Retinal Oxygen

Fundamental and Clinical Aspects

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We reviewed research on retinal oxygen (O₂) distribution and use, focusing on O₂ microelectrode studies in animals with circulatory patterns similar to those of humans. The inner and outer halves of the retina are different domains in terms of O₂. Understanding their properties can suggest mechanisms of and therapies for retinal diseases. Inner retinal PO₂ averages about 20 mm Hg. Effective O₂ autoregulation of the retinal circulation ensures that inner retinal PO₂ is relatively uninfluenced by systemic hypoxia and hyperoxia and increased intraocular pressure in healthy animals. Failures of the retinal circulation lead to tissue hypoxia that underlies the vasoproliferation in diabetic retinopathy and retinopathy of prematurity. Choroidal blood flow is not regulated metabolically, so systemic hypoxia and elevated intraocular pressure lead to decreases in choroidal PO₂ and photoreceptor O₂ consumption. The same lack of regulation allows choroidal PO₂ to increase dramatically during hyperoxia, offering the potential for O₂ to be used therapeutically in retinal vascular occlusive diseases and retinal detachment.

Oxygen (O₂) is essential for retinal function. As in other tissues, O₂ diffuses through the tissue passively from the circulation and is consumed in the oxidative production of adenosine triphosphate. However, several features make retinal oxygenation unique, and these have an impact on the pathogenesis and treatment of retinal diseases. Among the unique features influencing retinal oxygenation are the presence of a dual circulation, lack of metabolic regulation of the choroid, presence of O₂ regulation of the retinal circulation, and localization of mitochondria to photoreceptor inner segments. In most cases, it is useful to think of the retina as the following 2 domains: the avascular outer retina and the vascularized inner retina. The retina is also one of the most metabolically active tissues, consuming O₂ more rapidly than many other tissues, including the brain. Because the demand for O₂ is high, and because it cannot be stored in the retina or other tissues, a continuous O₂ supply is essential. This review focuses on vascularized retinas (eg, of the rat, cat, pig, monkey, and human) in which O₂ is delivered by the retinal and choroidal circulations. In some species, retinal vasculature is nearly or completely absent (eg, the rabbit and guinea pig). These retinas rely on the choroid almost exclusively and the inner retina is strongly glycolytic. A review focusing on avascular and partially vascularized retina has been presented elsewhere.

Retinal O₂ tension (PO₂) and other variables that provide an index of PO₂ have been measured in several ways, which have recently been presented by Hogeboom van Buggenum et al. Hemoglobin saturation in retinal vessels has been measured by several groups. Intravascular PO₂ has been measured with a phosphorescent dye, for which decay is inversely proportional to the PO₂. Berkowitz and coworkers have pioneered the development of magnetic resonance imaging techniques that measure absolute PO₂ in the preretal vitreous.
trode O2 consumption (QO2) is about the same as that the position of the intraretinal electrode.21,22 The inva-
retinal pigment epithelium, which allows verification of gram (ERG) and the transepithelial potential across the
trode has been used to record the local electroretino-
review. In some of this work, a second barrel of the elec-
will focus on these measurements in the first part of this
the retina with high spatial and temporal resolution. We
sitive nature of the microelectrode is the major disadvan-
of a single small cell. An electrode producing a current
significantly disturb the local O2 environment.25 Microelec-
3m LO2 per 100 g of tissue per minute.
6.2-µm cube of tissue in a region with a modest QO2 of
aged tissues of animals and humans using the fluorine signal
from perfluorocarbon droplets. They have also mea-
sured relative PO2 by using the dependence of the hydro-
gen wire, measures voltages generated in the retina (the intraretinal
electroretinogram and the transepithelial potential across the retinal pigment
epithelium). The copper wire from the other barrel is connected to a
polarizing voltage (−0.7 V) and a picoammeter to measure currents
proportional to PO2.
Oxygen recordings of the type presented herein are
obtained as shown in Figure 1. The microelectrode is
advanced with a microdrive through the retina in dis-
crete (eg, 3-µm) steps until it reaches the choroid. The
electrode is then withdrawn continuously, usually at a
constant speed of 2 µm/s in our work. The map of PO2
across the retina, an O2 profile, can be recorded during
microelectrode penetration or withdrawal, and these give
similar results. Data obtained during withdrawals are
preferable because one can be sure that the tissue and vas-
culature are not being compressed, and because a larger
number of data points can be recorded than during pen-
etration. In other experiments, the microelectrode has
been left stationary at a point in the retina or vitreous to
study responses to light,22,26 effects of altered inspired
gases,27-30 and spontaneous fluctuations in retinal PO2.31

THE NORMAL O2 PROFILE IN LIGHT AND DARK

Outer Retina

In vivo O2 profiles have been recorded from pigs,21,32
cats,22,33 rats,34 and monkeys.35 These studies have shown
similar O2 profiles everywhere except in the monkey fo-
vea. Under light-adapted conditions (ie, enough light to
saturate rod responses), the PO2 falls steeply between the
choriocapillaris (approximately 60 mm Hg) and the pho-
toreceptor inner segments and subsequently decreases
more gradually through the outer retina, as shown in
Figure 2. The direction of this gradient indicates that
all of the O2 used by the photoreceptors comes from the
choroid under light-adapted conditions. In darkness, the
PO2 in the outer retina is considerably lower,22,35 reach-
ing 0 mm Hg at the proximal side of the inner segments.
Sometimes much of the outer nuclear layer in the cat has
a PO2 of 0 mm Hg (Figure 2). This low value leads to a
reversal of the gradient of O2 proximal to the inner seg-
ment, so that in darkness, O2 also diffuses from the reti-
nal circulation to the photoreceptors.
The steep drop of PO2 from the choroid reflects the
high rate of O2 use by the photoreceptors.36,37 The high
choroidal PO2 is maintained by the unusually high flow
rate in the chorioidal circulation,38-40 which is necessary
for providing enough O2 to the retina.41

Figure 2. Intraretinal oxygen profiles across cat retina during light and dark
adaptations. The retina is shown schematically at the top. The 4 cell types
shown are (from left to right) retinal pigment epithelial cells, rod
photoreceptors, bipolar cells, and ganglion cells.

Figure 1. Schematic diagram of intraretinal oxygen measurements. A
double-barreled microelectrode is used as described elsewhere by
Linsenmeier and Vance20 and inserted into the vitreous through a 15-gauge
needle mounted onto a manipulator that allows gonioscopic motion. A boot
system is installed to maintain intraocular pressure and to prevent vitreal
leakage. The microelectrode is then connected to a microdrive that controls
movement in and out of the retina. One barrel, filled with isotonic sodium
chloride solution and connected to an amplifier via a silver/silver chloride
(Ag/AgCl) wire, measures voltages generated in the retina (the intraretinal
electroretinogram and the transepithelial potential across the retinal pigment
epithelium). The copper wire from the other barrel is connected to a
polarizing voltage (−0.7 V) and a picoammeter to measure currents
proportional to PO2.
Inner Retina

One or more O₂ peaks appear in the inner retina (Figure 2). These indicate O₂ sources and, therefore, the presence of nearby retinal vessels. The preretinal PO₂ in the vitreous is normally a good indicator of inner retinal PO₂, because the vitreous consumes very little O₂, and the inner retina is the source of vitreal O₂. The average PO₂ in the inner retina of the cat during dark adaptation was 18.5 mm Hg, which is in good agreement with the preretinal PO₂ in cats of 18.9, 20 to 30, 15 to 20, and 19 mm Hg. The similarity of retinal and vitreal P O₂ may break down under some conditions, however, including diabetes mellitus.

The values of inner retinal PO₂ cover a relatively wide range (Figure 3). The average PO₂ is a few millimeters of mercury lower in light adaptation. Oxygen extraction from the retinal circulation is high relative to that in many tissues, about 8 mL O₂ per 100 mL blood, resulting in a low venous PO₂ that contributes to making the average tissue PO₂ in the retina lower than in many organs.

Oxygen metabolism can be derived from material balances in vivo from blood arteriovenous differences or in vitro from reductions in PO₂ in a closed chamber. Oxygen consumption can also be obtained by fitting mathematical models of O₂ diffusion and consumption to PO₂ profiles. The use of material balances does not provide information on the spatial distribution of consumption, but was the first method to show that QO₂ was higher during dark than light adaptation in the whole retina. Further aspects of retinal energy metabolism, particularly the unusual amount of glycolytic metabolism, have been reviewed recently.

OXYGEN METABOLISM

Absolute values of photoreceptor QO₂ were computed by fitting a mathematical model of O₂ diffusion in the outer retina to the O₂ profiles. In the most satisfactory model, all the O₂ use is confined to the inner segment layer. It is important to realize that even though QO₂ is negligible in the outer segment and outer nuclear layers, linear gradients of O₂ still remain throughout these regions (Figure 2, Figure 4, and Figure 5). The evidence of a non-zero QO₂ is not the presence of a gradient but curvature in the profile, specifically a quadratic dependence of PO₂ on distance.

The Table shows that QO₂ of the photoreceptors during steady illumination is 36% to 68% of the value in the dark. This agrees with the effect of light found by other methods in the rat, rabbit, and pig. Above rod saturation, which is below-normal room illumination, no further changes in QO₂ are observed in the rod-dominated area centralis of the cat retina. Modeling also shows that the choroid is essential to photoreceptor metabolism, contributing approximately 90% of the O₂ consumed by the photoreceptors in darkness, and all of the O₂ consumed...
Oxygen Consumption Rate Measured From Animals With Vascular Retinas, During Dark and Light Adaptation in Outer and Inner Retina *

<table>
<thead>
<tr>
<th>Subject</th>
<th>Reference (Year)</th>
<th>Dark</th>
<th>Light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer retina</td>
<td>Medrano and Fox, 1995</td>
<td>3.7</td>
<td>2.3</td>
</tr>
<tr>
<td>Cats</td>
<td>Braun et al, 1995</td>
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<td>1.4</td>
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<td>Cats</td>
<td>Haugh et al, 1990</td>
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</tr>
<tr>
<td>Cats</td>
<td>Braun and Linsenmeier, 1995</td>
<td>4.9</td>
<td>2.6</td>
</tr>
<tr>
<td>Monkeys</td>
<td>Ahmed et al, 1993</td>
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</tr>
<tr>
<td>Rats</td>
<td>Medrano and Fox, 1995</td>
<td>2.4</td>
<td>2.3</td>
</tr>
<tr>
<td>Rats</td>
<td>Braun et al, 1995</td>
<td>3.5</td>
<td>3.7</td>
</tr>
</tbody>
</table>

*Data are expressed in milliliters of oxygen per 100 g of tissue per minute.

During light adaptation, the choroid contributes approximately 50% of the O2 consumed by the whole retina in dark adaptation, which is consistent with earlier estimates based on choroidal arteriovenous O2 differences and blood flow.

The Table gives values for QO2 as though the consumption were uniform across the whole outer retina. We have preferred to report the values this way, because it reduces the scatter in the measurements and makes values from modeling more comparable to those obtained by other methods. These values are somewhat misleading, however, since the QO2 is localized to the inner segments. The rod inner segments have an extremely high QO2 of 15 to 20 mL O2 (standard temperature and pressure) per 100 g of tissue per minute in the dark, consistent with the high density of mitochondria in this region.

Little is known about cone QO2. Light does decrease the QO2 of monkey fovea. Cones in the monkey have a volume of mitochondria that is about 10 times that of rods, but we have argued that QO2 of an individual cone could not be higher than that of a rod by a factor of 10, because the smaller number of mitochondria in rods already uses all the O2 available from the circulation.

Oxygen is used in photoreceptors for many processes, but the ones that appear to require the most energy are maintenance of the dark current, ie, pumping out the large load of sodium ion that enters through the light-dependent channels and generating guanosine triphosphate, from which cyclic guanosine monophosphate is produced. The dark current decreases dramatically during illumination, whereas the guanosine triphosphate turnover increases. These appear to be the only processes whose metabolic demands change significantly with illumination. The change in sodium ion pumping is larger, however, and, as a result, illumination causes a net decrease in photoreceptor QO2 in all species investigated to date.

Inner Retina

In the inner retina, the retinal circulation forms a 3-dimensional mesh, which makes mathematical modeling difficult. The retinal circulation must be occluded to allow the application of a diffusion model to the inner retina. Despite this limitation, various lines of evidence indicate that in the inner retina, no difference in QO2 exists between darkness and steady illumination (Table). The metabolism of the inner retina is higher when flickered light (4 Hz) is applied as opposed to steady light, presumably because flickered light leads to greater neural activation. Unfortunately, this increase is difficult to quantify.

RETNAL OXYGENATION UNDER ALTERED CONDITIONS

Hypoxemia

Figure 3 shows intraretinal O2 profiles from the dark-adapted cat retina during normoxia and hypoxemia, induced by adding nitrogen to the inspired gas. In the inner retina, tissue PO2 is effectively regulated across a wide range of PaO2. The decrease in preretal PO2 is only 0.14 to 0.18 mm Hg/mm Hg PaO2 when the PaO2 is above 35 mm Hg, and 0.2 mm Hg/mm Hg PaO2 in the miniature pig. When the PaO2 falls below 35 mm Hg, PO2 in the inner retina decreases more steeply, approximately 0.62 mm Hg/mm Hg PaO2. The good regulation at PaO2 of greater than 35 mm Hg results from vasodilation of the retinal circulation, which in cats can increase blood flow by more than a factor of 3 during hypoxemia.

In the outer retina, hypoxemia leads to a steep decrease in choroidal PO2 (Figure 4) of approximately 0.64 mm Hg/mm Hg PaO2. This occurs because the choroidal blood flow does not increase in hypoxemia (Melissa Fulfer, Jameel Ahmed, PhD, and R.A.L., unpublished data, June 2000). There would be little advantage of increasing choroidal blood flow, even if this were possible. The usual advantage of increasing blood flow to a tissue during hypoxemia is allowing the arteriovenous O2 difference to be reduced, which minimizes or prevents a decrease in venous PO2 during hypoxemia. In the choroid, the arteriovenous O2 difference is already very small, only about 1 volume percent, due to the high rate of blood flow. In relatively mild hypoxemia, arterial PO2 drops below the normoxic choroidal venous PO2. Reducing the arteriovenous difference could not prevent this. Because photoreceptor metabolism is O2 limited under normoxic conditions in darkness, even mild hypoxemia (PaO2 of 60 mm Hg) reduces the flux of O2 to the inner segments and, therefore, reduces photoreceptor QO2.

When hypoxemia occurs during light adaptation, choroidal PO2 also falls, but in this case, metabolism is not O2 limited, so photoreceptor QO2 is not affected.

Elevated Intraocular Pressure

When the intraocular pressure (IOP) is elevated, the effects on retinal PO2 are similar to those observed during hypoxemia. Decreased perfusion pressure does not affect PO2 in the inner retina owing to effective autoregulation in the retinal circulation. In contrast, when the IOP is elevated, the choroidal blood flow decreases, which leads to a reduction of choroidal PO2 of approximately 0.5 mm Hg/mm Hg of perfusion pressure. This reduction leads to a reduction in photoreceptor QO2.

An extension of this finding is that anything that decreases choroidal blood flow would be
expected to have a negative impact on retinal oxygenation and on photoreceptors. There is evidence that human and experimental glaucomas affect photoreceptors, possibly because they reduce photoreceptor O2 supply, and there is direct evidence of retinal damage by reduced choroidal blood flow in birds.

**Hyperoxia**

Figure 5 compares a normoxic profile to one obtained during hyperoxia (inspiration of 100% \( O_2 \)). Vitreal PO2 and inner retinal PO2,\(^{29,30,32,37,79}\) are well regulated during hyperoxia in the cat, pig, and rat and presumably in humans. This regulation is due to constriction of retinal vessels\(^{10,66,80}\) and reduction of retinal blood flow.\(^{30,66,83}\) In the miniature pig, PO2 in the inner retina has been reported to be perfectly regulated during hyperoxia in intervascular zones,\(^{29,32}\) but it increases by an average of approximately 40 mm Hg in juxta-arteriolar zones, the same value observed in cats.\(^{30,37}\) Adding 5% carbon dioxide to the inspired gas leads to larger increases in inner retinal PO2 during hyperoxia.\(^{16}\) The most striking change during hyperoxia is the increase in choroidal PO2 to about 250 mm Hg in cats\(^{28}\) and 220 mm Hg in miniature pigs.\(^{32}\) This increase is a consequence of the lack of metabolic regulation of choroidal blood flow.\(^{82}\)

It is sometimes thought that hyperoxic therapy has a limited potential for treating retinal disease. This is based on the idea that the blood is nearly saturated with O2 during normoxia, so that the small additional O2 content during hyperoxia should be of little benefit. The fundamental misconception is that the O2 supply to the tissue relies on O2 saturation, whereas, in reality, O2 moves into tissue by simple diffusion, which is driven by gradients of PO2, not saturation. The PO2 gradient from the choroid is much steeper during hyperoxia, and a greater portion of the retina can be supplied by the choroid during hyperoxia than during normoxia.\(^{30,32}\) as shown in Figure 5.

Despite an abundance of O2, higher photoreceptor \( QO_2 \) was not found in cat retina during hyperoxia.\(^{30,37}\) Pournaras and coworkers\(^{32,83}\) argued that \( QO_2 \) increased during hyperoxia in healthy retinas of the miniature pig, but not in retinas with a branch vein occlusion. Their conclusions were not based on calculations of \( QO_2 \), however, but only on the steepness of gradients, which cannot be used to infer values for \( QO_2 \). The gradient through the outer segments is steeper during hyperoxia, so the flux across them is larger, but they never use any O2.

For a few hours, at least, hyperoxia does not impair retinal electrical function in animals,\(^{77}\) but the issue of O2 toxicity to the retina has not been fully addressed. It is clear that O2 can be damaging to photoreceptors if combined with high levels of illumination.\(^{89,95}\)

**CLINICAL ISSUES**

**Diabetic Retinopathy**

Diabetic retinopathy is a disease predominantly of the retinal vasculature, leading first to capillary occlusion and then to vascular proliferation. For many years, tissue hypoxia has been suggested to be involved in the progression of diabetic retinopathy and retinal neovascularization in general.\(^{87}\) By the time that capillary nonperfusion is clinically observable, tissue hypoxia has likely occurred, but the exact time when hypoxia begins is as yet unknown. Retinal tissue hypoxia was not found in the early stage of diabetic retinopathy in animal models.\(^{46,88,89}\) However, intraretinal O2 measurements on cats with long-term (6-7 years) diabetes mellitus showed an abnormally low average inner retinal PO2 of 7.7 mm Hg (compared with 16.4 mm Hg in normal cats).\(^{47}\) This report, the only one to provide intraretinal measurements, stressed that tissue hypoxia was present in the diabetic retina before capillary dropout was evident and when only a few microaneurysms were present. Further evidence of hypoxia relatively early in the disease was provided by the observation that hyperoxia reversed early contrast sensitivity deficits\(^{86}\) and oscillatory potential reductions.\(^{94}\) In addition, Arden et al\(^{91}\) provided evidence of altered dark adaptation in diabetes mellitus, which was also suggested to be an effect of hypoxia.

Hypoxia may occur during diabetic retinopathy due to capillary occlusion. Leukocytes have been reported to be present in greater number and are less deformable in diabetic cats.\(^{93}\) Increased levels of endothelial cell/leukocyte adhesion molecules (intercellular adhesion molecule 1 and P-selectin) have also been observed in humans with diabetes mellitus (hereafter referred to as diabetic humans).\(^{94}\) These findings may explain the increase of leukocyte activation and adhesion in diabetic humans\(^{95}\) and rats,\(^{96}\) which are known to increase vascular resistance\(^{97,98}\) as capillaries become plugged. In addition, a hyperglycemic (hyperosmotic) environment increases the adhesion of leukocytes to retinal endothelial cells.\(^{99}\)

The findings of tissue hypoxia and occlusion of retinal capillaries by leukocytes are consistent with observations that retinal blood flow is reduced before and in the early stages of diabetic retinopathy.\(^{100-103}\) A subsequent increase in retinal blood flow occurs,\(^{102,103}\) possibly because lower-resistance shunt pathways develop and carry some of the flow\(^{102}\) and/or because vascular endothelial growth factor (VEGF) dilates vessels.\(^{103}\) The flow is reduced again in proliferative retinopathy.\(^{104,105}\) Complete consensus on this sequence of events has not been achieved, but they seem to explain most of the observations.

One of the puzzles about diabetic retinopathy has always been why it affects the microvasculature of the retina more than that of the brain.\(^{100}\) Arden et al\(^{92}\) suggested that dark adaptation aggravates hypoxia by depriving the inner retina of the small amount of O2 that diffuses from the choroid during light adaptation. They suggested further that avoiding a long period of dark adaptation (eg, during sleeping at night) could be an alternative therapy for diabetic retinopathy.

Hypoxia is thought to induce the synthesis of VEGF\(^{106,108}\) which is present in higher levels during proliferative diabetic retinopathy.\(^{109,110}\) Vascular endothelial growth factor has also been found in eyes with no retinopathy or only early signs of retinopathy.\(^{111-113}\) This initially suggested that factors besides hypoxia may induce the production of VEGF,\(^{114}\) but we now know that the retina is hypoxic early in the disease.\(^{47}\) Although VEGF...
is important, other angiogenic factors may also be involved. A detailed discussion on factors involved in intraocular neovascularization is available elsewhere. A competing theory of vasoproliferation implicated the mechanical aspect of vasodilation resulting from hypoxia or hyperglycemia. This theory has received little attention recently, but it seems prudent to recognize that mechanical factors could play a role in angiogenesis.

The O2 of the retina in diabetes mellitus has been a subject of controversy. In the intact cat retina, photoreceptor O2 was lower on average in subjects with diabetes than in healthy subjects, but O2 has a strong positive correlation with choroidal PO2, and the choroidal PO2 values tended to be lower in diabetic cats. Whether lower choroidal PO2 is a general feature in diabetes could not be determined with the number of animals available in that study. In vitro studies in rabbits suggested that the QO2 decreased in diabetes. In contrast, one study reported that QO2 in diabetic rats was greater. Little work has been done on the QO2 of the inner retina. Tiedeman et al found that retinal venous O2 saturation was lower than in healthy subjects, but QO2 has a strong positive correlation with choroidal PO2, and the choroidal PO2 values tended to be lower in diabetic cats. Whether lower choroidal PO2 is a general feature in diabetes could not be determined with the number of animals available in that study. Increased QO2 is difficult to reconcile with capillary dropout and inner retinal damage.

On the theory that one of the major problems in diabetic retinopathy is lack of O2 in the inner retina, therapies have been designed to improve the O2 supply. Panretinal photocoagulation is effective in treating proliferative retinopathy. Studies on animal models and in humans showed that PO2 in the preretinal vitreous is decreased in diabetes. These measurements provided little detail about O2 gradients in the retina, and were obtained mainly during hyperoxia, which is not the condition in which diabetic retinopathy develops or progresses. Nevertheless, they provide some evidence that photocoagulation reduces the QO2 of the photoreceptors, allowing more O2 to diffuse from the choroid to the inner retina.

At present, no treatment stops retinopathy apart from panretinal photocoagulation, but it seems reasonable that if early retinal circulatory changes preceding VEGF up-regulation could be detected clinically, then interfering with leukocyte adhesion or using other pharmacological techniques to increase retinal blood flow might be effective in preventing retinopathy.

Retinopathy of Prematurity

Retinopathy of prematurity (ROP), previously known as retrolental fibroplasia, develops in 3 distinct stages. First, the retina of premature infants is exposed to excess O2 as a result of breathing O2-enriched gas, which causes constriction and irreversible closure of retinal vessels and prevents new vessels from developing normally. As a result, hypoxia is thought to develop in the inner retina when the infant is returned to breathing air, and the extra supply of O2 from the choroid is lost. Finally, this hypoxic condition induces vascularization to counteract the hypoxia, but for some reason this vascularization is abnormal.

The current therapy for prevention of the disease is generally to titrate the infant’s blood to an adequate PaO2 but to prevent systemic hyperoxia. Moderate supplemental O2 with a target arterial saturation of 99% and a PaO2 of less than 100 mm Hg was found to decrease prethreshold ROP. Vitamin E supplementation may also be useful in decreasing the severity of ROP, possibly by minimizing auto-oxidative reactions that arrest the growth of normal blood vessels.

Other therapies, such as gradual reduction in O2 level (oxygen weaning) appear less promising.

Mechanistically, the disease is not as straightforward as the above discussion would suggest. As in diabetic retinopathy, it has been impossible to determine exactly what the oxygenation status of the human retina is at any time during the disease progression, and difficult even in the animal models. Ernest and Goldstick used O2 microelectrodes in the vitreous humor of kittens after O2 supplementation (80%-90% O2) and found that preretinal PO2 over the avascular retina was close to 0 mm Hg, but normal over the vascular retina. These remain the only direct measurements of PO2 in an ROP model. More recently, attention has shifted to a rodent model of ROP called ischemia-induced or O2-induced retinopathy. Retinopathy is produced most effectively by a regimen that cycles the inspired gas between a relatively hypoxic one and a relatively hypoxic or normoxic one for at least several days during retinal vascular maturation.

Surprisingly, well-controlled hyperoxia followed by normoxia produces ROP-like symptoms with less certainty than does O2 cycling. In O2-induced retinopathy, some evidence from novel magnetic resonance imaging measurements suggests that hypoxia plays a role early in the disease. Berkowitz and Penn based this conclusion on a blunted response of retinal oxygenation to breathing of carbogen (a mixture of 95% O2 and 5% carbon dioxide) in animals in which histological signs of the disease developed, rather than a direct demonstration of tissue hypoxia.

As in diabetic retinopathy, it is thought that hypoxia does not directly affect vessel growth, but instead influences the production of angiogenic factors in the retina, with the probable involvement of hypoxia-inducible factor 1 as an intermediate.

Vascular Occlusive Diseases

Work on complete ocular ischemia, produced in general with elevation of IOP, and work on the effects of anoxia on the retina in vitro have a long history. Although they are relevant to ocular ischemic syndrome, in which the retinal and choroidal circulations are compromised by corneal artery obstruction, they will not be reviewed herein. Of more interest from a clinical standpoint is the role of O2 in central and branch venous and arterial occlusions of the retinal circulation. Experimental venous occlusion in pigs leads to decreased inner retinal PO2 which can be followed by neovascularization. Work on cats shows that retinal arterial occlusion makes the entire inner retina anoxic and this causes a rapid failure of the ERG b wave.
in cats and monkeys. Even when the animal breathes air, the retina is reasonably tolerant to occlusion, surviving episodes of 100 to 120 minutes with full recovery of the ERG after the occlusion is reversed. Several studies have shown that retinal oxygenation can be partially or completely restored during the arterial occlusion by making animals hyperoxic. It is clear from $O_2$ profiles that this effect relies on an increased $O_2$ supply from the choroid during hyperoxia. Corroborating the importance of $O_2$ as the limiting factor during occlusion are a few studies that have used a model of total ischemia. Anderson and Saltzman have shown that if human subjects breathed $O_2$ before IOP elevation, their vision was sustained longer than if they breathed air. Blair and coworkers have shown that perfusing the vitreous with an oxygenated solution after total occlusion can maintain the structural integrity of the retina and the ERG. Despite this positive experimental data, the clinical experience with hyperoxia has been mixed, with only a few studies recommending hyperoxia as a treatment for vascular occlusion. Unfortunately, most clinical attempts to provide hyperoxia during occlusion have not adequately considered that it must be provided for large blocks of time and as soon as possible because $O_2$ is not stored and is used so rapidly. In addition, there have been two fears of using hyperoxic therapy. First, hyperoxia constricts retinal vessels, and this might impede the clearance of emboli. This may be an unnecessary concern, because the inner retina is acidic during occlusion (Gulnur Birol, PhD, N.D.W., Ewa Budzynski, MS, and R.A.L., unpublished data, October 2002), which would tend to counteract hyperoxic vasoconstriction. In addition, carbon dioxide can be added to the inspired gas to induce vasodilation. Second, extended $O_2$ breathing can be toxic, but this should not prevent hyperoxic therapy, because it is possible to breathe 60% to 70% $O_2$ for hours without toxic effects.

Other Diseases

Oxygen undoubtedly plays a role in other diseases. Several studies report that cystoid macular edema can be ameliorated by hyperbaric $O_2$ therapy. No clear theoretical reason for this exists. Depending on the mechanism of this effect, hyperoxia at normal atmospheric pressure may do just as well. This has not been tested, but should be, because it would allow application of the therapy to more patients.

Oxygen is likely to play a role in the dramatic vasodilatation of the retinal circulation that is associated with photoreceptor degenerations in humans and animals. This role can be understood on the basis of the normal gradients. When photoreceptors are lost, $O_2$ derived from the choroid that was once used by photoreceptors now diffuses all the way to the inner retina. In addition to the reduced size of the major vessels, capillaries in the inner retina are permanently lost in animals with photoreceptor degeneration. This capillary loss can be prevented by maintaining the animals under hypoxic conditions, which probably reduce the flux of $O_2$ from the choroid to the inner retina. Evidence also exists that $O_2$ can modulate the survival of photoreceptors early in the Royal College of Surgeons rat model of retinal degeneration. Hyperoxia is protective at early stages, whereas hypoxia induces death of photoreceptors. Up-regulation of basic fibroblast growth factor by hypoxia is not sufficient to prevent photoreceptor death, and later stages of the photoreceptor loss may not be caused by hypoxia.

Although a focus on mechanical causes of ganglion cell loss in glaucoma continues, many lines of evidence indicate that reduced ocular blood flow, and by implication reduced $O_2$, is a contributing factor in the retinal damage in glaucoma. This is a complex area in which most of the evidence is indirect, but support for a vascular hypothesis continues to accumulate. First, hyperbaric $O_2$, which does not change IOP, can improve visual fields in glaucomatous subjects. Second, photocoagulation, which destroys photoreceptors and presumably increases the inner retinal $O_2$ supply from the choroid, promotes survival of ganglion cells in glaucomatous monkeys. Third, cones, which should not be affected directly by mechanical stresses at the optic nerve, are swollen in eyes of humans with glaucoma and in primates with experimental glaucoma. Finally, systemic vascular variables, which have been known to be risk factors for glaucoma, help predict the degree of visual dysfunction in glaucoma.

Oxygen may also play a role in vision loss in retinal detachment. A recent argument has been made on a theoretical basis that hyperoxia may be useful in preventing photoreceptor damage. Detachment separates the inner segments from their $O_2$ supply, and although no consuming tissue is found under the retina, the increased distance reduces the flux of $O_2$ from the choroid to the photoreceptor inner segments. Hyperoxia should restore this flux, at least for detachments of moderate height, because increased choroidal $O_2$ can compensate for the increased distance. For large detachments, the photoreceptors may benefit more from increased amounts of $O_2$ in the retinal circulation than in the choroidal circulation. The protective effect of hyperoxia on detached photoreceptors has been shown experimentally in cats, in which hyperoxia was able to save photoreceptors and prevent the activation of retinal glia normally caused by detachment.

CONCLUSIONS

Most of the important features of normal retinal oxygenation are now known, and the status of retinal oxygenation in some prevalent diseases has been partially elucidated. Tissue hypoxia is believed to be important in regulating vascular growth factors, particularly in the vasoproliferation observed in diabetes mellitus and ROP. Hypoxia is also involved in the loss of photoreceptors during a retinal detachment and the loss of inner retinal neurons in retinal arterial occlusions. Evidence is emerging that hypoxia may damage photoreceptors and possibly other cells in glaucoma, and that $O_2$ modulates the survival of photoreceptors in photoreceptor degenerations. One of the most interesting aspects of retinal oxygenation is the important metabolic role of the choroidal circulation. Lack of regulation of the choroid by $O_2$ may be detrimental to the photoreceptors under conditions when choroidal $O_2$ or choroidal blood flow decrease (eg, glau-
coma), but this same lack of regulation provides an opportunity to use the choroidal therapeutically in treating cystoid macular edema, retinal arterial occlusions, and retinal detachment. Oxygen therapy only has a chance of working if it is sustained, however, and this has generally not been the mode in which it has been used.

The difficulty of measuring $O_2$ intraretinally in humans or in animal models of human diseases has prevented a more complete understanding of the role of $O_2$ in retinal diseases. Measuring retinal $O_2$ would be far better than measuring blood flow, because tissue $PO_2$ is the variable that is directly relevant to metabolism and presumably to visual function. Noninvasive measurements have been difficult, because of the spatial heterogeneity of $PO_2$ in the retina and the difficulty of accessing the choroid, but these techniques are important and need to be developed. Development of noninvasive optical techniques would allow one to study the timing of hypoxia with respect to biochemical and histological changes, and would serve as a way to follow disease progression and evaluate treatments.

Submitted for publication January 4, 2002; final revision received November 15, 2002; accepted December 6, 2002.

This study was supported by grant EY05034 from the National Institutes of Health, Bethesda, Md.

We thank Lissa Padnick-Silver, PhD, for useful feedback.

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