**Purpose.** This work assessed the hypotheses that (1) hyperoxia is preferable to air breathing during retinal arterial occlusion, (2) hyperoxia during occlusion is beneficial in promoting recovery from arterial occlusion, and (3) hyperoxia has value even if it is delayed relative to the onset of the occlusion.

**Methods.** Reversible branch retinal artery occlusion was produced by pressing with a glass probe onto an artery emerging from the superior part of the optic disc in the retina of anesthetized cats. During 2-hour occlusion episodes, the cats breathed 100% O₂, 1 hour of air and 1 hour of 100% O₂, 1 hour of air and 1 hour of 70% O₂, or air. Intraretinal ERGs were recorded before, during, and after the occlusion.

**Results.** Hyperoxia during occlusion preserved intraretinal b-wave amplitude at 86% ± 12% of normal; longer durations of increased oxygenation maintained the b-wave at higher levels during occlusion and increased the probability of b-wave recovery after occlusion; higher O₂ content in the breathing gas increased b-wave amplitude during recovery; and hyperoxia during occlusion decreased the time it took for the b-wave to recover after the occlusion.

**Conclusions.** Hyperoxia is preferable to air breathing during retinal arterial occlusion not only for maintaining b-wave amplitude during occlusion, but also for providing a shorter recovery time and better percentage recovery after the end of the occlusion. Even if it is not possible to begin hyperoxia at the onset of occlusion, it may still be valuable. (Invest Ophthalmol Vis Sci. 2004;45:3690–3696) DOI:10.1167/iovs.04-0062

Retinal artery occlusion is often transient, but it may lead to blindness by preventing delivery of nutrients and causing the accumulation of waste products. This leads to structural and functional damage in the inner retina. Experiments with cats and monkeys have revealed that the electroretinogram (ERG) b-wave is restored to levels of 1.5 hours to longer than 4 days. The clinical picture is complicated by several factors. First, occlusion may not be complete in all cases. Second, patients generally have various periods of breathing air before being treated with O₂. Third, O₂ has been used for only brief periods due to potential toxicity to the lungs.

Because of this variability, it is not only difficult to interpret the data that oxygen therapy was beneficial in previous work, but also very difficult to use prospective clinical studies to obtain evidence on whether hyperoxia during occlusion is beneficial for restoring vision after occlusion. Animal studies are needed to fill this gap, but no animal studies have been attempted to investigate whether oxygen administered during occlusion is better than air breathing in enhancing recovery in the retinal circulation. In the present work, therefore, we focused on this question, employing parameters that are relevant to the clinical situation. We investigated the intraretinal b-wave during occlusion, but were more interested in recovery after reperfusion (i.e., recovery time and percentage of recovery) than in the changes during occlusion. We allowed an episode of air breathing during occlusion before instituting hyperoxia, to mimic the clinical situation. We chose an occlusion period of 2 hours, because we knew that occlusions of 97 to 98 minutes or more in monkeys breathing air alone lead to irreversible loss of the ERG. Furthermore, we investigated whether 70% O₂ breathing, which is more clinically viable than 100% O₂ for long periods, would promote recovery. We tested the hypotheses that (1) hyperoxia is preferable to air breathing to levels that were normal or nearly normal and in restoring outer retinal O₂. This occurred because hyperoxia dramatically elevated the P O₂ in the choroid.

Although it is well-established that hyperoxia can restore retinal oxygenation during occlusion, clinical studies on the use of hyperoxia have reported mixed results. Investigators in some clinical studies have observed that administration of hyperbaric O₂ does not improve acuity, except in one case, in which visual ability returned to normal after reperfusion. However, other studies suggest that 100% O₂ or hyperbaric oxygenation may have a beneficial effect if applied early enough. Although these results are variable, administration of a mixture of 95% O₂ and 5% CO₂ or 100% O₂ for short intervals during occlusion has been recommended to prevent retinal functional loss.

The value of oxygen therapy is difficult to gauge from clinical studies, because cases have been reported in the literature in which patients have not been given O₂, but still have regained vision after central retinal arterial occlusions lasting from 1.5 hours to longer than 4 days. The clinical picture is complicated by several factors. First, occlusion may not be complete in all cases. Second, patients generally have various periods of breathing air before being treated with O₂. Third, O₂ has been used for only brief periods due to potential toxicity to the lungs.
during retinal arterial occlusion, (2) enhanced O$_2$ breathing is beneficial in promoting recovery from an episode of arterial occlusion, and (3) hyperoxia has value even if it is delayed relative to the onset of the occlusion.

**METHODS**

**Animal Preparation and Recording**

In these experiments, we adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The experimental methods followed procedures for occlusion and intraretinal recording from the intact feline retina described previously by Braun and Linsenmeier, with the exception that a single-barreled voltage microelectrode was used instead of an O$_2$ microelectrode. In one cat (no. 324), a double-barreled pH microelectrode was used instead, but only voltage measurements are reported herein. Animal preparation is also described in other recent publications. Twelve anesthetized adult male cats were used.

To record intraretinal ERGs, the microelectrode was oriented toward the part of the retina supplied by the superior artery or, more often, by the area supplied by a secondary artery arising from the disc and running temporal to the superior artery. This artery was not paired with a vein, so that a simultaneous venous occlusion could be more easily avoided. Intraretinal and vitreal ERGs in response to 2.5-second flashes of diffuse white light at or near rod saturation (0.5 to 0.7 mcds·m$^{-2}$·sec$^{-1}$) were recorded during dark adaptation every 60 μm for the first 180 μm during the penetration into the retina and every 30 μm thereafter, until the choroid was reached. After amplification, ERGs were displayed on a storage oscilloscope and sent to a chart recorder and a computer, running data acquisition software (Labtech Notebook; Laboratory Technologies Corp., Wilmington, MA) operated through a shell program (Visual Basic; Microsoft, Redmond, WA).

**Occlusion of a Retinal Artery**

An occluder was made by pulling a 0.7-mm glass capillary tube to a tip, followed by heating the tip until a ball of 0.5- to 0.7-mm diameter was formed. The occluder was inserted into the eye through a 19-gauge, thin-walled, sealed needle and was attached to a hydraulic microdrive (model 1207S; David Kopf Instruments, Tujunga, CA). To produce an occlusion, the ball was pressed onto an artery emerging from the optic disc. Figure 1A shows a schematic representation of the location of the occluder with respect to the vessels. Visual observation of gaps in the vessel was used to verify that an occlusion had been produced. The occlusion was confirmed by a very small or absent intraretinal b-wave while the animal was breathing air during occlusion. At the end of the occlusion period, the occluder was carefully pulled back to allow reperfusion, which was verified by visual inspection.

**Experimental Protocol**

Experiments were designed to investigate the effect of inspired O$_2$ level on recovery from a 2-hour retinal arterial occlusion episode. There were four protocols (Fig. 1B) based on the inspired O$_2$ during the occlusion: 2 hours of 100% O$_2$, 1 hour of air and 1 hour of 100% O$_2$, 1 hour of air and 1 hour of 70% O$_2$, and 2 hours of air. The number of experiments and blood gas values are given in Table 1. Table 2 summarizes the experimental conditions and results.

Once the occluder and the microelectrode had been placed in the eye, the vitreal ERG and several intraretinal ERG depth series were

---

### Table 1. Summary of Blood Gas and Blood Pressure during Occlusion

<table>
<thead>
<tr>
<th>Inspired Gas</th>
<th>Number of Cats</th>
<th>Arterial Values during Air Ventilation</th>
<th>Arterial Values during Increased O$_2$ Ventilation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PO$_2$</td>
<td>PCO$_2$</td>
</tr>
<tr>
<td>100% O$_2$ [2]*</td>
<td>4</td>
<td>100.9 ± 3.7</td>
<td>29.0 ± 1.5</td>
</tr>
<tr>
<td>Air [1] and 100% O$_2$ [1]</td>
<td>4</td>
<td>102.4 ± 8.1</td>
<td>29.5 ± 1.8</td>
</tr>
<tr>
<td>Air [1] and 70% O$_2$ [1]</td>
<td>3</td>
<td>107.0 ± 5.0</td>
<td>29.7 ± 0.8</td>
</tr>
<tr>
<td>Air [2]</td>
<td>2</td>
<td>103.0 ± 5.0</td>
<td>32.4 ± 2.2</td>
</tr>
</tbody>
</table>

Data are expressed as mean mm Hg ± SD.

* Bracketed numbers are duration of gas-breathing conditions in hours.
TABLE 2. Summary of Experimental Conditions and Results

<table>
<thead>
<tr>
<th>Inspired Gas</th>
<th>Cat</th>
<th>Occlusions (n)</th>
<th>Duration of Occlusion (h)</th>
<th>Recovery Time (min)</th>
<th>Relative b-Wave Recovery (% Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% O2 [2]†</td>
<td>304</td>
<td>1</td>
<td>2.0</td>
<td>0</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>306</td>
<td>1</td>
<td>2.0</td>
<td>0</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>309</td>
<td>1</td>
<td>2.0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>100% O2 [2.5]</td>
<td>324</td>
<td>1</td>
<td>2.5</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>Air [1] and 100% O2 [1]</td>
<td>304</td>
<td>2</td>
<td>2.0</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>Air [1] and 100% O2 [1.5]</td>
<td>305</td>
<td>1</td>
<td>2.0</td>
<td>0</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>306</td>
<td>2</td>
<td>2.0</td>
<td>0</td>
<td>73</td>
</tr>
<tr>
<td>Air [1] and 100% O2 [2]</td>
<td>307</td>
<td>1</td>
<td>3.0</td>
<td>243</td>
<td>41</td>
</tr>
<tr>
<td>Air [1] and 70% O2 [1]</td>
<td>312</td>
<td>1</td>
<td>2.0</td>
<td>101</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>329</td>
<td>1</td>
<td>2.0</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>Air [2]</td>
<td>352</td>
<td>1</td>
<td>2.0</td>
<td>281</td>
<td>48</td>
</tr>
<tr>
<td>Air [2]</td>
<td>358</td>
<td>1</td>
<td>2.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Air [3.5]</td>
<td>309†</td>
<td>2</td>
<td>3.5</td>
<td>86</td>
<td>50</td>
</tr>
</tbody>
</table>

† shows the cats excluded from statistical analysis.

Bracketed numbers are duration of gas-breathing conditions in hours.

Vitreal and Intraretinal ERG Analyses

The vitreal and intraretinal ERGs were analyzed by calculating b- and c-wave amplitudes. Typically, the maximum intraretinal b- and c-wave occurred at ~50% and ~90% retinal depths, respectively. Table 3 summarizes the amplitudes of retinal and vitreal b-waves in different experimental conditions. The b-waves reported were measured at the location of the maximum retinal pigment epithelium (RPE) c-wave in the subretinal space, called the distal b-wave, rather than at the depth of the maximum b-wave. This convention was adopted because horizontal cell recordings were often inadvertently obtained where the maximum b-wave was expected, making it impossible to isolate the maximum b-wave from the set of traces obtained across the retina. The relationship between maximum b-wave amplitudes and the distal b-waves recorded in the same penetration is linear (r² = 0.92), with the distal b-wave amplitude being approximately 77% of the maximum b-wave amplitude.24

Table 3. Intraretinal and Vitreal b-Wave Amplitude Averages in the Different Experimental Conditions

<table>
<thead>
<tr>
<th>Inspired Gas</th>
<th>Number of Cats</th>
<th>Relative Intraretinal b-Wave (% Control)</th>
<th>Relative Vitreal b-Wave (% Control)</th>
<th>Relative Intraretinal b-Wave (% Control)</th>
<th>Relative Vitreal b-Wave (% Control)</th>
<th>Recovery Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% O2 [2]†</td>
<td>4</td>
<td>76 ± 14</td>
<td>87 ± 19</td>
<td>86 ± 12</td>
<td>70 ± 20</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Air [2]</td>
<td>2</td>
<td>0 ± 0</td>
<td>80 ± 18</td>
<td>0 ± 0</td>
<td>80 ± 10</td>
<td>Never</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD.

Statistics

All results are reported as the mean ± SD. Statistical significance was determined with Student’s t-test and was defined as a P < 0.05. Statistical analysis was performed in cases in which there was total occlusion for 2 hours.

After hypothesis testing (one-tailed t-test) with a significance level of 0.05, power analysis was performed using commercially available software (Power and Precision; BioStat Solutions, Inc., Mt. Airy, MD) to support the results of the t-test. A true difference of 15% in the population mean scores between two conditions was deemed to be functionally significant and was used in the power analysis. (Most of the statistically significant differences reported are larger than 15%.) The SD of the population was estimated as the square root of the summation of the variance divided by the number of data points. The power is given in the text for each test result.

Twelve anesthetized adult male cats were used in the study. The criteria for inclusion in the statistical analyses were to have (1) a full occlusion and (2) a 2-hour occlusion. In some cases (cats 304, 306, 309, and 329), we were able to perform a second occlusion. The precocclusion b-wave amplitudes before the second occlusion were 65%, 74%, 100%, and 57% of the precocclusion b-wave amplitudes before the first occlusion and the time between the two occlusions were 479, 142, 79 and 269 minutes in cats 304, 306, 309, and 329, respectively. Cat 329 was excluded from the analysis because it had a partial second occlusion, whereas cat 328 was excluded from the analysis because the duration of occlusion was longer than 2 hours.
Terminology

In this article, recovery time refers to the time for the intraretinal ERG b-wave amplitude to increase and stabilize after the restoration of retinal circulation.

RESULTS

Typical Time Course

Figure 2 shows examples of intraretinal and vitreal b-wave amplitudes before, during, and after retinal artery occlusions in the dark-adapted retina. These recordings were obtained under the two extreme conditions of 2 hours of air breathing (Fig. 2A) and 2 hours of 100% O₂ breathing (Fig. 2B) during occlusion. For each experiment, before the occlusion, intraretinal ERGs were recorded in a few retinal penetrations while the animal was breathing air, to serve as the control. Figure 2 displays typical intraretinal ERGs in response to 2.5-second flashes of diffuse white light at or near saturation (~8.6 log quanta [555 nm/deg²·sec]) during dark adaptation. Unlike the b-wave amplitude, the intraretinal c-wave amplitude did not show any trend under air or oxygen breathing during occlusion, compared with its preocclusion level (data not shown). In an earlier study, it was reported that a paired t-test showed no difference between the RPE c-wave before and during occlusion while the animal breathed air. Because the origin of the c-wave is complex and it did not reveal information about inner retinal function, which was expected to be most affected by the occlusion, the results on the c-wave are not reported.

Effect of Air Breathing during Occlusion

Figure 4 displays intraretinal and vitreal b-wave amplitudes under all experimental conditions, obtained from data of the type shown in Figure 2. During the occlusion, intraretinal b-wave amplitude decreased significantly from 100% to an average of 0% during air breathing (Figs. 2A, 4A). The average reduction in the vitreal b-wave was approximately 10%, which is consistent with the relatively small fraction of the retinal area that was affected by the occlusion.

Effect of Increased Oxygenation during Occlusion

To test the hypothesis that hyperoxia is preferable to air breathing during retinal arterial occlusion, a series of experiments was performed in which the animal breathed either 70% or 100% O₂ for 1 hour preceded by 1 hour of air breathing during occlusion or 100% O₂ for the duration of the occlusion episode.

Table 2 summarizes the experimental conditions and results. Table 3 shows the intraretinal and vitreal b-wave amplitudes under these experimental conditions. In all cases, enhanced oxygenation at least partly maintained the intraretinal b-wave (Table 3, Fig. 4A). Although the intraretinal b-wave amplitude was reduced to 34% ± 27% and 32% ± 24% of its preocclusion level when the animal was breathing 70% and 100% O₂ for 1 hour during occlusion, respectively, the presence of the b-wave was indicative that some inner retinal function was maintained during occlusion. The intraretinal b-wave amplitude was larger during occlusion when the animal breathed 1 hour of 100% O₂ (P = 0.011, n = 4, power = 1.00) and 70% O₂ (P = 0.055, n = 3, power = 0.85) than when the animal breathed air during occlusion. The intraretinal b-wave amplitude was significantly larger (P = 0.024, n = 4, power = 1.00) during occlusion when the animal breathed 2 hours of 100% O₂, as opposed to 1 hour of 100% O₂. The changes in the

Figure 4 displays intraretinal and vitreal b-wave amplitudes under all experimental conditions, obtained from data of the type shown in Figure 2. During the occlusion, intraretinal b-wave amplitude decreased significantly from 100% to an average of 0% during air breathing (Figs. 2A, 4A). The average reduction in the vitreal b-wave was approximately 10%, which is consistent with the relatively small fraction of the retinal area that was affected by the occlusion.

Effect of Increased Oxygenation during Occlusion

To test the hypothesis that hyperoxia is preferable to air breathing during retinal arterial occlusion, a series of experiments was performed in which the animal breathed either 70% or 100% O₂ for 1 hour preceded by 1 hour of air breathing during occlusion or 100% O₂ for the duration of the occlusion episode.

Table 2 summarizes the experimental conditions and results. Table 3 shows the intraretinal and vitreal b-wave amplitudes under these experimental conditions. In all cases, enhanced oxygenation at least partly maintained the intraretinal b-wave (Table 3, Fig. 4A). Although the intraretinal b-wave amplitude was reduced to 34% ± 27% and 32% ± 24% of its preocclusion level when the animal was breathing 70% and 100% O₂ for 1 hour during occlusion, respectively, the presence of the b-wave was indicative that some inner retinal function was maintained during occlusion. The intraretinal b-wave amplitude was larger during occlusion when the animal breathed 1 hour of 100% O₂ (P = 0.011, n = 4, power = 1.00) and 70% O₂ (P = 0.055, n = 3, power = 0.85) than when the animal breathed air during occlusion. The intraretinal b-wave amplitude was significantly larger (P = 0.024, n = 4, power = 1.00) during occlusion when the animal breathed 2 hours of 100% O₂, as opposed to 1 hour of 100% O₂. The changes in the

FIGURE 2. A typical time course of an experiment when an animal breathed (A) air for 2 hours during occlusion (cat 358), and (B) 100% O₂ for 2 hours during occlusion (cat 506). Each symbol represents the b-wave amplitude for a single penetration.

FIGURE 3. Typical intraretinal ERGs from the dark-adapted retina in response to 2.5-second flashes of diffuse white light at or near rod saturation (~8.6 log quanta [555 nm/deg²·sec]). ERGs were recorded (cat 359) in different retinal penetrations from the distal retina before occlusion (left) and when the animal breathed 1 hour of air and 1 hour of 70% O₂ during occlusion (middle panels) and air during recovery (right).
occlusion when the animal breathed 2 hours of 100% O2 as opposed to 70% O2 (55% ± 13% and 48% ± 7% of the preocclusion amplitude respectively), but the difference was statistically insignificant (Fig. 6). The intraretinal b-wave amplitude recovered to 86% ± 12% of its preocclusion value when the animal breathed 2 hours of 100% O2, which was significantly more than with 1 hour of 100% O2 inspiration (P = 0.011, n = 4, power = 1.00).

Special Cases

Achieving an exactly reproducible occlusion and recovery is difficult, and a few special cases should be considered. In cat 505 (1 hour air and 1 hour 100% oxygen), both the superior artery and vein were occluded because they were very close to each other, and it was not possible to occlude only the artery. Furthermore, during the initial installation of the microelectrode, it hit the retina and caused some hemorrhages. At the end of the occlusion, some hemorrhages were observed from venous occlusion. These features may account for the longer recovery time and smaller percentage of recovery in this cat.

Percentage of Recovery of Intraretinal ERGs

The recovery of the amplitude of the intraretinal b-wave is summarized in Tables 2 and 3 as well as in Figure 6. In all cases of full occlusion, air breathing during occlusion led to a complete failure of the b-wave to recover. Inspiration of elevated O2 during occlusion helped to restore the intraretinal b-wave amplitude. The intraretinal b-wave amplitude recovery was significantly greater (P = 0.002, n1 = 3, n2 = 2, power = 1.00) when the animal breathed 1 hour of 70% O2 as opposed to air during occlusion. The b-wave amplitude was generally larger after occlusion when the animal breathed 1 hour of 100% O2 as opposed to 70% O2 (55% ± 13% and 48% ± 7% of the preocclusion amplitude respectively), but the difference was statistically insignificant (Fig. 6). The intraretinal b-wave amplitude recovered to 86% ± 12% of its preocclusion value when the animal breathed 2 hours of 100% O2, which was significantly more than with 1 hour of 100% O2 inspiration (P = 0.011, n = 4, power = 1.00).

A principal purpose of this study was to evaluate the recovery of the retina after occlusion. At the end of the 2-hour occlusion episode, the occluder was slowly withdrawn with the aid of a microdrive. The intraretinal and vitreal ERG amplitudes were recorded for up to 2 hours after the intraretinal b-wave amplitude leveled off and are summarized in Tables 2 and 3. As shown in Figure 5, the recovery time reported in Tables 2 and 3 was the time for the intraretinal ERG b-wave amplitude to increase and stabilize after the restoration of retinal circulation.

In the 12 cats used in the study, 17 occlusions were performed. In three cats (three occlusions), there was no recovery at all when the animals were ventilated with air during occlusion versus occlusion intraretinal and vitreal b-wave amplitudes were statistically nonsignificant during enhanced O2 breathing (Table 3).

Recovery Time of Intraretinal ERGs

In 13 occlusions (11 cats) involving hyperoxia, 8 reversed completely immediately after the removal of the occluder and showed significant recovery. These eight occlusions were performed when the animal breathed air plus 100% O2 (1 hour each), air plus 70% O2 (1 hour each), or 100% O2 (2 hours) for the duration of the occlusion. The average recovery times depended on the oxygen conditions during occlusion, as expected. They were 0 ± 0 min for 2 hours of 100% O2 breathing, 58 ± 115 minutes for 1 hour air plus 1 hour 100% O2 breathing, and 127 ± 142 minutes for 1 hour air plus 1 hour 70% O2 breathing during occlusion. Partial recovery was an expected result and supported our hypothesis that enhanced O2 breathing would help maintain retinal function and reduce the recovery time as well.

**Figure 4.** Effect of oxygenation during 2 hours of occlusion on b-wave amplitude. Distal b-waves were collected from each penetration, generally at approximately 8-minute intervals. (A) Filled symbols: the average intraretinal b-wave (% of control) in each cat; open symbols: averages of all cats in each experimental condition. (B) Filled symbols: average vitreal b-wave (% of control) in each cat; open symbols: averages of all cats in each experimental condition. Error bars, SD. The intraretinal b-wave amplitude was larger during occlusion when the animal breathed 1 hour of 100% O2 (P = 0.011, n = 4, power = 1.00) and 70% O2 (P = 0.055, n = 3, power = 0.85) than when the animal breathed air during occlusion (*). The intraretinal b-wave amplitude was significantly larger (P = 0.024, n = 4, power = 1.00) during occlusion when the animal breathed 2 hours of 100% O2 as opposed to 1 hour of 100% O2 (**).

**Figure 5.** Percentage change in b-wave amplitude in cat 512 (experimental condition: 1 hour air and 1 hour 70% O2 breathing during occlusion). Average recovery after occlusion was calculated by averaging the percentage of b-wave amplitudes after they reached a plateau. Recovery time was the time between the end of occlusion and the start of the b-wave amplitude plateau.
than in others in the same occlusion conditions. In cat 309 (air during occlusion), two arteries (the superior artery and one other) reperfused immediately, but the second one reperfused 90 minutes later. For this reason, the occlusion duration is reported as 3.5 hours (Table 2), and this animal has not been included in the statistical analysis.

In cat 307 (1 hour air followed by 100% O\textsubscript{2}), occlusion did not reverse until an hour after withdrawal of the occluder, so the animal was kept under 100% O\textsubscript{2} breathing for this additional hour. Because of the longer occlusion time, this cat was excluded from the statistical analysis, but it shows that supplementation with O\textsubscript{2} can allow partial recovery after a 3-hour occlusion (Table 2).

**DISCUSSION**

An effective treatment protocol for retinal arterial occlusions has yet to be developed. Administering enhanced oxygen (hyperbaric or 100%) to the patient has been recommended,\textsuperscript{7,15–18} but only for brief periods, and the clinical experience has been mixed. To date, no clinical study has evaluated the effectiveness of an extended duration of hypoxia.

The focus in previous animal experiments was on the situation during occlusion. Whether hyperoxia leads to an improved recovery after the end of the occlusion episode has not been explored in any previous experimental work. In the present study, therefore, we investigated the intraretinal b-wave during occlusion, but were more interested in what happens after reperfusion (i.e., recovery time and percentage of recovery). We focused on parameters that may be achievable in a clinical situation. Although we could not duplicate the case of human retinal artery occlusion exactly, we allowed an episode of air breathing before hyperoxia, because hyperoxic therapy might not be available immediately after the onset of occlusion. We chose to make reversible occlusions of 2 hours' duration, because occlusions of 97 to 98 minutes or more have led to irreversible effects on the ERG with air breathing.\textsuperscript{5} Furthermore, we investigated 70% O\textsubscript{2} breathing, which is clinically viable\textsuperscript{25} for prolonged use.

Consistent with previous experiments, hyperoxia was shown to be beneficial during occlusion, although full recovery could not be achieved.\textsuperscript{4} When the O\textsubscript{2} content of the breathing gas decreased to 21% (air), inner retinal oxygenation decreased and the b-wave amplitude disappeared within minutes. Even with enhanced oxygenation, the retina is acidic during occlusion,\textsuperscript{26} and there may be other factors involved in the lack of full recovery during hyperoxia.

Hyperoxia was also preferable to air breathing for allowing recovery of the b-wave after occlusion. Even if hyperoxic therapy was not initiated immediately, it still had substantial therapeutic value. This is evidenced by the experiments in which 1 hour of air breathing was followed by 1 hour of enhanced O\textsubscript{2} breathing (either 70% or 100%) during occlusion, which resulted in significant recovery.

Another important finding is that 70% O\textsubscript{2}, which can be inspired indefinitely without toxicity,\textsuperscript{25} was as effective in promoting recovery after occlusion as inspiring 100% O\textsubscript{2} for 1 hour. Recovery may, however, take longer with 70% O\textsubscript{2}. We also found that the duration of enhanced oxygenation was critical for the recovery. Experiments with 2 hours of 100% O\textsubscript{2} breathing as opposed to 1 hour of 100% O\textsubscript{2} breathing showed significantly greater recovery. Although we did not run experiments with 2 hours of 70% O\textsubscript{2} breathing, we anticipate that it is preferable to 1 hour. The conclusions were drawn from experiments with a small number of animals, but they had statistical powers of 80% to 100%.

We recognize the danger of generalizing from cat to human and that the treatment protocol used in this study may not be optimal in humans. However, it will probably never be possible to conduct a controlled clinical trial, because occlusions are infrequent, and because their natural history is different from one patient to another. The present work has provided a proof of concept, showing that supplemental oxygen is beneficial under conditions in which better control is possible than in the clinic, even if there is a delay in starting it. It is possible that the recovery observed in cats may be less than could be achieved clinically with hyperoxic therapy, for several reasons. First, the body temperature is higher in cats than in humans, and the tissues are therefore probably metabolically more active and may be damaged more by the absence of adequate oxygen. Second, the physical occlusion method used in the study may cause some trauma to the vessels that may limit recovery. This type of damage may not be present in all cases of human retinal artery occlusion. Third, in a clinical situation, retinal arterial occlusions are rarely complete,\textsuperscript{27,28} and retinal tolerance to an acute ischemic event is often several hours, rather than minutes.

Pure oxygen for an extended time is toxic and also causes vasoconstriction of the retinal vessels. The vasoconstrictive effect can be counteracted by adding 5% CO\textsubscript{2}, although this may cause hypercapnia. Atebara et al.\textsuperscript{29} performed a retrospective study of paracentesis and carbogen inhalation and concluded that such treatments offer minimal benefits. However, in that study, carbogen was administered to the patients by a breathing mask for only 10-minute periods, 1 to 2 hours apart, for a total of 48 to 72 hours. This is more O\textsubscript{2} than has been given in other clinical studies, but still would not be expected to maximize the benefit of O\textsubscript{2}. Earlier, we suggested ventilating patients with a mixture of 95% O\textsubscript{2} and 5% CO\textsubscript{2} for perhaps 75% of the time during the occlusion, recognizing that compensatory changes for respiratory acidosis would occur.\textsuperscript{3} On the basis of the present findings, we recommend 70% O\textsubscript{2} (with or
without CO₂ as being almost equally effective. This should be given for as long as it takes an occlusion to resolve and should be started as soon as the occlusion is detected.

In summary, the results obtained in this study suggest an alternative to current treatment of retinal artery occlusion by using 70% O₂, which produces almost the same benefits as 100% O₂, in terms of ERG recovery, reduces the toxic side effects of 100% or hyperbaric O₂, and can be administered for prolonged periods.

Acknowledgments

The authors thank Christina Enroth-Cugell, Christina K. Chung, and Shufan Wang for help during the experiments.

References