Nonuniformity of the Transmural Distribution of Coronary Blood Flow During the Cardiac Cycle

In Vivo Documentation by Contrast Echocardiography

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This study was performed to examine the transmural (endocardial vs. epicardial) heterogeneity of myocardial blood flow during the cardiac cycle (systole vs. diastole). Twenty-four contrast echocardiographic injections were performed in seven open-chest anesthetized dogs either into left anterior descending or circumflex coronary artery or into the aortic root. Two-dimensional echocardiography in short-axis view was performed and was digitized off-line into a 256×256 pixel matrix with 256 gray levels/pixel. All end-diastolic and end-systolic frames before and to peak contrast were analyzed. A region of interest corresponding to the most intensely opacified myocardial segment was traced, the mean videodensity measured, and the frame of initial contrast appearance detected. The region of interest was divided into three equal parallel layers corresponding to the endocardial, midcardial, and epicardial myocardium. When the echocardiographic contrast effect initially appeared in diastole, the increment in videodensity was greater for the endocardium (131±48%) than for the epicardium (71±37% of the increment in videodensity of the entire wall) (p<0.05). This inhomogeneity subsequently disappeared in the following end-systolic frame. When the initial echocardiographic contrast effect appeared in systole, intensity was higher in epicardium (136±83%) than in endocardium (60±60%) (p<0.05). However, in the following diastole, intensity was not significantly different for the two layers. Thus, myocardial contrast echocardiography demonstrates that coronary blood flow is primarily subendocardial in distribution during diastole and subepicardial during systole. (Circulation 1989;79:179–187)

Blood flow at the inlet of the coronary system varies during the specific phases of the cardiac cycle because of the generation of intramyocardial pressure by cardiac contraction. It is well recognized that arterial coronary blood flow is higher during diastole, when myocardial tension is low, than during systole. It has been proposed that the transmural distribution of coronary blood flow also varies during the cardiac cycle. Thus, studies in which myocardial flow distribution was assessed using radioisotope-labeled microspheres or 82Rb myocardial uptake have suggested that perfusion is primarily subendocardial in diastole and subepicardial during systole. However, these studies used complex and nonphysiologic experimental models in which blood flow was confined to systole or to diastole for a period of time long enough to generate a flow map by radionuclide methods.

Several recent studies have demonstrated the ability to evaluate the spatial distribution of myocardial perfusion by contrast echocardiography. Specifically, the accuracy of contrast echocardiography in assessing the extent of at risk and necrotic myocardial areas after coronary occlusion is high, and the intraobserver and interobserver variability of the measurements are low, and the findings are reproducible from injection to injection. Addi-
tionally, myocardial contrast echocardiography has been found to permit in vivo imaging of the transmural extent of myocardial infarction.\textsuperscript{14}

By virtue of the high temporal resolution of ultrasound instruments (up to 30 frames/sec), contrast echocardiography should enable the assessment of the myocardial distribution of contrast within a “short” time interval, such as a single cardiac cycle, thereby eliminating the need for complex experimental models. In the present study, we attempted a physiologic and direct approach to evaluating the temporal and spatial distribution of coronary blood flow during the cardiac cycle using echocardiographic detection of the early contrast distribution after upstream bolus injection.

\textbf{Materials and Methods}

\textit{Experimental Animal Preparation}

The study was performed in seven mongrel dogs weighing from 20 to 35 kg. The animals were anesthetized with either \textalpha;-chloralose (80 mg/kg i.v.) and morphine (3 mg/kg i.m.) or pentobarbital (25–30 mg/kg i.v.), intubated, and ventilated with room air by a Harvard respirator. A left thoracotomy was performed at the fifth intercostal space, the pericardium excised, and the heart exposed in a pericardial sling. The left anterior descending (two dogs) or circumflex (one dog) coronary arteries were dissected free from surrounding tissue, and an electromagnetic flowmeter and a hydraulic occluder were implanted on the vessels. Under fluoroscopic control, a 5F catheter, introduced via left carotid artery cutdown, was advanced to subselectively cannulate the isolated coronary artery and was used for contrast injection. A subselective cannulation was performed because of the difficulty in keeping the catheter in the left main coronary artery. To eliminate the possible influence of the intracoronary catheter on coronary perfusion pressure and blood flow, the echocardiographic contrast agent was injected into the aortic root by an 8F pigtail catheter in four additional dogs. To measure aortic pressure, a Millar catheter (5F) (Houston, Texas) was advanced into the abdominal aorta through the femoral artery. Before and during intracoronary injection of contrast, coronary blood flow, aortic pressure and an electrocardiographic lead were recorded on paper at a speed of 25 mm/sec. The presence of intact coronary reserve was confirmed by a hyperemic flow, exceeding 2.5 times resting coronary flow, after 20 seconds of total coronary occlusion, documenting that coronary flow was physiological during these studies.

\textit{Echocardiographic Examination}

The dogs were studied in right lateral decubitus position on a table with a cutout corresponding to the heart region to facilitate echocardiographic examination. Two-dimensional echocardiograms were obtained using two electronic sector scanners (PASS I, General Electric or Hewlett-Packard 77020) operating at 3.3 and 3.5 MHz, respectively. The transducer was positioned on the right side of the chest, at the point of maximal cardiac pulsation,\textsuperscript{13} and held in a fixed position of the optimal view by a mechanical sidearm. After having obtained a short-axis view of the left ventricle at the level of papillary muscles, the transducer was fixed by means of a mechanical arm. To minimize motion of the heart, the respirator was turned off immediately before injecting the echo contrast agent and restarted after few seconds. Gain setting controls were adjusted on the basis of a subjective analysis, maintaining the amplification low enough to avoid the saturation during contrast effect. Once an optimal gain setting was chosen for each dog, it was unchanged during each study. Echocardiographic images were recorded on 1/2-in. VHS or 3/4-in. U-Matic videotaperecorders for subsequent playback and analysis.

\textit{Echocardiographic Contrast Agent}

The polysaccharide contrast agent SHU-454 was used in all experiments.\textsuperscript{16} The agent consists of a powder formed primarily of galactose crystals dissolved in diluent to form a milky solution. This contains microbubbles of a median diameter of 3.2 \(\mu\)m, 97\% of which are less than 7.2 \(\mu\)m, and microparticles of 3-\(\mu\)m median diameter, 99\% of which are less than 12 \(\mu\)m.\textsuperscript{17} Four grams of powder were mixed with 8-ml diluent to form a 400 mg/ml solution. In three dogs, this solution was injected into the cannulated coronary artery by an electrocardiogram-triggered injector apparatus (Angiomat 3000, Cordis, Miami, Florida). The injections began with the R wave of the electrocardiogram and lasted 1 second at a flow rate of 2 ml/sec. In four dogs, 10 ml contrast were injected into the aorta in 1 second by a injector apparatus (Contrac, Conraves AG Zurich) without any electrocardiographic synchronization.

\textit{Digitization}

Echocardiographic images were digitized offline by means of an array processor–based system for medical image processing (Mipron, Kontron, FRG). A 256 \(\times\) 256 pixel matrix with 256 gray levels was used. Images were digitized at 25 or 30 frames/sec and stored in random access memory. A total of 256 frames in 8.5 seconds of imaging could be stored in the computer at one time. Digitization included at least four beats preceding visual myocardial contrast appearance up to peak contrast intensity for a total of at least 12 consecutive cardiac cycles. End-diastolic and end-systolic images were identified on the basis of the largest and smallest left ventricular cavity size and were ordered in two separate temporal sequences. End-diastolic and end-systolic images were also digitally subtracted from the same frames of subsequent cardiac cycles to confirm the data regarding initial appearance and transmural distribution of contrast.
Identification of Contrast Appearance

The two sequences of raw echocardiographic images, corresponding to end systole and end diastole, were reviewed in cine loops. The ventricular wall that showed the best myocardial opacification was identified. To test the intraobserver and interobserver variability in identifying this wall, the sequences of images were reviewed in cine loops by two different observers and by one of them on two occasions. The observers decided whether the best opacified myocardial wall corresponded to the perfusion territory of either left anterior descending or circumflex coronary artery or both.

A region of interest corresponding to this best opacified wall was traced in every end-diastolic and end-systolic image of each sequence. This region encompassed the actual area of myocardium opacified and always included at least 60° of the ventricular circumference. The same circumferential sector was maintained in both end-diastolic and end-systolic images. In cases in which the regions of interest corresponding to the anteroseptal and lateral wall, the outer epicardial and the inner endocardial edges were included in the videodensity measurements. In cases in which regions of interest extended throughout the entire ventricular circumference, the posterior interface of the left ventricle with the lungs (or the air in the open chest preparation) was avoided, and the signals from this interface were not included in the measurements. Myocardial regions corresponding to the area of lateral drop-out were not included in the measurements. When papillary muscles happened to be in the best opacified wall, they were included into the analysis. The mean videodensity inside the regions of interest was measured. To test the intraobserver and interobserver variability in videodensity measurements, regions of interest corresponding to the best opacified wall in 96 digitized echocardiographic images were traced once each by two observers and twice by one of them. Specifically, two end-diastolic and two end-systolic images were sampled in each injection, corresponding to baseline and to myocardial contrast effect. Intensity values were plotted against time to obtain time-intensity curves (Figure 2). For each

Figure 1. Short-axis echocardiographic left ventricular images obtained after circumflex opacification from a representative injection with initial contrast appearance in diastole. Left panel: Corresponds to the unaltered frame before contrast. Middle panel: Corresponds to the frame of initial contrast appearance. Right panel: Corresponds to the result of digitally subtracting these images. The initial distribution of the contrast effect can be seen to be primarily subendocardial in both raw and digitally subtracted images.
curve, a mean background value and its 99% confidence limits were calculated for both diastole and systole on the basis of the initial four beats. The upper confidence limit was used as threshold, and the first diastolic or systolic frame above threshold was identified and considered the one of contrast appearance.

Transmural Distribution of Contrast Effect

The frame of initial contrast appearance (diastole or systole) and the immediately preceding one of the same phase of the cardiac cycle (diastole or systole) were sampled. In these frames, the region of interest was manually divided into three parallel layers of equal thickness, corresponding to the subendocardial, midepicardial, and subepicardial myocardium. The thickness of layers ranged from 3 to 7 mm (mean, 4 ± 1 mm). The mean intensity value of each layer was measured. The increment in videodensity for endocardial, midcardial, and epicardial myocardium was calculated by subtracting, from the intensity values of the frame of contrast appearance, those of the previous frame of the same phase of the cardiac cycle. The increments were then normalized to the increment in videodensity of the entire wall and expressed as percent change. The maneuver enabled correction for the variable gray levels recorded by the individual instruments used in this study. The frame after that of initial myocardial opacification (systole in case of diastolic contrast appearance and diastole in case of systolic appearance) and the preceding one of the same phase of the cardiac cycle (systole in case of diastolic contrast appearance and diastole in case of systolic appearance) were then analyzed. The region of interest was divided into three layers, the mean intensity was measured, and the increment in videodensity of each layer was calculated. Figure 3 shows the temporal sequence of changes in videodensity for the three myocardial layers in a case of systolic contrast appearance. For display purposes, videodensity measurements were extended to several frames of the sequence.

Statistical Analysis

The intraobserver and interobserver variability in the identification of the best opacified wall was tested by χ² test. The variability in videodensity measurements was evaluated by the SEE obtained by least-squares linear regression analysis. The difference between increments in videodensity in the endocardial, midcardial, and epicardial myocardium in end-diastolic and end-systolic images was tested by ANOVA multiple comparison procedure. The same procedure was used to test the difference in changes in videodensity between the three intramyocardial layers in the frame after myocardial opacification.

Results

In the seven dogs, injections of echocardiographic contrast agent were performed either intracoronarily (three dogs) or into the aortic root (four dogs). All intracoronary contrast injections produced obvious myocardial echocardiographic enhancement by visual examination. However, ventricular dysynergy, electrocardiographic ST-T changes, and reduction in coronary reactive hyperemia provoked by short periods of coronary occlusion were occasionally observed after several intracoronary injections. Therefore, only the first two or three injections in each dog made directly into the coronaries were analyzed for a total of seven injections in three dogs. Good myocardial opacification was also obtained in 17 aortic injections in four dogs. When a suboptimal contrast effect was produced, it was attributed to the position of the catheter, which was readjusted for the subsequent injections. These suboptimal injections were not considered in this study. Therefore, the distribution of the echocardiographic contrast agent SHU-454 within the myocardium was studied for a total of 24 injections: seven intracoronary and 17 into the aortic root.

Contrast Distribution to the Ventricular Walls

The myocardial distribution of echocardiographic contrast effect in the initial cardiac cycle of appear-

![Figure 2](http://circ.ahajournals.org/)

**Figure 2.** Plot of full thickness myocardial time-intensity curve obtained at baseline and during the phase of contrast appearance. ▲, End diastole; ◇, end systole. The diastolic and systolic thresholds are delineated. In this injection, the initial appearance, identified as the first frame above threshold, was in diastole (arrow).
ane interval varied according to the site of contrast injection. When the contrast was injected subselectively into the left anterior descending coronary artery (five situations), the anteroseptal wall was the best opacified wall; when injection was performed into the circumflex coronary artery (two situations), the lateral wall was the best opacified one. Despite the site of injection, the echocardiographic contrast effect was distributed diffusely throughout the myocardium by the second cardiac cycle. This generalized distribution with intracoronary injections was attributed to the injection rate used (2 ml/sec), which probably exceeded the left anterior descending or circumflex coronary blood flow and caused reflux of contrast and enhancement of the myocardial areas perfused by the noncannulated vessel. In the case of injections into the aortic root, the anteroseptal and lateral wall were considered the best opacified walls in nine and three situations, respectively, whereas the entire ventricular circumference was opacified uniformly in five injections. Intraobserver and interobserver differences in identifying the best opacified wall were not statistically significant. An intraobserver and interobserver disagreement occurred in one and three out of 24 injections, respectively; these variabilities occurred in the cases of aortic root injections in which the anteroseptal wall and the whole ventricular circumference were selected, as best opacified wall, in different occasions. Using the algorithm described to detect the first echocardiographic frame of contrast appearance, initial opacification was identified during diastole in 12 injections and during systole in 12.

**Transmural Distribution of Contrast**

At initial appearance, the transmural distribution of myocardial contrast effect was different for the individual phases of the cardiac cycle. As shown in Figure 4 (upper panel), when the contrast initially appeared in diastole, the increment in videodensity was significantly higher in the subendocardium (131 ± 48%) than in the subepicardium (71 ± 37%) of the increment in videodensity of the entire wall) ($p < 0.05$ by ANOVA). The increment in videodensity of the midmyocardium was intermediate in magnitude but was not significantly different from either of the other two layers (119 ± 56%).

In the injections in which the contrast initially appeared in systole (Figure 4, lower panel), the subepicardium exhibited a higher increment in videodensity (136 ± 83%) than the subendocardium (60 ± 60% of the increment in videodensity of the entire wall) ($p < 0.05$ by ANOVA). For systolic frames, too, the increment in videodensity of the midmyocardium was not significantly different from either of the other two layers (80 ± 61%).

This dishomogeneity in the transmural distribution of contrast effect disappeared in the following

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**FIGURE 3.** Plot of time-intensity curves of myocardial contrast appearance for the three myocardial layers. The echocardiographic contrast agent was injected into the left anterior descending coronary artery, and the curve was derived from the interventricular septum. The initial contrast effect appeared in systole (arrow) and was recorded in the subepicardium (Epi). In the following diastole (arrow), the videodensity of the subendocardium (Endo) increased more than that of other myocardial layers. Mid, midmyocardium.

**FIGURE 4.** Bar charts of transmural distribution of the increment in video-Intensity in the frames of first echo contrast appearance. Upper panel: Corresponds to the cases of diastolic contrast appearance. Lower panel: Corresponds to the cases of systolic appearance. Each column represents mean ± 1 SD of the normalized increments in the subendocardium (ENDO), midepicardium (MID), and subepicardium (EPI).
phase of the cardiac cycle. As shown in Figure 5 (upper panel), in the diastole immediately after a systolic frame of initial contrast appearance, the distribution of increments in videodensities was not statistically different between endocardial and epicardial layers, indicating that coronary blood flow in this phase was primarily subendocardial. Similarly, in the systole after a diastolic contrast appearance (lower panel), the increment in subendocardial videodensity did not differ from the subepicardial, supporting the concept that myocardial perfusion during systole is predominantly subepicardial.

In the background frames before contrast appearance, no significant difference was detected between beat to beat changes in videodensity of the endocardial, midcardial, and epicardial layers for both diastole and systole, as shown in Table 1. The intraobserver and interobserver variability in videodensity measurements was low: 1.9% and 5%, respectively (SEE).

Discussion
The results of the present study demonstrate that the myocardial distribution of the echocardiographic contrast agent SHU-454 is heterogeneous both in time and space. In fact, the contrast distribution to the individual myocardial layers varied for the different phases of the cardiac cycle, being primarily subendocardial during diastole and primarily subepicardial during systole. Because previous studies have demonstrated that the distribution of the myocardial contrast effect reflects the distribution of myocardial perfusion, this study demonstrates the physiologic heterogeneity of coronary blood flow in the space and time domain during the cardiac cycle.

**TABLE 1. Background Variability: Changes in Videodensity Before Contrast Appearance**

<table>
<thead>
<tr>
<th></th>
<th>Wall</th>
<th>Endocardium</th>
<th>Midcardium</th>
<th>Epicardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diastole</td>
<td>1.7±5.9</td>
<td>0.4±7</td>
<td>3.2±5.8</td>
<td>1.5±4.9</td>
</tr>
<tr>
<td>Systole</td>
<td>1.8±4</td>
<td>2.1±5.8</td>
<td>1.5±4.2</td>
<td>2±2</td>
</tr>
</tbody>
</table>

Lists the difference in videodensity in grey levels between two consecutive end-diastolic and two consecutive end-systolic frames before contrast appearance. The differences in videodensity (mean±1 SD) in gray level per pixel are reported both for the entire myocardial wall and for the three myocardial layers. Differences in the transmural distribution of changes in videodensities are not statistically significant before contrast injection.

**Heterogeneity of Myocardial Perfusion by Labeled Microspheres**

Blood flow is inversely related to coronary resistance, an entity that in turn is composed of viscous resistance, autoregulatory resistance, and compressive resistance. The latter determinant is related to the compression of intramural vessels due to intramyocardial pressure and is characterized by both spatial and temporal heterogeneity. In fact, compressive resistance is three to four times higher during systole than in diastole, and it increases progressively from outer to the inner myocardial layers. As a result of compressive resistance, systolic perfusion would be anticipated to be predominantly subepicardial, and blood flow could even cease during systole in the inner portion of subendocardial layers.

Experimental studies, with measurements derived from radionuclide-labeled microsphere techniques performed over a number of cardiac cycles, have indicated that the blood flow per gram of subendocardial myocardium is equal to or slightly greater than blood flow per gram of subepicardial myocardium. The apparent contradiction between the equality of subendocardial and subepicardial blood flow despite greater compressive resistance on the inner myocardial layer may be related to reduced autoregulatory resistances or greater capillary density in the subendocardium.

In the animal models previously developed to study this issue by labeled microspheres, coronary perfusion was limited to the systolic phase by either creating acute aortic insufficiency, perfusing the coronary tree through the left ventricle, or limiting coronary perfusion to systole using electrocardiogram-triggered coronary occluders. These experimental models were necessary because the distribution of coronary perfusion can be analyzed by labeled microspheres for only the relatively long periods necessary for all microspheres to be trapped into coronary capillaries. The ability to extrapolate data from such nonphysiologic models is compromised. Moreover, limiting myocardial perfusion to either systole or diastole at the level of coronary arteries does not necessarily result in an exclusively systolic or diastolic microsphere distribution at the capillary level because of the capacitance of the large epicardial coronary vessels. In the present study, the use of...
contrast echocardiography obviated the need for complex experimental preparation.

**Heterogeneity of Myocardial Perfusion by Contrast Echocardiography**

Contrast echocardiography has several advantages in the study of the transmural distribution of myocardial perfusion. In vivo imaging is possible, whereas with the labeled microsphere technique, the analysis must be carried out on postmortem heart samples. In addition, the temporal resolution of contrast echocardiographic imaging is high: in fact, it matches the echocardiographic image rate of 30 frames/sec at the depth setting used in this study. This high temporal resolution makes it possible to study differences in the transmural distribution of coronary perfusion occurring within each cardiac cycle. Finally, as a tomographic imaging technique, echocardiography is particularly well suited to study different characteristics of the various layers of myocardium and is superior to the spatial resolution of in vivo nuclear medicine techniques. With these considerations in mind, no independent standard to measure coronary perfusion was used in this study.

The approach used in this study to examine transmural myocardial blood flow is based on the computerized analysis of the frames of initial contrast appearance. We have chosen to study contrast appearance, instead of contrast washout, to avoid the changes in coronary blood flow (reduction in flow and hyperemic effect) induced by echocardiographic contrast agents crossing the coronary microcirculation. As schematically shown in Figure 6, the myocardial distribution of an echocardiographic contrast agent assessed by subtracting the intensity values of two subsequent end-diastolic or end-systolic images should reflect the distribution of coronary blood flow during the entire cardiac cycle. The same subtraction, at the time of contrast appearance, reflects only the diastolic or systolic distribution of contrast and enables visualization of myocardial perfusion within the cardiac cycle, as shown in this study.

**Limitations**

Recent studies have shown that the distribution of myocardial opacification by contrast echocardiography may be influenced by the site of injection of the contrast agent. Thus, the area at risk was slightly but significantly larger when the contrast agent was injected into the occluded vessel (positive risk area) than when it was injected proximally to the occlusion (negative risk area). Such variability was not a factor in the present study, however, because the same site of injection was used to evaluate both subendocardial and subepicardial perfusion, which appeared as positive areas.

A series of factors intrinsic to ultrasound imaging technique and unrelated to the anatomic distribution of contrast agent may influence gray level variability in contrast-enhanced echocardiographic images. Prominent among these is the attenuation of the transmitted ultrasound energy as it traverses tissue structures. Attenuation did not contribute to our findings, however, because the transmural distribution of contrast followed the same pattern regardless of the segment perfused (septal, anterior, or lateral) and its spatial relation with the ultrasound beam. Ultrasonic artifacts and reverberations could have confounded the findings of this study, particularly in regard to the lateral wall of the left ventricle. However, such artifacts would be expected to be both systolic and diastolic.

Previous studies have shown that the relation between quantity of contrast agent into the left ventricle and peak intensity of echocardiographic contrast effect is better expressed by a logarithmic function than by a linear one. In this study, no attempt was done to correlate the echocardiographic intensity to quantitative measurements of coronary blood flow. However, if the same data were applied to this study, the recorded differences in videodensity between intramyocardial layers should be even more enhanced.

It is possible that the subepicardial contrast distribution, when appearing in systole at the first beat, might reflect opacification of large epicardial capacitance vessels. Whenever contrast effect was observed in the shape of a streak of opacification—suggesting the opacification of a major coronary vessel—the image was not considered for densitometric analysis. Furthermore, subepicardial perfusion was documented in systolic images after diastolic frames with primarily subendocardial opacification.

**FIGURE 6. Schematic diagram of myocardial contrast appearance, occurring in diastole (ED) (upper panel) and in systole (ES) (lower panel).**
An open-chest preparation, per se, might have partially influenced the transmural distribution of coronary blood flow during the cardiac cycle because of the lack of normal intrapericardial or intrapleural pressure. However, open-chest preparation has been used extensively in previous studies to evaluate myocardial blood flow. Also, the reported results are relative to the studied section of the left ventricle, at the level of papillary muscles, and do not necessarily apply to different sections in the apex-to-base direction.

We considered the possibility that the intracoronary position of the catheter or the injection pressure may have influenced the results we observed. However, analysis of images derived by aortic injection confirms that the heterogeneous distribution—both spatial and temporal—of the contrast effect is purely physiologic and independent of the methodology. We also considered that motion of the heart or ultrasound transducer during the cardiac cycle might have impacted on our measurements. However, suspending respiration and using a fixed transducer holding apparatus ensured a stable image. Finally, the subtraction of intensity values, rather than of the unprocessed images, should allow independence from problems related to image spatial alignment.

Several additional points relating to the methodology used in the present study warrant discussion. The contrast agent used, SHU-454, was chosen because it is superior to the usually used clinical agents in the production of contrast effect inside the cardiac chambers and has been shown to be more reproducible in contrast intensity created. However, the agent consists of a milky dispersion of saccharide microparticles (median diameter, 3 μm; 99% < 12 μm) and of microbubbles (median diameter, 3.2 ± 0.6 μm; 97% < 7.2 ± 1.6 μm). Accordingly, the agent itself may have been capable of influencing coronary flow by trapping of gas bubbles or microparticles. However, the latter effect would be expected to affect disappearance phenomena but not the appearance characteristics catalogued in this study. A similar consideration applies to the stability of the agent. In previous studies, the spontaneous disappearance of the contrast was studied in a closed, flowing, in vitro system, and 90% of the peak contrast activity was present for 6 seconds after injection. The time interval in which myocardial blood flow distribution was evaluated in this study (one cardiac cycle) is, therefore, well within the time of contrast dissolution. Additionally, in each injection, a new vial of agent was used, and the solution was injected immediately after preparation. Finally, the difference in contrast effect between endocardial and epicardial layers was relatively small, similar to findings by radionuclide techniques, and requires computer analysis of videointensity for accurate detection.

**Clinical Implications**

This study shows that the transmural distribution of myocardial perfusion varies with the phases of cardiac cycle and that this distribution may be assessed in vivo by contrast echocardiography. It has been demonstrated that intracoronary injection of echocardiographic contrast agents may be used to produce myocardial opacification in humans. The method described here would be amenable to these agents and might enable the examination of the transmural distribution of coronary blood flow in humans.

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