A Porcine Model of Selective Retinal Capillary Closure Induced by Embolization with Fluorescent Microspheres

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PURPOSE. To investigate the feasibility of creating an animal model of selective retinal capillary closure to mimic the capillary closure that occurs in diabetic retinopathy.

METHODS. Fluorescent microspheres of 10- or 15-μm diameter were delivered to one eye of anesthetized pigs via a customized cannula advanced through the carotid arterial system to the origin of the external ophthalmic artery that supplies blood to the eye in this species. After preliminary trials in 10 pigs, embolization was performed in one eye of 34 animals that were allowed to survive for 7, 14, or 28 days. Embolized eyes were assessed by fluorescein angiography, electroretinography (ERG), and, after enucleation, light (LM) and electron (EM) microscopy.

RESULTS. The microspheres were detectable in the retina immediately after embolization, were restricted to the nerve fiber layer of the retina, and remained thereafter within the retina for periods up to 28 days. They effectively occluded embolized capillaries and some precapillary arterioles. No systemic or cerebral adverse effects were noted, thus allowing survival and subsequent follow-up. Embolization caused a reduction in the b-wave amplitude and the oscillatory potentials of the rod–cone bright-flash ERG but did not affect the amplitude of the a-wave. Embolized microspheres caused extracellular and intracellular edema confined to the inner and mid retina, and as a result the retinas of embolized eyes were thicker than those of fellow, nonembolized eyes. The outer retina and RPE were unaffected.

CONCLUSIONS. This survival model of retinal embolization with microspheres should be useful in the study of the retinal effects of the capillary closure that may occur in diabetic eyes. (Invest Ophthalmol Vis Sci. 2010;51:6700–6709) DOI:10.1167/iovs.09-4870

A critical role for hypoxia in the etiology of diabetic retinopathy has been identified in many studies.1–5 Retinal hypoxia in diabetes is in part due to increased glyced hemoglobin levels in red cells, with a resulting reduced oxygen delivery to tissues.6

Many factors appear to be involved in the capillary closure that may occur in the diabetic eye. Important among these are basement membrane thickening, which causes narrowing of the lumen of retinal capillaries, and pericyte and endothelial cell loss.7 Blockage of retinal capillaries by circulating leukocytes8 is another factor. Adherence of circulating white blood cells to vascular endothelium results from a hypoxia-induced upregulation of intercellular adhesion molecules on retinal capillary endothelium and on circulating leukocytes.9 Not only does retinal hypoxia lead to cellular and molecular changes that favor retinal capillary closure, retinal capillary closure itself is a potent contributor to retinal hypoxia. In trying to differentiate between the hypoxia due to retinal capillary closure from that due to other factors induced by diabetes, it seemed useful to develop a model of retinal capillary closure in the nondiabetic eye.

There is an extensive body of literature on the functional and structural effects of ischemia and hypoxia in the retina of experimental animals (for review, see Osborne et al.10). In experimental animals, techniques used to induce retinal ischemia/hypoxia have commonly affected the whole thickness of the retina by reducing retinal oxygenation from both the retinal and choroidal circulations. Methods have included elevation of the intraocular pressure above systemic blood pressure,11–15 occlusion of carotid and/or vertebral arteries,16,17 ligation of the ophthalmic artery or central retinal artery,18–20 endovascular balloon tamponade of the ophthalmic and/or ciliary arteries,21 or systemic hypoxia induced by breathing an oxygen/nitrogen mixture with a low oxygen content.22–26

Most of these methods cause total retinal ischemia/hypoxia, and not surprisingly, total retinal ischemia results in damage to all retinal layers including ganglion cell damage (or death) in the inner retina, mid-retinal cell damage to Müller cells and structural abnormalities in the RPE and photoreceptors.14,27,28 These widespread abnormalities in all retinal layers do not resemble the pathologic changes associated with the retinal capillary closure that is commonly seen in the diabetic eye and that affects the retinal capillaries of the inner and mid retina, and so the effects of retinal capillary closure occurring in this condition are likely to be restricted to the inner retina without involvement of the outer retina.

The pig is a suitable species for the development of a survival model of inner retinal hypoxia and ischemia. The pig eye is similar in size to the human eye and has both rods and cones and a pseudofovea (visual streak).29 The pig retina, similar to the human retina, has a dual blood supply, the inner to mid retina being supplied by superficial and deep capillary vascular beds and the outer retina by the
choriocapillaris. Unlike the human eye, the pig eye receives its blood supply from the external, not the internal, carotid artery.\(^{29,30}\) The major blood supply to the pig eye is via the external ophthalmic artery, a branch of the maxillary artery that is itself a continuation of the external carotid artery.\(^{29,30}\) (Fig. 1).

The retinal blood supply in the pig is derived from the posterior ciliary arteries, and there is no central retinal artery. In the retina the arterioles supplying the upper and lower nasal and temporal quadrants of the retina lie very superficially on the retinal surface. Precapillary arterioles and those capillaries carrying arterial blood lie in the nerve fiber layer of the retina. Deeper layers of capillaries in the ganglion cell and the inner and outer plexiform layers of the retina are largely venular.\(^{30}\)

In terms of size, main retinal arterioles have diameters of 60.5 to 65.6 \(\mu\)m,\(^{31,32}\) whereas the diameters of precapillary arterioles range from 6.7 to 14.5 \(\mu\)m.\(^{32}\) In the pig, retinal capillaries are very thin (3-4-\(\mu\)m diameter).\(^{32}\)

The major blood supply to the brain in the pig, comes from the ascending pharyngeal artery.\(^{30}\) The internal carotid artery that contributes to the blood supply to the brain leaves the common carotid artery at some distance before the origin of the maxillary artery. After the maxillary artery gives rise to several branches (deep facial artery, anterior auricular artery, and lingual artery) and shortly beyond the origin of the external ophthalmic artery, the caliber of the maxillary artery becomes markedly reduced in its onward continuation as the inferior orbital artery (Fig. 1c).

We have developed a model of inner retinal capillary closure in the pig by selective embolization of the retina with fluorescent microspheres of 10- or 15-\(\mu\)m diameter. In this article, we give details of the methodology involved and present evidence confirming the feasibility of delivering microspheres selectively to the retinal capillary circulation in the pig, together with illustrative examples of the effects of capillary embolization on the ERG, quantification of retinal thickness postembolization, and preliminary data on the electron microscopic (EM) appearance of the embolized retina.

**METHODS**

All the experiments on pigs were performed in the Singhealth Experimental Medicine Centre (SEMC) located on the campus of the Singapore General Hospital (SGH). Pigs of a Yorkshire Landrace strain weighing 25 to 45 kg were obtained from a farm at Sembawang in rural Singapore that is a facility associated with SEMC. All surgical procedures were performed in a properly equipped surgical operating theater in sterile conditions and were approved by the Institutional Animal Care and Use Committee (IACUC) of the SEMC. The SEMC also is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). All experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Before experiments involving embolization of the ocular circulation, the vascular pattern in the strain of pig available to us was confirmed by dissection of latex-injected specimens (Figs. 1a–c). From these dissections it was concluded that it should be possible to deliver microspheres to the origin of the external ophthalmic artery and prevent their onward dispersion through the inferior orbital artery, thus ensuring the selective delivery of the microspheres to the orbital and ocular circulations.

Initial experiments to confirm the feasibility of the method were performed on one eye of 10 adult pigs.

The microspheres used in the initial experiments were polystyrene of 10-\(\mu\)m diameter, incorporating a fluorescent dye with an absorption peak at 505 nm and an emission peak at 515 nm (FluoSpheres; Molecular Probes, Inc., Eugene, OR). The absorption and emission spectra of the microspheres allowed them to be visualized in vivo with a fundus camera fitted with filters for fluorescein angiography. The 10-\(\mu\)m microspheres were supplied as a suspension containing \(3.0 \times 10^6\) per milliliter. In later experiments, we used microspheres of 15-\(\mu\)m diameter in a concentration of \(3.0 \times 10^6\) per milliliter.

To test sterility, samples of the microspheres were cultured in Luria broth agar medium at 37°C for 18 hours. Although the microspheres were not guaranteed by the manufacturers to be sterile, incubated samples failed to grow any organisms.
The pigs were premedicated with ketamine 10 mg/kg body weight and anesthetized with a 5% halothane/oxygen mixture for induction and a 2% halothane/oxygen mixture for maintenance, each delivered via an endotracheal tube. One carotid artery was exposed in the neck and the dissection carried upward to expose the external carotid artery beyond the origin of the internal carotid artery.

A customized cannula of 2.5-mm external diameter with a closed end and a side port 10 mm from the closed tip (Fig. 1d) was inserted into the external carotid artery via a small opening in the arterial wall and advanced until the closed tip engaged in the narrowing lumen of the inferior orbital artery when no further advance of the cannula was possible. At this point, it was estimated that the side port was in the vicinity of the origin of the external ocular artery.

Three milliliters of a suspension of microspheres of 10-μm diameter made up to 5 mL with sterile normal saline were injected via the cannula over a period of 3 to 5 seconds, followed by a washout injection of 10 mL of sterile normal saline. In later experiments, after a test injection of microspheres of 10-μm diameter had been delivered, a 3-mL suspension of 15-μm microspheres made up to 10 mL with sterile normal saline was injected, followed by a washout injection, as before. During the injection, the internal carotid and deep facial arteries were temporarily closed by the application of encircling sutures. Onward dispersion of the injected microspheres into the systemic circulation via the inferior orbital artery was prevented as the closed end of the cannula effectively occluded that artery, thus ensuring delivery to the orbital–ocular circulation.

The dosages of microspheres injected and their dilution were limited to those mentioned earlier, as a significant degree of iris atrophy, in addition to a large number of microspheres in the retina, developed in the preliminary series in one animal that received 3 mL × 10^6 microspheres diluted with only 3 mL of normal saline.

After the injection of the microspheres, the cannula was removed and the opening in the wall of the external carotid artery was closed with 10-0 nylon sutures. The neck wound was then closed in layers and skin closure completed with a continuous subcuticular suture. The ocular fundus of the relevant eye was then examined with a fundus camera fitted with filters for fluorescein angiography, to confirm that the fluorescent microspheres were visible. In initial experiments, shortly after the delivery of the microspheres, fundus fluorescein angiograms were recorded with a digital fundus camera (TRC-50EX; Topcon, Tokyo, Japan), after an injection of 0.5 mL of a 15% solution of sodium fluorescein was administered via an ear vein.

In later experiments, immediately after the delivery of the microspheres, the relevant ocular fundus was examined with the digital fundus camera, with the same excitation and barrier filters as was used for fluorescein angiography, and photographed without intravenous fluorescein. Microspheres in the retina with an emission spectrum similar to that of fluorescein could be easily identified. Formal fluorescein angiography was also performed in all animals before euthanatization.

The criterion for successful embolization was the presence of microspheres visible in the ocular fundus immediately after they were delivered. The nonembolized fellow eye acted as a control. In addition, where cannulation and injection of the microspheres failed to cause embolization, the treated eye acted as an additional control, to take account of the effects of temporary carotid artery occlusion during the delivery of the microspheres.

After wound closure, the animals were given a prophylactic intramuscular injection of a combined ampicillin/cloxacillin antibiotic (Amoxicillin 100 mg; GlaxoSmithKline, Uxbridge, UK) and allowed to recover under close observation in the SEMC. Once the model had been established and after the injection of 10- or 15-μm microspheres, the animals were allowed to survive for 7, 14, or 28 days, and electrophysiology and fluorescein angiography were performed in all animals before euthanatization induced by an overdose of pentobarbitone administered intramuscularly.

After euthanatization, the eyes were removed and bisected, and samples from one half globe were immersed in various fixatives (10% formalin for light microscopy [LM], 2% paraformaldehyde plus 3% glutaraldehyde for electron microscopy [EM], or 2% paraformaldehyde for immunohistochemistry [IHC]). The other half globe was immersed in liquid nitrogen and stored at −80°C for subsequent Western blot analysis and RT-PCR studies of growth factors and other factors thought to be implicated in the retinal reactions to induced hypoxia/ischemia.

Measurements of retinal thickness were made on histologic cross sections. Thickness was measured from the inner limiting membrane to the outer ends of the photoreceptor inner segments. Measurements to the tips of the photoreceptor outer segments were also made, but were less reliable, because of variations in the obliquity of the outer segments and the occasional separation of the retina from the pigment epithelium as an artifact created during histologic preparation. The mean of three measurements to the tips of the photoreceptor inner segments was accepted as a measure of retinal thickness (Fig. 2), and comparisons between embolized and fellow nonembolized retinas were made by Student’s two tailed test, as were comparisons between a- and b-wave ERG amplitudes. The amplitude of the oscillatory potentials, expressed as the root mean square (RMS), was calculated using the formula that follows, where \( R_E \) is the response amplitude at time \( t \), \( \mu \) is the average of the amplitudes from 0 to 60 ms, and \( n \) is the number of samples in the time period.

\[
\text{RMS} = \sqrt{\frac{\sum_{i=0}^{60} (R_E - \mu_{0-60})^2}{n}}
\]

**RESULTS**

In regard to the the immediate effects of the experimental procedure, no significant systemic adverse effects were noted. There was a brief disturbance of the ECG, as the microspheres were being injected, but it did not last longer than two heartbeats, after which the ECG remained normal. No significant alterations in blood pressure were noted, and oxygen saturation, as measured with an ear oximeter, was also unaffected. After recovery from the anesthesia, no animals demonstrated any abnormal behavior, and all appeared unaffected by the procedure. None of the embolized eyes showed any clinical evidence of infection or inflammation.

![Figure 2](https://example.com/figure2.png)

**FIGURE 2.** Histologic cross sections of the retina showing the technique used to measure retinal thickness. (a) Thickened embolized retina; (b) thinner fellow nonembolized retina. Measurements from the inner limiting membrane to the tips of photoreceptor inner segments were found to be more reliable than measurements to the tips of the outer segments.
In initial experiments, some of which involved the use of an open-ended cannula, successful retinal embolization was achieved in only 50% of the pigs (5 eyes in 10 animals). In subsequent experiments involving 34 animals, the success rate improved to 71% after the use of a redesigned cannula with the characteristics already described, including a closed end that prevented onward dispersion of microspheres into the inferior orbital artery.

Fundus photographs immediately after delivery of the microspheres showed numerous, small, bright, well-defined dots indicating fluorescence of the microspheres in the retina and more numerous, larger, less-well-defined spots indicating the presence, at that time, of numerous microspheres in the choroid (Fig. 5a). Later fluorescein angiograms (Fig. 5b) showed only well-defined dots of microspheres in the retina, with very few in the choroid.

The microspheres that were identifiable immediately after delivery were still visible in the same retinal locations on fluorescein angiography immediately before euthanatization (Figs. 4a, 4b), and it was our experience that the microspheres detectable in the retina immediately after delivery were still present in the same locations in the retina at the various survival time intervals before euthanatization, up to 28 days after delivery.

In the choroid, occasional microspheres appeared to be sequestered within macrophages. They were extravascular and did not appear to obstruct related choroidal capillaries (Fig. 7b).

**Structural Effects**

The structural effects of embolization of the retinal capillaries were assessed by LM and EM. The most notable feature in the embolized retinas was intracellular and extracellular edema restricted to the inner and mid retina. The edema affected all the inner retinal layers from the nerve fiber layer to the inner nuclear layer (Fig. 7c). The retina in the fellow nonembolized eyes frequently showed a minor degree of edema confined to the inner retina and characterized mainly as localized vacuolation of Müller cell cytoplasm limited to the area immediately subjacent to the inner limiting membrane. This feature was always much less than in the fellow, nonembolized eye. The amount of edema in the nonembolized fellow eye shown in Figure 7d is typical of that commonly found. The occurrence of a minor degree of inner retinal edema in the nonembolized eyes appeared to be associated with a longer duration of anesthesia in some animals. In assessing the effects of embolization, we always took the degree of abnormality in the fellow nonembolized eye into account.

In the embolized eyes, intracellular edema affected both Müller cells (Figs. 8a, 8d) and ganglion cells (Figs. 8a–c) and appeared in part to be the result of disorganization of mitochondria, but intracellular vacuolation apparently unassociated with mitochondria was also a feature (Fig. 8c). The inner plexiform layer showed marked abnormality of nerve fibers in the embolized eyes (Fig. 8c), but in general, the outer nuclear layer, outer plexiform layer, and photoreceptors retained their normal morphology, as did the retinal pigment epithelium, which in most cases retained its normal relationship with the retinal photoreceptor outer segments (Fig. 8f).

Measurements of retinal thickness on histologic sections showed a significantly increased retinal thickness in the embolized eyes (257.5 ± 86.7 µm, n = 17) compared with that in the fellow, nonembolized eyes (199.5 ± 60.0 µm, n = 17; P = 0.030, t-test). Mean retinal thickness of the embolized eyes at 1 to 2 weeks after embolization was 273.0 ± 79.2 µm, n = 9) and at 4 weeks was 262.0 ± 65.8 µm (n = 8). There is no significant difference in retinal thickness between these two time intervals (P = 0.8). It appears that the retinal edema associated with embolization did not diminish significantly up to 4 weeks after embolization compared with that at 1 to 2 weeks after embolization.

Despite the intracellular and extracellular edema visible on EM, the actual leakage of fluorescein visible on fluorescein

![Figure 3](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932969/)

**Figure 3.** (a) A highly magnified portion of a fundus photograph taken with a camera fitted with filters for fluorescein angiography but without intravenous fluorescein of an eye immediately after embolization with microspheres. The sharply defined hyperfluorescent dots are microspheres in the retina, whereas the larger, out-of-focus fluorescent spots are microspheres in the choroid. (b) An angiogram taken 2 weeks after embolization shows only well-defined emboli in the retina. The microspheres have virtually disappeared from the choroid.

![Figure 4](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932969/)

**Figure 4.** (a) Photograph of the fundus an eye immediately after embolization with microspheres. The original color image has been converted to grayscale and the contrast adjusted to match the fluorescein angiogram of the same eye (b) 2 weeks later. Microspheres can be identified in the same locations at both time intervals. The ellipses have been added to make comparisons easier.
angiography was a rare finding, being noted in only 3 of 30 angiograms of the embolized eyes studied (Fig. 9).

**Effect on Function**

The typical ERG responses before embolization and the effects of temporary closure of the carotid artery and embolization are shown in representative examples in Figure 10 and the group data in Table 1. Before embolization, the combined rod–cone bright flash ERG responses and the oscillatory potentials were similar in each eye (Fig. 10). Temporary closure of the carotid artery in animals in which embolization failed was not associated with any difference between the two eyes in the rod-cone response or the oscillatory potentials from as early as 1 week after injection onward. The eyes embolized with microspheres showed a significant reduction in the amplitude of the b-wave, but not of the a-wave amplitude (Fig. 10, Table 1), and thus a reduction in the b/a-wave amplitude ratio. There was also a decrease in the oscillatory potentials in the embolized eyes (Table 1).

The effects of capillary embolization on retinal function and on the molecular biology of the embolized retina will be reported in greater detail in separate papers.

**DISCUSSION**

In earlier reported studies of experimental retinal embolization, relatively large glass microspheres were used (e.g., 15–90 μm diameter), or microspheres of 53- to 60-μm diameter that were not fluorescent and not visible on funduscopy. The microspheres were delivered to the eye, either through the carotid artery or the lingual artery. In each of these studies, multiple arteriolar occlusions were a feature in the retina, thus resembling branch retinal arteriolar occlusions rather than the capillary occlusion and underperfusion that occurs in diabetes.

In our experiments, although a large number of microspheres were injected, only a small proportion actually embolized the retina, but in sufficient numbers to cause both structural and functional changes. The explanation as to why only a proportion of microspheres delivered to the orbital–ocular circulation reached the retina probably relates to the vascular anatomy of the pig eye and orbit and probably also to some aspects of the injection technique. Undoubtedly a proportion of the injected microspheres bypassed the ocular circulation and was diverted to the extraocular muscles and other intraorbital structures that are also supplied by the external ophthalmic artery. A further proportion must have passed through the...
choroidal circulation. In the pig, choroidal capillaries are supplied with blood via short, wide-bore precapillary arterioles that have a diameter of 19 to 22 μm, and unlike capillaries elsewhere, the choriocapillaris takes the form of a perforated vascular sheet with free communication between the elements making up this sheet.32

Choroidal blood flow is much greater than blood flow in the retina; indeed, it has been claimed that choroidal blood flow is greater than that in any other tissue on a weight-for-weight basis.38 There is little doubt that a large proportion of injected microspheres will be delivered to and, for the most part, pass through the choroidal vasculature. Immediately after the injection of the microspheres, a large number of microspheres were visible in both the retina and choroid on photography with filters for fluorescein angiography, whereas in later angiograms before euthanatization, the microspheres had disappeared from the choroid but remained visible in the retina.

On EM, we found occasional examples of microspheres lying extravascularly within the choroid, either singly or sometimes as chains of microspheres apparently sequestered within macrophages. This observation suggests that some microspheres had adhered to the choroidal vascular endothelium and excited a macrophagic reaction for their ingestion and disposal. Although after injection some of the microspheres were sequestered extravascularly in the choroid, most of those reaching the choroid would have exited the eye via the vortex veins and become distributed systemically. As most systemic arteries are not end arteries, the systemic distribution of microspheres is unlikely to have had any adverse systemic effects, and indeed none of the pigs showed any systemic effects after retinal embolization.

The origin of the external ophthalmic artery from the maxillary artery is not far removed from the origins of other branches of the maxillary artery, such as the anterior auricular artery, and there is a possibility that on some occasions, the side opening near the tip of the cannula was nearer the origin of this artery than that of the external ophthalmic artery, thus diverting microspheres to an area other than the eye or orbit. Another possibility is that, as the microspheres were injected under some pressure, there may have been slight, localized ballooning of the maxillary artery, sufficient to allow the microspheres to reach some of the branches of the maxillary artery other than or in addition to the external ophthalmic artery. In future experiments, the effect of reducing the rate at which microspheres are injected (with a consequent reduction of injection pressure) will be assessed to see whether this effect increases the delivery of microspheres to the retina.

In relation to whether only retinal capillaries were occluded by injected microspheres or whether precapillary arterioles were occluded, it is noteworthy that in the pig, major retinal arterioles that lie on the surface of the retina, bulging into the

**Figure 7.** Electron micrographs of the retina after embolization of one eye with fluorescent microspheres. (a) A microsphere filling the lumen of a retinal capillary with surrounding edematous retina. (b) A microsphere sequestered within a macrophage in the choroid. The neighboring choriocapillaris is compressed but not occluded. (c) Low-power EM of an embolized retina shows edema restricted to the inner and mid retina. (d) The fellow nonembolized retina shows a minor degree of edema only, largely restricted to the Müller cell cytoplasm adjacent to the inner limiting membrane.

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vitreous, as already noted, have a diameter of around 60 μm and thus are too large to be occluded by microspheres of either 10- or 15-μm diameter. Precapillary arterioles in the pig retina however are relatively long and have diameters ranging from 4.5 to 14.5 μm and may therefore be occluded, especially by the larger (15-μm) size of microsphere used.

In histologic cross sections of embolized retinas, microspheres were seen only in the inner retina, where capillaries carrying arterial blood are located, and not in the deeper layers of the retina where the capillary beds carry venous blood. This localization would be expected if the occlusions caused by the microspheres affected the precapillary arterioles or the capillaries they feed, for both lie in the nerve fiber layer of the retina.

It has been known for a long time that the occlusion of terminal precapillary arterioles leading to downstream nonperfusion of the capillary bed supplied by these vessels is a feature of the vascular changes in diabetic retinopathy. Although occlusion of precapillary arterioles may occur after delivery of microspheres to the retina, as previously noted examination of those fluorescein angiograms where the capillary bed is visible has shown in some areas (Fig. 6a) that microspheres are located in capillaries without any significant downstream areas of capillary nonperfusion. In other areas, evidence of precapillary arteriolar occlusion by microspheres can be detected (Fig. 6b) with evidence of capillary bed abnormality and downstream capillary nonperfusion. The lack of any sizeable areas of capillary nonperfusion in most embolized eyes, however, suggests that the emboli preferentially block capillaries rather than precapillary arterioles.

In the pig, retinal capillaries are of very reduced caliber having a luminal diameter of 3 to 4 μm. Microspheres are delivered to the eye and orbit under a significant degree of pressure, and there is a possibility that microspheres may be forced through the slightly wider precapillary arterioles, only to be trapped in the much narrower capillaries. Occlusion of precapillary arterioles in embolized eyes, however, would make the model closer to the situation in diabetic retinopathy, in which terminal precapillary occlusion is a known feature, than to pure capillary closure alone. Attempts to determine by confocal UV microscopy whether there is evidence of precapillary occlusion by microspheres have proved indecisive, and this question will only be answered by further studies using arterial perfusion with, for example, India ink, or in trypsin digest preparations.

The question of how long occlusion of the capillaries (or of precapillary arterioles) will last remains unanswered. As previously indicated and illustrated (Fig. 4), we have evidence of the persistence of microspheres acting as emboli in the same retinal locations from shortly after delivery to the embolized eye and at later time intervals up to euthanatization. As the microspheres were only demonstrable in the nerve fiber layer of the retina and were apparently unable to penetrate the inner retinal capillary bed to reach the venous capillaries of the mid retina, it would not be surprising that they would remain

within embolized capillaries or preretinal arterioles until undergoing degradation at a future time that has not yet been determined. Although microspheres in the choroid were occasionally seen extravascularly sequestered within macrophages, this phenomenon was not seen in the retina. As already noted, the retinas in the embolized eyes remained thickened to the same degree (presumably from edema) from 1 to 4 weeks after the embolization. Some microspheres that occluded the retinal capillaries or sequestered intracellularly in the choroid showed evidence of commencing degradation, with areas in their peripheries looking as if small bites had been taken out of them (Figs. 7a, 7b). Up to 28 days, however, damage of this sort to the periphery of embolizing microspheres was minimal and insufficient to restore patency to occluded vessels. Our conclusion is that, at least up to 4 weeks, there is no diminution in the number of microspheres in the retina and no diminution during the first 4 weeks in their effect on retinal structure, as indicated by measurements of retinal thickness.

It is a surprise that, although embolized retinas showed both extracellular and intracellular edema with a significant increase in retinal thickness, frank leakage of fluorescein visible on fluorescein angiography was an unusual feature. The explanation may lie in the degree of increased capillary permeability induced by embolization that was probably less than would be the case with widespread occlusion of terminal precapillary arterioles, where blood supply to groups of capillaries would be compromised. The localized areas of capillary closure induced by capillary embolization may cause fluorescein leakage insufficient to be visible on fluorescein angiography, but over time sufficient to increase the volume of extracellular fluid in the retina. The intracellular and extracellular edema that occurred in the embolized eyes may have been a manifestation of hypoxia induced by capillary embolization. The mitochondrial damage responsible for some of the intracytoplasmic vacuolation seen in ganglion and Müller cells would be an expected result of hypoxia. It has been shown that hypoxia can cause a breakdown in the inner blood–retinal barrier (iBRB), thus accounting for the extracellular edema seen in the embolized eyes. Hypoxic damage to Müller cells where the recorded potentials of the ERG b-wave are generated would be a likely explanation for the reduced b-wave amplitudes seen in the embolized eyes.

Although actual tissue oxygen levels have not been determined, the structural abnormalities in the embolized eyes, such as mitochondrial vacuolation, shrunken ganglion cell nuclei, and nuclear chromatin aggregation, were similar to those that

### Table 1. Effects of Embolization on Combined Rod-Cone Bright-Flash ERG Parameters and the OP

<table>
<thead>
<tr>
<th>ERG Parameters</th>
<th>Pre-op</th>
<th>Post-op without Spheres</th>
<th>Post-op with Spheres</th>
<th>P*</th>
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<td><strong>Maximum ERG a-Wave Amplitude, μV</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control eyes</td>
<td>264.4 ± 35.5 (n = 28)</td>
<td>264.7 ± 55.7 (n = 8)</td>
<td>260.5 ± 45.5 (n = 20)</td>
<td>0.972</td>
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<td>Studied eyes</td>
<td>264.4 ± 29.9 (n = 28)</td>
<td>271.4 ± 59.7 (n = 8)</td>
<td>252.1 ± 41.4 (n = 20)</td>
<td>0.650</td>
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<tr>
<td>P†</td>
<td>0.874</td>
<td>0.871</td>
<td>0.658</td>
<td></td>
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<tr>
<td><strong>Maximum ERG b-Wave Amplitude, μV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control eyes</td>
<td>769.9 ± 121.6</td>
<td>774.7 ± 127.0</td>
<td>768.8 ± 139.4</td>
<td>0.967</td>
</tr>
<tr>
<td>Studied eyes</td>
<td>782.2 ± 133.0</td>
<td>750.6 ± 135.5</td>
<td>520.0 ± 180.9</td>
<td>0.003</td>
</tr>
<tr>
<td>P†</td>
<td>0.875</td>
<td>0.815</td>
<td>0.004</td>
<td></td>
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<tr>
<td><strong>OP Amplitude, RMS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control eyes</td>
<td>37.8 ± 4.6</td>
<td>38.1 ± 6.3</td>
<td>38.2 ± 5.4</td>
<td>0.949</td>
</tr>
<tr>
<td>Studied eyes</td>
<td>38.5 ± 4.7</td>
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<td>27.8 ± 8.6</td>
<td>0.001</td>
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<tr>
<td>P†</td>
<td>0.965</td>
<td>0.980</td>
<td>0.001</td>
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Data are expressed as the mean ± SD.

* ANOVA.
† Student’s t-test.
have been described in experiments in which retinal or systemic ischemia/hypoxia was induced.14,25,36

In addition, the changes seen on electroretinography, with a reduction of the amplitudes of the b-wave of the combined rod-cone bright-flash ERG, were characteristic of retinal hypoxia. The fact that the a-wave of the ERG was not affected by embolization is further evidence that any hypoxia induced by embolization was restricted to the inner and mid retina. The a-wave, being generated in the photoreceptors would be affected only if embolization were to have significantly compromised the choroidal circulation; such an obstruction appears not to have occurred. The reduced amplitudes of oscillatory potentials in the embolized eyes were additional evidence of mid retinal dysfunction. Oscillatory potentials are thought to derive from the activities, interactions, and biofeedback mechanisms of cells within the interplexiform layers of the retina. Oscillatory potentials are also thought to reflect the function of the inner retina and are sensitive to changes in retinal circulation.42,45

By the use of microspheres of a size that can occlude retinal capillaries and probably terminal precapillary arterioles, but are too small to occlude wider-bore retinal arterioles or choroidal capillaries, we have been able to achieve selective closure of retinal capillaries. By using fluorescent microspheres, we have been able to visualize in vivo the presence and distribution of microspheres in the retina and thus have enabled an estimate to be made of the degree of vascular occlusion achieved. Currently, we are using this model to study the effects of retinal capillary embolization on intraocular growth factors, blood–retinal barrier integrity, and retinal function. We believe that the model is more useful for the study of the effects of capillary closure in the retina, such as may occur in the diabetic eye, than are earlier models that are characterized by the occlusion of major retinal arterioles or that involve hypoxic damage to all retinal layers, including the retinal pigment epithelium.

Acknowledgments

The authors thank Roger Beuerman for providing help and encouragement.

References


