LABORATORY INVESTIGATION
TISSUE CHARACTERIZATION

Sensitive detection of the effects of reperfusion on myocardium by ultrasonic tissue characterization with integrated backscatter*

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ABSTRACT We have recently shown that tissue characterization of myocardium with ultrasound reflects changes associated with contractile function throughout the cardiac cycle. To determine whether ultrasonic tissue characterization can sensitively detect the impact of ischemic injury and reperfusion on contractile properties of the heart, we studied the time course of change of backscatter after 5, 20, and 60 min of coronary occlusion followed by reperfusion in 15 dogs. The time-averaged integrated backscatter (IB) and the amplitude and phase of cyclic variation of IB (phase relative to the left ventricular pressure waveform) were measured. A novel ultrasonic index of acute injury was identified, the phase-weighted amplitude of cyclic variation, and calculated by weighting the amplitude of cyclic variation of IB with respect to the phase. We hypothesized that backscatter variables would change dramatically after occlusion and that their restitution after reperfusion would sensitively reflect the extent and time course of reversibility of ischemic injury. After coronary occlusion, segmental wall thickening decreased from approximately 55% to 5% regardless of the duration of ischemia. Changes in backscatter associated with this decrease included an increase in time-averaged IB of approximately 5 dB, a 5 dB decrease in cyclic variation, an 80 degree phase shift, and a 7 dB decrease in phase-weighted amplitude. Wall thickening after reperfusion immediately after the 5, 20, or 60 min occlusions recovered to 45%, 27%, and 12% of baseline values, respectively. Within 3 hr it recovered to 53%, 44%, and 22%. Time-averaged IB recovered initially by 89%, 61%, and 44% (all p < .05) and continued to recover subsequently although more slowly. Ultimate recovery was virtually complete. In contrast to the rapid recovery of time-averaged IB, phase-weighted amplitude recovered initially to only 72%, 41%, and -7% of baseline (all p < .05) and manifested slower and incomplete recovery when ischemia had been present for 20 or 60 min. After reperfusion, the time course of both cyclic variation and phase were reflected by changes in the phase-weighted amplitude. The backscatter variables assessed appear to sensitively delineate the duration, time course of recovery, and reversibility of ischemic injury in response to reperfusion. The results suggest that early recovery of time-averaged IB corresponds in part to the restoration of tissue ultrastructural integrity. Accordingly, ultrasonic characterization of reperfused myocardium should be useful in defining the myocardium’s response to reflow and to interventions such as thrombolysis designed to enhance salvage of ischemic myocardium.


CHARACTERIZATION of myocardium with ultrasonic integrated backscatter (IB) permits quantitative detection of pathologic alterations in heart muscle in experimental animals.1-5 In principal, the radiofre-
reflects regional, intramural myocardial contractile function.12-14 Furthermore, changes in the cyclic variation of IB reflect myocardial contractile dysfunction caused by reversible ischemic injury in experimental animals.15, 16

We recently developed an analog data acquisition system that displays the cyclic variation of IBM in real time in open-chest dogs and in human subjects.17 Although conventional echocardiographic procedures can detect reduced wall thickening or regional hypokinesis associated with ischemic injury, they are insensitive to transmural differences of contractile function and limited in defining the intramural and lateral borders of ischemic zones.18-20 Furthermore, the prognostic capabilities of two-dimensional echocardiographic techniques that depend on an assessment of regional wall thickening may be compromised for prolonged intervals after reperfusion despite persistent viability of reversibly injured but hypokinetic zones.21-25

The present study was performed to determine whether characterization of myocardial ultrasonic properties permits sensitive and quantitative delineation of the effects of duration of ischemia and subsequent reperfusion on the heart. Accordingly, we occluded coronary arteries in open-chest anesthetized dogs for 5, 20, or 60 min before reperfusion and characterized backscatter at selected intervals after occlusion and after reperfusion. Cardiac cycle-dependent changes in ultrasonic backscatter were found to reflect quantitatively the duration of ischemic injury, the early effects of reperfusion, and the time course of recovery of contractile function. The results obtained were consistent with predictions of a recently developed physiologically based model of the behavior of myocardial IB in terms of tissue elastic properties.14

Methods

Experimental preparation. Fifteen mongrel dogs (30 to 50 kg) were anesthetized with intravenous pentobarbital (20 to 30 mg/kg) and ventilated with room air supplemented with oxygen to maintain arterial blood gases in the physiologic range. A left thoracotomy was performed and a pericardial cradle employed to expose the left ventricular free wall. A short fluid-filled catheter was inserted into the left ventricular cavity through the apex to monitor left ventricular pressure and dP/dt. A 2 × 2 cm region of the anterior free wall was demarcated approximately two-thirds of the distance down from the circumflex artery toward the apex and five sites specified for ultrasonic data collection. Two to four distal diagonal branches of the left anterior coronary artery were dissected carefully from the myocardium and snared. Brief simultaneous occlusions (<30 sec) were used to ensure that the five sites specified for ultrasonic data collection were within the center of the ischemic zone, readily recognizable by the distribution of epicardial cyanosis. If the area rendered ischemic did not circumscribe the specified sites, a marginal branch of the circumflex artery was dissected distally and snared as well. A control, nonischemic region was demarcated approximately one-third of the distance down from the circumflex artery and at least 2 cm above the ischemic zone. Within it, five sites were specified for ultrasonic data collection. Ventricular electric stability was enhanced with the use of intravenous bretylium tosylate (2.5 mg/kg) administered slowly 60 min before acquisition of ultrasonic recordings. Lidocaine (1 mg/kg iv) was required only occasionally.

Ultrasonic data acquisition. IB was measured in real time as described previously.13, 17 In brief, a 5 MHz, 5 cm focused piezoelectric transducer mounted on a water-column standoff with a flexible latex tip was used to transmit and receive broad-band ultrasonic signals at a pulse rate of 1 kHz while oriented normal to the epicardial surface. A portion of the backscattered radiofrequency signal (3 μsec) was gated electronically from an intramural region beginning 3 mm subepicardially beneath the initial epicardial specular reflection and extending 2.25 mm toward the subendocardium. The gated and amplified signal was fed into an acoustoelectric energy detector that produced a peak output voltage directly proportional to the average energy contained in the signal over the frequency band of interest. Output voltages representing IB at each instant throughout the heart cycle were referenced to the voltage produced by backscatter from a stainless-steel reflector. The time-varying IB values were displayed in real time on a standard two-dimensional/M mode image screen to permit visualization of the cardiac cycle-dependent variation of IB. The IB values were fed also to an analog integrator with a 5 sec time constant to provide a cumulative display of time-averaged IB. This periodic IB signal was passed simultaneously to a lock-in analyzer (Ithaco Dyna- trac 3) and resolved to its fundamental frequency, defined by a period of one heart cycle, with in-phase (cosine) and out-of-phase (sine) components relative to the left ventricular pressure waveform. The lock-in output was processed further on-line to produce a real-time display of two variables: (1) the amplitude of the cyclic variation of IB and (2) the phase of the cyclic variation relative to the left ventricular pressure waveform. With the use of a 4 sec time constant for the lock-in analyzer, data from approximately 7 beats could be incorporated into the analysis of amplitude and phase at each site. We have shown previously that the amplitude of cyclic variation is 5 to 7 dB in normal myocardium depending on the specific intramural region studied.13, 17 In normal myocardium, phase with respect to the left ventricular pressure waveform is approximately 120 degrees, representing a decrease in IB with physiologic contraction.13, 14, 17 For each site, the backscatter waveforms, the values for time-averaged IB (from the analog integrator), and the amplitude and phase of IB (from the lock-in analyzer) were recorded on magnetic disks for later analysis. M mode echocardiograms at the same sites were recorded on ½ inch videotape for subsequent wall motion analysis.

Data collection and analysis. Three groups of five animals each were subjected to coronary occlusion for 5, 20, or 60 min with subsequent reperfusion. Baseline ultrasonic backscatter assessments and M mode recordings were obtained at each of the five specified sites within the region to be rendered ischemic. The snared arteries were occluded quickly. Data were recorded again at selected intervals after occlusion and after the onset of reperfusion. Corresponding data were obtained also from adjacent, normal, control zones in dogs subjected to occlusion for 20 min only because no changes were anticipated in these areas.

Ultrasonic data from individual sites in ischemic or control zones from each dog were averaged off-line to yield regional data at each interval. This procedure was employed to facilitate a more conservative analysis of dog-to-dog differences than that applicable to analysis referenced to individual sites. Regional
mean values were determined from all five sites for amplitude and phase of cyclic variation and for time-averaged IB.

In addition to the ultrasonic variables that were measured directly, a composite index of regional contractile dysfunction, the phase-weighted amplitude of cyclic variation, was calculated for each time period. Its physiologic implications are addressed in the Discussion. The index is based on a vector dot product and calculated by multiplying the postocclusion resultant amplitudes of cyclic variation by a phase-dependent weighting factor. The phase-weighting factors are strictly defined by:

\[
\text{weighting factor} = \begin{cases} 
1 & \text{if } 90^\circ \leq \theta < 180^\circ \\
-\cos(2\theta) & \text{if } 0^\circ \leq \theta < 90^\circ \\
-1 & \text{if } -90^\circ \leq \theta < 0^\circ 
\end{cases}
\]

where \( \theta \) represents the phase of cyclic variation. The weighting function is depicted graphically in figure 1. Phases of cyclic variation vectors for recordings after coronary occlusion at single sites that lay between 90 and 180 degrees were considered normal and assigned a weighting factor of 1. Phases between 0 and 90 degrees were assigned a weighting factor that varied smoothly from -1 to 1, and was 0 at 45 degrees, a value considered perpendicular to normal. Phases between 0 and -90 degrees were considered to be directed opposite from those vectors exhibited by normal tissue and were assigned a weighting factor of -1. No phases were observed between 180 and 270 degrees in this study.

Myocardial wall thickness was calculated offline from the videotaped M mode recordings. Thickness was measured at left ventricular end-diastole and end-systole at selected intervals after occlusion. Wall thickening was calculated as the percentage increase in thickness from end-diastole. Positive values signified thickening and negative values signified paradoxical thinning.

Statistical methods. Analysis of variance was used to determine the significance of differences over time in backscatter, hemodynamics, and wall thickness. Three comparisons for each data set were planned a priori to assess responses to both coronary occlusion and reperfusion. The significance of differences was determined with F tests. Comparisons included data acquired under the following conditions, with the notation employed to be used throughout the text: (1) baseline vs ischemia (immediately before reperfusion in each group), (2) ischemia vs reperfused-I (immediately after reperfusion in each group), and (3) baseline vs reperfused-F (final interval of data acquisition in each group, which was 120 min after occlusion for the 5 and 20 min occlusion groups and 240 min after occlusion for the 60 min group).

The responses of time-averaged IB and phase-weighted amplitude to reperfusion were evaluated analytically by curve fitting to an exponential function of the form \( IB = ae^{-tb} + c \), where a, b, and c are fitted coefficients, and t is time after reperfusion. The time constant of recovery is b and the asymptotic extent of recovery is c. Additional recordings of backscatter variables were made at intervals between the reperfused-I and reperfused-F periods expressly to provide sufficient data needed for the curve fits. Standard errors for the fitted coefficients were determined by analysis of covariance, and a multiple correlation coefficient was computed for the fit. All data are reported as means ± SE. The two-tailed significance level for F tests was determined with \( \alpha = .05 \).

Results

Physiologic responses to ischemia and reperfusion. Baseline left ventricular systolic pressure and heart rate averaged 132 ± 14 mm Hg and 118 ± 14 beats/min, respectively, for the three experimental groups and remained stable throughout the remainder of the experiment. Changes in the percentage of wall thickening over time in each group are shown in figure 2. Significant differences over time were demonstrated in each group by analysis of variance (p < .05 for f ratios). Wall thickening in ischemic areas decreased immediately after occlusion from approximately 55%
TABLE 1
Ultrasonic variables in the 20 min control group

<table>
<thead>
<tr>
<th>Ultrasonic variables</th>
<th>Baseline</th>
<th>Ischemia</th>
<th>Reperfusion-I</th>
<th>Reperfusion-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time-averaged IB (dB)</td>
<td>-52.0 ± 1.3</td>
<td>-51.6 ± 1.2</td>
<td>-51.5 ± 0.8</td>
<td>-52.3 ± 1.0</td>
</tr>
<tr>
<td>Cyclic variation (dB)</td>
<td>5.6 ± 0.6</td>
<td>5.0 ± 0.6</td>
<td>4.5 ± 0.6</td>
<td>5.5 ± 0.8</td>
</tr>
<tr>
<td>Phase (degrees)</td>
<td>119 ± 4</td>
<td>119 ± 10</td>
<td>125 ± 9</td>
<td>115 ± 8</td>
</tr>
<tr>
<td>Phase-weighted amplitude (dB)</td>
<td>5.6 ± 0.6</td>
<td>5.0 ± 0.6</td>
<td>4.5 ± 0.6</td>
<td>5.5 ± 0.8</td>
</tr>
</tbody>
</table>

...to 5% (p < .05 for baseline vs ischemia in each group). Recovery of wall thickening after reperfusion depended on the duration of the preceding occlusion. Reperfusion after 5 min of ischemia resulted in substantial, immediate recovery of wall thickening to 45 ± 5% (p < .05 for ischemia vs reperfused-I) and complete recovery within 2 hr (p = NS for baseline vs reperfused-F). Reperfusion after 20 min of ischemia resulted in more modest initial recovery of wall thickening to 27 ± 3% (p < .05 for ischemia vs reperfused-I). Recovery was still incomplete after 2 hr (36 ± 5%; p < .05 for baseline vs reperfused-F). Reperfusion after 60 min of ischemia resulted in only insignificant initial improvement in wall thickening to 8 ± 2% (p = NS for ischemia vs reperfused-I). Recovery was markedly attenuated even after 4 hr (22 ± 7%; p < .05 for baseline vs reperfused-F).

Significant differences in end-diastolic wall thickness over time were seen in the three groups (p < .05 for f ratios). A significant decrease in end-diastolic wall thickness of approximately 1.3 mm was observed in the ischemic zone immediately after occlusion in all three groups of dogs (p < .05 for each group). Diastolic wall thickness increased immediately after reperfusion in each group and approximated baseline values at the end of the experiment.

Ultrasonic variables in response to ischemia and reperfusion. Time-averaged IB, amplitude of cyclic variation, and phase of cyclic variation relative to the left ventricular pressure waveform at each of the intervals assessed (baseline, ischemia, reperfused-I, and reperfused-F) and statistical comparisons of values in the three groups of dogs studied are shown in tables 1 to 4. The time-courses of change in each of these variables after occlusion and after reperfusion are shown in figures 3 to 5.

Control regions. Backscatter variables were measured in control, nonischemic regions throughout the course of the study for each of the five animals subjected to 20 min occlusions (table 1). Analysis of variance indicated no significant changes in time-averaged IB, amplitude and phase of cyclic variation, or phase-weighted amplitude from baseline with ischemia or with reperfusion.

Changes in ischemic zones after 5 min occlusions. Generally, all ultrasonic variables assessed after 5 min of ischemia changed immediately after occlusion and recovered rapidly in concert after reperfusion (figure 3, table 2). Analysis of variance demonstrated significant differences over time in each of the ultrasonic variables...
TABLE 2
Ultrasonic variables in the 5 min occlusion group

<table>
<thead>
<tr>
<th>Ultrasonic variables</th>
<th>Baseline</th>
<th>Ischemia</th>
<th>Reperfusion-I</th>
<th>Reperfusion-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time-averaged IB* (dB)</td>
<td>-48.0±3.0</td>
<td>-43.6±2.5*</td>
<td>-47.5±2.7*</td>
<td>-48.6±3.4*</td>
</tr>
<tr>
<td>Cyclic variation* (dB)</td>
<td>6.0±0.8</td>
<td>2.7±0.4*</td>
<td>4.3±0.6*</td>
<td>6.0±0.9</td>
</tr>
<tr>
<td>Phase (degrees)*</td>
<td>120±6</td>
<td>123±13*</td>
<td>118±6</td>
<td>128±6</td>
</tr>
<tr>
<td>Phase-weighted amplitude (dB)*</td>
<td>6.0±0.8</td>
<td>-0.5±1.3*</td>
<td>4.3±0.6*</td>
<td>6.0±0.9</td>
</tr>
</tbody>
</table>

* p < .05 for f ratios by analysis of variance.
* p < .05 for baseline vs ischemia.
* p < .05 for ischemia vs reperfusion-I.

(p < .05 for f ratios). The time-course of change of the ultrasonic variables paralleled the time course of change of segmental contractile function as shown by comparing figures 2 and 3.

Changes in ischemic zones after 20 min occlusions. Despite the similarity of the rapid onset of changes in ultrasonic variables to that of changes seen after 5 min occlusions, recovery after 20 min occlusions was both delayed and incomplete (figure 4, table 3). Analysis of variance demonstrated significant differences over time in the measured ultrasonic variables (p < .05 for all f ratios). Time-averaged IB and phase appeared to recover more quickly and more completely than amplitude of cyclic variation. In general the pattern of recovery of ultrasonic variables resembled that of recovery of segmental contractile function as seen by comparing figures 2 and 4.

Changes in ischemic zones after 60 min occlusions. The immediate responses of ultrasonic variables after 60 min of occlusion were similar to those after occlusions of 5 or 20 min (figure 5, table 4). Analysis of variance demonstrated significant differences over time in all measured ultrasonic variables (p < .05 for f ratios). However, a significant immediate response to reperfusion was observed only for time-averaged IB, whereas only insignificant changes of amplitude or phase were observed initially. In addition, recovery of ultrasonic variables was delayed markedly and reduced substantially in comparison with results after 5 or 20 min of occlusion. The disparity between recovery of time-averaged IB after reperfusion compared with that of amplitude or phase was more pronounced after 60 min than after 20 min occlusions. Nevertheless, the overall trend of recovery for ultrasonic variables was consistent with the intensity and persistence of ischemic injury reflected by the comparably delayed and incomplete recovery of segmental contractile function seen by comparing figures 2 and 5.

Response of the phase-weighted amplitude. The response of the phase-weighted amplitude of cyclic variation to ischemia and reperfusion depended on the duration of occlusion (figure 6, tables 1 to 3). After occlusion of 5, 20, or 60 min, the phase-weighted amplitude decreased immediately (p < .05 for each group, for comparisons at baseline vs ischemia). However, the time-course of recovery of phase-weighted amplitude after reperfusion differed markedly. Reperfusion after 5 min of ischemia led to an immediate recovery of 4.8 dB (p < .05) and an ultimate recovery to baseline values. Reperfusion after 20 min of ischemia led to a more modest immediate recovery of 3.5 dB (p < .05) and a subsequent recovery at a slower rate.

FIGURE 4. Changes in backscatter variables after coronary occlusion for 20 min, followed by reperfusion (denoted by the arrow). Format as in figure 3.
to within 1.5 dB of baseline. Reperfusion after 60 min of ischemia led to only insignificant immediate changes (p = NS) and considerably attenuated ultimate recovery to within 4.1 dB of baseline values. The ultimate extent of recovery of phase-weighted amplitude corresponded to the recovery of wall thickening for each group: (1) 5 min occlusion, 100% (phase-weighted amplitude) vs 95% (wall thickening); (2) 20 min occlusion, 77% vs 62%; and (3) 60 min occlusion, 38% vs 40%. The time courses of change for phase-weighted amplitude and for wall thickening were comparable as shown in figures 2 and 6.

**Relationship between time-averaged IB vs phase-weighted amplitude.** Figures 7 and 8 illustrate the time course of recovery after reperfusion of time-averaged IB and phase-weighted amplitude of cyclic variation. The fitted exponential relationships for values with reperfusion after occlusion of 5, 20, or 60 min are shown as well. The multiple correlation coefficients for these fits ranged from .56 to .85 (all significant at p < .05). Time constants and asymptotes for these fits, shown in table 5, reflect the short-term rates and extents of recovery, respectively. In general, the fit curves indicate that ultrasonic indexes plateau after reperfusion at rates related to the duration of antecedent ischemia. Time-averaged IB recovers more fully than does phase-weighted amplitude.

With respect to time-averaged IB, duration of recovery time became more prolonged and extent of recovery declined progressively with increasing duration of occlusion before reperfusion (table 5). Thus recovery after 5 min of ischemia was more rapid than that after 20 min of ischemia (p < .05), in turn more rapid than that after 60 min of ischemia (p < .05). The overall extent of recovery of time-averaged IB after ischemia for 5 min slightly exceeded that after ischemia for 20 min (p < .05), in turn exceeding that after ischemia for 60 min (p < .05).

A similar correspondence was observed between the duration of ischemia, the prolongation of recovery time, and the decrease of extent of recovery with respect to the phase-weighted amplitude of cyclic variation. Recovery after ischemia for 5 min was more rapid than that after ischemia for 20 min (p < .05), in turn more rapid than that after ischemia for 60 min (p < .05). The ultimate extent of recovery of phase-weighted amplitude after ischemia for 5 min was significantly greater than that after ischemia for 20 min (p < .05).

**TABLE 3**

Ultrasonic variables in the 20 min occlusion group

<table>
<thead>
<tr>
<th>Ultrasonic variables</th>
<th>Baseline</th>
<th>Ischemia</th>
<th>Reperfusion-I</th>
<th>Reperfusion-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time-averaged IB (dB)</td>
<td>$-52.2 \pm 0.8$</td>
<td>$-47.6 \pm 0.7^b$</td>
<td>$-50.4 \pm 0.7^c$</td>
<td>$-51.9 \pm 0.6$</td>
</tr>
<tr>
<td>Cyclic variation (dB)</td>
<td>$6.6 ± 0.5$</td>
<td>$1.5 ± 0.4^b$</td>
<td>$2.7 ± 0.5$</td>
<td>$5.1 ± 0.6^p$</td>
</tr>
<tr>
<td>Phase (degrees)</td>
<td>$104 ± 3$</td>
<td>$32 ± 13^b$</td>
<td>$91 ± 6^c$</td>
<td>$130 ± 5^p$</td>
</tr>
<tr>
<td>Phase-weighted amplitude (dB)</td>
<td>$6.6 ± 0.5$</td>
<td>$-0.8 ± 0.7^b$</td>
<td>$2.7 ± 0.6^c$</td>
<td>$5.1 ± 0.6$</td>
</tr>
</tbody>
</table>

$^a$ p < .05 for F ratios by analysis of variance.
$^b$ p < .05 for baseline vs ischemia.
$^c$ p < .05 for ischemia vs reperfusion-I.
$^d$ p < .05 for baseline vs reperfusion-F.
< .05), in turn exceeding that after ischemia for 60 min (p < .05).

Relationship between wall thickening and backscatter. Although the relative change of phase-weighted amplitude appears superficially to be proportional to wall thickening, the relationship between the two was complex as shown in figure 9. It conformed to an exponential function with a correlation coefficient of .85 (p < .05).

Discussion

We and others have demonstrated that ultrasonic backscatter reflects structural and functional alterations associated with myocardial ischemic injury and necrosis in both experimental animals and human beings. We have also shown that regional intramural variations in contractile function are manifest in backscatter variables. Furthermore, ischemic myocardium can be characterized in vivo through the intact chest wall of dogs with use of IB by compensating for chest wall attenuation. Results from the present study indicate that serial assessments of IB sensitively differentiate responses of myocardium to reperfusion as a function of modest differences in the duration of the preceding ischemia and hence presumably the severity of ischemic injury sustained. Changes in time-averaged IB and in the amplitude and phase of cyclic variation (figures 3 to 5) correlate with the extent of recovery of segmental contractile function after reperfusion (figure 2). Changes in the phase-weighted amplitude of cyclic variation are strikingly, although nonlinearly, related to contractile function as measured by wall thickening (figure 9).

Factors contributing to changes in ultrasonic variables

Time-averaged IB. Tissue edema appears to be one determinant of time-averaged IB. However, the present results indicate that changes in time-averaged IB may occur very early, i.e., within 5 min after occlusion, and hence may precede the development of edema. The early increase in time-averaged IB may reflect reversible changes within myocardium, such as depletion of regional intravascular blood volume. Gaasch and Bernard have shown that a decrease of tissue blood volume is associated with a reduction of end-diastolic ventricular wall thickness early after coronary occlusion. In our study, diastolic wall thickness early after coronary occlusion was reduced correspondingly.
properties of myocardium that occur with ischemia include increases in both resting muscle length and regional myocardial wall stiffness. Diastolic wall thinning and sarcomere lengthening in ischemic myocardium have been ascribed to “systolic overstretched” of ischemic fibers. Myofibril stretching has been observed also early after the onset of ischemia by histologic analysis. Hess et al. recently demonstrated increased segmental stiffness in ischemic myocardial regions immediately after occlusion that was reversible with early reperfusion. The increased stiffness of ischemic tissue was thought to be related to reversible increases in the stiffness of parallel elastic elements as a consequence of passive fiber stretching.

Such modifications in tissue elastic variables are consistent with the observed changes in time-averaged IB and are in accordance with our previously proposed, physiologically based model for the behavior of ultrasonic backscatter. Backscatter at the cellular level may depend on the local acoustic impedance mismatch between series and parallel elastic elements. The series elastic element is stretched and stiffens with normal, physiologic contraction. This change elicits a reduction in the local acoustic impedance mismatch that may be responsible for the systolic decrease in backscatter observed by us and others. In contrast, passive mechanical left ventricular distention stretches the parallel elastic element and augments the local impedance mismatch, causing increases of backscatter. Coronary occlusion may elicit a similar degree of passive stretching of ischemic segments and thereby produce an immediate lengthening of sarcomeres and stiffening of parallel elastic elements. Time-averaged IB would initially increase under these conditions and subsequently decrease after the onset of reperfusion as diastolic wall thickness, sarcomere length, and wall stiffness were restored toward normal.

Some of the observed increase in time-averaged IB

### FIGURE 7
Effect of reperfusion on time-averaged IB. Time-averaged relative IB is plotted against time after the onset of reperfusion for 5 min (circles), 20 min (squares), and 60 min (triangles) occlusions. Specific times of reperfusion are designated as 5, 20, or 60 min above the appropriate fitted curve. Each data point represents an average value from five dogs. Error bars denote SEM. The fitted curves represent exponential functions of recovery.

The cyclic decrease in backscatter observed in myocardium undergoing physiologic, cyclic contraction and relaxation is replaced by a cyclic increase in backscatter in response to passive, mechanical distention of perfused left ventricular myocardium under conditions of diastolic cardiac arrest. Neither the amplitude of cyclic variation nor the time-average of IB produced by passive left ventricular distention is influenced by the abrupt discontinuation of coronary perfusion. Thus ultrasonic backscatter may be affected in part by alterations in passive structural properties of myocardium as well as by changes in coronary blood flow or volume. Alterations of passive physiologic

### FIGURE 8
Effect of reperfusion on the phase-weighted amplitude of cyclic variation. Phase-weighted amplitude is plotted against time after the onset of reperfusion for 5, 20, and 60 min occlusions. Specific times of reperfusion are designated as 5, 20, or 60 min above the appropriate fitted curve. Baseline phase-weighted amplitudes for each occlusion group are depicted in the upper left corner of the figure by the appropriate symbol and a horizontal line representing the control value. The format and symbols otherwise are similar to those in figure 7.

### TABLE 5
Recovery of time-averaged IB and phase-weighted amplitude after reperfusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Time constants (min)</th>
<th>Asymptotes (dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time-averaged relative IB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.1 ± 0.2</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>20</td>
<td>5.0 ± 0.3</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>60</td>
<td>40.6 ± 6.2</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Phase-weighted amplitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.4 ± 0.3</td>
<td>5.6 ± 0.8</td>
</tr>
<tr>
<td>20</td>
<td>6.0 ± 0.5</td>
<td>4.7 ± 0.1</td>
</tr>
<tr>
<td>60</td>
<td>47.3 ± 6.5</td>
<td>2.8 ± 0.2</td>
</tr>
</tbody>
</table>
after occlusion is attributable to an early reduction of the amplitude of cyclic variation (figures 3 to 5). Because IB typically decreases in systole under normal, physiologic conditions, any diminution in cyclic variation would result in an increase in the average of IB over the heart cycle. However, alterations of time-averaged IB do not appear to depend exclusively on changes in cyclic variation. After reperfusion, time-averaged IB changed more promptly than cyclic variation. After ischemia for 60 min, reperfusion elicited an immediate decrease of 2.4 dB despite a complete lack of recovery of cyclic variation. Recovery could not be accounted for by rapid formation of tissue edema after reperfusion because edema would result in an increase in time-averaged IB in contrast to the decrease that was observed. Thus, at least 2.4 dB of the original 5.4 dB change in time-averaged IB seen after ischemia for 60 min may be independent of changes in cyclic variation. The absolute contribution of the recovery of cyclic variation to time-averaged IB after ischemia for 5 or 20 min is difficult to ascertain because both ultrasonic variables responded so rapidly to reperfusion.

Cyclic variation and phase. The responses of backscatter parameters to reperfusion appeared to be markedly dependent on the duration of antecedent ischemia and hence the severity of ischemic injury sustained. The phase of cyclic variation changed substantially with ischemia and recovered in parallel with the amplitude of cyclic variation. These results, in concert with the relationship between cyclic variation and contractile function previously reported, suggest that both amplitude and phase of cyclic variation are related to dynamic aspects of contractile function.

Although an absolute decrease in cyclic variation with ischemic injury may be attributed to diminished contractile function, the finding of a residual 1 to 2 dB cyclic variation with a phase shift of nearly 90 degrees requires further explanation. Subepicardial segmental function may be relatively well preserved after coronary occlusion despite severe reductions of systolic wall thickening. The persistence of systolic wall thickening after transitory ischemia implies that contractile dysfunction and myocardial necrosis may be restricted to subendocardial layers. Nevertheless, segmental function in myocardial zones affected by...
ischemia is characterized by reduced velocities of wall thickening and fiber shortening and by "late systolic shortening" that may persist despite reperfusion. Because wall thickening was not abolished by coronary occlusion in our study (figure 2), contractile function must have been maintained to some extent. Therefore we hypothesize that myocardial fibers within the interrogated region of interest retain some contractile function but shorten relatively late in relation to global left ventricular systolic contraction. An absolute reduction of segmental shortening would explain the observed decrease in the amplitude of cyclic variation. A delay of shortening might account for a phase shift as well. A normal phase (approximately 120 degrees) signifies that the nadir of the IB waveform precedes the nadir of the left ventricular cavity pressure waveform. In other words, the typical systolic decrease in backscatter occurs well before the diastolic decrease in developed left ventricular pressure. A reduction in phase (e.g., closer to 0 degrees) indicates that the nadir of IB occurs closer in time to the diastolic decrease in left ventricular pressure and hence delayed relative to the normal systolic decrease in IB. Thus delayed fiber shortening may produce delayed decreases in IB relative to physiologic contraction indicated by the reduced absolute phase shifts relative to the nadir of the left ventricular pressure waveform.

Phase-weighted amplitude of cyclic variation. A significant result of this study is the characterization of a phase-weighted amplitude variable for cyclic variation that provides a single, composite index of contractile dysfunction that appears to reflect accurately the severity of ischemic injury. Several considerations led to the development of this index. First, the phase of cyclic variation appeared to reflect physiologic phenomena explicable in terms of myocardial elastic element behavior. Physiologic myocardial contraction is characterized by a systolic decrease in IB. Passive ventricular distention is characterized by an increase in IB. These phenomena are distinguished by a phase shift of approximately 180 degrees in the cyclic variation waveform. Ischemia produces a consistent shift in phase of approximately 70 to 80 degrees that is reversible with reperfusion as shown in figures 3 to 5.

The cyclic variation of IB can be represented as a vector quantity, with a magnitude (amplitude of cyclic variation) and an orientation (phase). Our procedure for phase weighting constitutes a vectorial approach to the determination of changes in dynamic backscatter variables. In contrast to the standard vector dot product, however, the computation of this index does not require knowledge of data from ischemic zones before occlusion. It can be computed based on the assumption that normal phase values lie within the range of 90 to 180 degrees. Data from well-perfused zones could of course be acquired to provide a normal reference for comparison because no significant changes occur in normal zones remote from zones rendered ischemic as seen by comparing tables 2 and 4. The lack of requirement for precoclusion backscatter data should greatly enhance the utility of this index with respect to the ultrasonic diagnosis of myocardial ischemic injury.

The nonlinear relationship between phase-weighted amplitude and wall thickening (figure 9) conforms to a similar, nonlinear relationship between the transmural extent of necrosis and segmental wall thickening in dogs reported by others. Normal wall thickening may depend more on inner wall than on outer wall contractile function. After occlusion, subepicardial flow and function may be relatively well preserved despite the presence of severe regional hypokinesis. Furthermore, ischemia confined to inner wall regions may substantially blunt or abolish transmural wall thickening. Frank systolic wall thinning occurs with transmural necrosis exceeding 25%. However, more extensive transmural necrosis does not elicit greater thinning. A similar threshold appears in the relationship between phase-weighted amplitude and wall thickening for severe hypokinesis (figure 9, 0 to 20% wall thickening range). Below this threshold, large decrements in the phase-weighted amplitude correspond with minor changes in wall thickening. Thus both the transmural extent of ischemic injury and the phase-weighted amplitude manifest similar nonlinear relationships to regional wall thickening. This concordance suggests that backscatter variables may contain additional information not available by a conventional analysis of regional wall thickening. Because the phase-weighted amplitude provides a direct assessment of regional intramural myofiber contractile performance in contrast to the indirect assessment by wall motion analysis, it may be possible to evaluate the effects of ischemia on discrete subendocardial vs subepicardial regions, which is not possible with simple wall motion analysis by conventional ultrasound.

Comparisons between responses of different ultrasonic variables. Our results demonstrate that backscatter variables as a group are sensitive to ischemia and that the recovery of specific variables after reperfusion depends considerably on the duration of ischemia and hence presumably the severity of ischemic injury. The restitution of time-averaged IB is rapid compared with that of phase-weighted amplitude, cyclic variation, or
phase. After 60 min coronary occlusions, the immediate recovery of time-averaged IB after reperfusion (2.4 dB) was not accompanied by any appreciable recovery of the phase-weighted amplitude of cyclic variation and was independent of any improvement in segmental contractile function (figure 2). Diastolic wall thickness was normalized after reperfusion regardless of the duration of ischemia, compatible with prompt restitution of tissue perfusion and coronary blood volume. Accordingly, the immediate recovery of time-averaged IB may reflect the restoration of myocardial blood flow after reperfusion, whereas the delayed recovery of phase-weighted cyclic variation may correspond more closely to the protracted recovery of segmental contractile function.

Contractile function improves slowly over the days to weeks with reperfusion after coronary occlusion of less than 3 hr duration in dogs. Therefore we expected the delayed and continuing recovery of backscatter variables after occlusions of 20 or 60 min. Because 20 min of ischemia is not likely to induce marked myocardial necrosis, we assumed that the incomplete short-term recovery of phase-weighted amplitude after occlusions of 20 min (to 77% of baseline) signified the presence of “stunned” myocardium that may ultimately recover completely. The incomplete short-term recovery of phase-weighted amplitude after 60 min occlusions (to 38% of baseline) may reflect a combination of reversible and irreversible myocardial ischemic injury.

The results of this study show that ultrasonic backscatter variables can distinguish the severity of ischemic injury in experimental coronary occlusion based on the response to reperfusion. They suggest that ultrasonic tissue characterization may provide a noninvasive tool for serial assessment of myocardial contractile dysfunction resulting from ischemia applicable in a critical care environment. The rapid delineation of ischemic myocardium at risk of necrosis and the documentation of recovery of myocardium after reperfusion may facilitate elucidation of the physiologic consequences of interventions such as coronary thrombolysis. The implementation of a real-time two-dimensional integrated backscatter imaging system offers promise for the clinical applications of the approach developed.

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