Oxygen Kinetics in the Vitreous Substitute Perfluorotributylamine: A $^{19}$F NMR Study In Vivo

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Perfluorocarbon (PFC) vitreous substitutes have physical properties which are advantageous for vitreoretinal surgery. Compared to water (vitreous is 97-99% water), they have a higher density which favors a downward tamponade on the retina, a high interfacial tension, a lower viscosity, and a similar index of refraction. Preliminary studies using PFC as vitreous substitute in retinal reattachment surgery have produced promising results. However, a physical property of PFC liquids in the eye which, to our knowledge, has not been explored in this context involves its oxygen handling capabilities.

Under typical surgical conditions, the PFC vitreous substitute perfluorotributylamine (FTBA) is in equilibrium with atmospheric PO$_2$ (150 mm Hg) prior to injection into the vitreous cavity. It would be useful to determine how long it takes the PO$_2$ in FTBA to return to typical vitreous oxygen tensions (<20 mm Hg) once placed in the eye. Generally, if the time constant of oxygen clearance from the FTBA is sufficiently long, then the FTBA might have a significant oxygen reservoir capacity due to its factor of 13 greater oxygen solubility than vitreous. Increasing the PO$_2$ above 150 mm Hg by preoxygenation of the FTBA with 100% O$_2$ immediately before injecting the compound into the vitreous cavity. The animal was continuously ventilated with room air. A single exponential profile was again obtained with a time constant of 69.6 ± 12.6 min (mean ± SD, n = 4) which does not differ statistically from the above mean (p = 0.4). This work represents the first to characterize oxygen kinetics in a perfluorocarbon vitreous substitute in vivo. These experiments provide a basis for future investigations to determine the possible beneficial effects of oxygenated PFC when used as vitreous substitutes during vitreoretinal surgical conditions. Invest Ophthalmol Vis Sci 32:2382-2387, 1991

Perfluorocarbon (PFC) vitreous substitutes yielded promising results in the surgical management of retinal detachments. This success is due primarily to their useful physical properties. However, oxygen kinetics in PFC in vivo have not been investigated. The oxygen flux in the vitreous substitute perfluorotributylamine (FTBA) was assessed in the rabbit eye by monitoring the partial oxygen pressure (PO$_2$) in real-time using Fluorine-19 nuclear magnetic resonance spectroscopy ($^{19}$F NMR). The spin-lattice relaxation rate ($T_1^{-1}$) of the CF$_3$ resonance of FTBA is a rapid and sensitive index of PO$_2$. $T_1$-derived PO$_2$ from the FTBA-filled rabbit eye was followed at regular time intervals under different oxygenation protocols. In the first series of experiments, FTBA in the vitreous space was oxygenated by ventilating the rabbit with a mixture of 95% O$_2$ and 5% CO$_2$. The oxygen uptake profile could be approximated by a simple exponential function with a time constant of 159 ± 110 min (mean ± SD, n = 3). A more reproducible correlate was obtained by performing an initial rate analysis on the first hour of ventilation with high oxygen levels. This analysis showed that the rate of increase in FTBA PO$_2$ was 2.34 ± 0.67 mm Hg/min (mean ± SD, r$^2$ = 0.99, n = 7). After the animal was removed from the 95% O$_2$/5% CO$_2$ gas and was ventilated with room air, the oxygen clearance profile could be approximated in all cases by a single exponential with a time constant of 59.8 ± 9.6 min (mean ± SD, n = 4). To assess whether or not ventilating the animal with 95% O$_2$/5% CO$_2$ could have influenced the O$_2$ clearance rate, FTBA was preoxygenated with 100% O$_2$ immediately before injecting the compound into the vitreous cavity. The animal was continuously ventilated with room air. A single exponential profile was again obtained with a time constant of 69.6 ± 12.6 min (mean ± SD, n = 4) which does not differ statistically from the above mean (p = 0.4). This work represents the first to characterize oxygen kinetics in a perfluorocarbon vitreous substitute in vivo. These experiments provide a basis for future investigations to determine the possible beneficial effects of oxygenated PFC when used as vitreous substitutes during vitreoretinal surgical conditions. Invest Ophthalmol Vis Sci 32:2382-2387, 1991

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mination of \( \text{PO}_2 \) in the vitreous space of the vitrectomized, FTBA-filled rabbit eye under steady state conditions.\(^5\) The NMR method provided values of \( \text{PO}_2 \) that were consistent with values previously determined for the central and posterior vitreous space.\(^5\) In contrast, \( \text{PO}_2 \) has traditionally been measured in various locations in the eye with an oxygen sensitive probe, eg, an oxygen microelectrode. The NMR method offers several advantages over this approach. Once the FTBA has been introduced into the vitreous cavity, no additional invasive procedures are required by the NMR method to determine \( \text{PO}_2 \). In addition, the \(^{19}\text{F} \) NMR method provides a rapid, absolute, and quantitative measure of \( \text{PO}_2 \) in real time, without the problems of drift, light-induced retinal changes (a potential disadvantage of the newer fiber–optic probe\(^6\)), oxygen consumption, or oxygen diffusion/stirring sometimes associated with oxygen sensitive probes.\(^5\)

The goal of this study was to investigate, using \(^{19}\text{F} \) NMR, temporal changes in \( \text{PO}_2 \) within the FTBA-filled vitreous space in the rabbit eye under different oxygenation protocols.

**Materials and Methods**

**Animal Preparation**

The 1–1.5 Kg male or female Dutch belted rabbits used in this study were treated in accordance with institutional guidelines and the Association for Research in Vision and Ophthalmology (ARVO) resolution on the use of animals in research. Each animal was anesthetized with a mixture of ketamine HCl (35 mg/kg) and xylazine HCl (5 mg/kg) intramuscular and the vitreous of one eye was gas-compressed with perfluoropropane gas (injected volume 0.3–0.4 ml).\(^1\) After 2–3 days, the expanded gas bubble was exchanged, under anesthesia, with commercially available neat FTBA, which had been passed through a 0.45 \( \mu \)m filter.\(^1\) On the day of the experiment, the animal was initially anesthetized as described above. Anesthesia was maintained by intravenous administration of ketamine (20–40 mg/kg/hr) via the auricular vein. Arterial access was obtained either by catheterizing the auricular or the femoral artery. The heart rate and pressure (typically 240–300 beats/min and 100–150 cm water, respectively) were continually monitored and these data were stored on floppy disk.\(^5\) A tracheotomy was then performed and the animal was artificially ventilated via a small-animal respiration pump (Harvard Apparatus, model 665, S. Natick, MA). While on the respirator, pancuronium bromide was intravenously administered to the animal (1.2 mg/kg/hr). The rectal temperature of the animal was maintained at 37–38°C via a circulating water blanket connected to a constant temperature bath. Blood gas monitoring (Instrumentation Laboratories, micro 13, Lexington, MA) was performed periodically to ensure proper status of the animal. Once the animal was physiologically stable (typical anesthetized blood gas values were pH \( \approx 7.4–7.5, \) \( \text{PCO}_2 \approx 20–30, \) \( \text{PO}_2 \approx 30–60 \)), it was gently placed in a home-built nonmagnetic cradle and secured. The low arterial \( \text{PO}_2 \) was partly the result of the anesthetic protocol used.\(^3\)

Two oxygenation protocols were followed. In experiment A, the animals were examined by \(^{19}\text{F} \) NMR between 5 and 48 hours after gas-fluid exchange. After the \(^{19}\text{F} \) NMR spectra (vide infra) and blood gas measurements indicated a stable preparation, the ventilation gas was switched from room air to 95% \( \text{O}_2/5\% \) \( \text{CO}_2 \) until the FTBA \( \text{PO}_2 \) approached the blood \( \text{PO}_2 \) (about 2–3 hours); the gas was then switched back to room air until the end of the experiment. In experiment B, the animal was prepared as above but the gas-fluid exchange was performed immediately before placing the animal in the magnet. In this case, the FTBA was passed through a 0.45- \( \mu \)m filter, bubbled with 100% oxygen for 5 min, and immediately used for gas-fluid exchange. The exchange procedure took approximately 5 min. At all times the animal was ventilated with room air. At the end of most experiments (A and B), the animals were killed and then both eyes were enucleated and weighed to estimate the extent of vitreous replacement. The average weight ratio of FTBA-filled to the normal fellow eyes was 1.45 \( \pm \) 0.13 (mean \( \pm \) SD, \( n = 11 \)).

**NMR Procedure**

All in vivo experiments were performed on a 4.7 T GE CSI horizontal bore system (GE, Fremont, CA). A round, 3-cm diameter, single-turn surface coil tuned to 188.2 MHz was positioned over the orbital region so that the eye protruded slightly through the center of the coil. Direct pressure on the eye was avoided. Centering of the animal in the magnet and shimming were performed on the fluorine signal. Typical linewidths on the CF \(_3 \) resonance varied from 40–50 Hz.

Inversion recovery (IR) \( T_1 \) measurements were performed in 10 or 15 min intervals during the entire time course of experiments A and B with hyperbolic secant pulses with pulse widths of 2 msec at approximately 75 watts of transmitter power.\(^3\) Ten delay values were used to determine the spin-lattice time constant: 10 \( \mu \)s, 50 msec, 100 msec, 200 msec, 300 msec, 400 msec, 900 msec, 1 s, 2 s, and 5 s; these values were alternated between long and short delays to help minimize systematic error. The delay between obtaining each \( T_1 \) point was 5 sec (approximately 4–5 \( T_1 \)). The data were acquired with a spectral width of 3000 Hz and 1K complex data points. The high signal-to-noise ratio (approximately 1400:1)\(^3\) in these experiments
permitted a single acquisition per delay point that resulted in an experiment time of 1 min/T₁ determination. These data were analyzed with software resident on the CSI computer (GE). Each T₁ value was converted, as previously described, to a PO₂ via a calibration curve obtained on a FTBA phantom at 37°C.

The time course of O₂ clearance in the FTBA in vivo was fit to a three-parameter exponential function of the form PO₂(t) = af + b*exp(−t/k₁); where af is the baseline PO₂ value, b + af is the PO₂ at time zero, and k₁ is the clearance time constant. This fit was performed starting with the T₁ point after the blood gas had returned to the control level. Only disappearance time courses which covered 2.5 time constant or greater were included in the data analysis (n = 4 for experiments A and B). The PO₂ uptake time profile could be approximated by a simple exponential of the form PO₂(t) = ([af-ao][t/k] + ao); where af is the FTBA PO₂ after ventilating the animal at high oxygen concentrations for a very long time, ao is the starting PO₂, and k is the time constant. When the (t/k) is 0.5 or less, this equation can be approximated by the linear equation PO₂(t) = ([af-ao][t/k] + ao) with an error of 20% or less. In these experiments, only three animals had oxygen uptake curves adequate for fitting to the exponential function. This was due to the relatively long time constant for oxygen uptake by FTBA. However, all animals in experiment A (n = 7) could be fit to the linear form of this equation and the resultant slopes were used to estimate the rate of oxygen uptake. Steady state PO₂ values were pooled from various experiments in which animals were ventilated with room air (n = 1). Statistical analysis was performed using student t-test.

### Results

Figure 1 is a representative time course of both the T₁-derived and blood PO₂ levels of an animal ventilated first with room air, then 95% O₂/5% CO₂, and finally room air again. This time course covers nearly 6 hr. In contrast to oxygen electrode measurements which can require an hour or more to yield a reproducible PO₂ value, once the probe has been introduced into the vitreous cavity, the NMR experiment yielded rapid and stable values once the animal was positioned inside the magnet and the static field homogeneity was optimized (≈10 min). The stability of the NMR experiment is shown in Figure 1, which also shows that during the control period of 30 min prior to the animal being ventilated with high oxygen concentration, no change in the FTBA PO₂ value was measured. The steady-state PO₂ value measured while breathing room air was 20.9 ± 5.1 mm Hg (mean ± SD, n = 11), which is in good agreement with the literature values of the central and posterior PO₂ in the rabbit eye, as shown previously. As expected, increased blood PO₂ resulted in increased FTBA PO₂ in the vitreous space. The mean uptake time constant was 159 ± 110 min (mean ± SD, n = 3). The large standard deviation could be attributed to unpredictable fluctuations in blood PO₂ at the high-inspired oxygen levels and to the somewhat truncated uptake curve, ie, much less than five time constants, both of which greatly reduced the precision of this determination. However, fitting the PO₂ increase during the first hour of ventilation with 100% O₂ to a straight line, ie, an initial rate analysis, yielded a reproducible correlate (the slope of the line) of the rate of oxygen uptake: 2.34 ± 0.67 mm Hg/min (mean ± SD, mean: r² = 0.99, n = 7).

Once the animal was taken off of the O₂/CO₂ mixture, the blood PO₂ returned to a relatively constant control level within 5 min. As seen in Figure 1, O₂ clearance from the FTBA was well-approximated by a single exponential. The average time constant for clearance of the PO₂ from FTBA in the vitrectomized...
space of the rabbit eye was 59.8 ± 9.6 min (mean ± SD, n = 4).

Figure 2 is a representative PO_2 time course of FTBA which had been bubbled with 100% O_2 immediately before gas-fluid exchange. Each animal was ventilated with room air and the blood PO_2 varied in the range of 34.8 ± 11 to 61.8 ± 21 mm Hg (mean ± SD, n = 4) during the 5-hr time course. The time constant of O_2 clearance was 69.6 ± 12.6 min (mean ± SD, n = 4) which is not statistically different from above (P = 0.4). Thus, the two clearance time constant values were pooled to yield a mean value of 63.8 ± 12 min (n = 8).

**Discussion**

In these studies, oxygen kinetics in FTBA in the vitreous space of the rabbit eye were investigated by 19F NMR under different oxygenation protocols. While detailed models of oxygen flux near the retina have been developed,8,9 such models are not readily applicable to this case where oxygen flux through the whole vitreous space in the vitrecomized eye is studied. To this end, a more comprehensive treatment of oxygen kinetics in FTBA in the rabbit eye is being developed and will be presented elsewhere. Nonetheless, conclusions can be made based on these data.

After a step change in ventilation gas from air to 95% oxygen, it took 2–3 hr for the entire FTBA PO_2 to approach blood PO_2 levels. This is in reasonable agreement with previously published values obtained in the cat eye with the oxygen microelectrode for the length of time required to reach steady-state PO_2 under conditions of breathing pure oxygen (1–2 hr).8 In these experiments, the observed oxygen uptake profile could be approximated by a simple exponential function. However, the uptake time constant determined in this manner had a large standard deviation. This is probably due to the unpredictable fluctuations in blood PO_2 during the long time (2–3 hr) each animal was ventilated with 95% O_2, and the relatively long uptake time constant. Thus, the observation time was in practice limited to approximately one time constant. To obtain a more precise measure of the oxygen uptake rate, we fit the data in the first hour after switching to 95% oxygen to a straight line, ie, an initial rate analysis. The linear correlate (mean r^2 = 0.99) of oxygen uptake during this time had a reproducible slope (vide supra). Using Henry’s law, the slope, expressed as a partial pressure per unit time, could be converted to a vol/unit time of 1.7 ± 0.51 μl O_2/min. To address the question of whether this rate of oxygen flux into the vitreous space is reasonable, we estimated the rate of oxygen delivery from choroidal blood under hyperoxia. In this estimate, we assumed no net release of oxygen from hemoglobin because this would only increase oxygen delivery. Based on a plasma–oxygen solubility of 0.03 μl O_2/ml blood/mm Hg,11 a blood PO_2 of 300 mm Hg, and a choroidal blood flow under hyperoxia of 1 ml/min,12 it is calculated that the choroidal blood flow can deliver 9 μl O_2/min. The whole rabbit retinal (100 mg) oxygen consumption is 5.7 μl O_2/min.13 If, as in the cat, retinal oxygen consumption is not altered by hyperoxia,10 and oxygen consumption of the choroid itself can be considered negligible in comparison, as much as 3.3 μl O_2/min might then enter the vitreous space. Thus, the value determined for oxygen influx into FTBA in this case seems to be reasonable.

The O_2 clearance in the FTBA-filled rabbit eye could also be approximated by a simple exponential function. This suggested a rate-limiting step that is dependent on the oxygen concentration. Several possibilities for the rate-limiting step may be considered: (1) oxygen transport across the FTBA/water interface; (2) choroidal blood flow; (3) retinal oxygen consumption; and (4) oxygen diffusion. If oxygen transport across the FTBA/water interface was rate-limiting, then the rate of the O_2 clearance in this study might be expected to have a different shape and clearance time constant than in a nonvitrectomized eye that does not contain FTBA. Briggs and Rodenhauser ventilated cats with 100% oxygen for several hours.14 The inspired gas was then switched to room air and the oxygen levels from different regions of the eye were measured as a function of time with an oxygen electrode.14

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**Fig. 2.** Representative PO_2 time course of oxygen clearance from FTBA, which had been preoxygenated with 100% oxygen immediately before being placed into the vitreous space. The animals in this experiment were ventilated on room air the entire time and the blood PO_2 values were essentially constant over the 5 hr time course. In this animal the blood PO_2 was 33.2 ± 7.8 mm Hg (mean ± SD, n = 10) averaged over the entire time course. The oxygen clearance was again well approximated by a single exponential with a time constant for this animal of 63.3 min.
The oxygen clearance time constant in the central portion of the eye could be estimated from their data and yielded a time constant of 68 min which is within reasonable agreement with the mean time constant of O₂ clearance determined in this study (63.8 ± 12 min, n = 8) obtained under similar conditions. This comparison suggests that the oxygen clearance in the vitreous space is probably limited by either the FTBA/water interface or species related differences in retinal structure, since the cat retina is thicker and has a more fully developed retinal vasculature than the rabbit retina.

Another possible rate-limiting step involves the choroidal circulation. The choroid has an extremely rapid blood flow and only minimally autoregulates with changes in the oxygen level. In these studies, the highest partial oxygen pressure in the FTBA was approximately 300 mm Hg, or 153 µl of oxygen/ml FTBA, at the time of the step change from high inspired oxygen levels to room air. Under normoxic conditions, hemoglobin can bind 200 µl O₂/ml blood. Given that the room-air blood PO₂ in this study was approximately 50 mm Hg (which translates to an arterial hemoglobin oxygen desaturation of approximately 20%), the choroidal circulation can remove roughly 40 µl O₂/min (neglecting dissolved oxygen). At this clearance rate, it would only take a few minutes to bring the FTBA PO₂ to baseline values. Even at a choroidal blood A-V difference of 3% (normoxia), it will only take about 1/2 hr for the FTBA PO₂ to approach baseline values. These calculations suggest that the choroidal circulation is probably not a rate-limiting step in oxygen clearance from FTBA.

A third possible rate-limiting step involves retinal oxygen consumption. Over 60 min, the amount of oxygen consumed by the whole rabbit retina (vide supra) would be 342 µl. This exceeds the amount of oxygen contained within 1.5 ml of FTBA at a PO₂ of 300 mm Hg (230 µl). Therefore, the time required for the retina to use the oxygen contained within the oxygenated FTBA, under the conditions of this study, would not be expected to exceed 40 min. However, 60 min after switching the ventilation gas from 95% oxygen to room air approximately 120–135 µl of oxygen is still present in the FTBA. This suggests that retinal oxygen consumption is probably not the rate-limiting step under these conditions.

Oxygen diffusion may also be considered as a rate-limiting step. To test this possibility, the clearance time constant of oxygen determined in this study was converted to a diffusion coefficient. This was done by first approximating the vitreous space to a sphere that contained a uniform oxygen concentration with a different, but constant, oxygen level at its surface. Under these conditions, the total amount of oxygen leaving the sphere, based solely on diffusion, is given by a sum of exponential terms. If we assume that the first term in the sum dominates, then the clearance time constant can be set equal to the exponent term Dπr²/t where D is the diffusion coefficient and r is the radius of the sphere. The volume of FTBA used in the gas-fluid exchange (~1.5 ml) allows the radius to be estimated as 0.71 cm (assuming a spherical volume). This calculation yielded an oxygen diffusion coefficient of 1.3 x 10⁻⁵ cm²/sec, which is in close agreement with published oxygen diffusion coefficients in pure FTBA of 2.0–5.8 x 10⁻⁵ cm²/sec. Previous studies showed that the oxygen diffusion coefficient (D) in FTBA and water (and thus, probably vitreous) are essentially the same under identical conditions. Reliable values for the oxygen diffusion coefficient through the retina (D retina) are unavailable. This calculation, however, suggests that oxygen diffusion may play a rate-limiting role under these conditions.

A possible source of error in our measurement arises because the T₁ observed do not come from a homogeneous sample, but from a solution of heterogeneous PO₂ values in exchange. If the exchange rate were not rapid, ie, slower than the shortest T₁ rate, a multicomponent T₁ data set would be observed. However, this was not the case in our study because the T₁ data set at each time point could be well approximated by a single exponential under both steadystate, ie, control conditions as well as during oxygen entry and clearance. In addition, preliminary images of FTBA-filled rabbit eyes in vivo did not show an oxygen gradient within the vitreous space during the time the animals were ventilated with 100% oxygen (data not shown). It appears that a somewhat rapid (relative to the shortest T₁ rate) re-equilibration of oxygen within the FTBA occurs in the vitrectomized eye. This hypothesis is further supported by previous oxygen electrode studies in which a re-equilibration of anterior chamber PO₂ to values similar to the vitreous was found after lensectomy and vitrectomy. These observations are attributed to increased convection currents within the intraocular fluid volume. These currents do not appear to introduce a T₁ measurement error (vide supra). Further, this argument supports the notion that the T₁ measurement of FTBA in the vitrectomized rabbit eye reflects the volume-weighted oxygen partial pressure from primarily the central and posterior vitreous. Although the preretinal vitreous space can have regions of relatively high PO₂ in nonvitrectomized eyes, the volume corresponding to this partial pressure is relatively small compared with the whole vitreous vol-
volume and thus the preretinal PO₂ contribution is probably relatively minor.

A related issue involves the possibility of using FTBA as an oxygen reservoir. In this case, once the FTBA is in the vitreous space and oxygenated, the PO₂ will decrease with a time constant of approximately 60 min (vide supra). In this situation, as opposed to conditions of retinal ischemia, choroidal blood flow is intact and the retinal metabolic needs for oxygen are largely met by the choroid. Nonetheless, excess oxygen from the FTBA may still be used by portions of the inner retina or, as discussed above, lost through diffusion. The question of how long FTBA could be expected to supply the metabolic requirements in retinal ischemia cannot be directly addressed by this work. However, we presume that this time would largely depend on the extent of ischemia, i.e., total retinal ischemia vs inner retinal ischemia such as seen in retinal vascular disease. In diseases such as diabetic retinopathy and branch retinal vein occlusion, retinal vascular insufficiency produces localized regions of inner retinal ischemia, whereas the more highly metabolic portion of the retina, i.e., the photoreceptor layer, is adequately supplied by the choroid. In these diseases, FTBA might supply the oxygen needs of the ischemic portion of the retina for a period of time on the order of hours as predicated based on the time constant in the present experiments. This time could be extended by fully oxygenating the FTBA to 760 mm Hg prior to its placement into the vitreous cavity. The utility of FTBA in treating chronic retinal ischemia is limited at the present time by the need for periodic recharging of the material with oxygen and by the retinal toxicity of FTBA which appears histologically two days after the FTBA is placed in the vitreous space.¹

Preliminary experiments in other laboratories have shown that supplying oxygen from the vitreous, either through an oxygenated perfusion medium (vitreoperfusion) or by inserting dialysis tubing with a steady flow of 100% oxygen, is successful in partially restoring ischemic retinal function.²⁰,²¹ We are investigating whether or not the oxygen provided from FTBA, introduced into a vitrecomized eye, will be efficacious in temporarily maintaining retinal function under hypoxic conditions.

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