EXPERIMENTAL STUDIES

Visually Discernible Myocardial Echocardiographic Contrast After Intravenous Injection of Sonicated Dextrose Albumin Microbubbles Containing High Molecular Weight, Less Soluble Gases

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Objectives. The central hypothesis of this study was that microbubble survival, and subsequent left ventricular and myocardial ultrasound contrast, could be improved by altering microbubble gas to consist of a higher molecular weight (less diffusible) and less soluble gas.

Background. Microbubble survival after intravenous injection is shortened because of rapid diffusion of blood-soluble room air gases (nitrogen and oxygen) across the permeable bubble membrane into blood.

Methods. Thirteen open chest dogs received intravenous injections of a constant dose of sonicated dextrose albumin that was incubated with either room air or 100% nitrogen, 100% helium or 100% sulfur hexafluoride. Nitrogen (100%) is less blood soluble than room air, whereas helium and sulfur hexafluoride are the least soluble. Sulfur hexafluoride has the slowest diffusion rate. To further decrease the diffusion rate, each sample was administered during inhalation of room air and again during brief inhalation of the same gas with which it had been incubated.

Results. The highest peak videointensity in the left ventricular cavity was produced by the sonicated dextrose albumin incubated with sulfur hexafluoride, the gas having lowest blood solubility and diffusion rate, while sulfur hexafluoride was briefly inhaled during the period of intravenous injection (peak videointensity 139 ± 10 vs. 54 ± 11 for room air-exposed sonicated dextrose albumin, p < 0.001). Myocardial contrast was visually evident in >80% of the intravenous injections of sulfur hexafluoride-exposed sonicated dextrose albumin when the agent was given as an 8-fold concentrated sample during brief inhalation of sulfur hexafluoride.

Conclusions. Visual myocardial echocardiographic contrast is possible after intravenous injection of sonicated dextrose albumin if the microbubbles contain a gas with low blood solubility and diffusivity.

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Sonicated albumin and sonicated dextrose albumin produce variable degrees of left ventricular chamber ultrasound contrast after intravenous injection (1-5). Because these microbubbles are small (4 to 6 μm), they are capable of swift transpulmonary passage. However, this small microbubble size is associated with high surface tension (6), which is associated with high internal pressure within the microbubble and, thus, with large internal concentrations of room air gases (nitrogen and oxygen). Therefore, these gases rapidly diffuse out of the microbubble down the concentration gradient after intravenous injection, causing shrinkage of the microbubble and loss of ultrasound reflective properties (6). Because these microbubbles appear to allow diffusion of gases across the microbubble membrane, we hypothesized that decreasing the microbubble gas solubility and gas phase diffusivity would both increase blood gas concentration and preserve microbubble size in blood. These effects would enhance left ventricular cavity contrast and could produce myocardial ultrasound opacification after intravenous microbubble injection.

Methods

Canine preparation. The study involved 13 mongrel dogs. Each dog underwent open thoracotomy after intravenous sedation with sodium pentobarbital and subsequent endotracheal intubation and ventilation initially with room air. A 3.5-MHz epicardial imaging transducer was placed in a warm water bath over the anterior portion of the heart and adjusted to produce a short-axis view of the left ventricle at the midpapillary muscle level.

Intravenous and intraarterial 7F sheaths were placed in the right and the left femoral artery and vein. In one venous sheath, a 7F pulmonary artery catheter was placed and advanced into the pulmonary artery under fluoroscopic or pressure waveform guidance. This catheter was used to monitor pulmonary artery pressures during intravenous injections of contrast medium. A 7F pigtail catheter was advanced into the left ventricular cavity for monitoring of left ventricular systolic and end-diastolic pressures during contrast injections. The entire study was approved by the Institutional Animal Care and Use Committee and was in compliance with the Position of the American Heart Association on Research Animal Use.

Table 1. Diffusion Coefficients and Blood Solubilities of the Inert Gases Used With Sonicated Dextrose Albumin in the Study

	Nitrogen	Helium	Sulfur Hexafluoride
Diffusion coefficients (cm²/s)	0.250*	0.725	0.101
Blood solubility	0.013	0.007	0.006

*On the basis of Graham's law (8).

Preparation of ultrasound contrast agents. The two sonicated solutions used for this study were 3- to 4-fold dilutions of albumin with either 5% or 50% dextrose. The mean size of these microbubbles during room air sonication is 5 to 6 μm (7). The following stepwise process was performed in formulating the different gas-exposed sonicated dextrose albumin microbubbles.

Step 1. Sonication of dextrose albumin. Five percent human albumin, 5% dextrose and 5% dextrose were obtained from the hospital pharmacy. Selected aliquots of albumin and 5% or 50% dextrose were measured into 35-ml syringes. A commercially available sonicator (Heat Systems Ultrasonics Corp., model XL2020) was utilized to create microbubbles. With the sonicating horn initially placed just below the surface of the albumin-dextrose mixture, continuous sonication proceeded at 20,000 Hz with power set to 30% maximal output for 80 ± 5 s. All samples were prepared in a sterile laminar flow room.

Step 2. Exposure of gas to sonicated dextrose albumin. Eight-milliliter samples of sonicated dextrose albumin were then left in room air or exposed to physiologically inert gases of varying blood solubility and gas phase diffusivity for 60 min (Table 1). These gases (100% nitrogen, 100% helium and 100% sulfur hexafluoride) were obtained from a commercially available distributor (Air Products). As can be seen in Table 1, helium is less soluble in blood than is nitrogen (8); helium and sulfur hexafluoride have similar solubility (9), but sulfur hexafluoride has a lower gas phase diffusivity (10). Separate 8-ml samples of sonicated dextrose albumin were mixed with 10 ml of each gas in a 35-ml syringe for ~60 min before intravenous injection. Continuous mixing of the gas with microbubbles was achieved by placing the syringe on a hematology rocker (Barnstead/Thermolyne Corp.).

Step 3. Decanting gas from sonicated dextrose albumin. The gas was decanted from the sonicated dextrose albumin after 60 min of exposure to the gas and immediately before intravenous injection. The syringe containing the gas and microbubbles was turned upside down, and the gas at the top of the syringe was ejected. The 8-ml sample of the remaining microbubbles was then injected intravenously immediately after this process.

Preparation of concentrated boluses of microbubble solutions. In four dogs, 16- and 32-ml samples of sonicated dextrose albumin were placed in an upright position for ~2 h, resulting in the formation of an upper white layer (~4 ml) and a lower clear layer. This clear layer was then decanted and the remaining 4-ml sample (thus a 4- and an 8-fold concentrated sample) was exposed to the gas with the highest molecular

weight and slowest gas phase diffusivity (sulfur hexafluoride) for 60 min (the same exposure period as that of the previous samples) before intravenous injection.

Microbubble analysis. The microbubble concentration of sonicated 5% dextrose albumin and the sonicated dextrose albumin samples exposed to 100% nitrogen, helium and sulfur hexafluoride were analyzed by using a model ZBI Coulter Electronics counter. The aperture diameter was 100 μm and the sample volume was 500 μl. Each sonicated dextrose albumin sample was analyzed three times with the use of three samples of each contrast agent. The mean microbubble concentration and standard deviation were then calculated for each dilution.

Microbubble size of a sample of sonicated dextrose albumin alone and of sonicated dextrose albumin exposed to the different gases was determined by using a hemocytometer slide. A drop of the sample was placed on the slide after decanting the gas from the syringe and immediately analyzed by light microscopy.

Study protocol. The dogs underwent intravenous injections of sonicated dextrose albumin solutions that had been exposed to the different external gas concentrations for 60 min before injection. Immediately before injection, the gas was decanted from an 8-ml sample of sonicated dextrose albumin. The 8-ml sample (or 4-ml concentrated sample) was then given as a bolus over 3 s in the femoral vein and followed with a 10-ml normal saline flush. Each agent-gas mixture was given twice to test reproducibility. This same mixture was then given during brief (five to six respiratory cycles) inhalation of the same gas with which the sonicated dextrose albumin had been incubated before intravenous injection. This procedure was used in an attempt to temporarily saturate the blood with the gas to further prevent diffusion and dissolution of the incubated gas during intravenous injection. The concentrated samples of sulfur hexafluoride-exposed microbubbles were given as 4-ml injections. Finally, each of the gas-exposed samples was readministered in each dog after the initial sequence of injections. This procedure ruled out the possibility that the gas from a previously injected gas-exposed microbubble sample contributed to the results of the subsequent gas-exposed sample.

The left ventricular cavity and myocardial contrast produced with these samples were analyzed off line at end-systole from videotape images with the use of contrast software on the Freeland Systems Prism Review Station. This software digitizes up to 30 frames at one point in the cardiac cycle and will quantify gray scale (0 to 255 shades of gray) from a 100-square pixel region of interest within the mid-left ventricular cavity and anterior myocardium. These values were used to calculate peak videointensity in each region.

Mean transit time of contrast appearance in the left ventricular cavity and myocardium was measured by using on-line software (Acoustic Densitometry; Hewlett Packard Sonos 1500 Ultrasound System). This software calculates mean transit time as the sum of background-subtracted videointensities multiplied by the time of appearance in the left ventricular

Table 2. Hemodynamic Measurements After Intravenous Injection of Sonicated Dextrose Albumin Exposed to Different Gases*

	Pressures (mm Hg)		
	Mean PA	LV Systolic	LV End-Diastolic
SDA RA	15.3 ± 3.5	118.0 ± 19.1	4.7 ± 3.2
SDA Nitro	16.7 ± 3.1	119.6 ± 13.6	6.0 ± 5.7
SDA Nitro + inh	14.8 ± 2.7	109.0 ± 6.1	3.5 ± 2.7
SDA He	16.6 ± 3.2	119.1 ± 16.9	4.5 ± 2.5
SDA He + inh	15.8 ± 3.2	116.6 ± 19.8	4.0 ± 3.8
SDA SF	15.9 ± 4.1	118.2 ± 17.5	6.3 ± 2.7
SDA SF + inh	15.0 ± 2.0	111.0 ± 15.1	4.7 ± 1.3
SDA SF Conc	14.3 ± 3.3	111.6 ± 10.8	6.0 ± 2.0

There were no significant differences between samples. All data are presented as mean value ± SD. He = helium; inh = inhalation with the incubated gas; LV = left ventricular; Nitro = nitrogen; PA = pulmonary artery; RA = room air exposure; SDA = sonicated dextrose albumin; SF = sulfur hexafluoride; SF Conc = 4- or 8-fold concentrated bolus of sulfur hexafluoride.

cavity or myocardium divided by the sum of all background-subtracted videointensities.

Visually discernible myocardial contrast in the anterior myocardium was also assessed by two experienced reviewers. The contrast was graded qualitatively as 0 = no contrast, 1+ = some visual contrast, 2+ = moderate contrast, and 3+ = marked contrast resulting in an appearance similar to that of cavity contrast.

Statistical analysis. The central hypothesis was that the highest left ventricular and myocardial videointensities will be produced by the contrast agent incubated with the least soluble gas with the lowest diffusion coefficient during simultaneous inhalation of that gas. Sulfur hexafluoride was the least soluble and diffusible of the gases chosen for this study (Table 1). Analysis of variance of the peak videointensities in the left ventricle and myocardium of each of the different gas-exposed contrast agents was used for comparison. A Student-Newman-Keuls multiple comparison procedure was used to compare groups. Reproducibility of left ventricular cavity or myocardial contrast was determined by calculating the coefficient of variation of the contrast produced by repeated intravenous injections of the same gas-exposed agent.

Results

Hemodynamic variables during intravenous contrast injections. Table 2 demonstrates the left ventricular systolic, end-diastolic and mean pulmonary artery pressures after the injections. There were no significant changes in pulmonary artery or left ventricular pressures during injection of any of the samples exposed to different gases.

Table 3 demonstrates mean microbubble size and concentration for sonicated 5% dextrose albumin alone and after exposure to nitrogen, helium and sulfur hexafluoride. Microbubble size was not different among the samples, but microbubble concentration of the helium- and nitrogen-containing samples was significantly less than those exposed to sulfur hexafluoride or room air alone.

Table 3. Microbubble Size and Concentration of Sonicated Dextrose Albumin Exposed to Different Gases

	SDA RA	SDA Nitro	SDA He	SDA SF
Bubble size (μm)	5.1 ± 2.4	5.6 ± 2.5	5.6 ± 1.9	5.5 ± 1.2
Bubble concentration (× 10⁹/ml)	4.1 ± 0.1	3.7 ± 0.4*	3.3 ± 0.2*	4.1 ± 0.2

*p < 0.05 versus sonicated dextrose albumin exposed to room air and sulfur hexafluoride. All data are presented as mean value ± SD. Abbreviations as in Table 2.

Differences in left ventricular cavity opacification. The total number of intravenous injections in the study was 229, and the average number of injections in the 13 dogs studied was 18. Table 4 displays differences in left ventricular cavity opacification *at end-systole* after each of the different gas-exposed sonicated dextrose albumin injections. There was a progressive increase in peak videointensity in the left ventricle as the blood solubility of the microbubble gas decreased (nitrogen, helium and sulfur hexafluoride). For each of these gases, the chamber videointensity produced with the sample was significantly increased *when the gas was also inhaled briefly during the period of intravenous injection*. The highest end-systolic left ventricular peak videointensity of the nonconcentrated samples was produced by the gas with the lowest blood solubility and highest molecular weight (the sulfur hexafluoride-exposed sonicated dextrose albumin microbubbles) when given during brief inhalation with sulfur hexafluoride (gray scale U 139 ± 10 vs. for 54 ± 11 sonicated dextrose albumin alone and 122 ± 8 for sonicated dextrose albumin exposed to sulfur hexafluoride but given during room air inhalation, p < 0.001). Figure 1 demonstrates the significant increase in left ventricular cavity opacification with sulfur hexafluoride-exposed microbubbles.

Differences in myocardial contrast with different gas exposure. Table 4 also demonstrates the myocardial contrast produced by sonicated dextrose albumin exposed to different gases. Although nonconcentrated sonicated dextrose albumin microbubbles exposed to 100% helium, 100% nitrogen and 100% sulfur hexafluoride and then given during inhalation of these same gases all produced significantly higher left ventricular cavity contrast, there was no visual and minimal quantifiable contrast in the anterior myocardium.

The only gas-exposed microbubbles that were concentrated were the sulfur hexafluoride-exposed sonicated dextrose albumin microbubbles. This procedure was followed because sulfur hexafluoride produced the highest left ventricular cavity videointensity of the different nonconcentrated gas-exposed microbubbles. The 8-fold concentrated sulfur hexafluoride-incubated microbubbles given during inhalation of sulfur hexafluoride produced significantly higher myocardial contrast (Table 4).

Visually discernible contrast of myocardial contrast medium was evident with the concentrated sulfur hexafluoride microbubbles. This visual myocardial contrast corresponded to peak videointensities (corrected for baseline) >15 U. The two

Table 4. Peak Videointensity at End-Systole in the Left Ventricular Cavity and Myocardium Produced by Intravenous Sonicated Dextrose Albumin Exposed to Different Gases

PVI	SDA RA	SDA Nitro	SDA Nitro + Inh	SDA He	SDA He + Inh	SDA SF	SDA SF + Inh	SDA SF Conc
LV	54 ± 11	78 ± 15*	100 ± 7*	82 ± 13*	103 ± 8*	122 ± 8*	139 ± 10†	152 ± 8‡
MYO	1.8 ± 1.9	2.7 ± 1.7	3.5 ± 2.5	4.0 ± 3.2	5.2 ± 2.2	8.9 ± 3.7*	11.8 ± 3.7*	17.4 ± 2.2‡

*, †, ‡p < 0.05 versus, respectively, sonicated dextrose albumin with room air exposure, all other nonconcentrated samples, all other samples, as assessed by analysis of variance. All data are presented as gray scale units. LV = left ventricular cavity; MYO = myocardium; PVI = peak videointensity; other abbreviations as in Table 2.

experienced reviewers detected 1+ or greater visual myocardial contrast in 9 (81%) of the 11 intravenous injections of the 8-fold concentrated bolus of sulfur hexafluoride microbubbles when given during brief inhalation of sulfur hexafluoride. There was visually discernible myocardial contrast in 6 (50%) of the 12 4-fold concentrated samples. The two reviewers were in agreement on the degree of uptake (within 1 degree) in all cases. However, in each of the dogs, the inferior and posterior walls could not be visualized because of acoustic shadowing due to the microbubbles in the left ventricular cavity. The mean transit time of contrast medium in the myocardium after administration of the concentrated sulfur hexafluoride–exposed sonicated dextrose albumin was 11.5 ± 1.5 s (range 8.4 to 13.2), whereas mean transit time in the left ventricular cavity of these concentrated microbubble solutions was 12.8 ± 1.5 s (Fig. 2). Figure 3 is an example of myocardial contrast after an intravenous injection of a concentrated bolus of sulfur hexafluoride-incubated microbubbles during inhalation of this gas.

Reproducibility of left ventricular cavity and myocardial contrast. Left ventricular cavity videointensity with the same amount of contrast medium and same gas exposure was tested

twice to assess reproducibility of the findings. The coefficient of variation for peak left ventricular cavity videointensity for the sulfur hexafluoride–exposed microbubbles in all dogs was 7.1%. The coefficient of variation for myocardial contrast of the concentrated samples of the sulfur hexafluoride–enhanced microbubbles in all dogs was 13.0%.

Discussion

These results demonstrate that intravenous sonicated dextrose albumin can reliably produce myocardial echocardiographic contrast when the microbubbles are filled with a high molecular weight (and thus less diffusible), less soluble gas before injection. The sonicated dextrose albumin incubated with sulfur hexafluoride, the gas of lowest blood solubility and diffusivity, produced the greatest left ventricular and myocardial contrast. Sulfur hexafluoride is a physiologically inert agent used as an inhaled gas because of its low diffusivity and solubility (11) and in intravitreal injections for retinal tamponade because of its low diffusivity (12). In each of these clinical applications, there has been no apparent toxicity. Similarly,
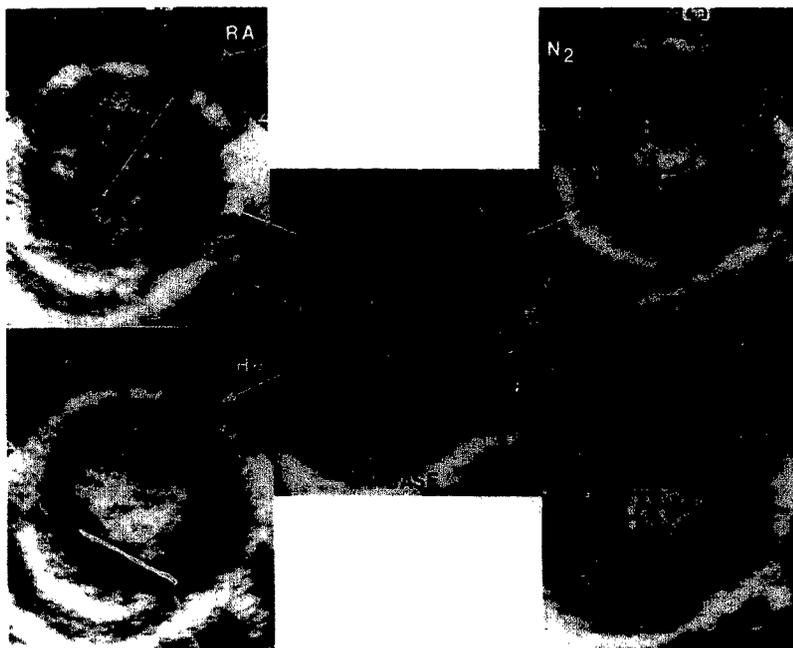

Figure 1. Epicardial short-axis images *at end-systole* of the left ventricular cavity at baseline (BASE, center image) and during injection of the same amount of sonicated dextrose albumin exposed to room air (RA), nitrogen (N₂), sulfur hexafluoride (SF) and helium (He). The sonicated dextrose albumin exposed to sulfur hexafluoride produced the greatest left ventricular opacification.
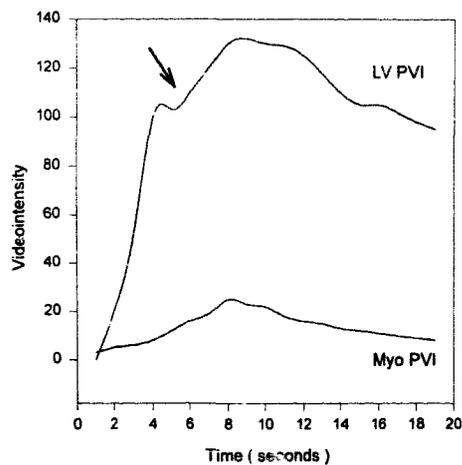
Figure 2. Time-intensity curves in the left ventriculai cavity (LV) and myocardium (Myo) after an intravenous injection of the concentrated sulfur hexafluoride–exposed sonicated dextrose albumin microbubbles. The left ventricular cavity videointensity was blunted at peak intensity because of acoustic shadowing (**arrow**). PVI = peak videointensity.

Figure 3. Epicardial short-axis images before (**left**) and after (**right**) femoral venous injection of concentrated sonicated dextrose albumin exposed to sulfur hexafluoride before injection and administered during brief inhalation of a partial mixture of room air and sulfur hexafluoride. Myocardial uptake is visually evident. BASE = baseline; SF = concentrated bolus of sulfur hexafluoride microbubbles given intravenously during brief inhalation with sulfur hexafluoride.

sulfur hexafluoride–exposed dextrose albumin microbubbles had no adverse hemodynamic effects in this study.

Both microbubble gas diffusivity and blood solubility appeared to be important determinants of the degree of left ventricular ultrasound opacification. The purpose of this project was to alter these two physical characteristics of the microbubble gas to prolong microbubble survival. Altering each of these known properties of gases produced microbubbles that could significantly enhance the clinical application of sonicated dextrose albumin.

Effect of microbubble gas diffusivity. It has been shown (13) that the rate of microbubble radius decay in blood is directly related to the diffusion constant for a given gas. The rate of diffusion of a gas is inversely proportional to the square root of the molecular weight (8). Sulfur hexafluoride has a molecular weight of 146 g/mol, in contrast to 4 g/mol for helium and 28 g/mol for nitrogen. Therefore, sulfur hexafluoride will diffuse more slowly down the concentration gradient from the microbubble into blood. This will prevent the microbubble from shrinking and preserve its reflective properties and explains why the best left ventricular ultrasound contrast after intravenous injection was obtained with the sulfur hexafluoride–exposed microbubbles.

In our study, inhalation of sulfur hexafluoride during the intravenous injection of sulfur hexafluoride–containing dextrose albumin microbubbles should have *decreased* the concentration gradient. We hypothesized that the inhalation would delay diffusion of sulfur hexafluoride gas from within the microbubble. Because the inhalation significantly improved left ventricular cavity and myocardial opacification, we assume that the delayed diffusion is one mechanism for this improvement. The opposite clinical results have been seen when the concentration gradient of smaller molecular weight gases
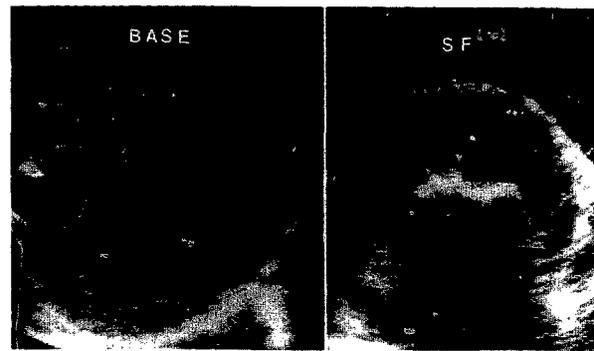
like nitrogen is increased. Wible et al. (14) demonstrated loss of left ventricular cavity opacification when predominantly nitrogen-containing albumin microbubbles were given intravenously during inhalation of 100% oxygen and 0% nitrogen. When we administered 100% nitrogen with the nitrogen-containing dextrose albumin microbubbles, we saw improved left ventricular videointensity by decreasing this concentration gradient (Table 4).

Effect of microbubble gas solubility. Exposing intravenously injected albumin microbubbles to gases like nitrous oxide that have increased blood solubility (15) results in a shorter duration of contrast effect (14). Sulfur hexafluoride and helium are gases with significantly lower blood solubility than that of nitrous oxide (16). In our study, both of these gases produced significantly higher left ventricular chamber opacification after intravenous injection than did the same amount of sonicated dextrose albumin exposed to room air (Table 4). Because helium-incubated microbubbles produced greater left ventricular cavity opacification than did room air–containing microbubbles, this study demonstrates the relative importance of blood solubility as well as diffusivity in determining microbubble survival. Helium has a molecular weight of only 4 g/mol and is therefore rapidly diffusible. However, it is less blood soluble than either nitrogen or room air (8). Because the microbubbles incubated in helium produced *greater* left ventricular opacification than did room air–containing microbubbles, blood solubility must be as important as diffusivity in determining microbubble survival in blood.

The low blood solubility of sulfur hexafluoride may also explain why inhaling a small amount of this gas during intravenous injection of the sulfur hexafluoride–containing microbubbles produced the greatest left ventricular chamber and myocardial opacification. If this inhalation saturated, or even supersaturated, blood with sulfur hexafluoride during intravenous injection, it would have prevented the gas from dissolving and preserved microbubble size (13).

Effect of bolus delivery of contrast agent. Villanueva et al. (1) have demonstrated that concentrated boluses of sonicated albumin produce visually discernible myocardial contrast in 50% of right atrial injections under baseline conditions (1). The concentration of albumin microbubbles required to achieve this were 4 to 5 × 10^8/ml. By concentrating the sulfur hexafluoride–exposed microbubbles 8-fold, we were able to produce visually discernible myocardial uptake in >80% of femoral venous injections under baseline conditions. Visual detection in the myocardium was not evident with smaller concentrations of the sulfur hexafluoride–exposed microbubbles, although videointensity was quantitatively increased. Visually discernible myocardial contrast did not occur until videointensity (corrected for baseline) exceeded 15 gray scale units (Table 4). The improved videointensity with highly concentrated boluses of these microbubbles is consistent with previous observations (16) that the gas-filtering effect of the lung becomes less efficient when exposed to high concentrations of the gas.

Limitations of the study. External exposure of sonicated dextrose albumin microbubbles to sulfur hexafluoride gas resulted in myocardial opacification after intravenous injection that was not produced with the same microbubbles exposed to room air, 100% nitrogen or 100% helium. Although this finding supports the hypothesis that this gas with lower diffusivity and blood solubility diffused across the microbubble membrane and prevented the microbubble from shrinking after intravenous injection, the proof we obtained for this hypothesis was inferential rather than direct. However, albumin microbubbles have been shown to be extremely sensitive to the surrounding gas (14), suggesting that the protein shell allows rapid diffusion of gases across its membrane. Because we assumed that sulfur hexafluoride diffused more slowly than the other gases, sonicated dextrose albumin was incubated with 100% sulfur hexafluoride for 60 min. However, we did not directly measure the partial pressure of sulfur hexafluoride within the microbubbles after incubation.

In addition to this theoretical limitation, there are other limitations in using this technique to noninvasively measure myocardial blood flow. First, despite excellent quantifiable opacification of the anterior myocardium in >80% of the injections performed at rest, posterior left ventricular attenuation prevented analysis of the posterior myocardium. This attenuation has also been seen with high concentrations of Albunex and has limited the ability of this technique to quantify blood flow in the posterior wall by using venous injections of contrast medium (1). One factor producing attenuation with albumin microbubbles appears to be the large number of small (<3 μm) microbubbles (13). These microbubbles produce very little reflection but still cause attenuation. Therefore, because of the rapid diffusion of gases out of the microbubble after intravenous injection, the majority of microbubbles will be in this size range by the time they reach the left ventricle after a peripheral intravenous injection. Even though we altered microbubble gas to make it less diffusible and less blood soluble than oxygen or nitrogen, there was still

considerable attenuation in the left ventricular cavity (Fig. 3). It is imperative to maintain microbubble size in the 5 to 6 μm range, because this size range produces a better reflection/attenuation ratio when transmitted frequencies are 2.5 to 3.5 MHz (13).

Second, for the sulfur hexafluoride–exposed sonicated dextrose albumin to produce myocardial contrast, the injection had to be given during simultaneous inhalation of sulfur hexafluoride. Although it may not be realistic to expect inhalation of sulfur hexafluoride gas combined with injection of sulfur hexafluoride-containing microbubbles to be a clinical approach to noninvasive perfusion imaging, *this finding supports the concept that altering microbubble gas composition to decrease diffusivity and blood solubility will improve the contrast effect in the left ventricular cavity and myocardium.*

Conclusions. In this study, sonicated dextrose albumin microbubble gas composition was altered by incubating the microbubbles with sulfur hexafluoride, a gas with lower levels of diffusivity and blood solubility than those of nitrogen or oxygen. Intravenous injection of a concentrated bolus of these microbubbles produced significantly higher left ventricular opacification *and visually evident myocardial opacification.* Because the injection also produced consistent acoustic shadowing of the posterior myocardium, further modifications are needed before this contrast agent is applied to the noninvasive study of myocardial blood flow. Other methods of improving the amount of these gases incorporated into the microbubble need to be explored. Clinically accepted techniques for doing this already exist (17). In addition, the intravascular rheology of these microbubbles must be examined to confirm that they act as red blood cell tracers and therefore can be utilized as indicators of myocardial blood flow. The mean transit time of contrast medium in the myocardium that we observed after the intravenous concentrated sulfur hexafluoride–incubated dextrose albumin microbubble injections indicates that they do behave as intravascular tracers. Therefore, altering microbubble gas composition represents an exciting new technique that may allow contrast echocardiography to be utilized in noninvasive studies of myocardial blood flow.

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