Perfluorinated Organic Liquid as an Intraocular Oxygen Reservoir for the Ischemic Retina

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Purpose. Liquid perfluorocarbons are used as temporary vitreous substitutes in the surgical management of complicated retinal detachment. The purpose of this study was to determine if physiologic benefits could also be derived from the high oxygen solubility of perfluorocarbons relative to vitreous, especially during retinal ischemia.

Methods. The normal vitreous humor of the rabbit eye was replaced with either perfluorotributylamine (FTBA) or balanced salt solution (BSS). Retinal ischemia was then induced by increasing the intraocular pressure above the peak systolic blood pressure for intervals of 10, 30, or 90 minutes.

Results. Over a 10- or 30-minute period of ischemia, during which electroretinographic (ERG) responses were recorded, FTBA-filled eyes and BSS-filled eyes showed decreases in the a- and b-wave amplitudes. However, wave amplitudes were significantly greater in FTBA-filled eyes at most times examined (P < .05). ERG responses were maintained throughout a 30-minute ischemic interval in oxygenated FTBA-filled eyes, but not in oxygenated BSS- or deoxygenated FTBA-filled eyes. When examined 1 day after a 90-minute interval of ischemia, oxygenated FTBA-filled eyes maintained 45% and 57% of the preischemic ERG a- and b-wave amplitudes, respectively, compared to a 5% and 3% retention of wave amplitudes in oxygenated BSS-filled eyes. On light microscopic examination of these eyes, FTBA-exposed retinas showed less ischemic damage than BSS-exposed retinas.

Conclusions. When used as a vitreous substitute, FTBA exerts a neuroprotective effect on the ischemic retina that appears to relate to an increased retinal oxygen supply compared to BSS.

Hypoxia is a major cause of cellular injury in ischemia because tissue stores of oxygen are limited relative to other substrates of metabolism. Investigations in humans have shown that loss of visual function occurs within several seconds of pressure-induced ischemia and that this time is increased 10-fold by hyperbaric tissue oxygenation before induction of ischemia. Animal studies have since demonstrated that oxygen provided from the vitreous space can prolong retinal function during ischemia. In the isolated perfused mammalian eye, high oxygen tension in the perfusate has also increased the duration of retinal light responsiveness. Based on these results, oxygen therapy has been proposed as a possible means of altering the course of ischemic retinal diseases. Traditional methods of oxygen therapy have varied from the use of high concentrations of inspired oxygen administered at atmospheric pressure to systemic hyperbaric oxygenation. More recent studies have focused instead on the use of local oxygen therapy. One such technique involves the use of a goggle for the administration of oxygen to the ocular surface. We have previously evaluated this approach and found that oxygen administered at atmospheric pressure for as little as 30 minutes can elevate preretinal oxygen tensions in the rabbit eye after removal of the lens and vitreous.
Advances in vitreous surgery have allowed the development of other, more direct methods of retinal oxygenation through the vitreous space.5,14

Removal of the vitreous humor (vitrectomy) has become standard in the treatment of diseases involving complex retinal detachment or vitreous opacification. In some instances, liquid perfluorochemicals (LPFCs) have been used as temporary vitreous substitutes to assist the surgeon in repositioning the detached retina.15–16 Properties that facilitate the use of LPFCs as surgical tools include their biologic compatibility, immiscibility in water, and high specific gravity (which provides a gravitationally directed force on the retina).17,18 However, a property that has received little attention by retinal surgeons is the high oxygen solubility of LPFCs relative to water.20–22

Increasing evidence suggests that LPFCs, because of their high oxygen solubility, can produce beneficial effects on the retina. Thoreson and Purple found that perfusion of the isolated cat eye with solution containing an LPFC-in-water emulsion improved retinal electrophysiologic responses.23 Braun et al found that an emulsion of perfluorooctylbromide, when introduced into the blood, could improve oxygenation of the retina in vivo.24 However, these applications were limited to the experimental use of perfluorochemicals as artificial blood substitutes, and no data were provided as to their effects on the ischemic retina.

We sought to determine whether a pure (non-emulsified) LPFC in the vitreous space could provide sufficient oxygen to alter the retinal response to ischemia. Previously, we examined the kinetics of oxygen clearance from an LPFC vitreous substitute, perfluorotributylamine (FTBA), in the normal rabbit eye and found an exponential time constant of 60 to 70 minutes.25 This result led us to speculate that oxygenated LPFCs could be useful in supplying oxygen to the ischemic retina.

In this study, we replaced the vitreous of the rabbit eye with FTBA and studied the effect of ischemia on retinal function, as measured by the electroretinographic (ERG) responses. In control eyes, the vitreous was replaced by balanced salt solution (BSS), an aqueous formulation used as a vitreous substitute during vitreous surgery. Electroretinographic and light microscopic data demonstrated a neuroprotective effect of oxygenated FTBA in retinal ischemia. This neuroprotective effect was reduced by decreasing the FTBA oxygen tension before use; alteration of the oxygen concentration in BSS produced little, if any, change in retinal responses to ischemia.

METHODS

Animals

Experiments were performed according to institutional guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Male Dutch-belted (pigmented) rabbits weighing 2 to 3 kg each were anesthetized with ketamine (20 to 40 mg/kg) and xylazine HCl (2 to 4 mg/kg) by intramuscular injection. During prolonged experiments, anesthetic induction was followed by a constant intravenous infusion (through the marginal ear vein) of ketamine and xylazine (80 mg/hour and 8 mg/hour, respectively). The pupils were dilated with cyclopentolate 1% and phenylephrine 2.5% drops. Topical anesthesia was induced with proparacaine 0.5% drops.

Continuous monitoring of heart rate and blood pressure and periodic blood gas measurements (approximately every 15 minutes) were performed from an auricular artery. Heart rates ranged from 180 to 220 bpm, and arterial pressures ranged from 80 to 100 mm Hg; peak systolic pressures did not exceed 105 mm Hg. Animals were mechanically ventilated with oxygen-enriched room air to maintain the arterial Po2 in the range of 80 to 120 mm Hg and pH in the range of 7.350 to 7.450. A water-circulating heating blanket was used to maintain normal core body temperature (approximately 39°C). After experimentation, anesthetized animals were killed with an intravenous injection of saturated KCl solution.

Vitreous Liquefaction

Cryotherapy to the inferior, peripheral retina was performed to produce a localized retinal scar through which to enter the vitreous safely in subsequent procedures. Two weeks later, vitreous liquefaction was produced according to the method of Thresher et al.26 Briefly, this method involves the injection of perfluoropropane, an expansible gas, into the vitreous cavity in a volume of 0.4 ml. The gas was given in two 0.2 ml injections, 15 minutes apart, to avoid prolonged elevation of intraocular pressure. During the next two weeks, the gas was allowed to expand and then dissolve without further manipulation. The effect of this procedure on vitreous fluidity has been reported.27

Vitreous–Fluid Exchange

Under a stereoscopic operating microscope, a 6-O silk limbal traction suture was placed at the 6-o’clock position and used to elevate the eye. The inferior conjunctiva and muscle attachments were incised to expose bare sclera. In protocols A and B (see Experimental Protocols), a single 19-gauge infusion cannula was inserted into a sclerotomy created with a 22-gauge needle at the 5-o’clock position (3 to 4 mm posterior to the limbus) and secured in position with 6-O silk suture. The cannula was attached to a infusion bottle of BSS elevated to 40 mm Hg pressure (hydrostatic pressure was confirmed at the eye level using a mercury manometer). A 6-O silk purse-string suture was then placed in the sclera at the 7-o’clock position,
after which a 25-gauge needle was used to create a small sclerotomy to allow slow egress of BSS from the eye. After 5 minutes of vitreous washout, either BSS or FTBA was injected through the 25-gauge sclerotomies. Two 25-gauge infusion cannulas were placed, one each at the 5- and 7-o’clock positions. Although one cannula was connected to an elevated infusion bottle of BSS as described, the other was closed with a stopcock. This configuration facilitated washout of FTBA or BSS from the eye at the end of the period of ischemia (see Protocol C below). After ischemia and washout, the cannulas were removed and the scleromies were closed with 8-0 polyglycan suture. Finally, topical antibiotic ointment was applied to the operated eyes.

When injecting vitreous substitutes, the volume of FTBA used (1.4 ml) was sufficient to fill the available vitreous space. However, because of the possibility of rapid exchange of BSS with endogenous water, larger volumes of BSS (as large as 30 ml) were injected to ensure adequate replacement. Any excess volume was allowed to reflux up the infusion cannula and was collected at a three-way stopcock between the cannula and the infusion bottle.

Oxygenation–Deoxygenation of Vitreous Substitutes

Oxygenation or deoxygenation of vitreous substitutes was accomplished by bubbling the substitute with either 100% oxygen or 100% nitrogen, respectively, for 10 minutes. Oxygenation was sufficient to elevate BSS P02 to >700 torr (measured on an AVL model 995 blood gas analyzer; AVL Scientific, Roswell, GA), which remained stable in capped syringes until use (solutions were used within 2 hours of preparation). In protocol C, oxygenated FTBA and BSS samples were heated to 39°C in a water bath before injection. Heating did not influence the P02 of BSS samples that were analyzed. Normoxic vitreous substitutes were used as supplied by the manufacturer after ventilating the containers to the room air.

Electroretinography

Electroretinograms were elicited using a flash photostimulator (Model PS22; Grass Instruments, Quincy, MA) placed 30 cm from the eye. The photostimulator produces a 1-μsec flash at G16 intensity. A corneal contact lens electrode and a needle electrode in the subcutaneous tissue over the superior orbital rim served as the active electrodes, whereas a second needle electrode inserted into the occipital scalp region served as the ground. Amplified signals (gain = 1000; band pass = 0.1 Hz to 1 KHz) were digitized using an IBM PC-based system running custom-designed software. Although room lights were off and instrument lights were minimized, these experiments were performed under mesopic conditions because 10 stimuli presented at a frequency of 1 Hz were averaged to obtain each ERG.

Experimental Protocols

Protocol A (10-minute Ischemia). The vitreous was exchanged with either FTBA or BSS (n = 4 eyes for each condition), both of which were room air equilibrated, as described. Forty minutes after exchange, the intraocular pressure was raised to 140 mm Hg by elevating the infusion bottle. Electroretinograms were acquired at 2-minute intervals for the next 10 minutes. The bottle was then lowered to control pressure (40 mm Hg), and ERG recovery was observed during the next 20 minutes (see Results).

Protocol B (30-minute Ischemia). The vitreous was exchanged with either oxygenated FTBA, deoxygenated FTBA or oxygenated BSS (n = 5 eyes for each condition). Forty minutes after exchange, the intraocular pressure was raised for 30 minutes in the manner described. Electroretinograms were recorded at frequent intervals during ischemia and reperfusion (see Results). Parallel 19F NMR studies were performed using either oxygenated or deoxygenated FTBA (see 19F NMR Experiments below).

Protocol C (Ischemia Recovery). Electroretinograms were obtained in triplicate after ocular cannulation but before injection of vitreous substitutes. The vitreous was then replaced with oxygenated FTBA in one eye and oxygenated BSS in the opposite eye of each of five animals. Five minutes later, the intraocular pressure was elevated in both eyes as described. After 90 minutes of ischemia, the pressure was lowered and fresh BSS was used to wash out the vitreous space of both eyes, removing most of the FTBA in the process. The next day, ERGs were repeated in triplicate.

19F NMR Experiments. These parallel experiments were performed to determine the oxygen content of FTBA vitreous substitutes used in the above studies and to determine the rate of oxygen clearance from oxygenated FTBA before, during, and after ischemia. The vitreous was replaced with either oxygenated or deoxygenated FTBA as described. As long as 40 minutes later, control FTBA oxygen tensions were obtained from the eyes using 19F NMR. In eyes that received oxygenated FTBA, the pressure was elevated.
and lowered as described in protocol B, but with the animal inside the magnet. Sequential NMR oxygen measurements were obtained throughout this process.

The details of the $^{19}$F NMR experiment have been described. Briefly, the animals were secured in a home-built plexiglass cradle with the eye of interest protruding slightly through the center of a 5-cm diameter surface coil. Physiologic monitoring and control were provided as described. The animals were placed in a 4.7 T GE CSI horizontal bore system (General Electric, Freemont, CA), and the coil was tuned to 18.8 MHz. $T_1$ experiments were performed using the inversion-recovery method and were iterated with temperature determinations that were based on chemical shift measurements. Each temperature was measured from a spectrum derived from 64 acquisitions. After correcting for temperature, $T_1$ values were converted to $P_O_2$ measurements as described.

**Histopathology.** Eyes from animals in protocol C were fixed in a solution of paraformaldehyde 2% and gluteraldehyde 2.5%. Both eyes of two representative animals were dissected, and a 5 mm x 7 mm specimen of retina, choroid, and sclera was obtained from an area approximately 2 mm inferior to the optic nerve. The specimens underwent automated tissue processing and were embedded in glycolmethacrylate. Sections (2-μm thick) were cut and stained with methylene blue and basic fuchsin. Tissue was then examined by a masked observer using light microscopy.

**RESULTS**

**Protocol A (10-Minute Ischemia)**

Figure 1 contains the ERG results of experimental protocol A, in which the effects of a 10-minute period of ischemia in eyes containing room air equilibrated FTBA or BSS were examined. Initial ERG amplitudes (a- and b-wave) were slightly lower in FTBA-filled eyes than in BSS-filled eyes, but this difference was not statistically significant ($P > .45$). After the imposition of retinal ischemia, a-and b-wave amplitudes decayed over time, but the ERG amplitudes were significantly greater ($P < .05$) in FTBA-filled eyes than in BSS-filled eyes at most points during the ischemic interval.

**Protocol B (30-Minute Ischemia)**

In these experiments, oxygen tensions of the vitreous substitutes were altered by bubbling with pure oxygen or nitrogen before use. The effect on FTBA oxygen tensions 40 minutes after intravitreal injection (a time corresponding to the initiation of ischemia in ERG experiments, described below) was assessed by $^{19}$F NMR, whereas the oxygen concentration in BSS was measured from vitreous samples. In NMR experiments, deoxygenated FTBA was found to maintain a low $P_O_2$ after the exchange procedure (22 ± 11 mm Hg, mean ± SD, $n = 3$). Similarly, oxygenated FTBA maintained a relatively high $P_O_2$ (270 ± 27 mm Hg, $n = 3$). Oxygenated BSS sampled from the vitreous of eyes at 40 minutes after injection had significantly higher $P_O_2$ values (101 ± 10 mm Hg, $n = 3$, $P < .001$) than those observed in deoxygenated FTBA and significantly lower ($P < .001$) values than those found in oxygenated FTBA.

Figure 2 contains the results of $^{19}$F NMR experiments. Eyes filled with oxygenated FTBA were subjected to approximately 50 minutes of pressure ischemia. During the interval examined, the profile of oxygen clearance was essentially linear. The rate of oxygen clearance from oxygenated FTBA did not change after initiation of ischemia, except for a brief reduction in the first 5 minutes. During this short interval, the FTBA $P_O_2$ actually increased after ischemia by an average of 19 ± 2.7 mm Hg (mean ± SD, $n = 3$; $P = .007$). The rate of oxygen clearance during the period of ischemia was 2.4 ± 0.0 mm Hg/minute. Based on this result and the known oxygen solubility of FTBA (40.2 ml O$_2$/100 ml FTBA), a volumetric oxygen clearance rate of 76 μl/hour·ml FTBA was calculated (106 μl/hour from an FTBA volume of 1.4 ml).

Figure 3 contains the ERG results of experimental protocol B, in which a 30-minute interval of ischemia was produced in eyes filled with oxygenated FTBA, deoxygenated FTBA, or oxygenated BSS. Initial ERG amplitudes were not significantly different between conditions, except for the comparison of eyes that received deoxygenated FTBA and those that received oxygenated BSS. In this comparison, a-wave amplitude was significantly lower ($P = .009$) in the FTBA-filled eyes. A similar effect was noted on b-wave amplitudes, but the difference was not statistically significant ($P = .06$).

At most times during the 30-minute period of ischemia, ERG amplitudes in oxygenated FTBA-filled eyes were significantly greater than those observed in BSS-filled eyes or in eyes that received deoxygenated FTBA. However, in the first 4 minutes of ischemia, a- and b-wave amplitudes of eyes that received oxygenated FTBA were not significantly different than they were in eyes that received deoxygenated FTBA ($P > .05$). After this interval, wave amplitudes in eyes filled with deoxygenated FTBA followed a time course of ERG decay similar to that observed in BSS-filled eyes.

**Protocol C (Ischemia Recovery)**

In protocol C, the effect of oxygenated FTBA on the retina was examined after a longer period of ischemia (90 minutes) than was used in protocols A and B. Opposite eyes filled with oxygenated BSS served as controls. The initial oxygen tensions of the vitreous...
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FIGURE 1. Absolute (A, C) and relative (B, D) ERG amplitudes in experimental protocol A (10-minute ischemia). In this protocol, the vitreous was exchanged with either room air-equilibrated BSS (B) or room air-equilibrated FTBA (C) 40 minutes before onset of ischemia at t = 0 minutes. No significant differences (P > .05) were noted in baseline ERG amplitudes (A). The ischemic interval (shown) was 10 minutes, during which significant differences (P < .05, Student's t-test) were noted in a- and b-wave amplitudes between groups (*). Error bars represent ± SEM for four eyes. ERG = electroretinogram; BSS = balanced salt solution; FTBA = perfluorotributylamine.

substitutes used in this experiment were comparable. For FTBA, the mean oxygen tension can be estimated from the data in Figure 2 and was 359 ± 28 mm Hg (mean ± SD, n = 3). In comparison, oxygen tensions measured directly from oxygenated BSS-filled eyes 5 minutes after injection were not significantly different (317 ± 93 mm Hg, n = 2; P = .48).

Electroretinograms were collected before and 1 day after ischemia, and the results are summarized in Table 1. The preischemic ERG amplitudes in FTBA- and BSS-filled eyes were not statistically different (P > .05). However, postischemic a- and b-wave amplitudes were significantly greater in the FTBA-filled eyes (P < .02). Post-ischemic ERG amplitudes in BSS-filled eyes were not significantly different from zero, whereas FTBA-filled eyes retained an average of 45% of control a-wave amplitude and 57% of b-wave amplitude.

Photomicrographs of representative eyes in protocol C are shown in Figure 4. Masked examination of these retinas confirmed greater ischemic damage in the BSS-filled eyes than in the FTBA-filled eyes, as evidenced by loss of ganglion cells and greater vacuolization of the inner nuclear and plexiform layers of eyes that were treated with oxygenated BSS. These differences were also found, to a lesser extent, in the outer nuclear layer.

DISCUSSION

In this study, we examined the effect of an oxygenated LPFC, FTBA, on the time-course and outcome of retinal ischemia. Retinal function during ischemia, as measured by the ERG, decreased less rapidly in eyes in which the vitreous was replaced with FTBA than in eyes filled with BSS, regardless of the initial oxygen tension of the FTBA. The effect of FTBA on ERG responses could be altered by changing the oxygen content of the compound. In ischemia-outcome studies (protocol C), oxygenated FTBA-filled eyes retained 45% to 57% of preischemic ERG responses 1 day after an ischemic interval that has been shown to extinguish ERG responses in the normal rabbit eye and that extinguished such responses in eyes filled with oxygenated BSS in the present study.
During the ischemic interval (shown), FTBA Po2 decreased at the same rate as noted before or after ischemia (2.4 ± 0.0 mm Hg/minute). However, at the start of ischemia, the Po2 increased slightly in all eyes by an average of 19 mm Hg (computed by extending the regression lines fitted to the data before and during ischemia to the start of ischemia at 48 minutes). The cause of this phenomenon is unknown (see Discussion). FTBA temperatures determined from chemical shift data decreased by an average of 0.8°C during ischemia (P = .02) but returned to control values during reperfusion (data not shown). This effect is consistent with an interruption of blood flow by elevation of the intraocular pressure. FTBA = perfluorotributylamine; NMR = nuclear magnetic resonance.

Stratified relative preservation of retinal structure in FTBA-filled eyes compared to BSS-filled eyes. These results appear consistent with our initial hypothesis that oxygenated perfluorochemicals in the vitreous space can serve as oxygen reservoirs for use by the ischemic retina.25 Several alternative explanations for the beneficial effect of FTBA in these studies were explored. First, differences between the thermal properties of LPFCs and aqueous vitreous substitutes may alter the amount of retinal cooling during ischemia. The neuroprotective effect of even small temperature reductions has been emphasized in studies of cerebral ischemia.32 To examine the possibility that the retina may have cooled more rapidly in FTBA-filled eyes than in BSS-filled eyes, we compared the thermal properties of FTBA with those of water. The specific heat capacity of FTBA is one fourth that of water at near-physiologic temperatures (1.05 J/g-K and 4.18 J/g-K, respectively), and the thermal conductivity is almost an order of magnitude less than that of water (66 mW/K-m and 615 mW/K-m) (data courtesy of PCR, Inc., Gainesville, FL). Taken together, these data imply that intravitreal FTBA acts as a thermal insulator to anteriorly directed heat loss from the retina and that reductions in temperature that accompany ischemia are not expected to occur more rapidly in the retina FTBA-filled eye than in the BSS-filled eye, given similar convection-related heat losses. In addition, oxygenated and deoxygenated FTBA would be expected to exhibit the same thermal response, which strongly suggests that thermal considerations do not account for ERG differences observed in protocol B.

We next considered the effect of carbon dioxide solubility of the vitreous substitutes used in this study. Assuming that FTBA, by providing an oxygen source for the retina, allows aerobic metabolism to proceed during ischemia (to the extent that energy substrates are available), it follows that carbon dioxide will be generated with attendant acidosis. Therefore, differences in carbon dioxide solubility or diffusivity might lead to differences in the neuroprotective effect of the vitreous substitutes described. However, the relative difference in carbon dioxide solubility of FTBA and water (isotonic saline) is much smaller (142 volume % versus 67 volume % at 37°C) than for oxygen (40.2 volume % versus 2.4 volume % at 37°C).20,33 and the diffusivities of carbon dioxide in water and in LPFCs have been reported to be similar.34 The 2.1-fold increase in carbon dioxide solubility of FTBA compared to water may have conferred some protection from acidosis in the FTBA-filled eyes. However, it still does not explain the ERG differences observed in FTBA-filled eyes at different oxygen tensions.

Fluid convection could serve to eliminate oxygen gradients within vitreous substitutes and promote the efficient diffusion of oxygen across the vitreo-retinal interface. The viscosity of a vitreous substitute is expected to play a large role in this regard. However, the viscosity of FTBA (2.8 centistokes at 25°C)19 does not differ greatly from that of water (0.9 centistokes at 25°C).35 Therefore, the effect of fluid “stirring” is not likely to favor oxygen transfer to the retina from either vitreous substitute in these experiments. Finally, energy substrate (i.e., glucose) availability might explain the effects of a vitreous substitute on the ischemic retina. Weiss has shown in the nonvitrectomized rabbit eye that intraretinal carbohydrate reserves are rapidly depleted during pressure ischemia, and that in the first hour of ischemia, two thirds of the substrate required to maintain anaerobic glycolysis is supplied by the vitreous.36 Other investigators have shown that supplemental intravitreal glucose exerts a large neuroprotective effect on the ischemic rat retina.37 Neither vitreous substitute used in this study contained glucose. However, it is possible that part of the neuroprotective effect of oxygenated FTBA is related to a shift in retinal carbohydrate metabolism from anaerobic to aerobic. This could reduce the rate of intraretinal carbohydrate depletion during ischemia.

As suggested earlier, a likely explanation for the observed differences between FTBA and BSS in this study relates to a large difference in their oxygen solubility. In a diffusion-dominated model, the rate of oxy-
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Relative A Wave Amplitude

Time (minutes)

FIGURE 3. Absolute (A, C) and relative (B, D) ERG amplitudes in experimental protocol B (30-minute ischemia). Vitreous was exchanged with oxygenated BSS (■), oxygenated FTBA (□), or deoxygenated FTBA (○) 40 minutes before the onset of ischemia at t = 0 minutes. At baseline (t = 0 minutes), no significant differences in ERG amplitudes was noted between groups except for the comparison of a-wave amplitudes in the oxygenated BSS-versus deoxygenated FTBA-filled eyes (P = 0.009). The ischemic interval (shown) was 30 minutes, during which significant differences (P < 0.05) were noted between oxygenated FTBA-filled eyes and BSS-filled eyes only (*) or both BSS-filled and deoxygenated FTBA-filled eyes (†). Note that after reperfusion, the increase in ERG amplitudes in FTBA-filled eyes appeared to exceed that of BSS-filled eyes at several times points. Error bars represent ± SEM for five eyes. ERG = electroretinogram; BSS = balanced salt solution; FTBA = perfluorotributylamine.

Gen clearance from an oxygen-containing vitreous substitute is expected to depend on the difference between retinal PO2 and the PO2 of the vitreous substitute, as well as on the diffusion coefficient of oxygen in the vitreous substitute. The oxygen diffusivities of water and FTBA are similar.34,38 However, because of the large difference in oxygen solubility, any given volumetric rate of oxygen clearance will result in a more rapid PO2 decrease in BSS than in FTBA. In this case, the higher oxygen solubility of FTBA would maintain a larger PO2 gradient at the vitreo-retinal interface during the intervals of ischemia examined in this study. This could explain why the ERG changes in eyes that were filled with FTBA at low oxygen tensions did not differ greatly from those filled with oxygenated FTBA during the first 4 minutes of ischemia (Table 2). Even at the lowest oxygen tension examined (22 mm Hg), FTBA still contains approximately

TABLE 1. Survival of Electroretinographic Responses 1 Day After a 90-Minute Interval of Ocular Ischemia in the Presence of Oxygenated Vitreous Substitutes

<table>
<thead>
<tr>
<th></th>
<th>A Wave Amplitudes (μV ± SD)</th>
<th>B Wave Amplitudes (μV ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FTBA*</td>
<td>BSS*</td>
</tr>
<tr>
<td>Before ischemia</td>
<td>195 ± 45</td>
<td>222 ± 49</td>
</tr>
<tr>
<td>24 hours after ischemia</td>
<td>88 ± 55</td>
<td>11 ± 9</td>
</tr>
<tr>
<td></td>
<td>145 ± 38</td>
<td>186 ± 38</td>
</tr>
<tr>
<td></td>
<td>82 ± 43</td>
<td>6 ± 6</td>
</tr>
</tbody>
</table>

* Eyes in which the vitreous was replaced with either FTBA or BSS (values represent the mean of 5 eyes).
† Paired t test (FTBA versus BSS).
FIGURE 4. Light micrographs of retinas from eyes of two representative animals in protocol C (ischemia-reperfusion), in which eyes filled with oxygenated FTBA (left) were compared to fellow eyes that were filled with oxygenated BSS (right). All eyes were subjected to 90 minutes of ischemia, followed by 24 hours of reperfusion. Note that the ganglion cell and other cell layers appear well preserved in eyes that were filled with oxygenated FTBA. In contrast, retinas exposed to oxygenated BSS during ischemia showed widespread vacuolization and cell death, which was most severe in the inner retina. FTBA = perfluorotributylamine; BSS = salt solution.

four times the amount of oxygen as the BSS used in protocol B (101 mm Hg).

In studies of the nonischemic rabbit eye, the kinetic of oxygen clearance from intravitreal oxygenated FTBA (2.8 mm Hg/minute) was consistent with a diffusion-limited process that was dependent on the diffusion coefficient of oxygen in FTBA. In the present study, a similar rate of oxygen clearance (2.4 mm Hg/minute) was found, and no sustained differences were noted in the rates of oxygen clearance before, during, or after the ischemic interval. Although cessation of blood flow did not appear to change the zero order kinetic of oxygen clearance from oxygenated FTBA (initial slope of Po2 decrease), it is difficult to assume that oxygen clearance will be identical in the perfused and nonperfused eye. When the Po2 of FTBA is large, the difference between the vitreo-retinal Po2 gradient in perfused and nonperfused eyes is proba-
TABLE 2. Changes in ERG Amplitudes During the First Four Minutes of Retinal Ischemia

<table>
<thead>
<tr>
<th>Vitreous Substitute</th>
<th>A Wave Changes (µV/min)</th>
<th>B Wave Changes (µV/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygenated FTBA</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Deoxygenated FTBA</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Oxygenated BSS</td>
<td>37</td>
<td>30</td>
</tr>
</tbody>
</table>

Based on data from Figures 3A and 3C. Numbers represent slopes of linear fits to first 4 minutes of data acquisition after induction of ischemia. All fits were satisfactory with $r^2 \approx 0.80$.

The rate of oxygen clearance appears the same. However, at a much lower FTBA oxygen tension (approaching that of the normal inner retina), the rate of oxygen clearance is expected to be slower in the perfused eye because oxygen efflux from FTBA equilibrates with oxygen influx supplied by blood flow.

The only change in the clearance rate of oxygen from oxygenated FTBA in the $^{19}$F NMR experiments appeared at the onset of pressure ischemia. At that point, an upward offset in PO$_2$ averaging 19 mm Hg was observed. Because there was no apparent biologic source for this additional oxygen, we speculate that intermolecular or intramolecular interactions due to the increased pressure may have slightly altered the relationship between the NMR-derived parameters and oxygen tension. In this case, the increase in PO$_2$ would be considered artifactual. Curiously, a similar reverse offset in PO$_2$ was not observed on lowering the pressure, as might have been expected if this were true. Therefore, the cause of this phenomenon will require further investigation.

The fate of oxygen cleared from the FTBA during ischemia is not certain. Whether all the oxygen is consumed by the retina or a portion is left to diffuse out of the eye cannot be determined from the ERG data. The ERG does not provide a direct measure of retinal metabolism, and it may be influenced by attendant features of ischemia, such as acidosis. However, if we assume that the oxygen that leaves FTBA is almost entirely consumed by the retina, it may be possible to compare the oxygen supply with retinal oxygen requirements on a theoretical basis.

As described above, the rate of oxygen efflux from 1.4 ml of FTBA was approximately 106 µl/hour. Ames and Li have recently determined the normal oxygen consumption of the whole rabbit retina in vitro. Under scotopic conditions, they reported a consumption rate of 128 nmol O$_2$/minute-retina, which translates to a volumetric consumption of 197 µl O$_2$/hour-retina at 1 atm pressure. In the avascular outer retina of the cat, other investigators have estimated that the oxygen consumption of the dark-adapted retina may be reduced by 40% during light adaptation. Tillis et al have provided evidence to suggest that the rabbit retina behaves in a similar fashion. Based on these results, we estimate that 1.4 ml of oxygenated FTBA can possibly supply 54% of the normal oxygen requirement of the rabbit retina under conditions of highest demand (dark adaptation) or perhaps a greater percentage under the mesopic conditions used in this study. Further investigation will be needed to determine whether the state of light adaptation modulates the neuroprotective effect of oxygenated FTBA.

During ischemia, the relative a- and b-wave amplitudes decreased at similar rates, as shown in Figure 5. This should not have been the case if all the oxygen supplied by the FTBA was insufficient to meet the demands of the ischemic inner retina, because oxygen must diffuse a greater distance from an intravitreal source to supply the photoreceptor layer than to supply the inner retinal layers. One explanation for this effect is that the oxygen requirement of the inner (and possibly outer) retina was reduced by pressure ischemia, perhaps by a reduction in temperature. Because the oxygen requirements of the different layers of the pressure-ischemic rabbit retina are not known, it is difficult to interpret this finding further.

The results of these experiments point toward the possible use of perfluoroochemical vitreous substitutes in ischemic retinal diseases. However, the protective effect of these compounds may be limited by the temporary nature of the oxygen reservoir or by other effects of ischemia that are not addressed by such vitreous substitutes. One feature of many ischemic retinop-
athies (i.e., diabetic retinopathy, ischemic retinal vein occlusions) that may permit a prolonged benefit of perfluorochemical vitreous substitutes is the focal nature of the process: Usually only small areas of inner retina are devoid of retinal blood flow. In most cases, areas of the retinal vasculature remain perfused as does the choroidal circulation, which supplies the outer retina. In this case, we can envision a process of facilitated oxygen movement whereby perfluorochemicals serve as an oxygen conduit between perfused and nonperfused retina. Such a process might provide benefit for focally ischemic retinas even after the oxygen in the LPFC has reached equilibrated levels. Although FTBA was the only LPFC used in these experiments, other LPFCs used clinically in retinal detachment surgery, also have high oxygen solubility and are expected to behave similarly.

There are several concerns about the use of oxygenated vitreous substitutes in humans. First, chemical toxicity and mechanical damage must be assessed and minimized. All LPFCs that have been evaluated in animal studies, including FTBA, have been shown to cause some degree of retinal damage when left in the vitreous cavity for extended periods. The cause of this damage remains uncertain. Second, the high oxygen concentrations provided by preoxygenated substitutes could promote oxidative damage to the retina. The extent to which this will occur in the normal or ischemic retina is unknown, but oxidative damage mediated by inflammatory cells has been cited as a possible mechanism of tissue injury associated with ischemia in various tissues, including the retina. These issues will require further evaluation before clinical application will be possible. In addition, the confirmation of these findings will be necessary in another animal model that, unlike the rabbit, possesses a fully vascularized retina. However, given future development, application of these findings to circulatory disturbances of the retina in humans appears feasible.

Key Words
perfluorochemicals, vitreous substitutes, rabbit, retinal ischemia, oxygen

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References
Perfluorotributylamine and Retinal Ischemia


