Perfluorocarbon emulsion improves oxygenation of the cat primary visual cortex

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Padnick, Lissa B., Robert A. Linsenmeier, and Thomas K. Goldstick. Perfluorocarbon emulsion improves oxygenation of the cat primary visual cortex. J. Appl. Physiol. 86(5): 1497–1504, 1999.—Tissue PO₂ was measured in the primary visual cortex of anesthetized, artificially ventilated, normovolemic cats to evaluate the effect of small doses [1 g perfluorocarbon (PFC)/kg] of a PFC emulsion (1 g PFC/1.1 ml emulsion; Alliance Pharmaceutical, San Diego, CA) on brain oxygenation. The change in tissue PO₂ (ΔPO₂), resulting from briefly changing the respiratory gas from room air to 100% oxygen, was measured before and after intravenous infusion of the emulsion. Before emulsion, ΔPO₂ was 51.1 ± 45.6 Torr (n = 8 cats). Increases in ΔPO₂ of 34.0 ± 26.1 (SD) % (n = 6) and 16.3 ± 8.4% (n = 6) were observed after the first and second emulsion infusions, respectively. The further increase in ΔPO₂ after the third dose (7.9 ± 10.5%; n = 7) was not statistically significant. The observed increases in tissue oxygenation as a result of the PFC infusions appear to be the result of enhanced oxygen transport to the tissue.


tissue oxygen tension; brain

Perfluorocarbon (PFC)-based artificial oxygen carriers have been proposed as a replacement for donor blood as well as a therapy for minimizing ischemic tissue damage (29, 38). The administration of a small dose (1 g PFC/kg body wt) of a PFC-based oxygen carrier (1 g perfluorobron/ml emulsion; Alliance Pharmaceutical, San Diego, CA) has been shown to enhance substantially oxygen transport in the retina of normovolemic cats while the animal was breathing 100% oxygen (5). This effect was surprising considering that the addition of such a small amount of PFC to the blood must have increased arterial oxygen-carrying capacity by 1% or less (Appendix A). To be efficacious in certain clinical applications, PFC emulsions would have to improve oxygenation of organs other than the retina, including the brain. Although the retina is often considered representative of the brain, the unique organization of the retinal vasculature (e.g., Ref. 3) may cause tissue oxygenation to differ in the two neural tissues.

The intravenous infusion of PFC emulsions has been shown to be beneficial in the treatment of stroke in cat (17, 26) and dog (13) models. Peerless et al. (26) concluded that the first-generation PFC emulsion, Fluosol (Green Cross, Osaka, Japan), had a protective effect on ischemic brain tissue in cats. Macrostrophic and histological tissue evaluations were consistent with the animals' neurological status after recovery from anesthesia. Fluosol-treated animals (5.25 g PFC/kg) were in better neurological condition, both histologically and clinically, than animals given an equal volume of an isotonic saline solution; however, differences between animals hemodiluted with Fluosol and animals hemodiluted with a mannitol solution were marginal. Kline et al. (17) also studied the effect of Fluosol on middle cerebral artery ligation in cats. After a 2-h ischemic period and a 2-h reperfusion period, animals were hemodiluted with Fluosol (5.25 g PFC/kg) or a dextran solution. The delay in treatment was meant to mimic a human clinical situation. Eight hours after reperfusion, cytochrome aa₃ in the ischemic penumbra was in a more oxidized state in the animals treated with Fluosol than in those left untreated or those treated with dextran. This suggested that the Fluosol-treated cats were in better condition, metabolically, possibly because of an improved cerebral oxygen supply. In a canine cerebral ischemia model (13), the magnitude of the auditory-evoked potential was used as the measure of brain stem function. Five hours after reperfusion, the animals pretreated with a PFC emulsion (1.5 g PFC/kg) exhibited an 88% recovery in evoked potential magnitude, whereas those animals given an equal volume of saline showed only a 23% recovery (13).

Infusion of PFC emulsions has also been shown to increase oxygen availability during hyperoxia in the brain of rabbits (9, 33) and cats (31). These studies measured oxygen availability rather than PO₂ because the investigators used relatively large cathodes. Large polarographic electrodes consume enough oxygen so that an artificial PO₂ gradient may be formed within the tissue, thus altering tissue PO₂. Actual brain PO₂ during hyperoxia has been reported to be increased in dogs hemodiluted with perfluorobron emulsion (1). Batra et al. (1) measured the average PO₂ in a fairly large region of the parietal cortex with a commercial oxygen electrode system (Eppendorf, Madison, WI).

The purpose of the present research was to directly examine the efficacy of a PFC emulsion (AF0104, 1 g perfluorobron/1.1 ml emulsion; Alliance Pharmaceutical) in enhancing brain PO₂ by recording locally from the visual cortex of the cat. Cortical tissue PO₂ was recorded during 2-min episodes of ventilation with 100% oxygen (hyperoxia), both before and after small doses of the PFC emulsion (1 g PFC/kg body wt) were delivered intravenously. By using small (5–10 μm) polarographic microelectrodes, we were able to obtain more local measurements than did most previous investigators. A unique aspect of this study was that visual-evoked
potentials, recorded from the same site as oxygen measurements, were used to examine cortical activity and to verify electrode penetration of the tissue (24).

METHODS

Animal preparation. The surgical procedures and experimental setup have been described previously (25). Three of the cats used in the previous study (25) were also used in this one, along with six additional cats for a total of nine animals. All measurements were made with double-barreled oxygen-voltage electrodes. Polarographic oxygen electrodes are not influenced by the presence of PFC emulsions (5, 6).

Experimental protocol. Tissue PO2 transients were measured during 2-min episodes in which the animal breathed 100% oxygen (hyperoxia). Two-minute periods were used to avoid any physiological changes that may be induced by long-term hyperoxia. These periods of hyperoxia were at least 10 min apart to allow the animal to completely return to its basal state. Each PFC dose consisted of 1.1 ml emulsion/kg. This dose resulted in the addition of 1 g PFC/kg. Each of the three emulsion doses contributed an incremental, theoretical fluorocrit (volume %PFC in blood) of 0.7% (assuming 70 ml blood/kg). All doses of the PFC emulsion were administered over ~3 min during ventilation with room air.

Before the PFC was administered, three control episodes of hyperoxia were used to check for reproducibility of the tissue PO2 transients and stability of the baseline. When the control transient responses were too variable, negative, or when only an immeasurably small PO2 change (ΔPO2) could be detected during hyperoxia, the electrode was repositioned in the brain. In some cortical locations, a positive-going oxygen transient in response to hyperoxia was not observed. Of all the intracortical locations sampled for a response to hyperoxia, approximately one-third of them had a negative-going oxygen transient or showed no measurable ΔPO2. These locations were not used to test the effect of the PFC emulsion on brain oxygenation. When no satisfactory intracortical location could be found, the electrode was positioned 50–150 µm above the brain surface, and the experiment was performed with the electrode in the chamber fluid (n = 4).

Data analysis. The effect of the first dose of the perfluorobron emulsion was determined by calculating the percent increase in the difference between tissue PO2 during ventilation with 100% oxygen and room air (ΔPO2). The transient immediately before and the transient immediately after emulsion infusion were used to calculate the percent ΔPO2. To determine whether inherent brain variability was influencing the results, the two values of ΔPO2 immediately before emulsion infusion were compared with each other in the same manner. All changes between consecutive transients were compared to zero with a two-tailed, paired Student’s t-test. Data were excluded from analysis in one cat because of baseline instabilities, and in another cat (cat 169) an outlying result for the second emulsion infusion (2.35 SD away from mean), in which the recording became unstable during or just after the recording of the postinfusion transient, was excluded. It was determined that a computer error resulted in loss of data for the third PFC infusion in one cat (cat 165).

The effectiveness of the second and third doses (each 1 g PFC/kg) was evaluated in the same manner as described above for the first dose. Because the same recording site could not always be used for all recordings, the cumulative effect of the second and third doses could not be obtained from direct comparison with the control PO2 transient recorded before the first emulsion infusion. Consequently, the hypothetical cumulative dose effects had to be calculated from the individual effects as described in APPENDIX B.

Statistics. Statistical significance was determined by a two-tailed, paired Student’s t-test except in the case in which the results from the present study and a separate retinal study were compared. Here, a two-tailed, unpaired Student’s t-test was performed. A P value of <0.05 was used as the criterion for statistical significance.

RESULTS

Response in tissue PO2 to ventilation with 100% oxygen (hyperoxia). Local responses in cortical PO2 to brief (2-min) episodes of hyperoxia were examined. As in previous studies (18, 19, 21, 23, 36), a variable response was observed. Different locations showed an increase, decrease, or no measurable ΔPO2 after the respiratory gas was changed from room air to 100% oxygen. In three of the cats, all three types of responses were observed in the same animal with the same electrode.

Figure 1A shows two responses from different locations in the same site recorded at a similar depth 2 h apart. As is demonstrated, intracortical areas that had
a higher baseline PO2 and a positive-going transient in response to hyperoxia tended to have a larger transient amplitude (∆PO2) than did areas with smaller baseline PO2 values. A significant correlation between baseline PO2 and ∆PO2 was found for intracortical (r2 = 0.665, P < 0.0001, n = 43 transients) and extracortical (r2 = 0.187, P = 0.009, n = 36 transients) data (Fig. 1B).

Effect of the PFC emulsion during ventilation with room air (normoxia). After the first 1 g PFC/kg infusion, while the animal was still breathing room air, the average increase in tissue PO2 was 8.2 ± 18.5 Torr (n = 7; PO2 during infusion not recorded in 1 cat). This increase was not statistically significantly different from zero (P = 0.29), probably due to the variability of the response (3 increases, 2 decreases, 3 no change in PO2). The top trace in Fig. 2 shows a slight increase in PO2 during emulsion infusion; the bottom trace shows a slight decrease in PO2 during emulsion infusion. The average changes in normoxic PO2 during the second and third infusions were 0.9 ± 3.3 Torr, respectively. Neither of these changes was significantly different from zero.

Infusion of the first emulsion dose seemed to affect the systemic blood pressure slightly. On infusion of the first dose, the blood pressure sometimes transiently decreased 5–10 mmHg (while the animal was still breathing room air). This decrease lasted for only a few minutes. All hyperoxic episodes were imposed after the blood pressure had recovered. The second and third emulsion infusions had no significant effect on the blood pressure.

Effects of the PFC emulsion during ventilation with 100% oxygen (hyperoxia). Figure 2 shows examples of oxygen transients recorded immediately before and after infusion of 1 g PFC/kg body wt. Figure 2A was recorded intracortically, and Fig. 2B was recorded slightly above the brain in the artificial cerebrospinal fluid. Often a trace with a stable baseline showed increased fluctuations during hyperoxia, as shown in Fig. 2A.

![Fig. 2. Consecutive oxygen transients separated by first PFC infusion in 2 cats. A: data from cat 169 were recorded intracortically at an electrode depth of 500 µm, B: data from cat 174 were recorded 150 µm above surface of brain.](image)

Table 1. Effect of the first 1 g PFC/kg dose of PFC emulsion

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Depth (µm)</th>
<th>Baseline PO2 (Torr)</th>
<th>∆PO2 Before Infusion 1 Torr</th>
<th>∆PO2 After Infusion 1 Torr</th>
<th>Increase in ∆PO2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>142</td>
<td>100</td>
<td>40.5</td>
<td>24.8</td>
<td>25.7</td>
<td>3.6</td>
</tr>
<tr>
<td>165</td>
<td>200</td>
<td>0.1</td>
<td>136.2</td>
<td>233.7</td>
<td>71.6</td>
</tr>
<tr>
<td>169</td>
<td>500</td>
<td>27.0</td>
<td>52.7</td>
<td>82.3</td>
<td>56.2</td>
</tr>
<tr>
<td>172</td>
<td>−100</td>
<td>24.4</td>
<td>46.8</td>
<td>54.0</td>
<td>15.4</td>
</tr>
<tr>
<td>173</td>
<td>400</td>
<td>3.3</td>
<td>2.6</td>
<td>3.6</td>
<td>38.5</td>
</tr>
<tr>
<td>174</td>
<td>−150</td>
<td>9.7</td>
<td>10.8</td>
<td>16.8</td>
<td>55.6</td>
</tr>
<tr>
<td>175</td>
<td>−100</td>
<td>11.7</td>
<td>35.0</td>
<td>45.6</td>
<td>30.3</td>
</tr>
<tr>
<td>179</td>
<td>−100</td>
<td>52.3</td>
<td>100.0</td>
<td>100.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Mean ± SD: 21.1 ± 17.2, 45.6 ± 70.3, 34.0 ± 26.1

Table 1 gives the effect of the first emulsion dose for each cat. Recording depths ranged from 150 µm above the cortex (negative) to 500 µm within the cortex (positive). The first PFC emulsion infusion always resulted in an increase in ∆PO2 (n = 8), but it was of variable amplitude. The mean increase in ∆PO2 was 34.0 ± 26.1 (SD) % (P = 0.008). On the other hand, the average ∆PO2 between the two transients taken immediately before emulsion infusion was not significantly different from zero. For some unexplained reason, there was, however, a nearly significant (P = 0.054) decrease in ∆PO2 of 17.8 ± 21.6% (n = 8) between the first and second transients after the first emulsion infusion.

Table 2 provides the baseline PO2 and average transient amplitudes, both before and after PFC infusion, for the second and third doses. The second PFC infusion (1 g PFC/kg) resulted in an increase in ∆PO2 of 13.7 ± 10.2% (n = 7, P = 0.004). The third emulsion infusion (1 g PFC/kg) resulted in an increased ∆PO2 of 7.8 ± 10.5% (n = 7), but this effect was not statistically significant (P = 0.074). Figure 3 shows the effects of all three individual doses.

It is important to note that the mean baseline PO2 reported in Tables 1 and 2, is not representative of average cortical tissue PO2. Because it was necessary to collect data at sites selected for sizable positive-going hyperoxic PO2 transients, our baseline PO2 was skewed toward a higher value (Fig. 1B). Average cortical PO2 has been reported to be 12.8 Torr (25). In addition, approximately one-half of the baseline values averaged for Tables 1 and 2 were obtained extracortically.

### DISCUSSION

Measurement of brain oxygen enhancement. The present study demonstrates the ability of a PFC emulsion (AF0104, Alliance Pharmaceutical) to enhance cortical...
oxygenation during transient hyperoxic conditions. Previous studies examining the effect of PFC infusions have used large electrodes to obtain a relative measurement of cortical oxygenation (19, 31, 33). The present study is one of the first to use small (5- to 8-µm tip) recessed-cathode microelectrodes inside of a sealed skull chamber. The smaller electrodes have several advantages. Polarographic electrodes consume oxygen in the process of measuring it, and the larger the electrode, the greater the oxygen consumption. Because of this phenomenon, larger electrodes create greater artificial oxygen sinks, and therefore gradients, making the PO2 in the presence and absence of the electrode different. For this reason, investigators using larger electrodes are forced to report oxygen availability rather than PO2. With recessed cathode microelectrodes, such a small amount of oxygen is consumed that tissue PO2 is not affected (30). In addition, a smaller electrode should compress the tissue and blood vessels less than a larger one. Also, smaller electrodes give very local measurements of PO2, whereas larger electrodes give spatial averages of tissue oxygenation. We attempted to make all measurements within the brain to exploit these advantages of our microelectrodes, but this was not always possible. Fortunately, measurements in the chamber fluid near the brain gave very similar results to intracortical ones. It is possible that the PFC effect may not be uniform at all cortical locations, and it would be worth investigating this with a technique in which PO2 could be measured in multiple cortical locations simultaneously.

Braun et al. (5) observed a substantial increase in retinal oxygenation after the intravenous administration of 1 g PFC/kg. The present study was done to determine whether this enhancement was similar in other neural tissue. To compare the PFC effect on the brain and the retina, we had to utilize similar analyses. Considering that in the preretinal vitreous only positive values of ΔPO2 were observed, we could only examine cortical locations with a positive ΔPO2 so that a fair comparison between the brain and the retina could be made.

Mechanism of the PFC-induced tissue oxygenation enhancement. After a single dose of 1 g PFC/kg, the brain, like the retina, exhibited an increase in tissue oxygenation that was much greater than expected from the minute increase in the oxygen-carrying capacity of the blood. Also, arterial blood PO2 does not change after emulsion infusions. In a previous experimental series, in which the effect of the emulsion on the retina was examined under virtually identical systemic conditions (5), no changes in normoxic or hyperoxic arterial PO2 were observed after emulsion administration. There was also no measurable effect of the infusion on hematocrit with the use of the standard evaluation methods (5). Apparently, the blood volume is regulated to be constant, by either renal mechanisms or blood and/or extracellular fluid shifts, so that the increase in oxygen-carrying capacity of the blood after the addition of 1 g PFC/kg to the blood is only 1.2% under hyperoxic conditions (Appendix A). If this regulation of blood volume did not occur, blood volume would increase by at most 1.66%, and the oxygen content in 100 ml of hyperoxic arterial blood would decrease by ~0.4% after the first PFC infusion. The observed enhancement of tissue PO2 must have resulted from something more than simply a change in the oxygen-carrying capacity of the blood.

Either a PFC-mediated increase in cerebral blood flow or a decrease in brain oxygen consumption could explain the enhancement. Experimental data suggest that cerebral blood flow and metabolism are not affected by the specific PFC emulsion used in this study. No experimental evidence has been published that shows vasoactivity of small doses of the PFC emulsion used here. Systemic hemodynamics do not change after intravenous infusion of the emulsion (e.g., Refs. 4, 20). Furthermore, it should be noted that increased oxygen transport has been observed in vitro in oxygenators, where flow was experimentally maintained at a constant rate (34). In addition, while the animal was breathing room air, neither brain PO2 nor electrophysiological activity (flash visual-evoked potential) was altered by the administration of the PFC, suggesting that metabolism was not affected. A previous study that used the same emulsion formulation as examined in the present work did not show any decrements in brain stem activity after emulsion infusion (13). Because systemic hemodynamics, cerebral blood flow, and cortical electrical activity appear not to be changed by the presence of small amounts of PFC, alternative explanations have been postulated.

Other possible mechanisms involve an increase in the overall mass transfer coefficient of oxygen from blood to tissue. Whereas it is not possible to quantify the contributions of these mechanisms, the basic ideas can be discussed. It is important to note that PFC particles essentially do not enter brain tissue. The PFC leaves the bloodstream unchanged through excretion by the lungs (29, 38). Only the transport within the
blood will be discussed, as it seems unlikely that transport characteristics of the tissue would be changed by the intravascular infusion of the emulsion.

The dominant resistance to oxygen transport from blood to tissue is thought to be in the plasma. There is comparatively little resistance to oxygen transport present in the red blood cell (RBC) (22). For vessels that are 100–200 µm in diameter, the identical PFC droplets as used in the present study (∼0.3 µm in diameter) have been found to be concentrated in an erythrocyte-free annulus near the wall (32). This phenomenon effectively decreases the overall resistance of the plasma phase to oxygen transport (8) by creating an annulus of PFC droplets that may act as “stepping stones” for oxygen (11). This radial separation of large and small particles with blood flow in a 200-µm tube was first shown with small latex beads that were 2.5 µm in diameter (10) and is known as the “near-wall excess” phenomenon.

The near-wall excess phenomenon, as it has been studied to date, applies only to arteriolar-sized vessels, but the brain is mostly oxygenated by a three-dimensional capillary mesh (27). It is, therefore, important to consider also mechanisms in capillaries that may be improving tissue oxygenation.

Capillary oxygen transport can be separated into two types. Some capillaries may be perfused only with plasma, preventing them from significantly contributing to tissue oxygenation. Assuming that a 1 g PFC/kg dose is evenly distributed in the plasma, the plasma would undergo a 28.0% increase in oxygen solubility (hematocrit = 43.7%, the average in 8 cats). Most of the oxygen would still be carried to the tissue by the RBCs, however, with the amount of oxygen dissolved in the plasma phase (plasma and PFC oxygen) only comprising 4.8% of the total amount of oxygen in the blood. In addition, only 3.6% of capillaries within the first 250 µm of the rat cortex were found to be cell free in an in vivo confocal laser microscopy study (35), so this mechanism is not likely to be important.

Most capillaries contain RBCs that flow in singlefile with spaces between single cells or groups of stacked cells (royleaux). In such a case, the cell-free gaps probably contribute little to oxygen transport. By adding PFC droplets to the circulation, however, oxygen transport can take place over the entire capillary surface area, increasing overall oxygen delivery to the tissues. This can be viewed as a “gap excess” of PFC droplets. In these capillaries, oxygen may first diffuse from the RBC to the PFC droplets within the plasma before diffusing to the tissue. Although some capillaries are smaller than RBCs, a cell-free annulus next to the vessel wall still exists where the tiny PFC droplets (0.3 µm in diameter) may create a near-wall excess, even at the microcirculatory level. Hochmuth et al. (14) have shown that flowing RBCs in 4- to 10-µm-diameter glass tubes display a cell-free annulus 0.6–1.9 µm thick.

Faithfull and Cain (11) believe that the PFC droplets also facilitate oxygen transport through the capillary endothelium, because PFC particles have been found residing within endothelial cells of both ischemic and healthy tissue. The trapped droplets may enhance oxygen transport by giving it a pathway of decreased resistance across the endothelium. Other mechanisms, unknown to us at this time, may also be operating.

Data from successive PFC doses further support a mechanism that lowers plasma oxygen transport resistance. Under control conditions, when there is no PFC in the bloodstream, the plasma is the dominant resistance to oxygen transport (22). Each dose of PFC lowers this resistance. At the point when the plasma oxygen transport resistance becomes approximately the same as other resistances to oxygen transport, further doses of PFC would show little effect. A calculation of the cumulative effect of multiple doses (APPENDIX B) is shown in Fig. 4. Saturation of the enhancement of transport was observed to occur between 2 and 3 g PFC/kg in the present study.

All of the above-mentioned mechanisms would also affect cortical Po2 during normoxia (ventilation with room air); however, the magnitude of the effect would be substantially lower than that observed under hypoxic conditions. In the present study, a statistically insignificant increase of 8.2 ± 18.5 Torr (n = 7 cats) was observed during the first 1 g PFC/kg infusion only. We believe that this increase reflects a slight PFC effect and that mechanisms that lower the plasma resistance to oxygen transport are operating. The variability among cats resulted in the statistical insignificance of the increase in normoxic cortical P02. As with the hypoxic results, the source of this variability is unknown. Again, a technique that measures brain Po2 at multiple sites simultaneously would be valuable in determining the effect of PFC during normoxia.

Magnitude of oxygen transport enhancement. Although the enhanced tissue oxygenation attributable to the PFC tended to be smaller in the cortex than in the retina (Fig. 5), the two results were not statistically significantly different. Retinal data in this figure were obtained from a previous study by Braun et al. (5). The tendency for a somewhat smaller effect in the cortex
may be due to differences in the vascular anatomy of the two organs. The retina is oxygenated by both arterioles and capillaries, as is apparent from the capillary-free zones surrounding retinal arterioles (e.g., Ref. 28). Also, the retina frequently has metarteriolar branches, which feed into the capillary network, that leave arterioles at a 90° angle. Plasma skimming may be facilitated by this branching pattern, and, as a result, it has been theorized that retinal capillaries have a lower hematocrit than do other capillaries (2), an idea supported by experimental observations (15). This lower capillary hematocrit, and, therefore, lower oxygen-carrying capacity, in the retinal capillaries may be the underlying reason for the somewhat larger enhancement of oxygenation by small doses of intravenous PFC in the retina compared with the brain. Another possibility may be that preretinal PO2 was influenced by oxygen diffusing through the vitreous humor from distant arteries and arterioles (7), in which the PFC effect may have been larger than in capillaries.

Whereas the mean effect on the brain was not statistically significantly different from the mean effect on the retina, the response to the PFC emulsion in the brain were more variable based on the coefficient of variation (0.77 in brain vs. 0.45 in retina). This may simply be a result of inherent brain variability. On the other hand, it may be that there is variability in the effect of PFC in different locations in each brain and that, if multiple locations were sampled simultaneously, the average effect would be more similar among cats. We could not detect a basis for variability, because the magnitude of the PFC effect was not correlated with recording depth, control transient variation, or hematocrit. The age of the emulsion was also considered because the small PFC droplets can enlarge over time. This also did not appear to influence the effect of PFC on tissue oxygenation.

The observed effect of the PFC on cat brain ΔPO2 was less than the comparable effect on rabbit brain oxygen availability (33). In that study, van Rossem et al. (33), using the same PFC emulsion as used here, measured the difference between oxygen availability during ventilation with 100% oxygen and room air in a manner similar to the present study. The change in oxygen availability nearly doubled with a single, larger 5.4 g PFC/kg dose. It is doubtful that the higher dose completely explains the larger effect observed, because the PFC effect appears to be nonlinear with dose (Fig. 4). In the present study, the calculated cumulative effect nearly saturated at 52% with a dose of 3 g PFC/kg, and it is unlikely that additional doses of PFC would have increased the effect. Two previous studies that used an earlier PFC emulsion (Fluosol), one in rabbits (9) and the other in cats (31), showed a very similar difference in emulsion effect (100 vs. 33%). Both of those studies (9, 31) measured oxygen availability and used similar PFC doses. Another study that used the same PFC emulsion as the present work, with a hemodiluted dog model, showed an increase in hyperoxic brain PO2 of 33% after emulsion infusion (1). It is, therefore, reasonable to conclude that the discrepancy between rabbits and higher mammals might simply be a species effect.

Treatment with a PFC before or after experimentally induced stroke has proven beneficial in dogs (12) and cats (9, 26). The increased oxygenation of the brain with the addition of small amounts of a PFC could explain the protective effects that PFC emulsions have been observed to have during stroke.

APPENDIX A

Oxygen-Carrying Capacity of the Blood and the Perfluorober Emulsion

Natural blood oxygen-carrying capacity. The overall blood oxygen-carrying capacity (O2blood) is the sum of the oxygen that is held by hemoglobin (Hb; O2Hb), the oxygen that is dissolved in the RBC (O2RBC), and the oxygen that is dissolved in the plasma (O2plasma) (36)

\[ O_{2\text{blood}} = O_{2\text{Hb}} + O_{2\text{RBC}} + O_{2\text{plasma}} \]

Hemoglobin fractional saturation, Y, is modeled by the Hill equation (12)

\[ Y = \frac{(P/P_{50})^n}{1 + (P/P_{50})^n} \]

where P50 is partial pressure of oxygen (in Torr) at which the Hb is 50% saturated (38.8 Torr for cat Hb) (16), P is oxygen partial pressure of the blood (in Torr), and n is the value of the exponent that most closely fits experimental data (2.95 for cat Hb) (16).

The amount of oxygen dissolved in the RBC and the plasma is determined by the oxygen solubility (4.7 × 10−3 ml O2/100 ml RBC or plasma/mmHg−1, and 2.9 × 10−3 ml O2/100 ml plasma/mmHg−1, respectively) and the PO2 of the blood (12, 36).

The total amount of oxygen in 100 ml of blood is therefore

\[ O_{2\text{blood}} = Y \cdot O_{2\text{max}} + 4.7 \times 10^{-3} \cdot V_{\text{RBC}} \cdot P + 2.9 \times 10^{-3} \cdot V_{\text{plasma}} \cdot P \]

where \( O_{2\text{max}} \) is maximum oxygen carrying capacity of Hb (100% saturation) (in ml O2/100 ml blood) and equals 0.45 × %hematocrit (5), \( V_{\text{RBC}} \) is the fractional volume of the RBC, and \( V_{\text{plasma}} \) is the fractional volume of the plasma.

Under hyperoxic conditions (PO2 = 500 Torr), the amount of oxygen carried by a 4-kg animal (70 ml/kg blood) with a hematocrit of 43.7 is 60 ml O2.

Fig. 5. Comparison of effect of first 1 g PFC/kg infusion in a previous retinal study (5) and the present cortical study. Both individual cat results (solid symbols) and mean effect (open symbols) are shown. Error bars represent 1 SD from mean. Difference in mean %increase of ΔPO2 was not statistically significantly different in the two studies (P = 0.083).
PFC emulsion oxygen-carrying capacity. The oxygen-carrying capacity of a PFC emulsion is determined by its solubility for oxygen. The emulsion used in the present work is able to hold $32 \times 10^{-5}$ ml O$_2$ ml emulsion$^{-1}$ mmHg$^{-1}$.

When a 1 g PFC/kg (1.1 ml emulsion/kg) dose is added to the blood of a 4-kg cat, the extra amount of oxygen carried by the blood at arterial P$_O_2 = 500$ Torr is 0.7 ml O$_2$. Assuming total blood volume remains constant, the increase in the amount of oxygen carried by 100 ml of blood is only 1.2%. The increase in the amount of oxygen carried by the plasma is increased to 4.8% from 3.7% of the total oxygen in the blood (increase of 30%).

If total blood volume does not remain constant, the volume of the emulsion would simply add to the initial blood volume of 280 ml, so the oxygen-carrying capacity of the blood would change to 60.7 ml O$_2$ in 284.4 ml blood, a 0.4% decrease in the blood of a 4-kg cat, the extra amount of oxygen carried by

### APPENDIX B

Calculations to Arrive at Cumulative Effects for the Second and Third PFC Doses

The following equations were used to obtain the hypothetical cumulative effects after the second and third doses from the data available. Let

\[
\Delta 1 = \frac{\text{post}(1) - \text{pre}(1)}{\text{pre}(1)}
\]

\[
\Delta 2 = \frac{\text{post}(2) - \text{pre}(2)}{\text{pre}(2)}
\]

\[
\Delta 3 = \frac{\text{post}(3) - \text{pre}(3)}{\text{pre}(3)}
\]

where \(\text{pre}(n)\) is \(\Delta P_O_2\) before the \(n\)th dose and \(\text{post}(n)\) is \(\Delta P_O_2\) after the \(n\)th dose of 1 g PFC/kg body wt.

Assuming that \(\text{post}(1) = \text{pre}(2)\), the hypothetical cumulative dose effects can be calculated from

\[
\text{cumulative}(2) = \frac{\Delta 2 \cdot \text{post}(1)}{\text{pre}(1)} + \Delta 1 = \frac{\text{post}(2) - \text{pre}(1)}{\text{pre}(1)}
\]

\[
\text{cumulative}(3) = \frac{\Delta 3 \cdot \text{post}(2)}{\text{pre}(1)} + \frac{\Delta 2 \cdot \text{post}(1)}{\text{pre}(1)} + \Delta 1 = \frac{\text{post}(3) - \text{pre}(1)}{\text{pre}(1)}
\]

where cumulative(2) and cumulative(3) are the hypothetical cumulative effects after the second and third doses, respectively. Because the electrode was sometimes moved between doses, the additional data manipulation is necessary to arrive at the above equations.

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