  $PO_2$  generally continued to increase monotonically for considerably longer when illumination was maintained. A series of responses to stimuli of 2–60 s in duration is shown in Fig. 2. A small change in  $PO_2$  was observed in response to the 5-s flash, and the amplitude continued to grow as the duration was increased over the range

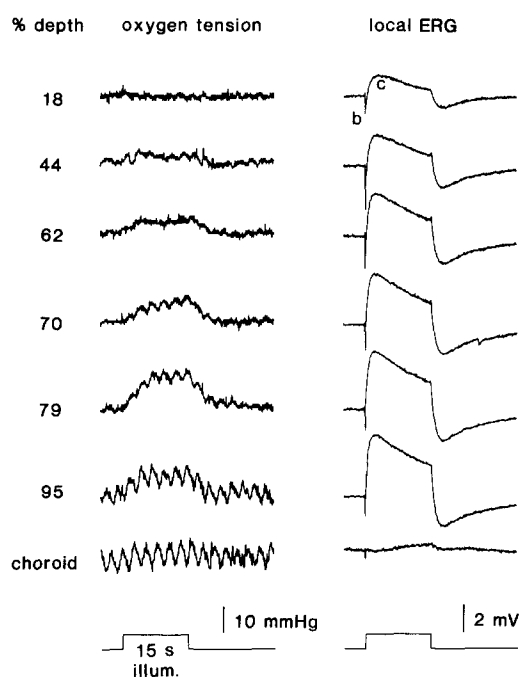


FIGURE 1. Oxygen changes and simultaneously recorded local ERGs as a function of depth in one microelectrode penetration of the dark-adapted cat retina. The stimulus was a diffuse white light 15 s in duration at  $8.6 \log$  quanta ( $555$ )/ $\text{deg}^2 \cdot \text{s}$  (approximately rod saturation). Cat 8.

shown. The time course cannot be easily described by a single number, since it often appeared to have a rapid and a slow component, probably seen most clearly in Fig. 4. To quantify the time course, the times for the  $PO_2$  to reach 50 and 90% of the final value were computed for all responses for which the stimulus duration was at least 60 s, and the illumination was at rod saturation. The average half-time was  $12.2 \pm 6.9$  s (17 responses in nine cats), and the time to reach 90% of the final value was  $42.0 \pm 14.7$  s.

Fig. 3 shows a response to 5 min of illumination recorded at the depth of maximal light-evoked oxygen responses. As in all such responses, the  $PO_2$  remained at a plateau level or recovered only slightly as the illumination was

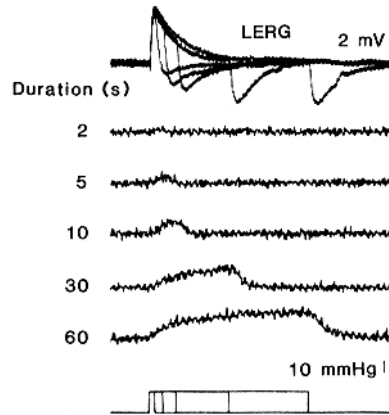


FIGURE 2. Oxygen responses to flashes of different durations all recorded near the location of maximal responses. The simultaneously recorded LERGs are shown superimposed at the top for comparison. The stimulus was a diffuse white light at  $8.6 \log q/\text{deg}^2 \cdot \text{s}$ . Cat 15.

maintained. It is apparent from all of the oxygen responses shown that their time course had no relation to any component of the ERG.

The recovery of  $PO_2$  at the end of illumination was more rapid than the increase at the beginning (Figs. 2 and 3), provided that the illumination was below rod saturation. The half-time of the recovery, calculated from the same responses as above, was  $5.9 \pm 3.5$  s, and the 90% time was  $21.0 \pm 21.7$  s. For stimuli at rod saturation, the recovery time did not depend on the duration of illumination.

*Illumination dependence of oxygen responses.* The amplitude of oxygen responses in the outer retina depended upon illumination, as shown in Fig. 4, which compares 60-s responses over 5 log units of illumination. The minimum illumination producing measurable oxygen responses was  $6.6 \log q/\text{deg}^2 \cdot \text{s}$ , although this may simply be the limit of detection and not the limit of the

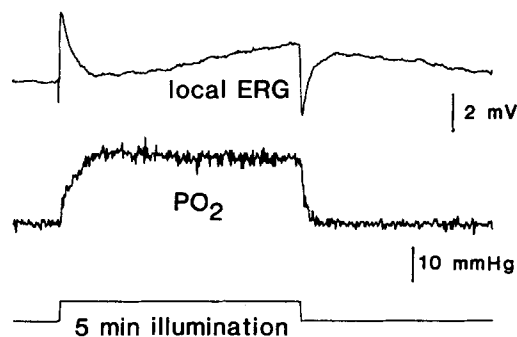


FIGURE 3. LERG and oxygen response to 5 min of illumination at  $8.6 \log q/\text{deg}^2 \cdot \text{s}$ . Responses were recorded at the position of the minimum dark-adapted  $PO_2$ . Cat 8.

underlying mechanism. The oxygen response was graded with illumination, and a summary of this dependence is provided in Fig. 5. For each of seven cats, the response to the maximum illumination was normalized to 100% and the responses to weaker stimuli were plotted relative to this. The stimulus durations were 15–120 s and all were the same for a given cat. The data are reasonably well described by a log-linear relationship.

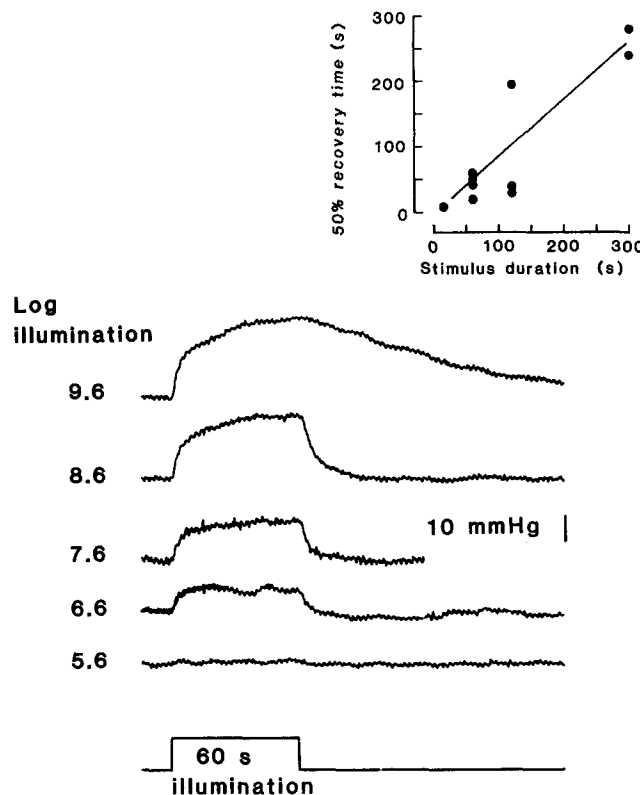


FIGURE 4. Oxygen responses to 60 s stimuli of varying illumination. The number to the left of each trace gives the illumination in  $\log q/\text{deg}^2 \cdot \text{s}$ . All responses were recorded near the position of the dark-adapted minimum  $PO_2$ . LERGs are not shown, since a single-barreled electrode was used for these recordings. Cat 15. The inset shows the time required for 50% recovery of the  $PO_2$  after 10 stimuli of different durations  $\sim 1$  log unit above rod saturation. Seven cats.

Stimuli above rod saturation (judged in these experiments from saturation of the c-wave amplitude) generally did not lead to an increase in the amplitude of the oxygen response, but instead to a prolongation of the plateau, analogous to the rod aftereffect first observed in horizontal cell responses (Steinberg, 1969*b*). Since rod aftereffects are observed in RPE, horizontal, and ganglion cells (Steinberg, 1969*a, b*; Schmidt and Steinberg, 1971), as well as in rods themselves (e.g., Cervetto et al., 1977), this does not directly implicate the rods as the source



of the oxygen responses to light. The failure to find an increase in amplitude above rod saturation does indicate, however, that cones and the cone system are not significantly involved in the observed responses, although it is possible that this is due to the paucity of cones rather than to a rod/cone metabolic difference.

As in horizontal cell responses (Steinberg, 1969*b*), the duration of the rod aftereffect depended upon the duration of illumination. As shown in Fig. 4 (inset), the half-time for recovery was approximately the same as the duration of the stimulus over the range shown, but the limits of this relation have not yet been tested.

*Inner retinal oxygen responses.* The major focus of the present work is on increases in  $PO_2$  that occur during illumination in the outer retina. In about half of the experiments, no change was detected in the inner retina, but in the other half, a decrease in  $PO_2$  was observed (Fig. 6). The decrease was maximal at

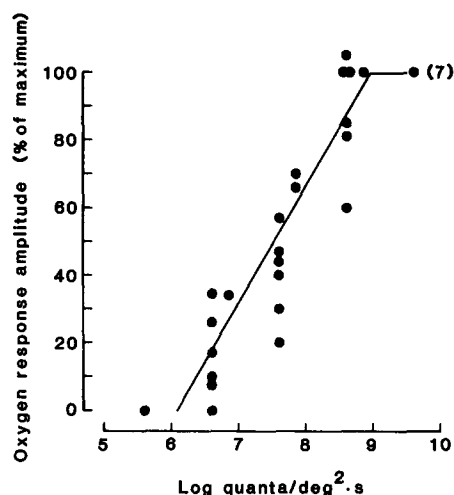


FIGURE 5. Dependence of relative oxygen response amplitude on illumination. For each of seven cats, the response to the strongest stimulus was taken to be 100%. The line was drawn by eye.

depths between 0 and 30%, and there was then a more distal region where no change in  $PO_2$  occurred, before the region of light-evoked increases. Inner retinal responses were generally smaller than the outer retinal oxygen responses. Their time course was very slow and they had a distinct latency of several seconds, in contrast to the outer retinal responses, which had no apparent latency when displayed on a similar time scale. They appeared to be maximal in response to  $\sim 30$  s of illumination.

It seemed that flickering light might elicit a larger change in the inner retinal  $PO_2$  than steady light by causing a higher average level of ganglion cell firing and therefore a higher oxygen utilization. Therefore, in one experiment, the effect of a steady light at  $8.3 \log q/\text{deg}^2 \cdot \text{s}$  was compared with the effect of repeated stimuli delivered at 1 Hz with the same time-averaged illumination. The oxygen response to these two stimuli was the same.

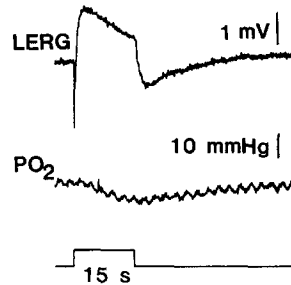


FIGURE 6. Inner retinal LERG and oxygen response to 15 s of illumination at rod saturation. This response was recorded at a depth of 10%. Cat 12.

### Oxygen Profiles

*Discrete penetrations.* The profile of  $PO_2$  across the retina during dark and light is important in assessing the relative roles of the retinal and choroidal circulations in supplying the outer retina. Dark- and light-adapted profiles, such as those in Fig. 7, were obtained from data like those shown in Fig. 1, where the circles denote the dark-adapted baseline values and the squares are the values at the end of each flash. These plots provided several pieces of information not obvious from the flash responses alone. First, the inner retinal  $PO_2$  was relatively

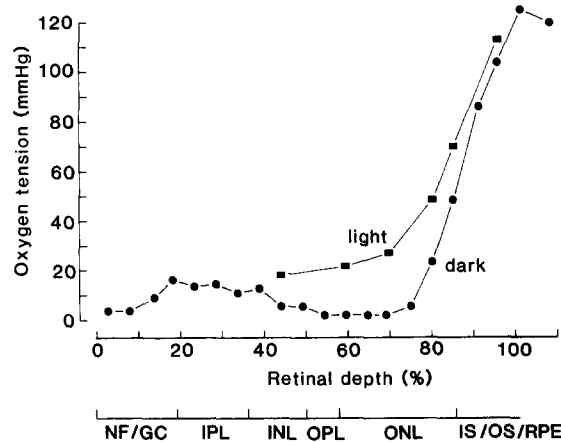


FIGURE 7. Profile of  $PO_2$  during a penetration of the dark-adapted retina (circles). The electrode was stopped at the points shown while the current stabilized, and with the electrode at selected depths, 10-s flashes (at  $8.6 \log q/\text{deg}^2 \cdot \text{s}$ ) were delivered. The maximum  $PO_2$  values during the flashes are plotted as squares. The time constant of oxygen responses in this animal was faster than the average, so the values during illumination are only slightly below those that would have been obtained if light adaptation had been complete. The lower abscissa gives approximate positions of retinal layers (Vogel, 1978): NF/GC, nerve fiber and ganglion cell; IPL, inner plexiform; INL, inner nuclear; OPL, outer plexiform; ONL, outer nuclear; IS/OS/RPE, photoreceptor inner and outer segments and retinal pigment epithelium. Cat 4.

uniform, generally averaging  $\leq 20$  mmHg, in agreement with the value reported by Alder et al. (1983). This part of the profile was rarely completely flat, however, often exhibiting a relative maximum in the inner retina, as shown here. Second, there was a minimum in  $PO_2$  in the dark-adapted retina near the depths where maximal oxygen responses were observed (65–85%). Surprisingly, the minimum dark-adapted  $PO_2$  in this region was very close to 0 mmHg. This statement is based not only on the in vitro calibration, but also on (a) the current recorded in the vitreous when the animal breathed nitrogen at the end of the experiment, and (b) the failure to detect any decrease in  $PO_2$  in this region during short episodes of severe hypoxia. The current measured at this position was very stable at the value corresponding to 0 mmHg, but since the resolution of the measurement was 2–5 mmHg (depending on the electrode), it is difficult to know whether the  $PO_2$  was zero or slightly higher. Since the minimum often occupied a substantial region, as in Fig. 7, it probably did reach zero at some point. Third, the  $PO_2$  rose steeply from the minimum to the choroid, where the  $PO_2$  averaged  $89.5 \pm 24.7$  mmHg ( $n = 23$  penetrations in nine cats). Fourth, during illumination

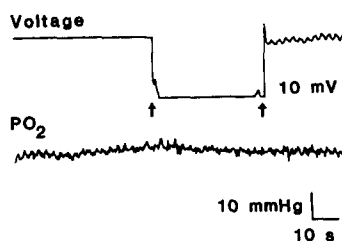


FIGURE 8. Voltage recorded with the microelectrode reference barrel and  $PO_2$  recorded with the oxygen barrel as the electrode penetrated the RPE (first arrow). At the second arrow, the trace was recentered, but the electrode was still in the choroid. Cat 10.

at rod saturation, no minimum in  $PO_2$  existed, and the  $PO_2$  rose monotonically between the inner retina and choroid.

Data presented in a study of oxygenation of the miniature pig retina (Tsacopoulos et al., 1976) suggested that there was a large change in  $PO_2$  across or behind the RPE, but this was not found in the present study or in that of Alder et al. (1983). Fig. 8 shows an example in which the RPE was penetrated cleanly (arrow). The drop in potential across the RPE (the TEP) was  $>15$  mV and saturated the tape recorder's input amplifier. At the second arrow, the voltage trace was recentered and the choroidal pulsations appeared. During this section of the penetration, no substantial change of  $PO_2$  occurred, so it can be concluded that the RPE is not a barrier to oxygen diffusion and does not consume an extraordinary amount of oxygen. This is consistent with the conclusion of Glocklin and Potts (1965), who found the oxygen consumption of the isolated bovine RPE to be 20–40% of that of the neural retina.

*Continuous withdrawals.* An individual penetration of the type shown in Fig. 7 sometimes took  $>1$  h, since several flashes were often given at one depth and time was allowed for recovery between flashes and for stabilization of the oxygen

current at each depth. Thus, changes in electrode position or time-dependent changes in  $PO_2$  could have contaminated these profiles. Furthermore, most of these profiles were obtained by using relatively brief (10 or 15 s) flashes, when oxygen had not reached steady state. To control for these potential problems, a separate set of experiments was done in which  $PO_2$  was recorded continuously as the electrode was withdrawn from the retina at a fixed speed. The protocol was to penetrate the retina stepwise during dark adaptation, delivering brief flashes to check that the LERG was normal. The withdrawal was performed relatively slowly,  $1.5 \mu\text{m/s}$ , during dark adaptation or after allowing the retina to adapt for several minutes to a steady light at rod saturation. The crossing of the RPE back into the retina was signaled by an increase in potential (the TEP) and a decrease in  $PO_2$ . In the dark-adapted withdrawals, brief flashes were given as the electrode neared the vitreous to determine when a vitreal ERG would be

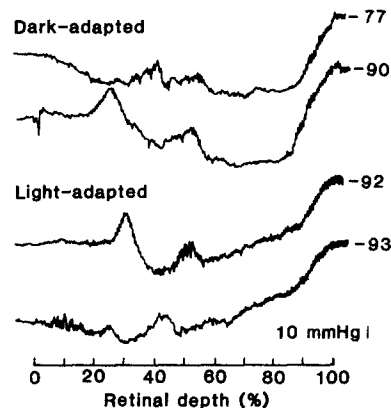


FIGURE 9. Continuous withdrawal of the electrode from the choroid to the vitreous during dark adaptation (top two traces) or light adaptation (maintained illumination at  $8.6 \log q/\text{deg}^2 \cdot \text{s}$ ). The top record was obtained first, followed by the two light-adapted records, followed in turn by the second dark-adapted record. The numbers on the right are the choroidal  $PO_2$ 's for each profile. Cat 12.

observed. The total distance of the withdrawals was greater than that of the penetrations by  $111.5 \pm 35.7 \mu\text{m}$ , or  $\sim 30\%$ . Most of this probably resulted from the electrode sticking to the tissue during withdrawal, and it is assumed that this resulted in a uniform distortion of the profile. This assumption was validated in some experiments in which the b-wave was recorded intermittently during the withdrawal.

Fig. 9 shows four separate withdrawals from one cat, two during dark adaptation and two during light adaptation, to illustrate both the similarities and differences observed in repeated measurements. These were recorded over a period of  $\sim 2$  h, with one dark-adapted profile being recorded before and one after the two light-adapted ones. These experiments revealed largely the same features as the stepwise penetrations. In the outer retina, there was a minimum  $PO_2$  of 0 mmHg in darkness, and a steeper increase from the minimum to the

choroid than during illumination. In these examples, the distance occupied by the dark-adapted minimum was about the same as that shown in Fig. 7, but in other examples it was smaller (Fig. 10). These profiles sometimes revealed a fine structure of the inner retinal  $PO_2$  that was not seen in the discrete penetrations. There were two local maxima in each of the withdrawals shown here, presumably corresponding to the different layers at which capillaries exist (e.g., Wise et al., 1971). A somewhat unusual feature of these particular profiles is the relatively high vitreal  $PO_2$  and the gradient from the vitreous into the retina. The significance of this will be considered in the Discussion.

*Oxygen consumption.* The oxygen profiles indicate that oxygen consumption ( $Q$ ) decreased during illumination in the outer retina. To attempt to evaluate this more fully, a model of steady state oxygen diffusion was fitted to the data. This analysis is possible only for the outer retina, where oxygen supply and consumption are spatially separated, and it cannot be applied to the vascularized inner retina at this time. The model assumes that diffusion of oxygen occurs only radially and that the curvature of the retina is small enough that the radial distance from the choroid can be represented by the variable  $x$  in a rectangular coordinate system ( $x = 0$  is the choroid, which is opposite to the way the data have been viewed so far). Oxygen consumption is assumed to be homogeneous over an interval  $\Delta x$ , but it may be different in the part of the avascular region near the choroid (region 1) than in the part near the retinal circulation (region 2). This steady state, two-region model is described by:

$$d^2P_1/dx^2 = (Q/Dk)_1 \quad \text{for } 0 < x < L1, \quad (1)$$

$$d^2P_2/dx^2 = (Q/Dk)_2 \quad \text{for } L1 < x < L, \quad (2)$$

with the boundary conditions:

$$P_1 = P_C \text{ at } x = 0,$$

$$P_2 = P_L \text{ at } x = L,$$

$$dP_1/dx = dP_2/dx \text{ at } x = L1,$$

$$P_1 = P_2 \text{ at } x = L1.$$

$P$  is oxygen tension (mmHg),  $Q$  is oxygen consumption (ml  $O_2$ /100 ml tissue·min),  $D$  is diffusion coefficient of oxygen ( $cm^2/s$ ),  $k$  is solubility (ml  $O_2$ /ml tissue·mmHg),  $L1$  is the boundary between the two regions of different  $Q/Dk$ , and the subscripts 1 and 2 denote the two regions.  $P_C$  is choroidal  $PO_2$  and  $P_L$  is the  $PO_2$  at the border of the avascular region,  $L$ . The actual value of  $L$  is not known for any particular penetration, since histological correlations were not done. Thus, the depth to be fitted was chosen in a somewhat arbitrary way, with the choice guided by assuming that the boundary was close to the point at which the profile reached a plateau in the inner retina. Choosing to fit slightly more or fewer data points did not have a significant effect on the fitted parameters, but it should be realized that the second boundary condition really involves an assumed value of  $L$  and the corresponding value of  $P$  at this point.

The solution to the equations above is:

$$P_1 = (Q/2Dk)_1 x^2 + Ax + P_C \quad \text{for } 0 < x < L_1,$$

$$P_2 = (Q/2Dk)_2 x^2 + Bx + C \quad \text{for } L_1 < x < L,$$

where

$$A = [(Q/Dk)_2 - (Q/Dk)_1]L_1 + B;$$

$$B = (P_L - P_C)/L - [(Q/Dk)_2 - (Q/Dk)_1]L_1^2/2L - (Q/Dk)_2L/2;$$

$$C = [(Q/Dk)_2 - (Q/Dk)_1]L_1^2/2 + P_C.$$

Since  $Q/Dk$  always occurs as a unit, it was not possible to determine the values of  $Q$ ,  $D$ , and  $k$  separately, so the fits gave the best values of  $Q/Dk$ . The variables obtained in the fitting were then  $(Q/Dk)_1$ ,  $(Q/Dk)_2$ , the boundary  $L_1$ , and  $P_C$  and  $P_L$ . The model equations were programmed in Fortran on a PDP 11/23 computer. In order to keep the fitting time reasonable,  $P_L$  and  $P_C$  were chosen and held fixed while the other parameters were allowed to vary in order to find the minimal total squared error between the model and data.  $P_L$  or  $P_C$  was then adjusted and the other parameters were allowed to vary again. In practice, the best-fitting values of  $P_C$  and  $P_L$  were close to the actual values at  $x = 0$  and  $x = L$ .

It was found that values of  $(Q/Dk)_1$ ,  $(Q/Dk)_2$ , and  $L_1$  could not be determined uniquely for a given set of data, presumably because of the noise in the data. It was possible, for instance, to keep the total squared error within a few percent of its minimum value while increasing both  $(Q/Dk)_2$  and  $L_1$  by as much as 20%. The parameter that was found to be more stable, and unaffected by variations in the individual parameters, was the weighted average  $Q/Dk$ , given by:

$$(Q/Dk)_{av} = (Q/Dk)_1(L_1/L) + (Q/Dk)_2(1 - L_1/L). \quad (3)$$

As individual parameters were varied,  $(Q/Dk)_{av}$  varied by about the same percentage as the error, and more confidence can be placed in its value than in the values of  $(Q/Dk)_1$  or  $(Q/Dk)_2$ .

Fits to one continuous light-adapted withdrawal and one dark-adapted withdrawal from the same retina are shown in Fig. 10. The data are shown as discrete points, where each point is the average of two successive digitized values. The fits were generally good, with an average error per data point of  $3.1 \pm 1.2$  mmHg ( $n = 33$  profiles). Not surprisingly, the fits were much better when two regions were assumed than when the entire avascular region was assumed to have a uniform consumption. In some cases, three-region fits would probably have been still better, but in view of the noise in the data and the limitations on the conclusions that can be drawn from individual parameters in the two-region fits, three-region fits were not attempted.

The means and standard deviations of parameters obtained from fitting 33 sets of data are summarized in Table I, which shows electrode penetrations and electrode withdrawals separately. The most important result of the modeling is shown in the bottom line.  $(Q/Dk)_{av}$  (Eq. 3) decreased by a factor of  $\sim 2$  during

steady state illumination at rod saturation. As one might expect, the reduction in  $(Q/Dk)_{av}$  during 10- or 15-s flashes was smaller than the reduction during several minutes of light adaptation. For the 10- or 15-s flashes given during electrode penetration,  $(Q/Dk)_{av,light}/(Q/Dk)_{av,dark}$  was 0.77, and for steady illumination (electrode withdrawals) it was 0.46. These average values are similar to those observed in each animal. The mean values of  $(Q/Dk)_{av,light}$  and  $(Q/Dk)_{av,dark}$  were significantly different ( $p < 0.001$ ) for the withdrawals. For the penetrations, these means were not different, since both the light and dark values varied

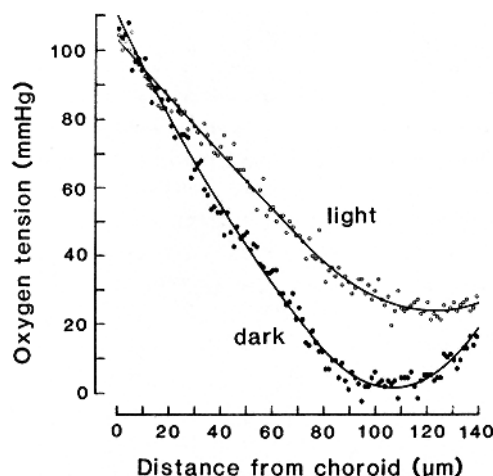


FIGURE 10. Fits of the oxygen diffusion model (solid lines) to the oxygen distribution in the outer half of the retina obtained in one dark-adapted (solid circles) and one light-adapted (open circles) withdrawal. Note that these data are plotted in terms of absolute distance, with the choroid at  $0 \mu\text{m}$ . A correction factor of 0.707 has been applied to the distance to account for the  $\sim 45^\circ$  angle between the electrode and the retinal surface. No correction for the drag between the tissue and electrode has been applied, but, as discussed in the text, such a correction would shorten the abscissa by  $\sim 30\%$  and increase the values of  $Q/Dk$  by  $\sim 69\%$ . The parameters for these fits, in the units of Table I, were, for the dark-adapted profile:  $P_C = 110$ ,  $P_L = 18$ ,  $L_1 = 71$ ,  $L = 139$ ,  $(Q/Dk)_1 = 0.47$ ,  $(Q/Dk)_2 = 1.76$ ,  $(Q/Dk)_{av} = 1.05$ . For the light-adapted profile:  $P_C = 103$ ,  $P_L = 26$ ,  $L_1 = 71$ ,  $L = 139$ ,  $(Q/Dk)_1 = 0.18$ ,  $(Q/Dk)_2 = 0.89$ ,  $(Q/Dk)_{av} = 0.44$ . The average error per data point was 3.00 mmHg for the dark-adapted and 2.64 for the light-adapted profile.

considerably. The value of  $(Q/Dk)_{av,light} - (Q/Dk)_{av,dark}$  computed for each animal and then averaged was different from zero, however, even for the penetrations ( $p < 0.01$ ). It seems unlikely that  $D$  or  $k$  could vary appreciably with illumination, so the changes in  $Q/Dk$  must be due predominantly to changes in  $Q$ .

The percentage reduction in  $(Q/Dk)_{av}$  obtained from the fitting is insensitive to errors in either the oxygen electrode calibration or the estimation of actual retinal depth, but all absolute values of  $Q/Dk$  are sensitive to such errors. An error of  $n\%$  in the slope of the oxygen electrode calibration would lead to an  $n\%$  error in the value of  $Q/Dk$ , but  $n$  is not likely to be very large. The major

error probably arises from the overestimation of retinal depth during the withdrawals. The dominant term in the solution to Eqs. 1 and 2 is  $(Q/Dk)x^2$ , and the values of  $x$  were probably at least 30% too high, as mentioned above. With a 30% smaller value of  $x$ , the value of  $Q/Dk$  would have to be 69% larger ( $1.30^2$ ), in order to keep  $(Q/Dk)x^2$  at the optimum value. The values for the withdrawals, corrected on this basis, would be  $(Q/Dk)_{av, dark} = 1.55 \times 10^{10}$  and  $(Q/Dk)_{av, light} = 0.71 \times 10^{10}$ . This brings the dark value close to that obtained during penetrations.

A strong conclusion cannot be drawn regarding the relative values of  $(Q/Dk)_1$  and  $(Q/Dk)_2$ . In 73% of the cases, the region closest to the choroid had a higher consumption, but in other cases this pattern was reversed. These parameters are rather sensitive to the details of the shape of the profiles, and even a minor distortion would cause this difference. On the average, in darkness it seems that region 1 has about twice the oxygen consumption of region 2 and that the border between the regions is about halfway through the avascular layer.

TABLE I  
*Parameters of Retinal Oxygen Diffusion Model*

	Penetrations		Withdrawals	
	Dark	Light	Dark	Light
<i>n</i>	7	4	15	7
$P_c$ (mmHg)	105±29	106±33	96±24	95±13
$P_L$ (mmHg)	10±6	14±4	14±6	18±7
$L_1$ (μm)	62±28	69±30	78±21	82±18
$L$ (μm)	127±19	128±23	149±8	149±9
$(Q/Dk)_1$	1.90±1.80	1.78±0.71	1.19±0.77	0.52±0.45
$(Q/Dk)_2$	1.24±1.00	0.28±0.29	0.53±0.68	0.53±0.62
$(Q/Dk)_{av}$	1.42±0.56	1.10±0.46	0.92±0.16	0.42±0.06

Fits of the diffusion model to data obtained in stepwise penetrations and continuous withdrawals (mean ± SD). Between 7 and 12 data points were fitted for the penetrations and between 75 and 120 for the withdrawals. The illumination was always 8.6 log q/deg<sup>2</sup>·s where light is indicated. For the penetrations, it was delivered as 10- or 15-s flashes, and for the withdrawals it was continuous. When multiplied by  $10^{10}$ ,  $Q/Dk$  has units of (ml O<sub>2</sub>/100 ml tissue·min)/(cm<sup>2</sup>/s) (ml O<sub>2</sub>/ml tissue·mmHg). When multiplied by  $1.67 \times 10^6$ ,  $Q/Dk$  has units of mmHg/cm<sup>2</sup>.

#### DISCUSSION

The measurements reported here indicate that the retinal oxygen tension changes dramatically with illumination in the intact cat retina, which is likely to be representative of mammalian retinas with similar vascular supplies. The changes in  $PO_2$  result from a light-induced decrease in oxygen consumption similar to that observed earlier in vitro (Sickel, 1972; Kimble et al., 1980; Zuckerman and Weiter, 1980) and, by indirect means, in vivo (Stefansson et al., 1983). These changes in consumption are relatively rapid and their amplitude depends on the log of stimulus illumination. Here I will consider the possible significance of these findings in light of retinal function, discuss two unexpected findings, compare these data with other estimates of retinal oxygen consumption, and suggest an interpretation for the time course of the responses.



During light adaptation, the choroid can supply about half of the retina with oxygen, but in darkness it can supply only about a quarter of the retinal thickness. In the dark, the  $PO_2$  is a few mmHg or less over part of the outer retina, even though the choroidal blood flow rate is very high (e.g., Alm and Bill, 1972; Weiter et al., 1973) and the choroidal circulation provides the retina with the highest possible  $PO_2$ . The high flow rate is essential in maintaining retinal oxygenation. If the flow rate were lower, more oxygen would be extracted per unit volume of blood, the average choroidal  $PO_2$  would decrease, and the anoxic region of the retina would be even larger.

The fraction of retina in which the  $PO_2$  is  $<5$  mmHg is considerably higher than the corresponding fraction for brain, which is only  $\sim 2\%$  (Lübbbers, 1977; Kessler et al., 1984). Based on a comparison of oxygen profiles with previously published anatomical descriptions (Vogel, 1978), it appears to be primarily the outer nuclear layer that tolerates the very low  $PO_2$ . This is possible probably because mitochondria and the enzymes of oxidative metabolism are concentrated in the photoreceptor inner segments (Lowry et al., 1956), where the  $PO_2$  is reasonably high. The energy needed by the outer nuclear layer may be derived partly from oxidative metabolism, since the appropriate enzymes are present in small amounts and since  $Q$  is probably constant until  $PO_2$  is quite low.  $K_m$  for  $Q$  in brain tissue is  $\sim 0.8$  mmHg (Buerk and Saidel, 1978), so that  $Q$  would still be  $\sim 80\%$  of normal when  $P_aO_2 = 4$  mmHg. The more hypoxic regions may also rely heavily on glycolysis (e.g., Glocklin and Potts, 1965; Winkler, 1981), since lactic acid dehydrogenase is localized in the outer nuclear and outer plexiform layers in monkey retina (Lowry et al., 1956). Interestingly, glycolysis decreases during illumination (Winkler, 1981), just as oxygen consumption does.

Light and dark differences in the oxygen profiles suggest a partial explanation for the sensitivity of the dark-adapted retina to mild hypoxia (Linsenmeier and Steinberg, 1984). Since the oxygen available under normoxic conditions is completely consumed, it is not surprising that metabolic processes in the photoreceptors would be affected by a very modest reduction in arterial  $PO_2$ . One of the main effects of hypoxia found by Linsenmeier and Steinberg (1984) was an increase in dark-adapted  $[K^+]_o$  in the subretinal space, which suggested that the rate of  $Na^+$  and  $K^+$  pumping by the photoreceptors had been reduced by the unavailability of energy. Hypoxia had a smaller effect during light adaptation, which was thought to result from the slowing of the pump during light adaptation and the smaller requirement for energy (e.g., Zuckerman and Weiter, 1980; Shimazaki and Oakley, 1984). This still seems a reasonable explanation for the changes in  $[K^+]_o$ , but the present work suggests that other processes in the outer retina that are not directly tied to the  $Na^+/K^+$  pump might also be less sensitive to hypoxia during illumination, when more oxygen is available.

Two other observations should be discussed here. The first is the oscillations of  $PO_2$  in the outer retina (e.g., Fig. 1). These oscillations were observed in every animal, but have not been reported in earlier work on mammals. The possibility that these oscillations were artifactual was considered. It is possible that electrode bending could somehow change the oxygen current, but the subretinal space is the region where the most stable recordings can usually be obtained, and oscillations were not observed in the voltage signals. It also seemed possible that,

since the gradient of  $PO_2$  in the outer retina is so steep, small oscillatory movements of the electrode relative to the tissue could produce this effect. However, oscillations were also observed in the choroid, where there is no gradient, and during illumination at the point of the dark-adapted minimum, when there is also a very shallow gradient. Thus, at the moment, these oscillations are interpreted as actual changes in  $PO_2$  of a few mmHg. As pointed out in the Results, oscillations were recorded only distal to the position of the minimum during dark adaptation. At the minimum, oscillations often appeared only during illumination (e.g., 70 and 79% in Fig. 1). No oscillation would be expected during darkness if the oxygen had been completely consumed by this point and the minimum  $PO_2$  were 0 mmHg, as suggested above for other reasons.

Another interesting observation was that the vitreal  $PO_2$  sometimes exceeded the inner retinal oxygen tension, which is contrary to the view that the oxygen in the vitreous must diffuse from the retina. There are two possible explanations for this. First, as Molnar et al. (1985) have shown, the gradient of  $PO_2$  in the vitreous is not always in the same direction, but depends on the proximity to the larger blood vessels. Near arteries, oxygen diffuses into the vitreous, but near veins, it may diffuse in the opposite direction. Second, the oxygen diffusion coefficient of the medium influences the measured oxygen current, and it is possible that the retina and vitreous have different diffusivities. This would lead to differences in oxygen current even if the  $PO_2$ 's are similar. If the diffusion coefficient in the vitreous were higher, higher currents would be expected there (Schneiderman and Goldstick, 1978). This error should be small if the recess in the electrode is long enough and contains the same material at all times.

The modeling leads to values of  $(Q/Dk)_{av}$  that suggest a maximal light-induced decrease in oxygen consumption of a factor of  $\sim 2$ . If a value for the product  $Dk$  is assumed, an absolute value of  $Q$  can be obtained. Measurements of  $Dk$  have not been made for mammalian retina, but several values have been reported for mammalian brain at 37°C:  $5.04 \times 10^{-10}$  ml  $O_2$ /cm·s·mmHg (Thews, 1960),  $3.30 \times 10^{-10}$  (Ganfield et al., 1970),<sup>1</sup> and  $5.09 \times 10^{-10}$  (Clark et al., 1978). If the average of these values (4.48) is used along with the corrected value of  $Q/Dk$  discussed in the Results, then  $Q$  in the outer retina during dark adaptation is estimated to be 7.0 ml/100 g·min. During light adaptation, it is 3.2. The values for whole mammalian retina, obtained in a variety of ways and converted to these same units, are 5.7 for pig (Tornquist and Alm, 1979), 5 for rat (Reading and Sorsby, 1962) (illumination not specified), and 5.7 for dark-adapted rabbit (Ames and Nesbett, 1981). Only the general agreement among these values should be noted, since outer retinal values cannot necessarily be compared to whole retinal values, and since species and illumination differences may play a role.

As far as light-dark differences are concerned, comparisons can only be made with in vitro data on the amphibian retina. Zuckerman and Weiter (1980)

<sup>1</sup> The reported value was 2.85, which, in their method, relied directly on a value for  $Dk_{water}$  at 37°C. Ganfield et al. (1970) used instead a value appropriate for  $Dk_{water}$  at 20°C. The use of more appropriate values ( $k_{water} = 3.15 \times 10^{-5}$  ml/ml·mmHg and  $D_{water} = 2.8 \times 10^{-5}$  cm<sup>2</sup>/s) results in the value of 3.3 used here.

reported that the light-adapted oxygen consumption was ~43% of the dark-adapted value for whole aspartate-treated frog retina. This is rather different from the 4% reduction reported by Kimble et al. (1980), and the ~10% reduction reported by Sickel (1972). The values reported in the present work are the only ones that are specifically for the outer retina. If they could be converted to a whole-retina basis, they would probably lie somewhere between these extremes.

It was hoped that the modeling could reveal regional differences in  $Q$  within the avascular layer, but the noise in the data and the minor distortion of the profile caused by the electrode have so far prevented a definitive conclusion. Other strategies for modeling, such as using a Michaelis-Menten function when the  $PO_2$  is low, or attempting to have only a narrow band of high consumption corresponding to the inner segments, may prove useful.

The time course of light-evoked oxygen responses must be determined by three factors: the time course of the metabolic change itself, the time for oxygen gradients to readjust after a change in metabolism, and the time constant of the electrode. The response time of the electrode is <200 ms, so it cannot play a significant role. The time for gradients to readjust after a local change of consumption is a matter of diffusion and is also fast. When one elevates the inspired oxygen concentration, the change in the outer retina has a time constant of only a few seconds (Linsenmeier, R. A., and C. Yancey, manuscript in preparation), which includes not only retinal diffusion, but also the time constant of the circulation and lung. Another way to look at the diffusion component is to consider a step change in  $PO_2$  at the boundaries of a slab 100  $\mu\text{m}$  thick and having a diffusion coefficient of  $1.4 \times 10^{-5} \text{ cm}^2/\text{s}$ . The time constant for the change in  $PO_2$  at the center of the slab would be only ~1 s (Crank, 1975). This time is much shorter than the observed time constant for light-evoked changes in  $PO_2$ , so it appears that the time course of light-evoked responses directly reflects the rate of change of oxygen consumption. The response times observed here are shorter than those in the work on frog (Kimble et al., 1980), which would be expected from the difference in body temperature, but in both frog and cat, the decrease in consumption at the onset of light appears to be slower than the increase at the end of illumination.

The cellular processes underlying the change in consumption cannot be specified completely at this time. Certainly, as Zuckerman and Weiter (1980) have emphasized, changes in the rate of the  $\text{Na}^+/\text{K}^+$  pump play a major role. The presence of some light-dependent change apart from the pump was demonstrated by Kimble et al. (1980), who found that in the aspartate-treated retina, one can reveal a light-evoked increase in oxygen consumption after the administration of ouabain. A possible mechanism for this might be the replenishment of GTP during illumination (e.g., Goldberg et al., 1983).

The present experiments have also revealed for the first time a decrease in oxygen tension in the inner retina during illumination. It is tempting to conclude that this implies an increase in inner retinal oxygen consumption resulting from increased activity of inner retinal neurons. Such a conclusion must be tentative, since the other mechanism for a decrease in oxygen tension, a decrease in oxygen supply, cannot yet be ruled out. Future work will be devoted to revealing the

mechanisms of changes in oxygen consumption in the intact cat retina during illumination.

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