Formation of bubbles in tissue and vasculature from a sudden reduction in ambient pressure is likely an underlying cause of the clinical symptoms of decompression sickness (DCS). Thus, tools detecting bubbles in the vasculature may be important for evaluating DCS. Sheep were air-compressed to 6.0 ATA (30 minutes bottom time) then rapidly decompressed to the surface. A fundus camera was quickly positioned for continuous observation of the retinal vasculature. Bubbles were observed in the retinal vasculature of 25.8% (n=31) of the sheep. Bubble onset time ranged from 5-22 minutes post-chamber and lodge time ranged from 0-70+ minutes. Bubbles were visualized mostly in the arteries of the retinal circulation.

Severe vasoconstriction was captured using red-free angiography in two sheep. In two other sheep, fluorescein angiography demonstrated occluded blood flow caused by arterial gas emboli. This study demonstrates that retinal angiography is a practical tool for real-time, noninvasive detection of bubbles in the retinal circulation, a visible window to the cerebral circulation. Thus retinal angiography may prove invaluable in the early detection of arterial gas emboli in the cerebral circulation, the resolution of which is imperative to favorable neurological outcomes. This study also presents for the first time images of bubbles in the retinal circulation associated with DCS captured by a fundus camera.

INTRODUCTION

Decompression sickness (DCS) is associated with the formation of bubbles in blood and tissue as a result of a sudden decrease in ambient pressure, usually as a result of surfacing from a dive without adequate decompression or a sudden high altitude loss of pressurization (1-3). Bubbles are typically formed in the venous circulation and can result in venous gas emboli (VGE) (2).

DCS is a mixed compendium of symptoms with a highly variable presentation, ranging from vague joint and muscle pains to cardiopulmonary incapacitation, neurological sequelae, and death (1, 2, 4, 5). The number of gas emboli, size of gas bubbles, movement in the circulation and ultimately which vital vascular structures are occluded by gas emboli are likely responsible for both symptoms and outcome.

Arterial gas emboli (AGE) are also associated with DCS albeit less frequently than VGE (2). There are several ways AGE can arise. Normally, bubbles do not develop de novo in the arterial circulation due to equilibration with alveolar gas and high arterial pressure (2). However, bubbles can cross from the venous to arterial circulation via intrapulmonary arteriovenous shunts, a patent foramen ovale, pulmonary barotraumas, or from the venous bubble load being too great for the pulmonary capillary bed to completely filter (2, 5).

As of 2001, there were more than nine million certified scuba divers in the United States alone (5). There are approximately 1,000 reported incidents of diving injuries per year requiring recompression therapy and a mortality rate of 90 divers per year (5, 6). Often, diving injuries requiring recompression occur at a site remote from a definitive recompression chamber, necessitating air evacuation, which can exacerbate DCS (7). Thus, with recreational divers in the United States, combined with military and commercial divers, as well as caisson workers, methods for detecting bubbles in
the vasculature, especially in the cerebral circulation where a bubble lodged for 10 minutes can result in significant brain damage, may be important tools to aid in the clinical assessment of potential DCS and the need for immediate treatment.

An ideal device for assessing the bubble load in the circulation should be a real-time monitor that is sensitive, easy to use (requiring little training) and noninvasive. Highly sensitive transcranial Doppler ultrasound and transesophageal echocardiography (TEE) are currently employed in assessing total bubble load (8). However, these techniques are either highly invasive (TEE) or require extensive expertise (Doppler) for operation and interpretation (8).

Other noninvasive techniques such as electrocardiogram, end-tidal N₂, or oxygen saturation have either very limited sensitivity for detecting bubbles or low availability (8).

A fundus camera is a real-time, widely available, and relatively easy-to-use routine ophthalmologic tool. The noninvasive visualization of ocular structures, including venous and arterial vasculature of the retina, is easily carried out with fundus cameras (9). Red-free, color and fluorescein retinal angiograms generated by a fundus camera are used to assess ocular and vascular integrity, blood flow, and changes due to disease such as diabetes (9).

This manuscript demonstrates that retinal angiography is a viable tool for monitoring bubbles in the vasculature as a result of rapid decompression. Presented in this publication are the first known noninvasive pictures of bubbles in the retinal vasculature circulation associated with DCS.

METHODS

All animal use procedures were in strict accordance with the National Institutes of Health Guide for the Care and use of Laboratory Animals and approved by the Department of Defense and Virginia Commonwealth University’s Institutional Animal Care and Use Committee. Juvenile, 15-20 kg, Dorset sheep of either sex (Robinson Services, Inc., Mocksville, N.C.) were housed in USDA- and AALAC-approved animal facilities in social flocks with free access to food and water on a 12-hour light-dark cycle. Flocks were acclimated for a minimum of three days after shipment to allow for hematocrit stabilization and veterinary inspection for any disease.

Male, 2-3 kg, New Zealand White Rabbits (Robinson Services, Inc., Mocksville, N.C.) were housed in AALAC-approved animal facilities with free access to food and water on a 12-hour light-dark cycle. Animals were acclimated for a minimum of three days prior to any procedure.

Sheep dry dive procedure

Forty-eight hours prior to the dive, a sheep was muzzled to prevent access to food and allowed free access to water while remaining with its social flock. On the morning of the experiment, anesthesia was induced with an intramuscular injection of 20.0/2.0 mg/kg ketamine/xylazine and the animal placed supine on a surgical table.

All animals were immediately intubated with a 9.0 mm internal diameter endotracheal tube and ventilated (Servo Ventilator 900C, Siemens Medical Solutions Inc., Malvern, Pa.) with 50/50 nitrogen/oxygen at a rate and tidal volume approximately 12 ml/kg adjusted to maintain arterial CO₂ tension at 40 ± 5 mmHg and pH at 7.40 ± 0.05. An orogastric tube was placed into the rumen to discharge any vomitus and allow excess gas accumulated during the dive to escape during decompression.

Anesthesia was maintained via continuous infusion of an anesthetic cocktail (triple drip) consisting of ketamine 2.0 mg/ml, xylazine 0.1 mg/ml and guaifenesin 50 mg/ml in 5% dextrose at a rate of 2.0 ml/kg/hr through a catheter via the right external jugular vein.

When the achievement of an appropriate surgical anesthetic plane was reached, the right femoral artery was exposed and cannulated for monitoring of blood pressure and arterial blood sampling. The right femoral vein was cannulated for the infusion of fluids and a pulmonary artery catheter (Swan-Ganz Continuous Cardiac Output/SvO₂/End Diastolic Volume Thermodilution Catheter 774F75, Edwards Lifesciences LLC, Irvine, Calif.) was placed via the left femoral vein for monitoring cardiac output, pulmonary arterial pressure,
central venous pressure and central venous blood sampling. The sheep’s left neck area was shaved in the proximity of the jugular vein. An Emboli Detection and Classification (EDAC, Luna Innovations, Hampton, VA) probe, a pulse echo ultrasound-based device for assessing venous bubbles, was coated in ultrasound jelly and affixed to the skin using surgical sutures directly above the jugular vein. Exhaled respiratory gases (O\textsubscript{2}, CO\textsubscript{2}, N\textsubscript{2}) were measured using mass spectrometry (MGA 1100, Perkin-Elmer; Norwalk, CT).

Following surgical manipulations, the sheep was stabilized for a minimum of 30 minutes prior to diving. Inclusion criteria during stabilization on the ventilator was arterial O\textsubscript{2} tension above 200 mmHg, arterial CO\textsubscript{2} tension approximately 40 mmHg, and mean arterial blood pressure above 60 mmHg.

Once stabilized, the sheep was weaned off the ventilator until capable of spontaneous respiration, disconnected from all monitoring equipment, and placed in the hyperbaric research chamber (Model 17-48-100, Reimers Systems, Inc., Springfield, VA). During the dive procedure, the animal breathed room air and anesthesia was maintained with triple drip. Unless otherwise specified, sheep were subjected to the following dive protocol.

Starting at 1 atmosphere absolute (ATA), the animal was initially compressed at a rate of 1 ATA/min to a pressure of 2.0 ATA then compressed at a rate of 2 ATA/min to a final pressure of 6.0 ATA (73.5 psi or 165 feet of sea water). The animal remained at 6.0 ATA for 27 minutes (30 minutes bottom time). The sheep was then decompressed at a rate of 2 ATA/min back to 1 ATA.

Upon full decompression (considered time zero) the sheep was quickly removed from the chamber and reconnected to all monitoring equipment. The animal was also immediately reconnected to the ventilator to receive one of four breathing gas mixtures (part of another study) and administered intravenous pancuronium (0.1 mg/kg) for muscular paralysis necessary for accurate determination of respiratory gases via mass spectrometry. The sheep was then monitored for 90 minutes following decompression.

**Sheep cerebral arterial gas emboli procedure**

Anesthesia was induced as described above and the animal placed in the supine position on a surgical table. Anesthesia was maintained via continuous infusion of triple drip through a right external jugular vein catheter. The right common, internal and external carotid arteries were exposed and a second catheter (PE 50) was advanced retrograde from the external carotid artery back into the common carotid artery. A syringe was then used to administer 5 cc of air over two minutes, followed by 1 cc bolus of normal saline to ensure all of the air entered the internal carotid circulation.

**Rabbit cerebral arterial gas emboli procedure**

Anesthesia was induced with an intramuscular injection of 35.0/2.0 mg/kg ketamine/xylazine and the animal placed in the supine position on a surgical table. Anesthesia was maintained with an hourly intramuscular dose of 23.0/1.4 mg/kg ketamine/xylazine as needed. A catheter was then inserted for administration of cerebral arterial gas emboli as described for sheep above. A syringe was then used to administer a 2 cc bolus of air followed by 1 cc of normal saline to ensure all of the air entered the internal carotid circulation.

**High-resolution retinal angiography**

All retinal angiographic procedures were performed using a Topcon TRC 50EX Fundus Camera (Topcon Medical Systems, Inc., Paramus, N.J.) outfitted with Ophthalmic Imaging Systems’ WinStation 5000 digital workstation, including an ultra-high resolution CCD camera (2400 x 2048 pixels) and WinStation XP 10.2.44 image capture and enhancement software. Unless otherwise specified, all images were captured using a 50-degree angle of coverage, 100 ISO, variable flash intensity (ranging from 12 W/s for color to 150 W/s for fluorescein images), diopter compensation set to 0, and illumination diaphragm set to normal.

In all cases, the sheep were supine and images captured from the sheep’s right eye. Prior to all retinal photography, 1 drop of atropine sulfate (0.1 mg/ml, Abbott Laboratories, Chicago, Ill.) was administered to achieve adequate mydra-
sis. The eyelids were held open with a V. Mueller Williams OP-60 eyelid speculum (Cardinal Health, San Carlos, Calif.) and adequate corneal moisture maintained with Refresh Liquigel (1.0% carboxymethylcellulose sodium, Allergan Inc., Irvine, Calif.) as needed.

For red-free angiograms, the green filter (Topcon proprietary) was engaged prior to capturing images. For fluorescein angiography, both exciter and barrier filters (Topcon proprietary) were engaged prior to capturing images. An intravenous bolus dose of 8 mg/kg fluorescein sodium (25% AK-FLUOR, 250 mg/ml, Akorn Inc., Buffalo Grove, Ill.) was administered followed by a 10-cc normal saline flush.

All baseline images were captured during the stabilization period following surgical manipulations and prior to the dry dive procedure. Following compression/decompression of the sheep, the fundus camera was positioned for the visual assessment of the retinal circulation within five minutes of the animal exiting the chamber and observations continued throughout the entire 90 minutes post-chamber protocol. Red-free and color images were taken throughout the post-dive period. Fluorescein angiograms were captured between 20-30 minutes following the dry dive procedure unless otherwise specified.

**Image manipulation**

WinStation XP 10.2.44 image capture software has on-screen capture controls for enhancing images captured by the CCD camera that cannot be disabled. The settings for the WinStation 5000 camera interface were as follows for contrast, brightness and gamma levels, respectively: 5 – medium-low (fluorescein); 1 – low-low (color); and 3 – medium-low (red-free). Images were exported as uncompressed TIFF files. Photoshop 7.0 (Adobe Systems Inc., San Jose, Calif) was used to convert color RGB images to grayscale, resize and compile images, add labels and arrows and create compressed TIFF files using the LZW algorithm. For some of the red-free and fluorescein images, highlight, midtone, and shadow levels were manipulated to create better balance between the bright tapetal and dark non-tapetal fundus revealing more vasculature detail. No other image manipulations were performed unless otherwise specified.

**Results**

Color angiograms can be used to assess when bubbles form in the vasculature following the dive, location (arterial, venous, formed *in situ*), bubble size, dwell time and rate of bubble flow through veins and arteries. Figure 1 (below) depicts a representative color angiogram (converted to grayscale) taken during the stabilization period prior to diving the animal, revealing several characteristics of the ovine ocular fundus (10).

The yellow tapetal area (lower, lighter area), unpigmented nontapetal area (upper, darker area), and distinct horizontal blue line of demarcation were easily discerned. The optic disc was elliptical in shape with white myelin fibers protruding into the tapetal and nontapetal retinal areas. Several central retinal arteries protruded from the optic disc as opposed to a single central retinal artery that branches after entering the globe. The presence of pairs of retinal arteries and veins twisting upon themselves were also present and visible in Figure 1. Finally, Winslow Stars [end on capillaries (10)] were visible in the yellow tapetal palette.
Fluorescein angiograms can be used to determine blood flow and reveal vascular hyperfluorescence, indicative of a damaged endothelial cell barrier resulting from exposure to gas emboli (11). The different phases of a fluorescein angiogram taken during the stabilization period prior to diving the sheep are shown in Figure 2 (above).

Figure 2A reveals the late choroidal flush / early arterial phase characterized by the presence of fluorescein first in the choroid followed by dye beginning to appear in the arteries. The time required for the onset of fluorescein appearance during the stabilization period prior to the dry dive procedure when the dye was injected into the jugular vein was 10.5 ± 0.5 sec (SEM, n=13).

In Figure 2B, the arterial phase, fluorescence in the choroid intensified with near-complete filling of the arteries and lack of dye in the veins.

During the arterial-venous phase (Figure 2C), arteries were filled with fluorescein and the dye began to appear in the veins.

Next, in the venous phase depicted in Figure 2D, the dye had moved mostly into the veins with little residual fluorescein in the arteries.

Finally, in Figure 2E, the late phase, fluorescein had circulated out of the vasculature but still remained in the choroid.

Shortly following the dry dive profile, the fundus camera was repositioned for observation of the ovine retina. Observations were made for the entire 90-minute post-dive procedure for signs of gaseous emboli in the retinal vasculature. Figure 3 (next page) combines color and fluorescein angiography to demonstrate lack of blood flow caused by an AGE. Bubbles in the arterial vascular tree of the retina were readily visible in a color angiogram taken approximately 20-28 minutes after the sheep surfaced (Figure 3A).

Roughly 28.5 minutes after surfacing, fluorescein was administered resulting in the angiogram depicted in Figure 3B. Lack of dye in the vasculature where a bubble was present in Figure 3A indicated no arterial blood flow resulting from an AGE. Presence of fluorescein in the vasculature where bubbles were present in Figure 3A demonstrated that the previously noted AGE had moved, dissolved or was not obstructing flow. Following the fluorescein angiography series, another color
angiogram was acquired at approximately 36.5 minutes following the dry dive protocol (Figure 3C). The lodged AGE responsible for the lack of blood flow demonstrated by the fluorescein angiogram in Figure 3B was still present.

The final diagnostic property of the fundus camera utilized in these studies was the capture of red-free angiograms. Red-free images can be utilized to examine vascular integrity including vascular collapse, vasoconstriction and vasospasm.

Figure 4 (below) reveals representative red-free angiograms from different regions of the sheep ocular fundus obtained during the predive stabilization period.

The central retinal region including the optic disc is displayed in Figure 4A. The ventral-nasal region of the ovine ocular fundus containing vasculature in the nontapetal palette is shown in Figure 4B. The vasculature tree in the tapetal area of the dorsal-nasal region is revealed in Figure 4C.

**FIGURE 3.** Post-dive color and fluorescein angiograms. A) Color image captured at 20-28 minutes. B) Fluorescein angiogram initiated at 28.5 minutes. C) Color image captured at 36.5 minutes. Arrows indicate lodged and mobile bubbles.

**FIGURE 4.** Regional pre-dive red-free angiograms. A) Central retinal region. B) Ventral-nasal region. C) Dorsal-nasal region. D) Dorsal-temporal region. E) Dorsal-temporal periphery region. Arrows reveal orientation of Figures 4B-4E with respect to Figure 4A.
Extensive branching of the arteries and veins in the tapetal region was visible in the dorsal-temporal region (Figure 4D). Finally, branching of the vascular tree into the microcirculation was demonstrated in the periphery of the dorsal-temporal region (Figure 4E).

Following a dive, reactive vasoconstriction can result from the lack of blood in the vasculature trailing gaseous emboli. Figure 5 (left) is a red-free angiogram captured between 8-23 minutes following the sheep dive procedure. Severe vasoconstriction was visible in the arterial tree in several places trailing the movement of gas bubbles into the microcirculation.

The data revealed in Figures 1 to 5 demonstrate that retinal angiography can be used to visualize bubbles in the retinal vasculature, assess the effect of dye, and thus blood, flow as a result of lodged bubbles, and discern reactive vasoconstriction associated with DCS.

It is well known, yet poorly understood, that the degree of DCS associated with rapid decompression is highly variable from mild pain to death (1, 2, 4, 5). Retinal angiograms associated with one extreme example of DCS that lead to the death of the sheep is presented in Figure 6 (below). In this case, the animal was compressed starting at 1.0 ATA at 0.5 ATA/min to a depth of 6.8 ATA. The animal remained at 6.8 ATA for 30 minutes (41.6 minutes bottom time) before being decompressed.

**FIGURE 5.** Post-dive red-free angiogram. Arrows indicate areas of vasoconstriction after exposure to gas emboli.

**FIGURE 6.** Extreme case of DCS. Pre-dive color (A) and fluorescein (B) angiograms. Post-dive color (C) and fluorescien (D) angiograms. Arrows demonstrate loss of blood flow. Post-dive color image (E) with 20-degree angle of coverage. Arrows indicate vasospasm and vasoconstriction.
back to 1.0 ATA at 2.0 ATA/min. Baseline color and fluorescein angiograms (Figure 6A and 6B respectively) were taken during the stabilization period prior to the dive.

The images revealed healthy color and blood flow represented by the arterial-venous phase of the fluorescein angiogram. Soon after surfacing, visual inspection of the ovine retina revealed a bubble load that filled every vessel, visible by the fundus camera, resulting in the displacement of all blood (Figure 6C).

Shortly after the color image was captured, fluorescein was administered in order to assess the effect of the bubble load on blood flow. The fluorescein angiogram in Figure 6D revealed that dye flow, and thus blood flow, was all but absent in the retinal vasculature except for a small amount of dye penetrating the choroid of the dorsal-nasal region.

Finally, Figure 6E is a color angiogram that depicts the microcirculation in the periphery of the dorsal-temporal region captured at a higher magnification. Vasoconstriction and vasospasm as a result of the massive air embolic load were readily visible.

A total of 31 sheep (23 males and eight females) dove to 6.0 ATA. Out of the 31 sheep, there were bubbles observed in eight (25.8%). Out of the eight animals for which bubbles were observed in the retinal circulation, there was a ratio of 50:50 male:female. However, 50% of the females that entered the chamber had retinal vasculature bubbles, while only 17% of the males that entered the chamber had retinal vasculature bubbles. The bubbles observed were mostly arterial and were not formed in situ, entering the retinal circulation via the central retinal arteries.

In all cases where retinal arterial bubbles were observed, the EDAC probe detected a significant load of bubbles in the sheep’s left jugular vein. One sheep had exclusively venous bubbles (not shown) lodged in the two largest central retinal veins where the vasculature protruded from the optic disc. All other venous bubbles observed (Figure 6) were the result of bubbles passing through the capillary bed and into the venous circulation.

Bubble onset time assessed through visual observation of the eight sheep that dove to 6.0 ATA with the fundus camera ranged from five to 22 minutes post-dive chamber. Bubble lodge time, also assessed through visual observation with the fundus camera, ranged from 10 to 70+ minutes, with the exception of one sheep where bubbles did not lodge.

Necropsies were performed following all experimental procedures. Hearts were dissected and closely examined for structural defects, including
a patent foramen ovale. Patent foramen ovales were not found in any of the nine sheep reported in this study having retinal arterial bubbles. Upon examining the chest cavity, it was found that two of the nine had severe pulmonary barotrauma resulting from local gas-trapping that caused bubble arterialization. In addition, two other sheep of the nine had foam in the right ventricle, including the sheep with retinal vasculature devoid of blood (Figure 6) that also had a massive bubble occluding the superior vena cava.

The remaining five sheep had extensive amounts of bubbles in the vasculature and right heart. Thus, arterialization was possibly due to overwhelming the ability of the pulmonary capillary bed to filter the bubbles. Since only the surface of the lungs were examined, it is possible that arterialization of bubbles in any of the sheep could be due to barotrauma deeper inside the lung parenchyma or the presence of pulmonary arteriovenous shunts.

While AGE is less frequently associated with DCS than VGE (2), AGE is also a concern in many modern clinical procedures, including craniotomies, laparoscopic procedures and cardiac surgery (8).

AGE was experimentally created by administering 5 cc of air over two minutes into the internal carotid artery of a sheep as described in Materials and Methods (see Figure 7, facing page).

Figure 7A reveals a normal-looking ocular fundus with healthy blood flow indicated by blood color captured just prior to administration of air emboli. Within minutes of the air injection, the color image in Figure 7B was captured. A large amount of air was visible in the major retinal arteries. The ability of the Topcon fundus camera to capture retinal images of a rabbit was also tested using a model of AGE.

Figure 8 (above) shows a series of red-free and fluorescein angiograms representing a temporal profile of events captured by the fundus camera after the administration of a 2cc air bolus into the internal carotid artery. A red-free image of the rabbit ocular fundus captured prior to the animal receiving the air insult is shown in Figure 8A. Five seconds after the air insult, the red-free image dis-
played in Figure 8B was captured. Notice that the presence of air was first observed in several choroidal vessels.

In the red-free image shown in Figure 8C, captured 21 seconds post-insult, air had filled several of the branches of the main retinal arterial tree, while there was a substantial increase in the number of choroidal vessels filled with air.

Fluorescein was then administered, and a fluorescein angiogram captured one minute, 25 seconds post-air insult is presented in Figure 8D. A considerable portion of the choroid area was lacking dye, and thus blood flow, as well as a complete absence of dye in the major retinal vessels. At approximately four minutes post-air insult, the fluorescein angiogram depicted in Figure 8E revealed that a large portion of the choroid layer was still absent of dye, while the major retinal vessels were just beginning to demonstrate blood flow.

At approximately 13 minutes following the air insult, the fluorescein angiogram (Figure 8F) revealed that several major retinal vessels were still lacking in dye and thus blood flow.

DISCUSSION
The fundus camera is a powerful and commonly used diagnostic tool in the field of ophthalmology. Serial retinal images are used to assess the progression of glaucoma (9). Fluorescein retinal angiography is useful in the diagnosis of diabetic retinopathy and age-related macular degeneration, among other diseases (9). Indocyanine green angiography is used to visualize the choroid vasculature not visible behind the retinal pigment epithelium during fluorescein angiography (9).

The fundus camera is also useful in nonophthalmologic disciplines. During cardiopulmonary bypass procedures, fluorescein angiography is used to monitor the retinal circulation for the presence of microemboli generated by the bypass circuit (12). Fluorescein angiography was also used in an experimental animal model of cardiopulmonary bypass to demonstrate the neuroprotective effects of perfluorocarbons added to the bypass prime (11).

The retina is thought to be a visible window to the microcirculation of the brain. It is an essential part of the central nervous system during development and, unlike the brain and spinal chord, the retina is readily visible and easily examined using noninvasive techniques. Similar to other components of the nervous system, the retina is formed from the neural tube during vertebrate embryonic development (13). The central portion of the neural tube forms the brain, while the protrusions are optic vesicles that later develop into optic cups and eventually the retina. Thus, the retina actually develops as part of the central nervous system.

The retina and brain also have similarities with regards to blood flow. The internal carotid artery is the main supply line of blood to cerebral tissue. The ophthalmic artery, which leads to both the ciliary artery that feeds the choroid and the central retinal artery that feeds the retina, is the first branch of the internal carotid artery (14). Also, retinal capillaries, like those of the brain, have very thick basement membranes and are embedded in a neuronal-glial matrix (15). This may decrease the ability of these vessels to expand in respond to an air embolus.

Finally, the retina has blood retinal barriers analogous to the blood brain barrier, both comprised of tight junctions between endothelial cells that line the vasculature (16), giving both structures similar permeability. Therefore, any embolic event affecting the circulation and tissue integrity of the retina, should parallel embolic events affecting the circulation of the cerebral tissue.

Since the retinal circulation is a noninvasive window to the cerebral circulation and retinal bubble loads may represent bubble loads in the cerebral vasculature, the retinal camera could be a useful tool to aid in clinical evaluations predicting neurological outcome as a result of DCS. A significant bubble load in the retinal vasculature for an extended period of time (in the case of neuronal tissue, this can be as little as 10 minutes) may mean not only a significant load of bubbles in the cerebral circulation but also the potential
for a substantial level of immediate brain damage. This would also necessitate further monitoring for evidence of delayed neuronal cell death that can exacerbate complications arising from immediate neuronal necrosis.

In addition, the retinal camera may be a useful instrument in the development and refinement of dive tables. Since the retinal camera could be a useful aid for predicting neurological outcome, the device may be useful for developing dive tables that minimize neurological DCS. Also, studies are currently under way by this group to correlate retinal bubble load with neurological damage as measured behaviorally, immunohistochemically and with biomarkers of cell death in models of both induced AGE and DCS. These studies will also monitor for bubbles in the jugular vein and carotid artery to determine correlations between venous bubble load, arterialization, and visualization of bubbles in the retinal vasculature.

The dive profiles utilized in this study were optimized for the study of severe experimental cardiopulmonary DCS with anesthetized large animals. A part of this cardiopulmonary DCS study was to develop and refine methods for detecting bubbles such as the retinal camera and EDAC device. Much like clinical DCS in humans (1, 2, 4, 5), the presentation of symptoms as observed through the fundus camera was extremely variable and unpredictable, ranging from retinal vasculature completely devoid of blood and overwhelmed by bubbles (see Figure 6) to no bubbles observed throughout the entire post-dive chamber procedures.

It is possible that the percentage of sheep observed to have bubbles in the retinal circulation would increase in a model of severe neurological DCS. It is widely accepted that interruption of blood flow and thus O₂ delivery to the brain for as little as 10 minutes can lead to significant cell death of sensitive neuronal tissue. Since an embolic occurrence in the retinal circulation is likely to parallel embolic events in the cerebral circulation, the sheep experiencing blocked retinal blood flow in this study would likely have some degree of brain damage as a result of the experimental DCS.

The normal ophthalmological characteristics of the sheep ocular fundus were recently depicted using a fundus camera (10). Photographs presented in this study (Figures 1 and 4) revealed structures characteristic of the sheep ocular fundus such as lack of a single central retinal artery, myelinated fibers over the nontapetal zone, and shape and myelin distribution of the optic disc consistent with those described in the Galan study (10).

Fluorescein angiographic studies of normal ovine ocular fundus were also recently described (17). Fluorescein angiographs (Figure 2) captured in this study reveal identical angiographic phases as previously observed in the Galan study (17). In addition, Galan et al., demonstrated that the onset of fluorescein appearance was 9.54 ± 2.18 sec when the dye was injected in the jugular vein of upright, nonsedated sheep (17). The value is similar to that reported in this study of 10.5 ± 0.5 sec when the dye was injected in the jugular vein of supine, anesthetized sheep.

Finally, the Galan study utilized 20 mg/kg of fluorescein sodium (17) while the current protocol in this paper called for 8 mg/kg. This discrepancy is easily explained by the use of black-and-white 35mm film in the former study and the use of digital imaging in the current manuscript. Less dye is required for digital imaging because of the increased sensitivity of ccd cameras compared to film, the decreased amount of “noise” in digital imaging compared to film, and the ability to digitally manipulate brightness, contrast and gamma levels in real time with the digital imaging system used for this study.

Bubbles in the eye as a result of a rapid loss of pressure have been previously documented. The first observation of ocular involvement was by Sir Robert Boyle in 1670 when he noticed bubbles in the anterior chamber of an eye of a viper that had been subjected to a vacuum (18, 19). In addition, anterior chamber bubbles in the eyes of a frog were observed as a result of altitude exposure (18, 20). Also, utilizing slit-lamp photography, N. Murray observed bubbles in the corneal epithelium of an Australian abalone diver who had exceeded accepted dive standards and further exacerbated the
condition by immediately taking a plane ride in an unpressurized cabin (21).

Evidence also exists linking bubbles in the retinal circulation with rapid decompression (22). Utilizing monkeys and a dive profile similar to the one described in this manuscript, Cockett and colleagues visualized bubbles in retinal vasculature post-dive chamber (23). However, images were not captured because the emboli moved too rapidly through the retinal vasculature to be photographed. The authors also found bubbles in the cerebral circulation of monkeys with retinal bubbles (23). This further emphasizes the potential of using the retinal circulation as a window for monitoring the cerebral circulation to aid in predicting the potential for deleterious neurological sequelae.

The current manuscript describes for the first time pictures of bubbles in the retinal circulation associated with DCS captured by a fundus camera. Color images are presented clearly revealing bubbles in the retinal microcirculation. Red-free pictures show severe vasoconstriction of the microcirculation caused by bubbles resulting from rapid decompression. Color images combined with fluorescein angiography demonstrate that bubbles arising from rapid decompression block arterial blood flow feeding retinal tissue. Previously, it was reported using fluorescein angiography that retinal pigment epithelial abnormalities in divers were the result of gaseous emboli associated with decompression (18, 24). However, these findings were based on the observation that the abnormalities were indistinguishable from those seen in choroidal ischemia and not direct observation of bubbles.

In conclusion, this manuscript describes for the first time the use of a fundus camera as a tool for the real-time, noninvasive detection of bubbles in the retinal circulation arising as a result of rapid decompression in experimental sheep. The camera is relatively easy to use and portable, making its use in DCS and AGE research practical and its use as an aid in predicting potential neurological damage feasible.

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