Assessment of Myocardial Perfusion Abnormalities
with Contrast-enhanced Two-dimensional Echocardiography

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SUMMARY A new echocardiographic contrast agent, gelatin-encapsulated microbubbles, that produces an intramyocardial contrast effect, was evaluated as a marker for the detection of regions of abnormal myocardial perfusion in nine open-chest dogs. The gelatin-encapsulated microbubbles were injected into the aortic root under control conditions and during circumflex coronary artery occlusion. Myocardial perfusion was simultaneously assessed with radioactive microspheres. Echocardiographic contrast enhancement (ECE) was measured in footlamberts (Ft-L) from the videoscreen of an off-line playback system, using a commercially available light meter. A single short-axis section of the left ventricle was divided into octants to analyze myocardial perfusion. The equivalent regions of the echocardiographic image were analyzed for contrast enhancement and wall motion. An ECE > 0.3 Ft-L was seen in all 120 octants analyzed before circumflex coronary artery occlusion and in 48 of 51 (94%) octants with > 50% of the normal zone flow during circumflex artery occlusion. An ECE ≤ 0.3 Ft-L identified 19 of 21 octants (90%) with ≤ 50% normal zone flow and all 13 octants with ≤ 25% normal zone flow during coronary artery occlusion. In contrast, wall motion abnormalities (akinesis or dyskinesis) were seen in 13 of 51 octants (25%) with > 50% normal zone flow, and normal wall motion was seen in two of 21 octants (10%) with blood flow ≤ 50% of normal zone flow during circumflex coronary artery occlusion. We could not demonstrate a linear correlation between ECE and the absolute level of myocardial blood flow. We feel this was due to the limitations imposed by imaging an open-chest animal preparation, variation in the number of gelatin-encapsulated microspheres used for each injection and variations in the echocardiographic gain settings among experiments. We conclude that contrast-enhanced two-dimensional echocardiography with gelatin-encapsulated microbubbles can accurately identify ischemic regions of the left ventricular myocardium. This technique is more accurate than wall motion analysis for detecting myocardial ischemia.

A NEW CLASS of ultrasound contrast agents has been developed that offer the possibility of determining the distribution of myocardial blood flow with two-dimensional echocardiography. These agents are encapsulated gas microbubbles, which are highly reflectant and are the source of ultrasonic contrast effect.1 When in-

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Materials and Methods

Echocardiographic Contrast Agent

The echocardiographic contrast agent used for this study was a suspension of gelatin-encapsulated microbubbles, 76 ± 1 μm in diameter, that contained nitrogen. This contrast agent was provided by the manufacturer (Berliscan) in 3.0-ml vials or in 0.75-ml dye tubes that contained 1.8–3.8 × 10⁹ microbubbles. The gelatin-encapsulated microbubbles were stored under refrigeration and were prepared for use by warming for 60 seconds in a 60°C water bath. Once in fluid form, the microbubbles were injected over 1–2 seconds through a standard angiographic catheter using sodium diatrizoate (Renografin-76) as a flush solution. Sodium diatrizoate was used because it is a viscous liquid capable of clearing the microbubbles from the dye tubes and moving the contrast agent through the catheter as a bolus. It was also selected because of its availability, record of safety and lack of toxicity in the doses used (3–5 ml).

Animal Preparation

Under halothane or pentobarbital anesthesia, 10 mongrel dogs that weighed 18–25 kg were prepared. Respiration was maintained with a Harvard respirator or an Ohio volume veterinary respirator. A median sternotomy was performed and the heart was suspended in a pericardial cradle. Catheters were placed in the left atrium, the left brachial artery, the left femoral artery and the jugular vein. A #7F angiographic catheter with a 6-cm terminal curve was advanced retrogradely from the right femoral artery into the aortic root. The catheter had a single end hole and six side holes within 1.0 cm of the catheter tip. Both direct echocardiographic visualization and contrast injections (saline) were used to confirm the catheter position. A hydraulic occluder was placed on the circumflex coronary artery distal to the first large marginal branch. A pulsed Doppler flow velocity transducer was placed immediately proximal to the coronary occluder. The ECG, arterial pressure, and Doppler flow velocity signal from the coronary artery were continuously recorded on a multichannel recorder throughout the experiment.

Echocardiographic Examination

A commercially available, mechanical, two-dimensional echocardiographic recorder with a 5-MHz transducer was used. Gain settings were adjusted at the beginning of each experiment and were not changed throughout each experiment. The echocardiogram was recorded on videotape for later analysis. A single short-axis scan of the left ventricle was recorded at the level of the tips of the papillary muscles. The transducer was placed directly on the free wall of the right ventricle throughout the entire experiment and made secure by clamping to a stand affixed to the procedure table. A conventional acoustic standoff was not used.

FIGURE 1. The short-axis two-dimensional echocardiogram before (A) and after (B) injection of contrast material. The intensity of the echocardiographic image increases in all areas of the left ventricle after the injection. The white lines are the dividing points for the octants and the faintly reproduced numbers are the preselected sampling points for echocardiographic contrast enhancement (ECE) determination.

The schematic outlines the results of the radioactive microsphere perfusion study and ECE determination. All octants have normal ECE values and normal myocardial perfusion. CBF = coronary blood flow from the radioactive microsphere perfusion study; the values represent the percentage of the normalized CBF for each octant.
Experimental Procedure

After preparation of the dog and placement of the echocardiographic transducer, a baseline two-dimensional echocardiogram of the short axis of the left ventricle was recorded. Radioactive microspheres were then injected into the left atrium for determination of myocardial perfusion. Forty-five seconds later, and during continuous recording of the two-dimensional echocardiogram, a 0.75-ml bolus of gelatin-encapsulated microbubbles was injected rapidly into the aortic root through the angiographic catheter. The catheter was immediately flushed with 3–5 ml of sodium diatrizoate to ensure complete delivery of the contrast agent.

After completion of the baseline studies, the circumflex coronary artery was occluded, and the Doppler flow velocity signal was monitored to document the absence of flow. A continuous two-dimensional echocardiogram was monitored during the occlusion and recorded on videotape. After the artery had been occluded for 4 minutes, microspheres bearing a second radioactive label were injected for measurement of myocardial perfusion. Forty-five seconds after injection of the radiolabeled microspheres, gelatin-encapsulated microbubbles were again injected as a bolus into the aortic root during continuous two-dimensional echocardiography. After completion of the two-dimensional echocardiogram, the circumflex occlusion was released. In seven dogs, the circumflex artery was occluded a second time, and the contrast echocardiogram repeated; in four of these, myocardial perfusion was assessed by the radioactive microsphere technique.

After the experiment, spinal needles were passed along the plane of the two-dimensional echocardiographic examination to mark the area of the left ventricle for perfusion determination by the radioactive microsphere technique (fig. 2). The heart was arrested with potassium chloride and removed with the spinal needles in place. After flushing with saline, the left ventricular cavity was packed with gauze and fixed in 10% formaldehyde for 24 hours. A single 1-cm slice of the left ventricle corresponding to the plane of the echocardiographic examination was then taken for study. This slice was sectioned into octants, using the posterior interventricular groove as a landmark.

Analysis of Echocardiographic Data

The videotape of the two-dimensional echocardiogram was analyzed on a commercially available off-line system (Micro Sonics, Inc.). This system has a self-contained videodisc that allows for presentation of a stable, flicker-free image to determine video intensity. The off-line system is equipped with a graphics display format that allowed the tracing of a diastolic endocardial contour on the video screen, which was retained as a reference during systolic wall motion studies. Numeric characters were entered for overlay onto the echocardiographic image. This overlay allowed for the predetermination of 18–21 points around the circumference of the left ventricle at which video intensity measurements and wall motion analysis could be made.

After review of the entire tape, a 10-second interval was selected that incorporated a baseline echocardiogram and the injection of the gelatin-encapsulated microbubbles; this interval was transferred to the videodisc. The image recorded on the disc could be played back repeatedly in real-time, slow motion, and frame-by-frame for a detailed analysis of video intensity and wall motion. Wall motion was analyzed immediately before injection of the contrast agent by freezing an end-diastolic frame on the video screen and then outlining the endocardial contour using the graphics capability of the off-line system. Motion of the endocardial border during systole was characterized as normal, akinetic or dyskinetic using standard criteria.4

Contrast enhancement was measured with a commercially available light meter with a digital readout (Minolta Inc., 1° spot meter). All readings of video intensity were taken at end-diastole, which was defined as the onset of the QRS on the ECG. Both the baseline and contrast video intensity were measured from the same 10-second segment, which was recorded on the videodisc. The baseline measurements were taken from each of the cardiac cycles immediately preceding injection of the gelatin-encapsulated microbubbles, and the contrast measurements were taken from an end-diastolic scan on the same 10-second segment. The actual cardiac cycle evaluated for contrast effect was selected as the diastolic frame demonstrating peak echocardiographic contrast effect when analyzed visually. This occurred within five cardiac cycles after injection of the contrast agent. The video image controls of the playback system and video monitor remained constant throughout analysis of each experiment. Using the video overlay capabilities of the off-line system, a series of 18–21 points around the perimeter of the left ventricular short-axis image was established for each dog. For each preselected point on the short-axis image of the left ventricle, two to four video intensity readings, in foot-lamberts (Ft-L), were averaged. Readings were taken of the full thickness of the myocardium excluding the bright epicardial echo.

![Figure 2. Short-axis two-dimensional echocardiogram of the left ventricle after passage of spinal needles (arrows) through the heart to mark the plane of the echocardiographic examination. M = myocardium.](image)
Use of the video overlay system allowed for analysis of the same points at baseline and during occlusion, both before and after injection of the contrast agent. As each octant served as its own control for contrast enhancement, differences in the baseline echo intensity due to location within the short-axis image were not reflected in the echocardiographic contrast enhancement (ECE) measurement. Each of the sampling points along the short-axis of the left ventricle was then assigned to one of the octants of the anatomic specimen for comparison with myocardial perfusion determined by the radioactive microsphere technique. Using this method of analysis, each octant contained one to three video intensity sampling points; all but four octants in the series consisted of two or three sampling points. A mean video intensity at baseline, both before and after contrast injection, and during circumflex coronary artery occlusion, also both before and after contrast injection, was then calculated for each octant. The ECE was calculated as the difference between video intensity at baseline and after contrast enhancement.

Myocardial Blood Flow Determination

Myocardial perfusion was measured with radioactively labeled 15-μm-diameter microspheres labeled with 51Co, 57Cr, 113Sn, 103Ru, 59Nb or 46Sc (New England Nuclear). Between 1.9 × 10⁶ and 5.0 × 10⁶ microspheres, suspended in 10% dextran, were injected into the left atrium for each flow determination. Reference arterial blood was withdrawn from two arteries at the rate of 2.06 ml/min with a Harvard pump, beginning 1 minute before injection and continuing for 2 minutes after injection of radioactive microspheres.

After fixing and cutting the heart, the myocardial segments were weighed to the nearest milligram and placed in plastic scintillation vials. Reference blood samples and myocardial segments were then counted in a gamma scintillation counter and standard techniques were used for separation of isotopic activities. Myocardial perfusion was calculated using the formula: \( BF_m = (C_m \times 100 BF_r) / C_r \), where \( BF_m \) = blood flow to the myocardium, \( C_m \) = myocardial counts per gram of tissue, \( BF_r \) = rate of withdrawal of reference blood and \( C_r \) = counts in reference blood sample. Data were discarded if the counts in the two reference arterial samples differed by more than 15%. For baseline studies, perfusion was normalized to the mean of all myocardial segments. For studies performed during circumflex artery occlusion, the perfusion for each octant was normalized to the mean of the value for octants 2–4, which were perfused by the patent left anterior descending artery and were not underperfused in any experiment.

Statistical Analysis

A Spearman rank-order correlation was performed for all octants with both echocardiographic enhancement values and flow data available. A one-way analysis of variance was used to compare the amount of ECE with quartiles of normalized myocardial blood flow. If analysis of variance yielded a \( p \) value < 0.05, multiple comparisons with control of overall size were performed. Statistical significance was assumed if \( p < 0.05 \). Data are mean ± SD.

Data from nine dogs were available for analysis. Data from one dog were not accepted because of a large tumor attached to the left ventricular wall that was felt to alter the echocardiographic characteristics of the myocardium. Myocardial perfusion measurements by the radioactive microsphere technique were technically acceptable in six dogs. Echocardiographic data were available for 120 octants at baseline (15 measurements in nine dogs) and for 128 octants during circumflex artery occlusion (16 measurements in nine dogs). Simultaneous perfusion studies were available for 80 octants at baseline (10 measurements in six dogs) and for 72 octants during circumflex artery occlusion (nine measurements in six dogs).

Results

Infusion of gelatin-encapsulated microspheres into the aortic root consistently produced a visible contrast effect within the myocardium both at baseline (fig. 1) and during circumflex artery occlusion (figs. 3 and 4). The visible contrast effect reached a maximum within five cardiac cycles, and persisted up to 2 minutes. During occlusion, nonperfused segments of myocardium were visible as areas that failed to increase in echo intensity after injection of the contrast agent.

ECE ranged from 0.8 to 11.2 Ft-L (mean 3.5 ± 1.8 Ft-L) for 120 octants analyzed at baseline (fig. 5A).

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** Baseline (A) and contrast-enhanced (B) echocardiograms of a short-axis scan of the left ventricle during circumflex coronary artery occlusion. (A) The area from 10 o’clock to 4 o’clock (arrows) had normal wall motion. (B) Image intensity increased after contrast enhancement (arrowheads) only in this area of myocardium.
Figure 4. Results of echocardiographic contrast enhancement (ECE) and myocardial perfusion studies during circumflex artery occlusion in the same dog as in figure 1 — the short-axis two-dimensional echocardiogram of the left ventricle before (A) and after (B) injection of the contrast agent. The white lines in panel B outline the octant divisions; the faintly reproduced numbers are the sampling points for measuring ECE. The white lines in panel B outlining sampling points 13–19 and 1–2 does not increase in image intensity, whereas points 3–12 show a normal increase in image intensity after injection of the gelatin-encapsulated microbubbles. The schematic outlines results of ECE and the myocardial perfusion study. Octants 6, 7, 8 and 1 have markedly reduced myocardial perfusion and abnormal ECE values. CBF = coronary blood flow.

For the 80 octants with baseline microsphere perfusion data available, the mean coronary blood flow, determined by the radioactive microsphere technique, was 119 ± 64 ml/min × 100 g. During baseline studies, all octants with perfusion measurements had normal perfusion (>75% of the normalized flow). During circumflex artery occlusion, the mean myocardial blood flow of the normal flow zones was 120 ± 73 ml/

Figure 5. The distribution of echocardiographic contrast enhancement (ECE) values at baseline and during circumflex artery occlusion as a function of quartiles of normalized coronary flow. Each symbol represents one octant.
min x 100 g and the relative perfusion ranged from 4–138% of the normal zone flow in the 72 octants in which microsphere perfusion studies were performed. The ECE ranged from –3.0 to 9.1 Ft-L in the same 72 octants. The distribution of ECE values during occlusion, as a function of the percent normal zone flow, is presented in figure 5B, and comparisons among quartiles of flow are presented in table 1. There was a statistically significant (p < 0.001) difference between the ECE of the octants with flows ≤ 50% of normal zone flow when compared to the octants with flow > 50% of the normal zone flow. There was also an association between ECE and myocardial perfusion when values from baseline and occlusion studies were combined (r = 0.55, p < 0.05).

After inspection of the ECE values from baseline octants and from octants with preserved flow during circumflex artery occlusion, an ECE of 0.3 Ft-L was arbitrarily assigned as the best discriminator between normal and abnormal perfusion. Of the 51 octants with myocardial blood flow > 50% of the normal zone flow during occlusion, all but three (94%) had an ECE > 0.3 Ft-L. All three of these octants were located in border zones adjacent to areas of marked hypoperfusion and all three were dyskinetic on wall motion analysis. Of the 21 octants with myocardial blood flow ≤ 50% of the normal zone flow, all but two (90%) had an ECE ≤ 0.3 Ft-L. All 13 octants with myocardial blood flow ≤ 25% of the normal flow zone flow had ECE < 0.3 Ft-L. The two octants with a normal value for ECE but reduced flow had normal wall motion, and were in border areas between octants with normal and markedly reduced flow. Thus, contrast echocardiography with gelatin-encapsulated microbubbles could correctly separate low- and normal-flow regions, although the range of ECE in normally perfused segments was great.

At baseline all octants had normal wall motion. During circumflex artery occlusion, 75 octants had normal wall motion, 12 akinesis and 41 dyskinesis. Myocardial blood flow and ECE as a function of wall motion is presented in table 2. Results from a single experiment are presented in figure 6. An ECE > 0.3 Ft-L was seen in 18 of 53 akinetic or dyskinetic octants, and a myocardial blood flow > 50% of the normal zone flow in 13 of 32 akinetic or dyskinetic octants for which microsphere studies were available. Therefore, wall motion abnormalities did not serve as well as contrast echocardiography as a marker for low- and normal-flow regions.

We noted no significant adverse hemodynamic or electrocardiographic effects directly attributable to the infusion of gelatin-encapsulated microspheres. We consistently noted transient hypotension and ST-segment depression after each injection, which was reproducible with injection of sodium diatrizoate alone. Typically, systolic blood pressure fell 20 mm Hg, but returned to normal within 10 seconds. Injection of sodium diatrizoate alone (5 ml) into the aortic root produced no appreciable segmental wall motion abnormalities and only an occasional faint, patchy area of intramyocardial contrast effect that lasted less than 5 seconds. We noted no effect on coronary flow velocity from injection of this dose of sodium diatrizoate alone.

**Discussion**

The new information provided by this study is that gelatin-encapsulated microbubbles injected into the aortic root enhance the myocardial image and reliably identify regions of poorly perfused myocardium. During circumflex coronary artery occlusion, ECE correctly identified 48 of 51 octants (94%) with > 50% of the normal zone flow and 19 of 21 octants (90%) with reduced coronary flow. The ECE data were considerably more accurate than wall motion analysis for assessing the state of regional perfusion. Our finding of an overestimation of the ischemic region size by wall motion analysis is in concurrence with previous studies.6, 7

Although we accurately detected regions of reduced perfusion, correlation between ECE and myocardial perfusion by the radioactive microsphere technique was poor (r = 0.55, p < 0.05). There may be several reasons for the wide scatter of ECE values, which occurred most prominently in the higher flow pieces. The most important is probably that although the echocardiographic gain and contrast settings remained constant throughout each experiment, they differed from experiment to experiment. The gain setting was adjusted to provide the best image at the beginning of each experiment and thereafter was not changed. Additionally, the number of bubbles injected for each contrast determination varied. Differences in anatomy among the dogs and slight differences in the echocardiographic transducer position among the experiments probably introduced further variability. Because we did not use a standoff for the echocardiographic transducer, variations in image intensity related to the area of the left ventricle sampled were created. In future

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**Table 1. Comparison of Echocardiographic Contrast Enhancement (ECE) and Myocardial Perfusion Determined with Radioactive Microspheres**

<table>
<thead>
<tr>
<th>Normal zone flow</th>
<th>n</th>
<th>ECE (Ft-L) (mean ± SD)</th>
<th>ECE (Ft-L) (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–25%</td>
<td>13</td>
<td>-0.9 ± 0.6*</td>
<td>-1.9 to 0.1</td>
</tr>
<tr>
<td>26–50%</td>
<td>8</td>
<td>0.3 ± 1.9*</td>
<td>-3.0 to 3.2</td>
</tr>
<tr>
<td>51–75%</td>
<td>9</td>
<td>2.8 ± 1.5</td>
<td>0.0 to 5.8</td>
</tr>
<tr>
<td>&gt; 75%</td>
<td>42</td>
<td>3.0 ± 1.8</td>
<td>-0.8 to 9.1</td>
</tr>
</tbody>
</table>

* p < 0.001 compared with octants with flow > 50% of normal zone flow.

**Table 2. Wall Motion Characteristics as a Function of Myocardial Blood Flow and Echocardiographic Contrast Enhancement**

<table>
<thead>
<tr>
<th>MBF (% normalized flow) n = 72</th>
<th>ECE (Ft-L) n = 128</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wall motion</td>
<td>&gt; 50%</td>
</tr>
<tr>
<td>Normal</td>
<td>38</td>
</tr>
<tr>
<td>Akinetic or dyskinetic</td>
<td>13</td>
</tr>
</tbody>
</table>

Abbreviations: ECE = echocardiographic contrast enhancement; MBF = myocardial blood flow.
studies, standardization of the gray scale and gain settings, standardization of the number of bubbles injected and the use of a closed-chest preparation may reduce the variability of ECE values with respect to myocardial perfusion.

Because we used the aortic root as an injection site, the contrast agent may not have mixed uniformly with coronary blood. Therefore, the distribution of the contrast agent may not have been the same as the blood perfusing the myocardium. Also the rheology of the gelatin-encapsulated microbubbles is not precisely known. These factors may influence the distribution of the contrast agent, particularly in regard to its distribution across the left ventricular wall. Generally uniform contrast effect in all regions of the left ventricle in our baseline studies was observed, which suggests that the distribution of the contrast agent is relatively uniform. Visually, the distribution of the contrast agent was approximately the same in the subepicardium as in the subendocardium. Because of the potential parallax error introduced by measuring video intensity with a light meter through the glass of a cathode ray tube, we did not attempt to record the epicardial video density separately from the subendocardial density. The interfacing of a video densitometer with the playback unit might eliminate this difficulty. Despite these limitations, we found this new technique to be an accurate means of identifying normal and ischemic regions in the myocardium.

DeMaria et al. demonstrated that the intracoronary injection of a suspension of 30-μm microbubbles increased the image intensity of the ventricular myocardium. Subsequently, Bommer et al., using a saccharide microbubble preparation, showed that the contrast effect was diminished, and the disappearance time of contrast effect was accelerated in areas of the myocardium rendered ischemic by occlusion of a coronary artery. An independent means of determining myocardial perfusion was not used, and the contrast agent was delivered by direct intracoronary injection. We used radiolabeled microspheres to validate our conclusions about the relationship between ECE and myocardial perfusion. We used an aortic root injection rather than a left atrial or left ventricular injection to avoid opacifying the left ventricular cavity and obscuring the endocardial border. Also, an aortic root injection exposes the entire coronary arterial tree to the contrast agent with a single injection, and both ischemic and nonischemic areas could be assessed simultaneously without having to correlate information from several injections.

Analysis of distribution of left ventricular perfusion with gelatin-encapsulated microbubbles or similar echocardiographic contrast agents may have potential if these agents are proved to be safe and effective. Obviously, extensive toxicologic and pharmacologic testing is required before use in humans. Specifically, the question of allergic reactions to the gelatin solution or adverse effects on organ systems other than the heart were not evaluated in our study. At this time, the gelatin-encapsulated microbubbles should be considered as a prototype of a new class of echocardiographic contrast agents. The results of our investigation validate the technique of contrast echocardiography for evaluating regional myocardial perfusion.

Compared with current techniques such as thallium scintigraphy, contrast echocardiography appears to have several advantages for assessing the distribution
of regional myocardial blood flow. Echocardiography has a relatively higher resolution than nuclear medicine techniques and serial two-dimensional echocardiographic studies of the ventricular blood flow could be performed as often as every 3–5 minutes over several hours. Thallium scintigraphy or similar nuclear medicine procedures can be performed only once during this period. Finally, all equipment used for the recording and analysis of the echocardiographic studies is commercially available and has other uses.

Contrast echocardiography could be performed in conjunction with cardiac catheterization to assess the location and functional significance of obstructive arterial lesions discovered by coronary arteriography. Selective injections of the contrast agent into coronary artery bypass grafts could help assess their patency and region of distribution. Clinical determinations of the extent of myocardial infarction could be accomplished using this technique. In addition to its potential clinical use, this technique may be useful in experimental animal models of myocardial infarction in which there is no nondestructive technique available for the serial determination of the size of the ischemic region. Therefore, it may be useful in studies designed to alter the size of an ischemic region.

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