OXYGEN THERAPY AND INTRAOCULAR OXYGENATION*

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INTRODUCTION

ISCHEMIC CONDITIONS OF THE EYE CAN DAMAGE OCULAR TISSUES WHEN TOXIC products of metabolism accumulate, important nutrients fail to reach the tissue, or hypoxia exists. The damage that hypoxia effects on the anterior segment of the eye is dramatically exemplified in patients with sickling hemoglobinopathies who develop traumatic or postoperative hyphemas.[1-3] In these eyes, the normally hypoxic environment of the anterior chamber (pO2 values approximately 40 to 60 mm Hg compared with approximately 100 mm Hg in arterial blood) results in the sickling of erythrocytes in the presence of homozygous sickle cell disease (SS), hemoglobin SC disease, hemoglobin S β-thalassemia, and even sickle cell trait.[2] Important work by Goldberg has demonstrated that high ascorbic acid levels, relative acidosis, and higher than normal pCO2 levels also may contribute to the sickling of erythrocytes in the anterior chamber.[1-3] The inflexible sickled erythrocytes then cause a logjam in the trabecular meshwork, which produces an increase in intraocular pressure. This secondary glaucoma results in further hypoxia by contributing to the ischemia, thus establishing a vicious cycle that leads to severe glaucoma. The glaucoma also contributes to the development of corneal bloodstaining and can damage the posterior ocular structures. The optic nerve and retina in these patients are more susceptible to damage from the elevated intraocular pressure because the intravascular erythrocytes in these structures tend to sickle.

Increased delivery of oxygen to the anterior chamber may help in the treatment of hypoxic anterior segment diseases, including sickle cell hyphema. A previous report[4] demonstrated that hyperbaric exposure of

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adult albino rabbits to two atmospheres of 100% oxygen for 2 hours increased the pO2 in the aqueous humor from a baseline value of 63.5 mm Hg to over 500 mm Hg. This elevated pO2 in the aqueous humor was sufficient to prevent or reverse the sickling of injected intracameral human erythrocytes containing sickle hemoglobin. Two hours after the production of experimental sickle cell hyphema with the exposure of the animals to hyperbaric oxygen, the percentage of sickled erythrocytes in the anterior chamber decreased from 35.7% in rabbits breathing normobaric room air to 4.1% in rabbits exposed to 100% oxygen at two atmospheres. From this work, it was clear that hyperbaric 100% oxygen could raise the pO2 in the aqueous humor dramatically and reverse sickling of erythrocytes. This methodology could potentially be used to treat patients with sickle cell disease and hyphema.

One important question raised by this earlier work was whether the oxygen reached the anterior chamber of the eye directly through the cornea or primarily through the vascular system. The present series of experiments was formulated to study in detail if oxygen could be delivered under normobaric conditions through the cornea of a live animal (or patient) and significantly raise the oxygen levels in the aqueous humor. If this were possible, patients with sickle cell hyphema and other ischemic ocular disease could be treated with transcorneal oxygen delivery under normobaric conditions. In addition, the present studies were carried out to determine further if hyperbaric conditions would facilitate the transport of oxygen across the cornea. As an offshoot of this work, experiments were performed to determine whether or not hyperbaric conditions in primates could raise the oxygen level in the preretinal vitreous cavity (and thus the inner layers of the retina) to possibly therapeutic levels if arterial or venous obstructive disease produced ischemic insults to the inner layers of the retina. The experiments in part I of this thesis relate to anterior segment oxygen delivery and ischemia. Those in part II describe oxygen delivery to the preretinal vitreous body.

PART I — ANTERIOR SEGMENT ISCHEMIA

The devastating picture of anterior segment necrosis is undoubtedly, at least in part, related to anterior segment hypoxia. Ischemia of the anterior segment may occur after scleral buckling operations, the removal of multiple extraocular muscles, or in patients with vasculitis or vascular obstruction.[5] Clinically, the ischemia often produces corneal epithelial edema and stromal thickening, flare in the anterior chamber, hypotony, a sluggish or nonreactive pupil, and cataract. A frequent sequela is rubeosis iridis.

Rubeosis iridis is a sight-threatening complication associated with a variety of ischemic ocular conditions including diabetic retinopathy, ischemic central retinal vein occlusion, retinal arteriolar obstructions, and carotid artery disease.[6] In the majority of these situations, rubeosis iridis develops from ischemic disease of the posterior segment of the eye, although occasionally it occurs secondary to anterior segment disease (such as anterior uveitis). Although many investigators believe that rubeosis iridis is a response to a biochemical angiogenic stimulus (a so-called factor X synthesized by ischemic tissue), others believe that it is caused by anterior segment hypoxia with resulting iris vascular dilation.[7,8] This latter theory states that a compensatory dilation of iris vessels occurs in response to a fall in the aqueous humor pO2, from whatever cause (eg, following vitrectomy and lensectomy). This dilation then is a further stimulus to the development of rubeosis iridis. If this theory is correct, chronic, and presumably milder hypoxia of the aqueous humor will cause rubeosis iridis rather than the picture of anterior segment necrosis. Moreover, the delivery of oxygen to the eye, either transcorneally or through the vasculature, might play an important role in preventing or treating rubeosis iridis.[7,8]

A series of experiments was constructed to determine if the oxygen tension in the aqueous humor could be increased through transcorneal or systemic administration of oxygen under normobaric and hyperbaric conditions.

METHODS AND MATERIALS

Adult albino rabbits, weighing 2 to 3 kg each, were used for the initial portion of the experiment. Swimming goggles were modified (Figs 1 and 2) to fit snuggly on a rabbit's head, yet maintain a relatively tight seal surrounding the eyes. A single port in the goggle for each eye delivered a humidified gas independently to the surface of each cornea. Gases egressed from the surface of each eye through a second port. In the majority of the experiments, 100% oxygen or 100% nitrogen (0% oxygen) was delivered. In the remainder of the experiments, air (21% oxygen) was utilized. The gases were bubbled through water to humidify them. The flow rate in the goggles was 1 to 2 l/minute. The flow of gas at the egress port was checked by observing a change in the O2 concentration with an O2 analyzer head. In all experiments, precautions were taken so that the environment of the animals was sufficiently ventilated to prevent contamination of the inhaled oxygen concentration by any spillover of gas from the goggles. This was monitored intermittently by placing an oxygen

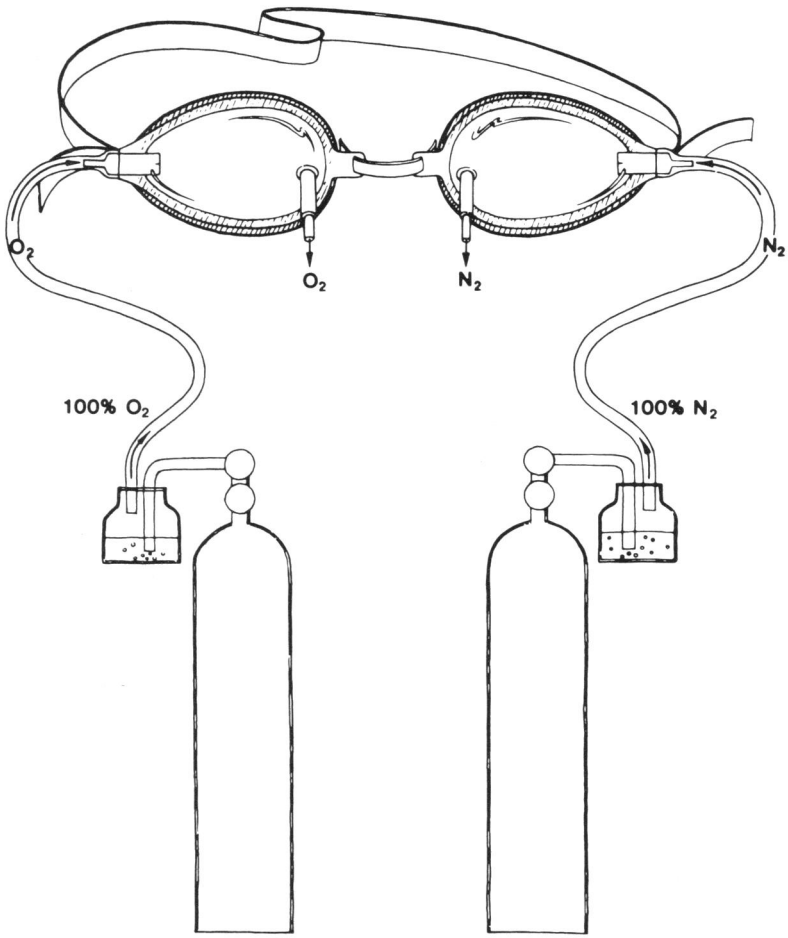


FIGURE 1
Illustration depicting swimming goggles used to control pO2 at the corneal surface.

sensor adjacent to the rabbit's nose. The delivery of gas to each eye was independent; there was no potential mixing of the gases.

Experiments were performed under normobaric or hyperbaric conditions. Normobaric experiments were done outside the hyperbaric chamber. The hyperbaric experiments were carried out in a large walk-in chamber (Fig 3; Vacudyne, Chicago Heights, IL) pressurized to two atmospheres of pressure absolute (14.7 pounds/sq in gauge pressure, PSIG). The four individual pressure gauges (Crosby 0-90 PSIG, Crosby Manufacturers, Boston, MA) were calibrated against a mercury manom-

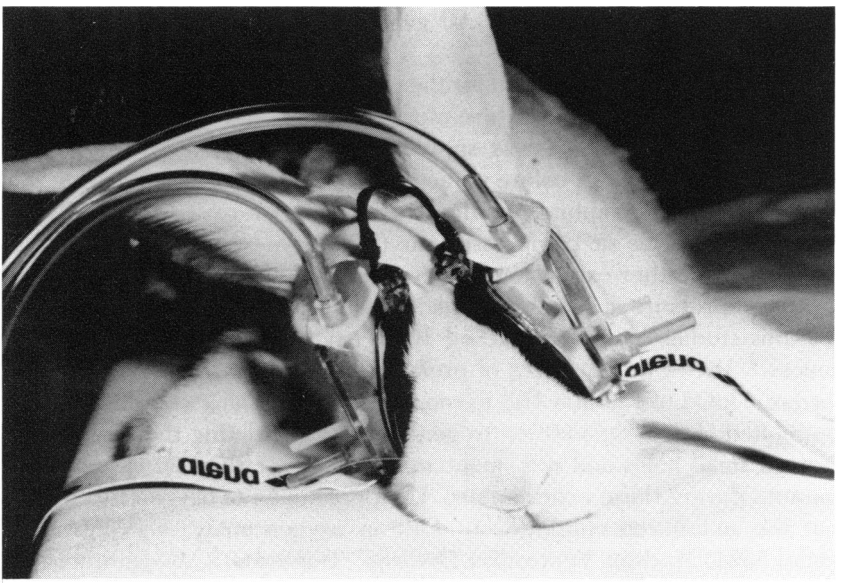


FIGURE 2
Modification of goggles to fit albino rabbit.

FIGURE 3
Walk-in hyperbaric chamber used in experiments.

eter at 5 PSIG (259 mm Hg). All gauges read within 0.5 PSI of one another at 14.7 PSIG.

All rabbits were anesthetized for the experiments with a 1 ml intramuscular injection of a 1 to 5 mixture of acepromazine (10 mg/ml) and ketamine (100 mg/ml), which was supplemented as necessary. Additional anesthesia was achieved before paracentesis with topical proparacaine. In some experiments, rabbits breathed room air (21% oxygen) under normobaric conditions or compressed air (21% oxygen) under hyperbaric conditions. In other experiments they inhaled 10% oxygen at two atmospheres of pressure in the chamber administered through an oxygen tent. Previous studies had administered 100% oxygen under similar circumstances.[4] At two atmospheres of pressure, 10% oxygen is approximately normoxic (pO2 of 150 mm Hg) to room air at normobaric conditions. The humidified 10% oxygen/90% nitrogen mixture ventilating the tent exited through small holes and was maintained at a flow rate of 30 to 45 actual l/minute during these experiments. The percentage of oxygen inside the tent was monitored continuously with an oxygen analyzer (Ventronics model 5524, Hudson Ventronics Division, Temecula, CA) calibrated at 21% and 100% oxygen at ground level pressure. When the tent was used, gas was sampled continuously through a plastic tubing that was fed through a port in the hyperbaric chamber hull to the outside oxygen analyzer. With this technique, the oxygen concentration in the tent was maintained easily at 10% throughout the entire experiment.

When a specimen of aqueous humor was necessary to determine anterior chamber pO2, a paracentesis with the rabbit under topical anesthesia was performed immediately (5 to 10 seconds) after the removal of the goggles (when used) or after the removal of the animal from the oxygen tent (when used). The aqueous humor was obtained with a 150-μl heparinized capillary tube, which was modified using a short (⅝ inch) 25-gauge needle attached with epoxy glue. Following the aspiration of aqueous humor, both ends of the tube were sealed using Critoseal, and the capillary tubes were placed on ice. If the chamber was used, the samples were passed from the chamber through a decompression lock. All aqueous humor (and blood) samples were then immediately analyzed (within 10 minutes) for pO2 values at ground level pressure using a pH blood gas analyzer (IL System 1303, Instrument Lab System, Lexington, MA).

Several experiments were done to determine the effect of oxygen levels on sickling of human sickled erythrocytes in the rabbit anterior chamber. In these experiments, blood from a patient with sickle cell β-thalassemia was collected by venipuncture. The donor blood was immediately anticoagulated with ethylenediaminetetraacetic acid, maintained at room tem-

perature, and injected into the rabbit anterior chambers within 1 hour. After 0.15 ml of aqueous humor was removed by paracentesis, 0.15 ml of anticoagulated blood ws injected into the rabbit anterior chamber through the limbus with a 30-gauge needle and a tuberculin syringe to create a hyphema. Gentle tamponade was applied to the paracentesis tract for 15 seconds which did not leak detectable aqueous. To determine the percentage of erythrocytes that was sickled in the anterior chamber (or blood) after 2 hours, samples of aqueous humor (or blood) were collected and were fixed immediately in 2% buffered glutaraldehyde. These samples were coded and prepared using a Cytospin technique. Counts to determine percentage of cells in the sickled configuration were done in a controlled fashion by a masked examiner.

In separate experiments, pO2 levels in monkey aqueous humor were assessed using two adult female cynomolgus monkeys weighiig approximately 3.4 to 4 kg each. Before performing these experiments, the monkeys were made to fast for 12 to 24 hours. Initially, the animals were anesthetized with an intramuscular injection of ketamine hydrochloride (10 to 15 mg/kg), after which an intravenous line was established. Anesthesia was maintained using 2% thiamylal sodium intravenously (2.5 mg/kg). The anesthesia was supplemented as necessary with additional thiamylal. Paracentesis and sample collection were performed in a similar manner to the rabbit experiments for pO2 level analysis.

To test the assumption that the various treatments with nitrogen or oxygen under normobaric or hyperbaric conditions had no effect on the pO2 or the percentage of sickled cells in the aqueous humor of rabbits, Student's *t*-test (two-tailed) for paired observations (experiments 1, 2, 4) and the Wilcoxon Rank Sum test (experiment 3) were used. The tests were done to compare each experimental group with a normal group of rabbit eyes treating the control group as the true population. Data are presented as mean ± standard deviation. A *P* value of 0.05 was considered statistically significant.

EXPERIMENTS

EXPERIMENT 1

Eight albino rabbits were kept under normobaric conditions breathing room air (pO2, 150 mm Hg). The specially modified goggles (Figs 1 and 2) were placed on the rabbits, and a flow of 100% oxygen (pO2, 760 mm Hg) was delivered to one eye, and 0% oxygen (100% nitrogen; pO2, 0 mm Hg) was administered to the second eye. After 30 minutes, samples of aqueous humor were obtained and analyzed for pO2 levels.

EXPERIMENT 2

Seven rabbits were placed in the hyperbaric chamber and were breathing compressed air (21% oxygen) at two atmospheres of pressure (pO2, 300 mm Hg). The goggles were used to give one eye of each rabbit 100% oxygen (pO2, 1520 mm Hg) and the second eye compressed air (21% oxygen; pO2, 300 mm Hg). At the end of 30 minutes, the pO2 levels in both eyes of each rabbit were measured.

EXPERIMENT 3

A total of 14 rabbits were allowed to breath compressed air (21% oxygen) at two atmospheres of pressure in the hyperbaric chamber. Bilateral hyphemas with sickle cell blood were created in each rabbit, as described. One eye of each rabbit was given 100% oxygen (pO2, 1520 mm Hg) through the swimming goggles, while the opposite eye received 100% nitrogen (pO2, 0 mm Hg). Two hours later, paracentesis was performed. Samples were measured for pO2 levels and for the percentage of sickled erythrocytes (as previously described). In some situations, insufficient amounts of aqueous humor were available to measure both pO2 and sickled cell counts, so that the total number of samples (n) in all groups do not equal 14.

Two additional rabbits (four eyes) were similarly injected with sickle cell blood. These rabbits breathed compressed air (21% O2) at two atmospheres of pressure for 2 hours before the pO2 levels and the percentage of sickled cells were determined.

EXPERIMENT 4

Eight rabbits were kept in the gas tent breathing a normoxic mixture of 10% oxygen at two atmospheres of pressure in the hyperbaric chamber. One eye received 100% oxygen (pO2, 1520 mm Hg) and the second eye was given 100% nitrogen (pO2, 0 mm Hg) through the goggles. Sickle cell blood was injected in each eye, as described. Two hours later, samples of aqueous humor were obtained for determinations of pO2 levels and percentage of sickled erythrocytes.

EXPERIMENT 5

This experiment was repeated on two different days with the same two anesthetized cynomolgus monkeys. The goggles were modified to fit snuggly on the monkey face under normobaric conditions (Fig 4). One eye received 100% nitrogen (pO2, 0 mm Hg) while the other was given 100% oxygen (pO2, 760 mm Hg). After 30 minutes, samples of aqueous humor were obtained and the pO2 levels were measured.

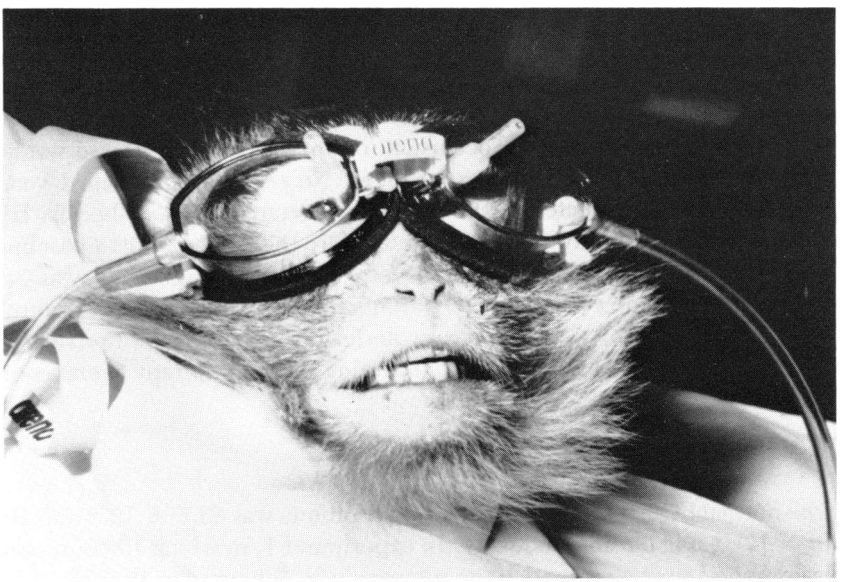


FIGURE 4
Modification of goggles to fit cynomolgus monkey.

EXPERIMENT 6

Anesthetized albino rabbits were used to determine the effect of increased oxygen levels in the aqueous humor on iris fluorescein angiography. Baseline fluorescein angiograms were performed on one eye of ten rabbits. One milliliter of 25% sodium fluorescein was injected in an ear vein. Several days later the swimming goggles were utilized to deliver 100% oxygen to the same eye while the animals breathed room air under normobaric condition for 30 minutes. The angiograms were repeated immediately after the goggles were removed and were compared with the baseline views.

EXPERIMENT 7

Volunteer subjects were used after giving their informed consent. This study was approved by our Institutional Review Board. Two normal subjects were fitted with the modified goggles. One eye of each volunteer underwent iris fluorescein angiogram without goggles to obtain baseline views. The same week, iris fluorescein angiography was performed in the same eyes immediately after the subjects breathed room air under normobaric conditions for 30 minutes while receiving 100% oxygen through the goggles. The angiograms for each eye were compared.

EXPERIMENT 8

The experiment included four patients with stable rubeosis iridis. These patients had residual rubeosis iridis with leakage following panretinal photocoagulation for proliferative diabetic retinopathy (three patients) or ischemic central retinal vein occlusion (one patient). The disease was in the inactive phase during the experiment. No recent (more than 1 year) change had been detected in the clinical appearance of the rubeosis. Iris fluorescein angiography was performed before the treatment as a baseline for comparison. Within the next several days, the angiogram was repeated on the same eyes after the patients received 100% oxygen through the goggles while breathing room air under normobaric conditions for 30 minutes. The angiograms before and after oxygen therapy were compared.

RESULTS

The normal pO2 of the aqueous humor in rabbits was 63.5 ± 12.3 mm Hg (n = 12), using our methodology. In experiment 1, in which 100% oxygen was applied to one eye and 100% nitrogen was delivered to the other eye through goggles under normobaric conditions, the value of the aqueous pO2 rose to 139.5 ± 32.4 mm Hg (n = 8) in the eyes receiving oxygen. This was significantly greater than our baseline values ($P = 0.001$). The eyes receiving 100% nitrogen showed no change in pO2. The measured value of 68.8 ± 10.1 mm Hg (n = 8) was not significantly different from the baseline but was significantly less than eyes receiving 100% oxygen ($P = 0.001$) (Tables I to III).

In experiment 2, corneal oxygen delivery with goggles and hyperbaric pressure were combined to raise the pO2 level in rabbits. Following 30 minutes of exposure to two atmospheres (breathing compressed air, pO2, 300 mm Hg) and receiving 100% oxygen to one eye and compressed air (21% oxygen) to the other eye, the rabbit eyes receiving the 100% oxygen had an aqueous humor pO2 level of 295.2 ± 132.4 mm Hg (n = 7). This was significantly greater than the normal value under normobaric conditions ($P < 0.01$). The seven eyes receiving two atmospheres of compressed air at the corneal surface also had higher than normal pO2 values. The mean value of 134.5 ± 24.4 mm Hg was significantly greater than normal ($P < 0.001$). Eyes receiving the two atmospheres of 100% oxygen had a greater pO2 value than those receiving compressed air ($P = 0.01$), indicating that at least some of the increase in aqueous pO2 was due to transcorneal oxygen delivery.

In experiment 3, rabbits inhaled two atmospheres of compressed air (21% oxygen; pO2, 300 mm Hg). Sickle cell hyphemas were created in all

TABLE I: AQUEOUS pO2 VALUES (mm Hg) IN RABBITS WITHOUT HYPHEMAS

TREATMENT	DURATION (HR)	% CORNEAL OXYGEN	% INSPIRED OXYGEN	ATMOSPHERES OF PRESSURE	pO2 (n)*
Control group	—	21	21	1	63.5 ± 12.3 (12)
Experiment					
1	1/2	100	21	1	139.5 ± 32.4 (8)
	1/2	0	21	1	68.8 ± 10.1 (8)
2	1/2	100	21	2	295.2 ± 132.4 (7)
	1/2	21	21	2	134.5 ± 24.4 (7)

*n is the number of observations.

TABLE II: AQUEOUS pO2 VALUES (mm Hg) IN RABBITS WITH EXPERIMENTAL HYPHEMA

EXPERIMENT NO.	DURATION (HR)	% CORNEAL OXYGEN	% INSPIRED OXYGEN	ATMOSPHERES OF PRESSURE	pO2 (n)*
3	2	100	21	2	143.9 ± 49.5 (11)
	2	0	21	2	89.6 ± 41.7 (7)
	2	21	21	2	84.2 ± 12.2 (4)
4	2	100	10	2	80.8 ± 33.7 (8)
	2	0	10	2	62.7 ± 30.2 (8)

*n is the number of observations.

TABLE III: pO2 VALUES (mm Hg) IN MONKEY AQUEOUS HUMOR

	% CORNEAL OXYGEN	
MONKEY NO.	0	100
Day 1		
32	50.3	140.5
38	41.7	85.3
Day 2		
32	72.0	87.1
38	59.5	128.5

eyes. The corneas were administered either 100% oxygen or 100% nitrogen at two atmospheres of pressure. Two hours later, the pO2 value in the eyes receiving oxygen was 143.9 ± 49.5 mm Hg (n = 11). This was significantly above the normal value of 63.5 mm Hg ($P < 0.001$), but below the pO2 values measured in eyes without hyphemas (experiment 2). The data are not entirely comparable because the animals were in the hyperbaric chamber for 30 minutes in experiment 2 and for 2 hours in experiment 3.

The eyes receiving two atmospheres of 100% nitrogen (pO2, 0 mm Hg) at the corneal surface had a pO2 value of 89.6 ± 41.7 mm Hg (n = 7). This was somewhat increased over the normal value but the difference was not statistically significant ($P = 0.15$). This value was significantly less

than for those eyes receiving 100% O2 ($P = 0.03$). Experiment 3 also showed that transcorneal entry of oxygen was important in raising the pO2 value.

The blood initially injected intracamerally had approximately 20% to 61% of the cells sickled, although this value varied from experiment to experiment. The percentage of sickled erythrocytes in the anterior chamber of the eyes receiving oxygen ws 6.6% ± 3.9% (n = 14) compared with 13.7% ± 8.5% (n = 12) in the eyes receiving nitrogen. This difference was significant ($P = 0.014$).

The four eyes receiving only two atmospheres of 21% oxygen at the cornea for 2 hours had a pO2 value of 84.2 ± 12.2 mm Hg (n = 4). This was significantly less than the 100% oxygen group ($P = 0.04$) and not different from the 100% nitrogen group. The percentage of sickled cells in this group of eyes was 8.3 ± 3.3 (n = 4). This did not differ significantly from either the nitrogen or oxygen groups.

Experiment 4 was performed to determine whether the pO2 value in the aqueous humor could be increased transcorneally in eyes of rabbits that did not have heightened pO2 values in the blood. These eight rabbits were breathing a normoxic 10% oxygen mixture at two atmospheres of pressure, which was roughly equivalent to breathing one atmosphere of air (21% oxygen; pO2, approximately 150 mm Hg). One eye was given 100% oxygen (pO2, 1520 mm Hg) and the other eye was given 100% nitrogen (pO2, 0 mm Hg). The eyes receiving oxygen had a pO2 value of 80.8 ± 33.7 mm Hg (n = 8), compared with the 62.7 ± 30.2 mm Hg (n = 8) measured in the eyes receiving nitrogen. These values were not significantly different, although they suggested that the oxygen group may have had higher values ($P = 0.10$) than the nitrogen group. The measurements of either group did not differ statistically from the normal aqueous pO2 values. The percentage of sickled erythrocytes in the anterior chamber was 18.1% ± 9.0% in the eyes receiving oxygen and 16.6% ± 6.4% in the eyes receiving nitrogen. A comparison of these values also showed no statistically significant difference.

In experiment 5, the cynomolgus monkeys were tested in a manner similar to that used for the rabbits in experiment 1. Baseline pO2 values in monkey aqueous humor were approximately 50 to 60 mm Hg by our techniques. Under normobaric conditions, 100% oxygen at the corneal surface (pO2, 760 mm Hg) demonstrated increased aqueous pO2 values compared with eyes receiving 100% nitrogen (Table III). Our findings showed that it is possible to delivery oxygen transcorneally under normobaric conditions in monkeys as well as rabbits.

In experiment 6, iris fluorescein angiograms of excellent quality were

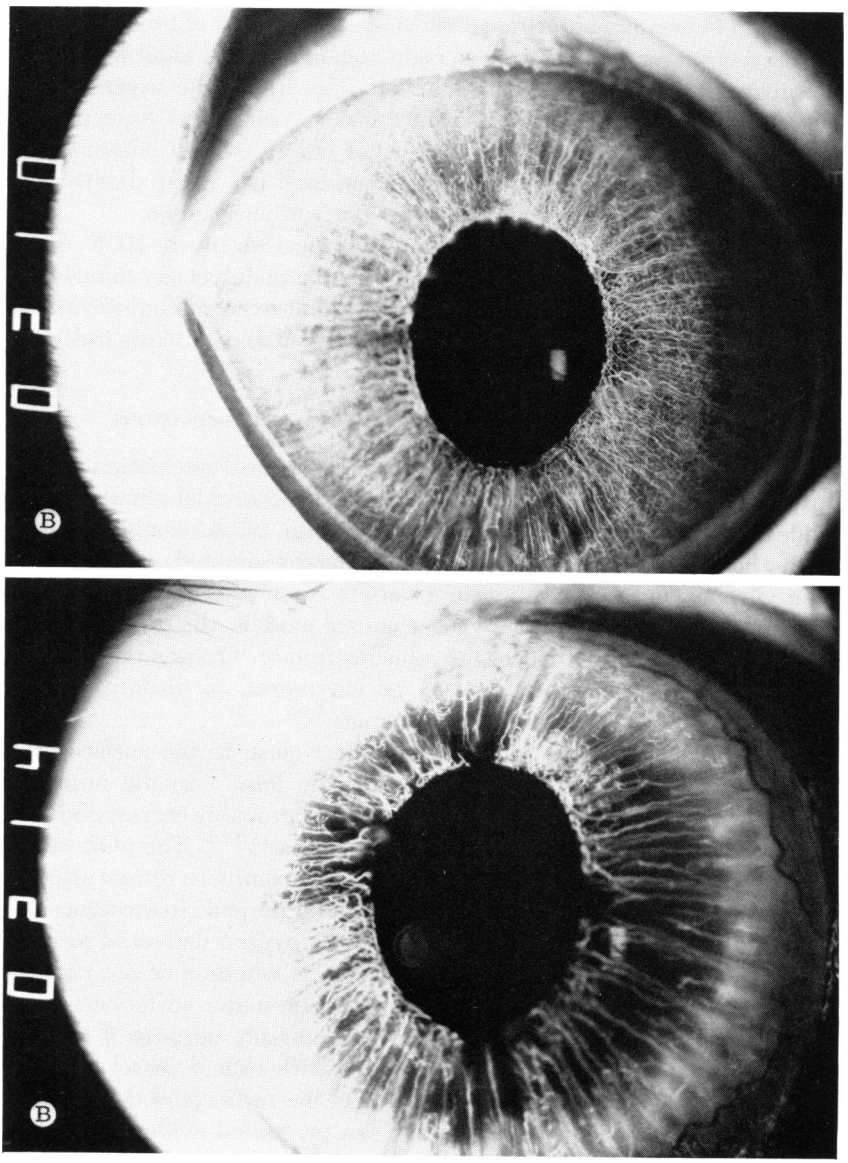
obtained before and after transcorneal oxygen in eight of ten rabbits. The masked observer evaluated these eight angiograms and identified the eye receiving transcorneal oxygen in seven. The iris in the oxygen-treated eyes showed delayed filling of the arteries, veins, and especially the capillaries compared with the same eyes before oxygen administration (Fig 5). In the eighth eye, the oxygen-treated iris filled slightly more rapidly with fluorescein than the same eye without oxygen.

In experiments 7 and 8, human volunteers received 100% oxygen transcorneally for 30 minutes. We were unable to detect any difference in the iris fluorescein angiograms with or without oxygen administration in the two normal subjects or four patients with stable rubeosis iridis.

PART II — POSTERIOR SEGMENT OXYGEN DETERMINATIONS

The primate retina has two circulations. The retinal circulation supplies oxygen to the inner half of the retina and the choroidal circulation provides oxygen to the retinal pigment epithelium, photoreceptors, and the outer half of the retina. Numerous studies have evaluated oxygen levels in the vitreous and inner retina in a variety of animals including rabbits, pigs, cats, and monkeys.[9-30] In these animal models, the pO2 level of the vitreous is lower than that of the aqueous humor. Oxygen does not pass from the aqueous to the vitreous, or vice versa, in phakic animals,[28] although this can occur in aphakic animals.[7,25]

The pO2 level in the vitreous is highest close to the surface of the retina, then decreases anteriorly toward the lens.[15] In the immediate preretinal area, the pO2 levels in the vitreous probably correspond closely to those in the innermost layers of the retina.[11,17,19] The pO2 value in the preretinal vitreous is increased in close proximity to retinal arterioles and decreased near retinal venules.[12,20] Under normal circumstances, the pO2 value in the preretinal vitreous reflects oxygen delivered primarily by the retinal circulation. The controversy is whether or not choroidal oxygen contributes to inner retinal oxygenation under normobaric conditions.[12,14,16,18,21-23,25-30] The answer is important, because if choroidal oxygen reaches the inner retina, patients with retinal vessel occlusions could receive oxygen to the inner layers of the retina (and the preretinal vitreous) from the choroid. Landers[23] has presented evidence in the cat and monkey to support the contention that breathing 100% oxygen increases choroidal oxygen delivery to the inner retina and prevents damage to the retina after closure of the retinal circulation. Flower and Patz[22] have suggested that breathing 100% oxygen alone is ineffective in protecting the inner cat and monkey retina from ischemia. Other investigators,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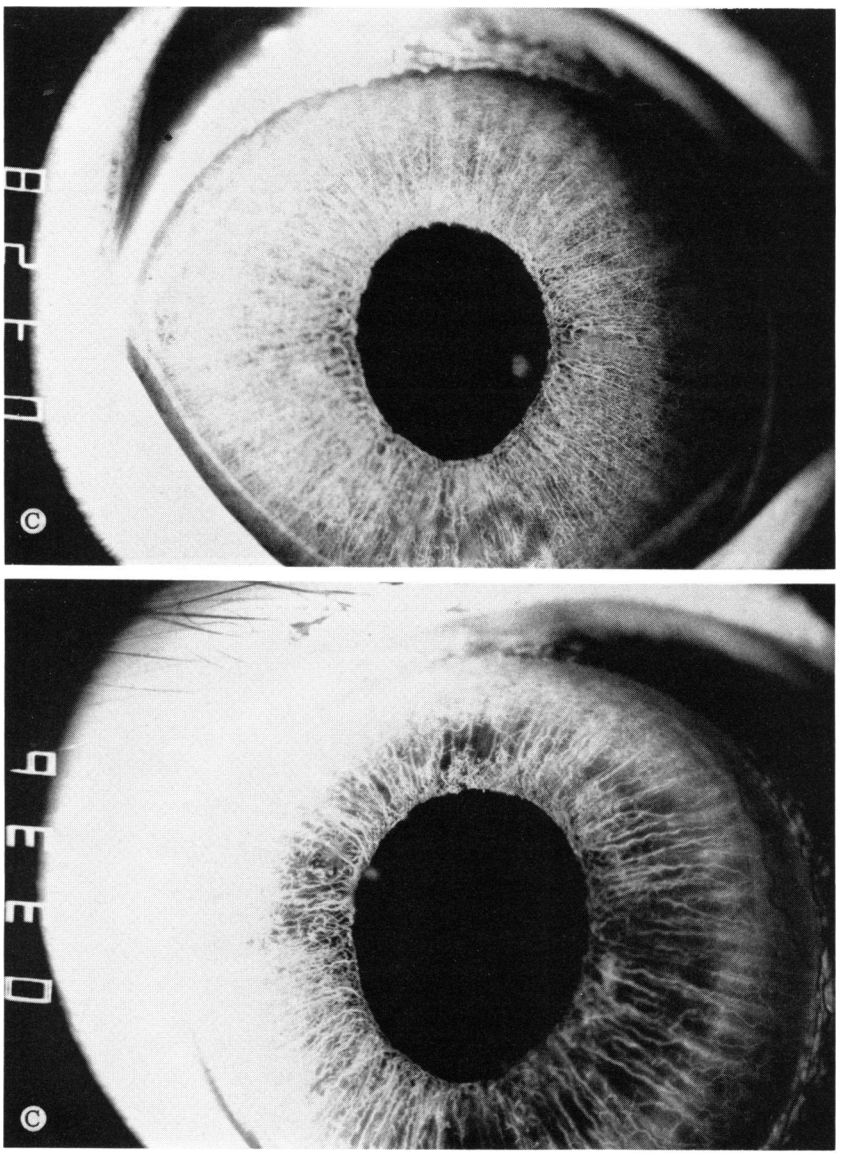

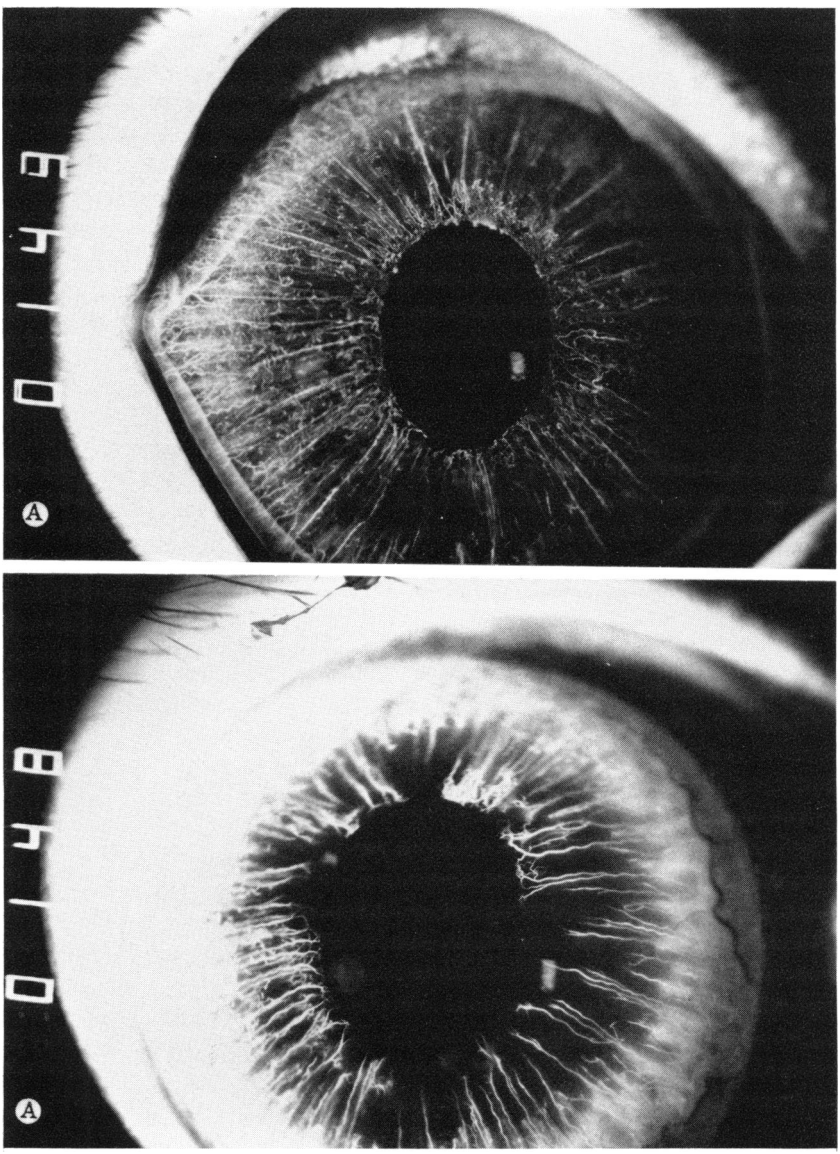

FIGURE 5

Iris fluorescein angiogram from a representative albino rabbit. A: Comparable frames of same eye (top) under normal conditions or (bottom) following 30 minutes of transcorneal oxygen. Filling of vessels is markedly delayed with oxygen administration. B: Later-phase angiogram still shows delayed filling in oxygen-treated eye (bottom). C: Angiogram at 32 to 33 seconds still discloses diminished capillary filling in oxygen-treated eye (bottom).

based on a sampling of pO2 levels in the retina under normal conditions and after the inhalation of elevated oxygen concentrations, believe that the increase in oxygen observed in the inner layers of the retina results from retinal circulatory delivery of oxygen rather than choroidal contributions.[12,30]

The use of hyperbaric conditions[21,22,31-34] to deliver greater amounts of oxygen through the choroidal and/or retinal circulation may be of value in treating selected patients with acute retinal vascular obstructions. A series of experiments was designed, therefore, using cynomolgus monkeys to determine if the oxygen tension in the preretinal (premacular) vitreous could be increased by having the monkey breath oxygen under normobaric and hyperbaric conditions.

METHODS AND MATERIALS

Two adult female cynomolgus monkeys, weighing 3.4 to 4 kg each, were anesthetized with ketamine hydrochloride and thioamylal sodium, as previously described. To facilitate the sampling of preretinal vitreous in a species with formed vitreous, bilateral central core vitrectomies were performed on the monkeys under sterile conditions. The pupils were dilated with 1% tropicamide and 10% phenylephrine hydrochloride. After the placement of a lid speculum, a lateral canthotomy was made and a fornix-based conjunctival flap was created on the temporal side. Traction sutures of 4-0 silk were placed beneath the tendons of the inferior and lateral rectus muscles to maintain mobilization of the eye. Sclerotomies were made with a 51 S Beaver blade just above and below the level of the insertion of the lateral rectus muscle. A 3 mm infusion cannula was placed in the superior sclerotomy and held in place by a 5-0 silk mattress suture. A mechanical vitreous cutter, 0.89 mm in diameter, was inserted through the lower sclerotomy. The infusion fluid was balanced salt solution. A vitrectomy was performed using a binocular indirect ophthalmoscope for visualization. The entire central vitreous was removed to the macula and disc. No attempt was made to remove the anterior cortical vitreous or the retrolental vitreous. The sclerotomies were closed using 6-0 polyglactin 910 (Vicryl). The globe was then reformed with balanced salt solution injected with a 30-gauge needle through the pars plana. The canthotomies were repaired. The eyes were inspected at the termination of the procedure to ensure that the retinas were undamaged and attached. The surgical time was approximately 1 hour. Topical corticosteroids and cycloplegics were administered. The eyes were allowed to heal for more than 2 weeks to allow any detectable intraocular inflammation to resolve.

By this time the eyes were not inflamed and the retinas appeared ophthalmoscopically normal.

To obtain preretinal vitreous samples in the now liquid vitreous, a special sampling needle was made. A 25-gauge 1½-inch long hypodermic needle was removed from the hub and attached to a heparinized glass capillary tube, 150 μl in size (Fig 6). Air- and water-tight seals were produced with epoxy glue. The needle was cut from a butterfly infusion set, and the end of the plastic catheter tubing was connected to the distal end of the glass capillary tube. Air- and water-tight seals were produced here as well. A 3 ml plastic syringe was next attached to the end of the infusion cannula to permit suction (under careful control) of liquid vitreous for pO2 determinations. When sampling was necessary, the animals were anesthetized and their pupils dilated. A lid speculum was placed. After a temporal conjunctival peritomy was performed, silk sutures were placed under the tendons of the inferior and lateral rectus muscles. The temporal sclera was punctured 4 mm posterior to the limbus with the tip of the needle (Fig 7). The needle was advanced into position under indirect ophthalmoscopic control, until it was just in front of the macular area, less than 1 mm from the retinal surface. The needle did not touch the retina. When the needle was in position, a sample of liquid vitreous was taken by applying controlled suction with the plastic syringe to fill the capillary tube slowly. When the tube was filled, the needle was removed

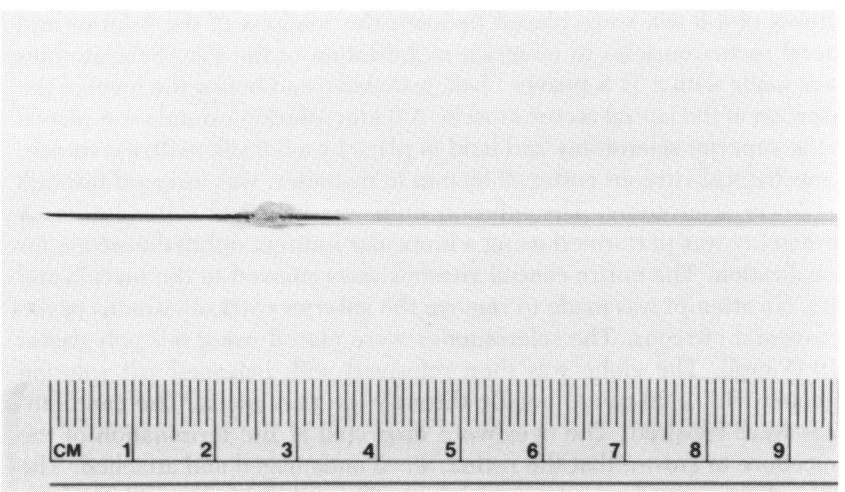

FIGURE 6
Needle developed to obtain liquid vitreous samples for the determination of preretinal pO2 values.

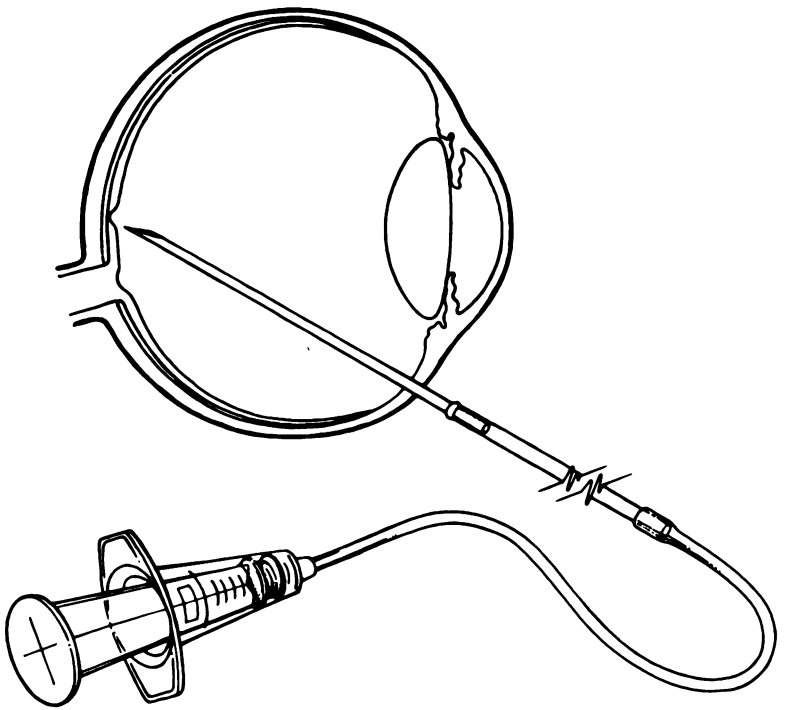

FIGURE 7
Drawing showing system for the aspiration of vitreous.

from the globe, and the capillary tube was sealed immediately with Critoseal and placed on ice. The pO2 levels were then analyzed, as previously described. The needle tracks sealed without leakage of vitreous following removal of the needle. The eye was then re-formed with an injection of balanced salt solution using a 30-gauge needle through the pars plana.

The same two monkeys were used in the following series of experiments:

EXPERIMENT 9

To determine normal arterial blood pO2 values, blood pO2 values upon breathing 100% oxygen, and pO2 values breathing 100% oxygen at two atmospheres pressure, the monkeys were anesthetized as previously described. Baseline arterial blood gas levels were obtained by femoral artery puncture or placement of an indwelling arterial catheter. Blood gases were measured while the animals were under normobaric conditions

breathing room air, under normobaric conditions breathing 100% oxygen in an oxygen tent for 30 minutes, and under hyperbaric conditions (two atmospheres of pressure absolute) breathing 100% oxygen in an oxygen tent for 30 minutes.

EXPERIMENT 10

The oxygen tension in the premacular vitreous of the monkeys was determined under the following conditions on three separate days: (1) The animals breathed room air under normobaric conditions. (2) The monkeys were allowed to breathe 100% oxygen (in an oxygen tent) under normobaric conditions for 30 minutes. (3) The animals were given two atmospheres of 100% oxygen (in an oxygen tent) for 30 minutes.

RESULTS

Baseline arterial blood pO2 values in the two monkeys (Table IV) ranged between 58.3 and 131.7 mm Hg. These fluctuations were thought to be related to the level of anesthesia, as blood pressure and respirations were not monitored or regulated in this study. On breathing 100% oxygen, the pO2 values obtained in the monkeys were 300.5, 518.3, and 441.5 mm

TABLE IV: pO2 VALUES (mm Hg) IN MONKEY ARTERIAL BLOOD

	INSPIRED GAS		
MONKEY NO.	ROOM AIR	100% O2	2 ATMOSPHERES 100% O2
Day 1			
32	58.3	518.3	—
38	91.3	300.5	—
Day 2			
32	119.9	—	—
38	131.7	441.5	> 999.9

TABLE V: pO2 VALUES (mm Hg) IN MONKEY PRERETINAL VITREOUS

	INSPIRED GAS		
MONKEY NO.	ROOM AIR	100% O2	2 ATMOSPHERES 100% O2
Day 1			
32	46.0	58.6	159.3
38	76.2	69.2	211.6
Day 2			
32	44.7	58.8	123.9
38	54.3	39.6	80.2
Day 3			
32	47.3	63.4	120.1
38	58.8	45.7	86.5

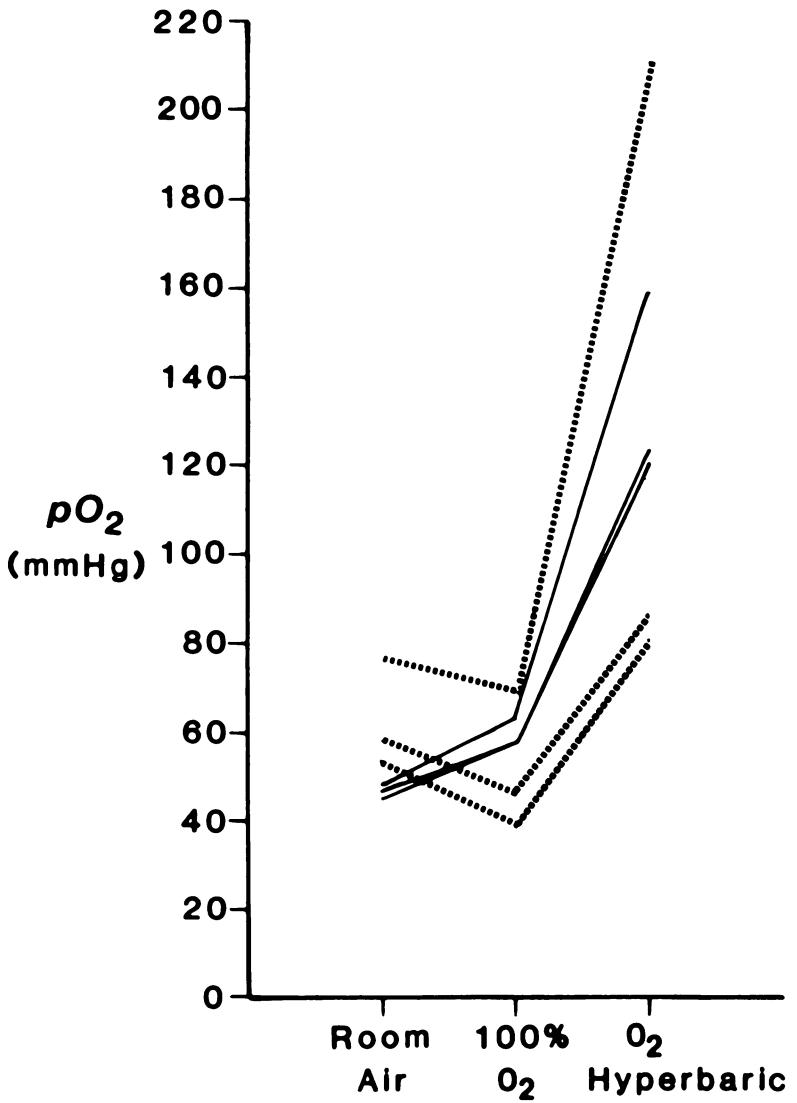

FIGURE 8
Preretinal pO2 values in three separate experiments on monkeys 32 (*solid line*) and 38 (*broken lines*). Two atmospheres of 100% oxygen raised the pO2 values, whereas inhaled 100% oxygen alone did not appreciably increase the pO2.

Hg. On breathing 100% oxygen at two atmospheres, the pO2 value was greater than 999.9 mm Hg in the single determination made.

The measurements of preretinal vitreous pO2 values in the six determinations on the two monkeys breathing normobaric air (21% O2) ranged from 44.7 to 76.2 mm Hg (n = 6) (Table V, Fig 8). After breathing 100% oxygen, the same two monkeys (three experiments each) showed a range of pO2 values from 39.6 to 69.2 mm Hg (n = 6), which were no different from normal. After breathing 100% oxygen at two atmospheres for 30 minutes, the pO2 values in the vitreous increased markedly to 80.2 to 211.6 mm Hg (n = 6). These data also are illustrated in Fig 8. Thus, breathing 100% O2 under normobaric conditions did not raise the preretinal vitreous oxygen partial pressure appreciably in these animals. However, breathing 100% oxygen under hyperbaric conditions markedly increased the preretinal vitreous oxygen partial pressure.

DISCUSSION

OXYGEN TENSION IN ANTERIOR CHAMBER AQUEOUS HUMOR

A variety of techniques has been used to measure aqueous humor oxygen tension in the anterior chamber including polarographic electrodes in the eye and the withdrawal of aqueous samples. Studies have been made on a variety of species including rabbits, cats, dogs, and humans. After reviewing the various values obtained, Stefansson et al[7] have reported the mean pO2 level in the rabbit anterior chamber, in most cases, to be between 31 and 72 mm Hg. Our techniques yield a value of 63.5 ± 12.3 mm Hg.

In a recent publication,[4] the pO2 of the aqueous humor reportedly increased after two atmospheres of 100% oxygen was administered for 2 hours to rabbits that had had experimental hyphemas induced by the injection of human sickle cell blood. The pO2 values rose from 63.5 to 620 mm Hg in saline-injected eyes or to 503.7 mm Hg in eyes injected with human sickle cell blood. These increases in pO2 were significantly different from normal values in each situation, although the eyes with hyphemas had lower pO2 values than the saline-injected eyes. In addition, increased pO2 values were associated with a decrease in the percentage of sickled human erythrocytes in the anterior chamber from 35.7% in rabbits exposed to room air to 4.1% in rabbits exposed to hyperbaric 100% oxygen. Two routes were possible for the entry of oxygen into the anterior chamber in these rabbits. Transcorneal oxygen transfer could have accounted for the increase, as there was 100% oxygen on the epithelial side of the cornea at two atmospheres of pressure. Moreover, the intravascular

partial pressure of oxygen was markedly increased by the hyperbaric 100% oxygen, which also could have contributed to the increased aqueous humor pO2 values through the vasculature of the uveal tract (iris and ciliary body). We designed a series of experiments to determine which of these possibilities was correct.

Under normobaric conditions, we demonstrated a definite increase in the pO2 of the aqueous humor in rabbit and monkey eyes in which the cornea was exposed to 100% oxygen using modified swimming goggles. The pO2 value in the rabbit rose to approximately 140 mm Hg, more than double the normal value. A similar rise was seen in monkeys, confirming that even under normobaric conditions, oxygen can cross the cornea and enter the aqueous humor. It is unclear how much oxygen normally found in the aqueous humor diffuses across the cornea. Previously, Heald and Langham[35] and others[36,37] had suggested that oxygen crosses the cornea from the epithelial side to the endothelial side and increases the aqueous pO2 values. However, other authors have stated that the aqueous humor oxygen level, under normal circumstances, reflects uveal intravascular oxygen delivery rather than transcorneal oxygen delivery.[38-40] Results in the literature offer conflicting evidence regarding transcorneal oxygen delivery under normobaric conditions. One group found the highest aqueous humor pO2 values at the corneal endothelial part of the anterior chamber. These values decreased thereafter as measurements were taken deeper into the anterior chamber.[37] In contrast, a second group showed a uniform level of pO2 in the whole anterior chamber.[40] We noted that when the rabbit cornea was exposed to 100% nitrogen (0% oxygen) under normobaric conditions for 30 minutes (experiment 1), the pO2 level in the aqueous humor was unchanged from our normal values. This finding differed from a previously reported suggestion[36] that 100% nitrogen on the epithelial side of the rabbit cornea resulted in decreased aqueous pO2 values (from a normal 32 mm Hg to 9 mm Hg). Similar reductions in aqueous humor pO2 were seen by these authors following the placement of a contact lens or cellophane on the cornea or after lid closure. Because cellophane is permeable to oxygen and because lid closure should not reduce the pO2 values as dramatically as 100% nitrogen, there were inconsistencies in these data. In addition, it is uncertain what effect the placement of a corneal contact lens would have on aqueous humor pO2 values. Stefannson et al[41] and Fatt and co-workers[39] observed reduced levels in cats, in contradistinction to previous work by Friedenwald and Pierce[42] in rabbits.

In our experiments, the anterior chamber pO2 values with 100% nitrogen on the epithelial side of the cornea under normobaric conditions were

within normal limits after 30 minutes. This suggested that, under normal conditions, most or all of the oxygen in the aqueous humor results from vascular delivery of oxygen to the uveal tract (iris and ciliary body). However 100% O2 at the corneal surface caused an increase in aqueous pO2 values in the rabbit. Our experiments with monkeys (experiment 6) also demonstrated that under normobaric conditions oxygen could also be delivered across the primate cornea into the anterior chamber.

Using hyperbaric conditions, with 100% oxygen placed on the epithelial side of the cornea while the rabbit breathed compressed air (21% oxygen, pO2, 300 mm Hg), we could increase the pO2 values in aqueous humor even higher than in experiment 1. For seven rabbit eyes, the mean value of the aqueous oxygen tension was 295.2 ± 132.4 mm Hg. This was much higher than under normobaric conditions, but lower than that observed in rabbits with corneas exposed to 100% oxygen while breathing 100% oxygen at two atmospheres.[4] In the latter situation, the oxygen was delivered in part transcorneally and in part intravascularly. Eyes exposed to air on the epithelial side of the cornea at two atmospheres of pressure with the rabbit breathing air also at two atmospheres showed an increase in aqueous pO2 (134.5 ± 24.4 mm Hg), which is significantly higher than normal.

Two experiments tested the value of transcorneal oxygen in reversing the sickling of erythrocytes in the anterior chamber (experiments 3 and 4). In experiment 3, the rabbits breathed 21% oxygen at two atmospheres of pressure, but wore goggles that delivered 100% oxygen to one cornea and 100% nitrogen to the other. After 2 hours, the pO2 in the aqueous humor of the oxygen-treated eyes injected with human sickle cell blood rose to 143.9 ± 49.5 mm Hg, whereas the nitrogen-treated eyes with hyphema reached only 89.6 ± 41.7 mm Hg. The percentage of sickled cells in the anterior chamber of the oxygen-treated eyes was decreased to 6.6% ± 3.9%, in contrast with 13.7% ± 8.5% in the nitrogen-treated eyes. This clearly showed that even while compressed air is inhaled, the pO2 in the aqueous humor can be increased by exposing the corneas to 100% oxygen and the sickling of the erythrocytes in the anterior chamber can be reversed. It should be noted that these rabbits were breathing 21% oxygen (air) at two atmospheres of pressure, which increases arterial blood pO2 levels above normal.

Interestingly, by having the rabbits breathe 10% oxygen under two atmospheres of pressure (experiment 4), we were prevented from demonstrating a statistically significant difference in pO2 values between eyes administered 100% nitrogen versus eyes delivered 100% oxygen on the corneal epithelial surface (although the pO2 level of the oxygen-treated

eyes was higher). In addition there was no difference in the sickling of erythrocytes between these situations. Thus, some increased delivery of oxygen by the vascular system is necessary for the transcorneal route to increase the oxygen levels markedly in the aqueous humor. This information suggests that therapy for anterior segment ischemia under normobaric conditions with corneal oxygen delivery alone might not effectively raise aqueous pO2 values, as the vascular system in this situation is probably delivering virtually no oxygen.

Consistent with the increased pO2 values that we demonstrated in the rabbit eye under normobaric conditions with the cornea exposed to 100% oxygen, we were able to show constriction of the rabbit iris vasculature with fluorescein angiography in rabbits. The iris vesels thus responded with autoregulation to the elevation of the aqueous pO2 value from approximately 63.5 mm Hg to approximately 140 mm Hg. This finding was in agreement with a previous photographic documentation illustrating autoregulation of the iris vasculature in guinea pigs,[43] and should be confirmed by more quantitative techniquees of determining iris blood flow (such as with radioactive microspheres). In the two normal patients and the four patients with residual stable rubeosis iridis, we were unable (with the relatively crude technique of iris fluorescein angiography) to effect a change in the blood flow patterns or leakage in these eyes after exposure to 100% oxygen for 30 minutes. Possibly patients with newly developed rubeosis might respond differently, but such patients were not studied in the present protocol.

SIGNIFICANCE

Hypoxia and secondary changes in the anterior segment occur commonly in human disease. The most extreme example is anterior segment necrosis, which develops after a small percentage of scleral buckling procedures or a larger percentage of similar procedures in patients with sickling hemoglobinopathies, after strabismus surgery involving the removal of multiple extraocular muscles from the globe, and occasionally in patients with vascular insufficiency caused by collagen vascular disease, temporal arteritis, or atherosclerosis. It is possible that when anterior segment ischemia and necrosis are of concern, as in a patient with a sickling hemoglobinopathy and a hyphema, or in one requiring extensive scleral buckling and the removal of multiple rectus muscles, the delivery of 100% oxygen through the cornea with goggles could increase aqueous humor pO2 values (even under normobaric conditions) thus protecting the eye during a critical period. It is noteworthy that hyperbaric oxygen

has been used as an adjunct for scleral buckling procedures in patients with sickling hemoglobinopathies to prevent anterior segment ischemia.[44] Transcorneal oxygen therapy appears safe, as no side effects occurred in either our patients or animals given oxygen through the goggles. No patient experienced conjunctival injection, corneal edema, or an effect on vision.

Of even more potential importance is the possibility that oxygen delivered transcorneally might effectively treat or prevent rubeosis iridis. Stefansson et al[7] and Wolbarsht and Landers[8] have strongly suggested that decreased aqueous humor pO2 levels result in dilated iris vessels, which could stimulate the development of rubeosis iridis and rubeotic glaucoma. Increased vascular delivery of oxygen or transcorneal oxygen under normobaric or hyperbaric conditions might be effective in reducing iris vessel dilation and thus prevent rubosis iridis. The length and frequency of treatments and necessary partial pressure levels of oxygen are highly speculative. In addition, if developing rubeosis is not treatable by standard therapies (such as panretinal photocoagulation) because of cataract, vitreous hemorrhage, or other conditions, oxygen therapy administered transcorneally or intravascularly (with or without hyperbaria) might be of value in retarding the progression (or even causing regression) of the rubeosis. We anticipate further studies in this area, using a more sensitive quantitation of iris blood flow with and without transcorneal and systemic oxygen administration.

Our studies confirm previous work on the rabbit and, for the first time, demonstrate in a primate (cynomolgus monkey) model that oxygen can be delivered transcorneally to the aqueous humor and thus to the anterior segment of the eye. Clearly, increasing the partial pressure of oxygen by inhalation or transcorneally augments oxygen delivery to the anterior segment and might be valuable in a patient with sickling hemoglobinopathy who develops a hyphema. We have shown that it is possible to prevent sickling of erythrocytes in the rabbit anterior chamber if oxygen levels in the aqueous humor can be raised. It is realistic that a similar increase in aqueous humor pO2 levels in patients could result in a similar unsickling of erythrocytes, which might improve the secondary glaucoma caused by logjammed erythrocytes in the trabecular meshwork. Should other therapies, such as early surgical intervention,[45] not be of value for sickle cell hyphema, a randomized trial of hyperbaric and/or transcorneal oxygen would seem appropriate.

Ischemic disease of the inner retina causes visual loss even more commonly than anterior segment ischemia. Patients with central and branch retinal artery occlusions, central and branch retinal vein occlusions, and a

variety of other acute vascular insufficiencies of the retina develop inner retina ischemia with resulting loss of visual function. Also, these diseases can be associated with the development of neovascularization of the disc, retina and iris.[46] Previous work has suggested the possibility of delivering oxygen to the inner retina by choroidal oxygen transport.[18,23] Studies have used both 100% oxygen inhalation with human patients or animals or hyperbaric oxygen delivery. To date, it is unclear just how much of the inner retinal oxygen requirement is satisfied by the choroid. Some research groups believe that the increased levels of oxygen seen in the inner retina in hyperoxic situations (eg, breathing 100% oxygen) represent an overflow from the retinal vessels despite autoregulation of the retinal vasculature.[12,30] These authors and others[22] suggest that choroidal blood flow is incapable of delivering significant oxygen to the inner retina. Other groups, however, have offered evidence strongly suggesting that oxygen levels in the inner retina are influenced by choroidal oxygen transport and that increased concentrations of oxygen in inhaled air or through hyperbaric measures can result in increased oxygen delivery to the inner retina.[18,23]

We have shown that the pO2 values in the blood can be markedly increased by having monkeys breathe 100% oxygen under normobaric conditions. The pO2 increases even more if the animals breathe 100% oxygen at two atmospheres of pressure. Also, it is possible to increase the preretinal vitreous oxygen tension in monkeys by exposing the animals to two atmospheres of 100% oxygen. The pO2 values in the preretinal vitreous rose from the normal range of 45 to 76 mm Hg to markedly increased values of 80 to 211 mm Hg after 30 minutes. Simply breathing 100% oxygen in this model did not increase the pO2 value in the preretinal vitreous despite higher blood pO2 levels. An increase in the preretinal vitreous oxygen tension indicates higher inner retinal oxygen levels. If humans exhibit the same rise as the monkeys, then two atmospheres of 100% oxygen could potentially be used in patients with acute vascular occlusions to temporarily protect the retina. If true, then the preservation of retinal function might be possible for ischemic conditions that are self-limited, such as central retinal artery occlusion (which frequently is transient). Clinical trials using hyperbaric oxygen delivery acutely in these patients would be of value. Based on prior work by Hayreh and Weingeist,[47] it has been indicated that the healthy primate retina can tolerate retinal vascular shutdown for approximately 90 minutes before permanent damage occurs. The institution of hyperbaric oxygen therapy in this interval might allow preservation of inner retinal function, as manifested by the maintenance of vision and a normal elec-

troretinogram. Whether the oxygen delivered in this situation spills over from the retinal vessels or from the choroid is not an academic point. If the choroid is the source of the oxygen then hyperbaric therapy would be very helpful in patients with central retinal artery occlusion. If the retinal vessels supply the increased oxygen, then one would expect a much reduced effect of hyperbaric oxygen in increasing inner retinal oxygen levels and preserving inner retinal function. Resolution of the controversy in regard to delivery of oxygen from the choroid to the inner retina should follow now that detailed polarographic methods[24,30] are being used, including simultaneous intraretinal recording of electroretinograms and oxygen levels.

SUMMARY

When delivered to the corneal surface of rabbits or monkeys, 100% oxygen can significantly increase the pO2 in the aqueous humor.

Under hyperbaric conditions (two atmospheres), an observed rise in the aqueous pO2 in rabbits breathing room air can be increased further by exposing the rabbit cornea to 100% oxygen. The high oxygen levels under hyperbaric conditions are mediated by intravascular and transcorneal delivery of oxygen. The increase in the pO2 levels in the aqueous can prevent sickling of intracameral human erythrocytes containing sickle hemoglobin. Thus, oxygen therapy transcorneally or systemically could potentially be used to treat a sickle cell hyphema.

The exposure of rabbit eyes to 100% oxygen at the corneal surface is followed by autoregulation (constriction) of the iris vasculature. We could demonstrate no constriction in the eyes of two normal human volunteers or of four patients with chronic stable rubeosis iridis.

Preretinal vitreous pO2 levels can be significantly raised by exposing monkeys to hyperbaric 100% oxygen. This procedure may be of value in treating acute, reversible ischemic inner retinal diseases.

Transcorneal or vascular delivery of oxygen to the eye under normobaric or hyperbaric conditions may be effective in treating ischemic diseases of the anterior segment, such as anterior segment necrosis or rubeosis iridis, or ischemic inner retinal diseases.

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