Angiographic and Histological Evaluation of Porcine Retinal Vascular Damage and Protection With Perfluorocarbons After Massive Air Embolism

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Background and Purpose—We have previously shown that perfluorocarbon emulsions (PFEs) reduce the severity of cerebral injury (indicated by infarct, reduced blood flow, and depressed EEG) induced by air embolism during cardiopulmonary bypass (CPB). This study used retinal fluorescein angiography to define the mechanisms of cerebral injury and to determine the efficacy of PFEs in cerebral protection. These angiographic findings were correlated to previously reported histologic findings.

Methods—Twenty domestic pigs underwent CPB with a prime of standard crystalloid or PFE (5 mg/kg) and crystalloid. After 10 minutes on CPB, a single (5 mL/kg) or double (2 × 2.5 mL/kg) bolus of room air or saline (control) was delivered via the right carotid artery. Retinal fluorescein angiograms were captured at 4 time points: baseline, air insult, postbypass, and postreperfusion. Following euthanasia, both eyes were removed and the retinas isolated for histological analysis with horseradish peroxidase (HRP), as previously reported.

Results—In control pigs, postreperfusion angiograms showed small nonperfused areas, and retinal whole mounts demonstrated vascular damage as previously reported. In 5 PFE-primed animals, postreperfusion angiograms showed hyperfluorescence, but angiograms and HRP mounts were otherwise not significantly different from baseline. Severely hyperfluorescent vessels observed angiographically also showed a correlation with HRP extravasation but were not consistently indicative of severe vascular damage.

Conclusions—Retinal fluorescein angiography and retinal staining with HRP indicate that mechanisms of cerebral air embolism include nonperfusion, vascular leakage and spasm, red blood cell sludging, and hemorrhage. Priming with PFE prevented many of the sequelae associated with air embolism. (Stroke. 1998;29:2396-2403.)

Key Words: air embolism ■ angiography ■ cardiopulmonary bypass ■ perfluorocarbons ■ pigs

Stroke and lesser neuropsychological impairments have been recognized as significant dangers in cardiothoracic procedures for many years. Although incidence of these complications varies with patient age, type of operation, and sensitivity of the assessment tests, estimates indicate that stroke occurs in 0.9% to 8% of cardiopulmonary bypass (CPB) patients.1-3 The lesser neuropsychological changes, although varied, include memory loss and attention deficits1 and may result in a permanent cognitive disability. The etiology of these impairments is not thoroughly understood, but cerebral embolism (air, microparticulates, or atherosclerotic debris)1-5 or cellular injury secondary to ischemia1-4 are frequently implicated.

Previous attempts to treat the effects of cerebral air embolism have included barbiturates and steroids, hyperbaric oxygen therapy, and retrograde venous cerebral perfusion. However, none of these has had a profound impact on the incidence or outcome of cerebral air embolism, Recently, perfluorocarbon emulsions (PFEs) have been investigated as a potential means of cerebral protection when used as a CPB prime. PFEs are unique for their ability to carry and absorb nitrogen and oxygen and therefore protect from gaseous emboli while enhancing oxygen delivery. Additionally, the emulsion’s small particle size (0.1 to 0.6 μm in diameter) as compared with that of red cells (7 to 8 μm) results in an increased surface area for gas exchange, improved oxygen diffusion compared with plasma,5-6 and potentially enhanced oxygen transport beyond occluded vessels or into areas of sludged erythrocytes.

Because many of the neuropsychological effects of CPB are subtle, and the etiology somewhat uncertain, they are difficult to quantify. Many CPB patients undergo a series of neuropsychological tests both before and after CPB procedures in an attempt to better understand the origins and
long-term effects of cerebral impairment after CPB. It would therefore be beneficial to be able to observe microembolic events in the central nervous system during CPB to better identify both their origins and potential means of prevention. One method of observing emboli in the retinal circulation of the eye in patients undergoing CPB is retinal fluorescein angiography. During embryonic development, the retina develops from the neural tube, a primitive section of the brain. Therefore, the blood-retina barrier is comparable structurally and functionally to the blood-brain barrier and parallels may be drawn between the two. In addition, the ophthalmic artery supplying the retina is the first branch from the carotid artery that delivers blood to the brain; therefore, the retina and the brain should experience similar embolic loads. The retina is an integral part of the nervous system of vertebrates and one that is highly visible and easily examined with noninvasive procedures; therefore, mechanisms and sequelae of cerebral air embolism in the retina can be used as a model for similar interactions in the brain.

The following study attempted to further our investigations evaluating the efficacy of a 40% v/v second-generation PFE in cerebral protection during CPB. Retinal fluorescein angiographic images of the retinal vasculature captured during CPB were compared with horseradish peroxidase (HRP) histological stains of the retina in an attempt to identify and correlate angiographic and histological indications of retinal vascular damage following air embolism. This comparison also aided us in understanding the potential mechanisms of injury from air embolism and vascular protection afforded by the PFE.

**Materials and Methods**

The experiments were performed in strict accordance with the Guide for Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Research Council (National Academy of Sciences, revised 1996) and were approved by the University of Washington Institutional Animal Care and Use Committee.

**Surgical Preparation**

Twenty domestic pigs (aged 2 to 4 months; mean weight 20.5 kg) were sedated with an intramuscular mixture of ketamine (22 mg/kg), acepromazine (1.1 mg/kg), and atropine (0.05 mg/kg). Anesthesia was induced by mask inhalation of halothane in 100% oxygen, followed by tracheotomy and endotracheal intubation. Anesthesia was maintained with halothane in 100% oxygen. Muscle relaxation during thoracotomy was achieved with pancuronium bromide (1 mg IV).

All animals were monitored with continuous ECG from peripheral limb leads. Transfemoral polyethylene catheters positioned at the midabdominal level were used for fluid administration and arterial sampling. Bitemporal EEG tracings were monitored continuously. Rectal temperature readings were used to maintain normothermia. Fluid maintenance was controlled with lactated Ringer’s solution (4 mL/kg per hour), and arterial pH was maintained with sodium bicarbonate. Rectal temperature readings were used to maintain normothermia. Fluid maintenance was controlled with lactated Ringer’s solution (4 mL/kg per hour), and arterial pH was maintained with sodium bicarbonate.

**Experimental Protocol**

The 20 swine were randomized into 1 of 6 experimental groups to determine prime and insult. Ten animals received a prime of PlasmaLyte-A (crystalloid), with 4 receiving single air insults, 4 receiving double insults, and 2 receiving saline insults as controls. An additional 10 animals received the PFE in conjunction with the crystalloid prime and were divided similarly for insult.

Two different air insults were utilized in this study. Animals in the single air bolus (SAB) group received 5 mL/kg air over a 30-second period. Those in the double air bolus (DAB) group initially received 2.5 mL/kg air over a 30-second period, then a cross-clamp was placed on the left carotid artery for 15 minutes (to prevent the return of air to the heart). An additional 2.5 mL/kg air was then delivered over 30 seconds and allowed to flush out of the vessels at its own rate. The total volume of air delivered in each case was 5 mL/kg.

The porcine heart was cannulated and placed on bypass with either a combination prime of crystalloid and PFE (5 mg/kg) or crystalloid only for a 10-minute stabilization period. Next, in the air insult groups, the air embolism was delivered to the right carotid artery as either a single or double bolus, as described. The 4 control swine received a single 5-mL/kg bolus of saline.

From the time of initial insult, normothermic bypass was continued for 1 hour. The animal was weaned from the bypass pump and allowed to reperfuse spontaneously for 1 hour while remaining under general anesthesia. After reperfusion, an HRP staining preparation (type II, 180 mg/kg in 5 mL buffered saline) was injected intravenously and allowed to circulate for 15 minutes. At the end of the experimental period, the animal was euthanized with concentrated pentobarbital (90 mg/kg). Both left and right eyes were removed following euthanasia and placed in cold buffered saline until dissection and staining.

**Methods of Measurement**

**Retinal Fluorescein Angiography**

Retinal angiographic images were acquired with a specially redesigned and vertically mounted Zeiss FF3 fundus camera. Standard angiograms were captured with a blue filter after injection of fluorescein dye. With use of a green filter, red-free angiograms were used to capture the transit of the air insult within the retinal vessels. A 35-mm camera (TopCon Inc) was mounted above the Zeiss camera to allow the operator to select the angiographic field of view. The 30° field of view of this camera is standard; however, it captures only a portion of the retina (approximately 55 mm², or 8% to 10% of the retina). This field of view produces an image of the retinal vasculature magnified (×2.5) at the film plane. The computer used for capture and digitization of the angiograms was a 486DX-33 with a DFC-1024 angiographic acquisition system (Ophthamlic Imaging Systems). This system was capable of capturing up to 40 sequential images.
images at a rate of approximately 1 frame every 1.5 seconds following an injection of fluorescein. Retinal images were automatically digitized at a resolution of 1024×1024 pixels. All angiographic series were saved to an optical disk (Panasonic model LMD702W).

A wire ophthalmologic speculum was required to separate the swine’s eyelids for angiography. The sclera was sutured to the lid in 3 places to maintain fixation of the eye, and a saline drip line was placed to maintain the moisture of the corneal surface. This line was temporarily removed before initiation of an angiographic series.

To capture the baseline angiographic series, the camera was positioned with the objective lens approximately 2 inches from the corneal surface of the right eye and focused to the level of the retinal vessels (just above the nerve fiber layer). Fluorescein dye was injected systemically (1 mL 10% sodium fluorescein), at which time the angiographic frames were initiated. Images were captured every 3 seconds until fluorescein appeared in the retinal vasculature, and then every 1.5 seconds until full arteriovenous filling was achieved.

To capture angiographic series at time points following the baseline series, the camera position was adjusted to recover the original field of view. One red-free angiographic series was initiated starting at the moment of air insult to record the transit of the air and continued until the air disappeared from the vessels in the camera’s field of view. Additional fluorescein angiography series were captured at termination of bypass (1 hour after insult) and following 1 hour of reperfusion (2 hours after insult), in the same manner as the baseline series.

Quantification of Angiographic Images
Capillary Dropout. Angiographic images were analyzed for the appearance of capillary dropout (nonperfusion) and hyperfluorescence with ALICE and the Digital Image Processing Station (DIP-Station) (Hayden Image Processing Group). Following acquisition of the images, 1 frame from each of the 3 angiographic series was chosen as a representative baseline, postbypass, and postreperfusion angiogram. These images were chosen on the basis of clarity, field of view similarities, and degree of fluorescein filling. These criteria were held as constant as possible among animals. Quantitative comparisons were made between baseline and postbypass images as well as baseline and postreperfusion images.

Before quantitative comparison of the images with image processing methods, all images were first normalized to compensate for variations in image brightness and were then spatially registered.10,11

The postbypass and postreperfusion images were processed in a manner similar to the baseline images with 2 exceptions. Before applying the 65% Sobel enhancement procedure (to highlight the edge structures), producing an image that was approximately 20% vasculature (80% of pixels represent background, 20% represent vasculature). Twenty percent was determined to be an appropriate value by evaluating half of the images to determine what level of segmentation encompassed most of the vessel structures yet eliminated much of the background. This value of 20% also agrees well with Jagoe and Arnold’s value of 13% vasculature11 and Jagoe and Blauth’s value of 27% vasculature12 from angiograms of clinical patients. Finally, using a thresholding technique, the baseline images were converted to binary images (black and white) and skeletonized to produce a unit line thickness tracing representative of the vascular structure.

The postbypass and postreperfusion images were processed in a manner similar to the baseline images with 2 exceptions. Before applying the 65% Sobel enhancement, the sign of the image was changed. This has the same visual (but not mathematical) effect as inverting the image: black vasculature on a white background. The next steps of Sobel enhancement and segmentation followed the baseline image procedure. For these images, the vasculature was not skeletonized after thresholding to produce a binary image. The technique of skeletonizing the baseline image and not the postinsult image being compared with it accommodates for the fact that vessel diameters change from image to image because of phases in the cardiac cycle and the degree of fluorescein filling at each time point. This technique is more forgiving of variations in vessel diameter during comparison.

The processed baseline image and either the postbypass or postreperfusion processed image were then compared with a Boolean logical AND operation to identify pixel segments potentially representing segments of capillaries occluded during embolism.11,12 A Boolean AND operation is a logic operation used to combine pixel values in binary images. Each AND operation requires 2 binary images as input and produces a single image as a result. The AND operation is performed pixel by pixel to combine 2 pixel values into 1. For example, 1 AND 1 equals 1, and 1 AND 0 equals 0. In this study, a binary baseline image contained white (pixel value 1) vasculature on a black background (pixel value 0). This image was combined with a binary postinsult image containing black (0) vasculature on a white (1) background. Vessels that were not perfused after insult did not contain fluorescein; therefore, these vessels did not become part of the vasculature portion of the image following the thresholding step. These nonperfused vessels were considered to be binary terms “white” on a white background. The image produced from the AND operation exhibited a black (0) background with white (1) vasculature. Any vessels common to the 2 images were black (0) and were not visible against the background. Vessels present in the baseline image but absent in the postinsult image appeared white on the combination image. False-positives were identified and discarded after comparing the logical AND resultant image to a blended image of the baseline and postinsult images (a layered composite image that retains all portions of both images).

Hyperfluorescence
Hyperfluorescence of the retinal vessels was quantified by first determining the percentage of the image composed of pixels associated with vascular structure. Using the DIPStation’s histogram features, the number of pixels in the image associated with vasculature could be determined for baseline, postbypass, and postreperfusion images. Hyperfluorescence of the vessels causes the pixels adjacent to the affected vessels to be brighter in postinsult images; these pixels then have a brightness value closer to that of the vessels than of the background pixels. This change in vascular fluorescence was expressed in terms of percent increase in pixels associated with vasculature from baseline to postinsult time points.

Air Residence Time
The angiography acquisition software recorded the time in minutes and seconds on each image captured within a series, reflecting the time passed since the first image was captured. The first red-free image during the air insult series was captured at the exact moment the air was introduced into the right carotid artery. Therefore, the time marked on the last image in the series that shows air within the vasculature represents the time required for the air to be flushed from the vessels within the angiographic field of view. These times were measured only in larger (100- to 200-μm) vessels.

HRP Histological Staining
Retinal vascular damage following massive air embolism was assessed with histological staining of the retinal tissues with HRP as described in detail in a previous communication.5 Tissues were examined for evidence of HRP extravasation, vascular spasm, and RBC hemorrhages.

Correlation of the fluorescein angiography images and the histologic sections was achieved with the digitized angiographic images and photomicrographs of the histologic stains. The peroxidase-stained tissues were mounted on slides and evaluated at magnifications of ×12.5 to ×125 with a microscope (Olympus, model BH-2).6 The region captured during angiographic imaging was located on the retinal whole mount, and photographs were acquired of the region at several magnifications. These images were then carefully compared with fluorescein angiograms to find histological extravasation of HRP or vascular or perfusion abnormalities in specific vessels that
corresponded to angiographic hyperfluorescence or capillary dropout in the same vessel.

**Statistical Methods**
Results were analyzed for statistical significance with factorial ANOVA and unpaired t tests (StatView, Abacus Concepts, Inc), with \( P < 0.05 \) considered significant. A 2 × 3 factorial ANOVA was performed to find significance for the effect of prime (PFE versus crystalloid) or insult (saline, SAB, or DAB). Unpaired t tests were performed for comparison of prime effect within a given insult group. Data are presented as mean ± SEM.

**Results**

**Fluorescein Angiography**
Visual inspection of the postbypass and postreperfusion angiograms for all animals indicated few macroscopic areas of capillary dropout. However, computerized comparison of digitized baseline and postinsult images revealed that there were microscopic areas of nonperfused vessels after air insult.

In both the postbypass and postreperfusion images, there were no significant differences in dropout area between the PFE- and crystalloid-primed groups when analyzed for effects of prime and insult (Tables 1 and 2). The mean areas of dropout for the PFE-primed animals, however, were less than the mean area determined for the crystalloid-primed animals in both postbypass and postreperfusion images. An example of capillary dropout in an animal receiving a crystalloid prime and SAB is shown in Figure 1.

Hyperfluorescence of the retinal vessels was limited to 5 of the PFE-primed animals (Table 3). One animal receiving a saline prime showed a <2% increase in fluorescence from baseline to postbypass, with no additional increase in fluorescence as reperfusion time passed. The 2 animals receiving an SAB showed a marked increase in hyperfluorescence following insult when compared with baseline. One animal demonstrated an increase of nearly 125% from baseline to postbypass; postreperfusion data were not available for this animal due to failure of the camera flash. The second animal showed an increase in hyperfluorescence with the passage of time, increasing from 54.8% at postbypass to 130.5% at the end of reperfusion. However, the 2 animals receiving a DAB did not show hyperfluorescence results consistent with one another. While one animal showed a decrease in fluorescence from 7.7 to 2.5% from postbypass to postreperfusion, the other showed the most severe hyperfluorescence observed in this study: an increase in fluorescent pixels of 193.8% at postbypass and 472.2% at the end of reperfusion. An example of this severe hyperfluorescence in retinal vasculature is found in Figure 2.

There was also no significant effect of prime on the time required for the air to disappear from the retinal vasculature. Regardless of type of air insult, the air residence time within the vessels was not significantly different for the PFE-primed animals compared with the crystalloid-primed animals (\( P = 0.71 \)) (Table 4). As expected by the design of the experiment, it did take significantly longer for the double air insult than for a single bolus insult to be removed from the vasculature (\( P < 0.0001 \)). An example of red-free angiography used to determine air residence time is found in Figure 3.

**HRP Staining**
Specific histology results are as described in detail in a previous communication. In brief, the PFE-primed animals demonstrated significantly less lengths of vasculature with HRP extravasation than the crystalloid-primed animals (\( P < 0.05 \) ANOVA). An example of HRP extravasation in an animal following air embolism is found in Figure 4. Prime did not have a significant effect on the appearance of vascular spasm or RBC hemorrhages.

**Correlation of Angiographic and Histological Results**
Although other investigators have attempted correlations between retinal fluorescein angiography and histological

<table>
<thead>
<tr>
<th>Prime</th>
<th>Area ± SEM, mm²</th>
<th>Area ± SEM, pixels</th>
<th>( P )</th>
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<tr>
<td>PFE</td>
<td>0.192 ± 0.041</td>
<td>149 ± 31.5</td>
<td>0.47</td>
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<tr>
<td>Crystalloid</td>
<td>0.310 ± 0.115</td>
<td>238 ± 87.6</td>
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<th>Prime</th>
<th>Area ± SEM, mm²</th>
<th>Area ± SEM, pixels</th>
<th>( P )</th>
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<tbody>
<tr>
<td>PFE</td>
<td>0.140 ± 0.031</td>
<td>127 ± 19.4</td>
<td>0.32</td>
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<tr>
<td>Crystalloid</td>
<td>0.262 ± 0.101</td>
<td>201 ± 77.6</td>
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**Table 2. Mean Areas of Capillary Dropout for Postreperfusion Angiograms**

<table>
<thead>
<tr>
<th>Prime</th>
<th>Area ± SEM, mm²</th>
<th>Area ± SEM, pixels</th>
<th>( P )</th>
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<tr>
<td>PFE</td>
<td>124.8</td>
<td>130.5</td>
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<td>PFE/SAB</td>
<td>7.7</td>
<td>NA</td>
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<tr>
<td>PFE/DAB</td>
<td>193.8</td>
<td>472.2</td>
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**Table 3. Percent Increase in Number of Pixels Associated with Vasculature Relative to Baseline**
preparations, angiographic images have not been compared with histological tests with a tracer molecule (HRP) capable of providing information regarding endothelial integrity. For the 4 PFE/air insult animals whose angiograms demonstrated fluorescein extravasation, the correlation with HRP varied. The most severely hyperfluorescent vessels showed the best correlation with histological HRP extravasation, while those vessels showing less extensive “point” hyperfluorescence seemed to correlate histologically to RBC sludging and nonperfusion within the same vessels. There was no apparent correlation between the type of air insult and the appearance of fluorescein extravasation, since 2 animals from each of the SAB and DAB groups exhibited the phenomenon. An example of HRP extravasation corresponding to an extremely hyperfluorescent vessel is found in Figures 2 and 4.

Discussion

Capillary dropout, also called capillary nonperfusion, is indicative of vascular occlusion and has been ascribed to embolic events. Baseline angiograms typically demonstrate normal fluorescence of all retinal vessels, since these swine have no retinal pathology. However, angiograms after air insult may indicate regions of retinal vasculature that are no longer perfused by fluorescein (Figure 1). This pattern is indicative of vascular occlusions that prevent the perfusion of segments of retinal vessels. Our study did demonstrate areas of capillary dropout after air embolism, although differences in nonperfused areas between the PFE and crystalloid groups were not significant. The mean area of dropout in the PFE-primed groups for both the postbypass and postreperfusion series was 30% to 59% less (P<0.47 and P<0.32) than the mean area of dropout for the crystalloid animals; these differences might become significant if more animals were included in the study. The results from this study, however, do support a trend that the perfluorocarbon tends to protect the retinal microvasculature from dropping out during an embolic event.

These angiographic findings are comparable to those of Blauth and Smith in clinical studies of CPB patients. These investigators evaluated areas of nonperfusion that ranged from 0.01 mm² to 1.23 mm². Our study showed dropout areas from 0.02 mm² to 1.17 mm². This area represented approximately 0.03% to 2.1% of the visible retinal field that has potentially “dropped out.” A clinical study by Arnold and Jagoe found that the average number of pixels missing in human postbypass images was 337; for this porcine study, the

<table>
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<th>Experimental Group</th>
<th>Mean Dissolution Time ± SEM, min</th>
<th>P (Unpaired t Test)</th>
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<tr>
<td>Crystalloid/SAB</td>
<td>5.14±1.07</td>
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<td>PFE/SAB</td>
<td>7.24±2.64</td>
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<td>Crystalloid/DAB</td>
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<td>PFE/DAB</td>
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<tr>
<td>Crystalloid/saline</td>
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<td>NA</td>
</tr>
<tr>
<td>PFE/saline</td>
<td>NA</td>
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Figure 2. Postreperfusion angiogram indicating severe hyperfluorescence of an entire vessel within the camera field (arrow). This same vessel was previously exposed to the experimental air embolism (see Figure 3).

Figure 3. Red-free angiogram showing air insult within the arterial side of the retinal vasculature (indicated by bright white lines and arrow).

Figure 4. HRP stain of retinal vasculature demonstrating HRP extravasation (arrows) along the same vessel affected by the air insult in Figure 3 and exhibiting hyperfluorescence in Figure 2 (original magnification ×12.5; scale bar, 5 mm).
average was 173 pixels (138 for PFE animals, 220 for crystalloid animals). These data represent a 59% decrease in pixel loss in PFE-primed animals compared with crystalloid-primed animals. This concordance in data also shows that the angiographic and image processing methods used for this study do produce results comparable to other studies. However, our study was not designed with enough power to demonstrate the efficacy of PFE in protecting retinal angiographic pixel loss.

Vascular hyperfluorescence appears brighter than normal fluorescence because fluorescein molecules that are not bound to proteins or screened by hemoglobin fluoresce more intensely.14 Hyperfluorescence can involve either discrete points, which likely indicates selected breakdown of the inner blood-retinal barrier, or an entire length of vessel that has been in contact with the air insult, which is thought to represent more massive compromise of the endothelial cell barrier. As noted, hyperfluorescence appeared only in 5 of the PFE-primed animals. Although the DAB animals showed the greatest percent increase in fluorescence from baseline to postinsult of the 5 animals displaying hyperfluorescence, there was no obvious correlation between air insult and angiographic hyperfluorescence. Ultimately, 5 of the PFE/air insult animals and all 10 of the crystalloid/air insult animals did not demonstrate this phenomenon.

It was not anticipated that only PFE-primed animals would demonstrate hyperfluorescence, which is thought to indicate altered permeability of the blood-retinal barrier. In fact, on analysis of the HRP-stained retinal tissues, the PFE-primed animals demonstrated significantly less total vascular leakage of HRP than those primed only with crystalloid, even though 4 animals exhibited vascular hyperfluorescence. This may be an indication that angiographic hyperfluorescence is not an accurate indicator of vascular damage following massive air embolism. One possible explanation returns to the analysis of the HRP stains and the relatively poor correlation between moderate (point) hyperfluorescence and extravasated HRP. Because fluorescein (1.1 nm) is a smaller molecule than the HRP molecule (2.5 to 3.0 nm), fluorescein may be able to move from the vasculature to the surrounding tissue with only a very small change in the size of the endothelial junctions (0.8 nm),7 such as those mediated by complement activation during CPB. Fluorescein is also injected at 3 time points throughout the experiment, providing more opportunities for it to escape the vasculature whenever those changes occur. However, HRP is injected only at the end of the experiment and would then only extravasate when the morphological changes to the endothelial junctions are more dramatic or permanent. This difference may also help to explain the inconsistent correlation between the fluorescein and HRP.

A second potential explanation returns to the PFE’s extremely small particle size. Dissection of the retinas of PFE-primed animals revealed the tissues to be very soft and milky in color when compared with those of the crystalloid animals, indicating that PFE was present in the tissue. PFE may subsequently interfere with the fluorescence of the fluorescein molecules or may simply distort the edges of the vessel such that they falsely appear hyperfluorescent along particular lengths. This theory is supported by the apparent slight increase in vessel diameter from baseline to postbypass angiograms of 1 of the PFE/saline animals. This variation in vessel diameter was initially quantified as hyperfluorescence; however, the percent increase (1.9%) was much lower than those determined for other animals. This vessel diameter increase, then, may potentially be an artifact of the PFE within the tissue surrounding the vasculature; there was no apparent diameter increase for the vessels of crystalloid/saline animals. However, the presence of the PFE in the surrounding tissue, although potentially interfering with fluorescein studies, might still enhance oxygen transport in the tissue surrounding the vasculature. The presence of PFE could be vital to oxygenating ischemic tissue.

Although hyperfluorescence has been a reliable marker of increased vascular permeability in studies of diabetic patients,15 it has never before been interpreted in the presence of PFEs. In this study, hyperfluorescence may indeed indicate altered permeability, but it is not consistently indicative of severe vascular damage. Extravascular leakage of fluorescein is often observed clinically but has also been previously observed without any accompanying recognizable anatomic cellular changes.16 In fact, Blauth and Arnold1 indicated that focal extravasation of fluorescein, although indicative of endothelial cell damage and breakdown of the blood-retinal barrier, often occurs with gaseous embolic events but is unpredictable and is often not present or observed. Thus, fluorescein leakage has generally not been considered an essential or consistent diagnostic feature in retinal angiography associated with CPB.37 An observation which is supported by the results of this study.

Air residence time within the vasculature was evaluated in an attempt to elucidate the PFE’s mechanism of vascular protection. Angiographic information indicated that the perfluorocarbon did not enhance removal of the massive air insults from the vasculature compared with the crystalloid prime in these pigs. The residence time was evaluated in only the larger (100- to 200-μm) vessels for 2 reasons. First, these vessels appeared more consistently in the angiographic field of view. Second, it was often extremely difficult to discern whether the smaller vessels still had air within the lumen. Although the air was not removed from the vasculature more quickly in the PFE-primed animals, the air bubbles may still have been broken down into smaller bubbles or dissolved directly; neither the angiographic nor the histological techniques used in this study allowed this to be discerned. PFE’s enhanced solubility of both oxygen and nitrogen could promote the decomposition of air bubbles into smaller bubbles that are less threatening; the smaller bubbles could be removed from the vessels more quickly than if PFE were not present in the vasculature. Prior work has demonstrated that the emulsifying agents alone do not protect animals from massive air embolism.39 The lack of difference in dissolution time does not necessarily disprove nitrogen absorption as a potential mechanism. The massive air insults may have overwhelmed the PFE’s capacity for absorption of nitrogen at the PFE/bubble interface. This interface may become saturated with nitrogen, and without blood flow, new, nonsaturated PFE would not be made available for gas absorption. The PFE’s ability to handle microemboli may be more...
pronounced than its effect on the massive air embolism utilized in this study, and data from routine bubble oxygenator CPB would suggest that this is true.

In addition, PFEs may be unable to protect weak spots in the vessel wall that are compromised by the air insult, thus allowing the release of RBCs into the surrounding tissue. However, PFEs may protect against minor changes in permeability, such as those that allow the release of HRP, but not the more severe, traumatic changes sufficient to allow extravasation of red cells. The RBC hemorrhages may also be a consequence of cell death, while HRP extravasation is the result of endothelial dysfunction. In this case, the PFE appears to have a greater ability to protect the integrity of the endothelial lining than to reduce cell death following a massive air embolism.

**Experimental Challenges Associated With Angiography in a CPB Model**

One reason that fewer discernable angiographic dropout areas were seen in our study than in other studies may be attributed to our use of the pig as a model, while other studies have used humans.11–13,19 or dogs as models for angiographic studies. These studies in humans and dogs have focused the angiography and image processing quantification around the macula, because the individual capillary structure of the retina is clearly identifiable only in the region surrounding the fovea, where retinal thickness is a minimum.11 Since the pig lacks a macula, it is more difficult to identify microvascular structure and consistently image the equivalent region from animal to animal. Also, when the angiography images are not centered on the macula, where most of the vessels are very similar in size, larger and much brighter vessels will dominate the angiogram. When the thresholding step is then applied, these large, bright vessels overwhelm the image, and many of the smaller vessels (which are perhaps of more interest when evaluating microembolic phenomena) are segmented to the background because they are less bright than the large vessels. In this study, retinal fluorescein angiography was integrated into an established CPB/massive air embolism model used previously to evaluate the performance of the PFE Oxyfluor, so the animal model was not altered.

**Technical Aspects of Angiography**

The technical difficulties of acquiring angiograms during CPB also often preclude acquiring images of the highest quality. One problem is that of positioning the camera in the midst of numerous other pieces of surgical equipment. Another difficulty is camera movement, since the camera must be withdrawn from the head of the operating table after the acquisition of each series. When the camera is not aligned at exactly the same point on the cornea, the image will be distorted to some degree, even when the camera is only a fraction of a millimeter off alignment. Other factors affecting image quality and quantification include fluorescein dye concentration and degree of fluorescein filling, camera flash intensity, and background accumulation of fluorescein during multiple angiographic series.

**Summary**

Based on evaluation of angiographic and histological images of retinal tissues, the mechanisms of cellular damage in the retina due to air embolism and affected by the presence of PFE were represented by capillary dropout (nonperfusion) and vascular leakage of HRP molecules. Vascular spasm, RBC hemorrhages, and air residence time did not appear to be influenced by use of PFE as a CPB prime. In addition, select retinal vessels that appeared severely hyperfluorescent in PFE-primed animals during angiography also exhibited HRP extravasation upon histological evaluation, although hyperfluorescence did not appear to be consistently indicative of vascular damage when evaluated by HRP extravasation. Less hyperfluorescent vessels correlated with vascular nonperfusion and cell sludging.

The results of this study indicate that the PFE Oxyfluor does protect the retina from the effects of a massive air embolism during CPB procedures by limiting vascular endothelial damage and improving perfusion. Although not all results were statistically significant, the trends consistently point to the efficacy of the PFE Oxyfluor in cerebral protection. Further work in human CPB is required before the effects of PFE (protection or treatment) in either micro or massive air embolism can be embraced as a therapy.

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**References**

Cardiopulmonary bypass and other surgical procedures can be associated with formation of air embolism, resulting in decreases in cerebral blood flow and brain injury. In this study, the authors used the retina as a model and tested effects of perfluorocarbons on retinal injury following air embolism. Earlier studies suggested that perfluorocarbons may be protective because of their ability to absorb molecular nitrogen and oxygen and to promote oxygen delivery. The results of the present study suggest that the retina can be protected from injury due to air embolism by perfluorocarbons. The mechanism of protection may relate to improved perfusion, reduction in vascular injury, and attenuation of increases in vascular permeability. Because the retina may be a useful model for brain, the present results suggest that a similar approach with perfluorocarbons may be neuroprotective.

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