Effects of Myocardial Ischemia on Quantitative Ultrasonic Backscatter and Identification of Responsible Determinants

JAMES W. MIMBS, DANIEL BAUWENS, RANDOLPH D. COHEN, MATTHEW O'DONNELL, JAMES G. MILLER, AND BURTON E. SOBEL

SUMMARY Quantitative characterization of myocardial properties represents a rapidly emerging area of echocardiographic investigation. Because measurement of the ultrasonic integrated backscatter is theoretically applicable to analysis in vivo with reflected ultrasound, this study was performed to develop and evaluate a suitable method for measurement of quantitative backscatter in vivo. In view of the desirability of characterizing ischemic myocardium noninvasively, the study was performed with animal preparations simulating myocardial ischemia in humans. In one series of open-chest dogs, integrated backscatter among 22 ischemic regions was increased by 200% ($P < 0.01$) compared to values in control rgions within 1 hour after coronary occlusion and by 400% (-45.1 ± 0.7 dB compared to -50.9 ± 0.4 dB) ($P < 0.001$) within 6 hours. In a second series of open-chest dogs, ischemia was quantified with ^{141}Ce microspheres, and mean integrated backscatter was found to increase (280% of control) ($P < 0.01$) in regions with flow less than 20% of control 2 hours following coronary occlusion. Additional studies with perfused hearts revealed two determinants of the increased ultrasonic backscatter observed: (1) an increase in cardiac fluid content reflected by the wet-to-dry weight ratio, and (2) the contributions of formed elements in whole blood. The results indicate that ultrasonic integrated backscatter distinguishes severely ischemic from nonischemic myocardium in vivo in open-chest animals. Because it was possible to obtain these results in the reflection mode, potential extension of the approach to clinical applications is promising. *Circ Res 49: 89-96, 1981*

THE value of ultrasound for quantitative assessment of cellular and structural characteristics of myocardium has been demonstrated recently. Ultrasonic properties potentially applicable to characterization of tissue include attenuation, absorption, scattering, and velocity (Linzer, 1976, 1977, 1978). Although previous investigations have successfully differentiated ischemic form nonischemic myocardium (Mimbs et al., 1979) and elucidated some mechanisms underlying alterations in ultrasonic attenuation of scarred tissue (Mimbs et al., 1980), a method for quantitative measurement of ultrasonic characteristics in vivo has not been available. Indeed, theoretical considerations of measurements restricted to either attenuation measured in transmission or quantification of echo amplitude alone suggest that such approaches would be seriously limited. To achieve clinical noninvasive assessment of cardiac properties by ultrasound, a method for ultrasonic characterization of tissue should be sensitive and quantitaive and should rely upon reflected rather than transmitted ultrasound.

From the Cardiovascular Division and Department of Physics, Washington University, St. Louis, Missouri.

This work was supported in part by National Institutes of Health Grant HL 17646, SCOR in Ischemic Heart Disease.

Address for reprints: Burton E. Sobel, M.D., Director, Cardiovascular Division, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, Missouri 63110.

Received September 11, 1980; accepted for publication January 14, 1981.

This study was designed to use and evaluate reflected ultrasound to characterize quantitative ultrasonic backscatter in vivo. Promising results with simpler biological systems and measurement of attenuation and backscatter coefficients in vitro provided part of the foundation and impetus for the present approach. However, the present approach entails a qualitative difference, i.e., the application of reflected rather than transmitted ultrasound in vivo. Theoretical considerations of ultrasonic scattering processes in myocardium suggest that alterations in cellular structure should be detectable by measurement of the backscatter coefficient (Fields and Dunn, 1973; Shung and Reid, 1977). Because previous studies have documented that biochemical concomitants of myocardial infarction occur beginning within minutes after coronary occlusion, hearts subjected to acute ischemia were studied. In the first series of experiments, two groups of dogs were studied to characterize (1) the time course of alteration in the ultrasonic backscatter during the first six hours following coronary occlusion, and (2) the relationship between the ultrasonic backscatter and regional myocardial blood flow. In the first group, seven hearts were studied in vivo with the use of an open-chest dog preparation. Results of quantitative ultrasonic backscatter measurements obtained from 22 ischemic regions in seven dogs were compared to results obtained from 23 nonischemic regions 1, 2, 4, and 6 hours after coronary occlusion.

In the second group, a total of 77 regions from nine hearts were studied precisely 2 hours after coronary occlusion. In addition to measurement of the ultrasonic backscatter in vivo, myocardial blood flow was measured in each of the 77 regions with ^{141}Ce-labeled microspheres.

In order to elucidate potential mechanisms responsible for alterations in the ultrasonic backscatter observed in canine hearts studied in vivo, a second series of experiments was conducted with a different preparation. Perfused rabbit hearts were used to measure the backscatter coefficient under a number of selected conditions of perfusion. In these experiments, 86 regions from 47 hearts were analyzed to determine whether variability in water content of the heart (estimated on the basis of wet-to-dry weight ratio) or variability in the concentration of formed blood elements were significant factors affecting the ultrasonic backscatter.

Ultrasonic Methods

Quantitative measurements of the ultrasonic backscatter were obtained over a broad bandwidth with the use of an index designated the integrated backscatter. Integrated backscatter is related to the frequency average of the backscatter transfer function over the bandwidth of the insonifying transducer (O'Donnell et al., 1979). If this index is obtained over a sufficiently broad bandwidth, spatially localized measurements of the intrinsic ultrasonic scattering properties of myocardium can be obtained. Consequently, the ultrasonic backscatter was measured with the use of a broadband, focused piezoelectric transducer (1.3 cm diameter, 5 cm focal length, 5 MHz nominal center frequency). For measurements conducted on canine myocardium in vivo, the transducer was mounted in a water-filled, latex-tipped sleeve constructed so that its length matched the focal length of the transducer. This mounting arrangement permitted acoustical coupling to the beating heart effected by application of the flexible latex tip to the epicardial surface of the heart. In addition, the sleeve ensured that the myocardial segment of interest would remain within the focal zone of the transducer. For measurements conducted with rabbit myocardium in vitro, a transducer of similar design was employed, and all measurements were obtained in a 0.9% saline bath by means of techniques similar to those described previously (Mimbs et al., 1977). For these experiments, each myocardial sample was placed in the bath so that the zone under study lay within the focal zone of the transducer.

The system used to obtain the integrated backscatter was the same for both canine and rabbit myocardium. The transducer was excited with an impulse, using a broadband driver circuit. The ultrasonic wave backscattered from the tissue was detected by the same transducer. The output of the transducer was amplified and gated to permit analysis of the myocardial segment of interest. After the first gate the signal was relayed to an oscilloscope which acted both as an rf amplifier and trigger generator. The oscilloscope produced a trigger pulse corresponding to the arrival of the echo from the epicardial surface of the heart. The amplified output of the oscilloscope entered a second gate of 4 μsec in duration initiated at a fixed interval after the trigger pulse. This arrangement was designed for measurements in vivo so that echoes emanating from the same region of intramural myocardium could be analyzed throughout the entire cardiac cycle. The output of the second gate was squared and integrated to obtain a signal proportional to the total pulse energy backscattered from the cylindrical volume of tissue defined approximately by the beam width at 5 MHz (approximately 3-mm beam width at the 3-dB point) and the gate length (approximately 3 mm). Although the backscattered signal reflects each cardiac structure in the beam path, integration is performed only on a 3-mm gated section of intramural myocardium adjacent to the transducer. After rf detection, the signal was amplified logarithmically, peak detected, and stored in the memory of the microprocessor control system.

To obtain measurements of the integrated backscatter, the total pulse energy backscattered from the myocardial region of interest was normalized to the total pulse energy measured when the tissue was replaced by a planar, nearly perfect ultrasonic reflector. Thus, the integrated backscatter provided a measure of the energy efficiency of acoustic backscatter in the sample. Although the numerical magnitude of the integrated backscatter depends to some extent upon the characteristics of the transducer beam pattern and upon the gate length, measurements of this index for regions positioned within the focal zone of the same transducer with the same gate length are independent of the specific properties of the transducer (O'Donnell et al., 1979). Consequently, changes in the value of this index reflect variation in the intrinsic scattering properties of the sample.

Experiments with Canine Hearts in Vivo

In the first series of experiments, the ultrasonic backscatter was measured in hearts from two groups of dogs. Fifteen to 25 kg adult, mongrel dogs were anesthetized with sodium pentobarbital (25/mg per kg, iv) intubated, and ventilated with a respirator supplemented by oxygen. The chest was entered through an incision in the left 5th intercostal space, the pericardium incised, and the beating heart suspended in a pericardial cradle. Prior to coronary ligation, specific regions of the left ventricle were designated as likely to become ischemic, i.e., those within the distribution of supply of the coronary artery chosen for ligation, based on delineations in previous studies (Mimbs et al., 1980). Ultrasonic analyses were performed in these regions and in additional regions remote from the potentially ischemic zone. The cross-sectional area of

each region characterized ultrasonically was approximately 1.0 cm^2. Prior to any manipulation of the coronary artery, ultrasonic backscatter measurements were obtained from two to six regions within the anteroapical zone of potential ischemia and from two to six regions remote from the zone. In preparation for coronary occlusion, the left anterior descending coronary artery was dissected free immediately distal to the first ventricular branch. To ensure reproducibility in determination of regions of ultrasonic measurement, both photographs and drawings of the exposed beating heart were used.

In group A of this series of experiments (seven dogs), a sequence of backscatter measurements was obtained to evaluate the progression of alterations in backscatter within the first 6 hours following coronary occlusion. After completion of backscatter measurements at time zero (prior to coronary artery dissection), the left anterior descending coronary artery distal to the first ventricular branch was ligated completely in two stages over 5 minutes with administration of lidocaine (1 mg kg iv) to diminish ventricular dysrhythmia. The ultrasonic backscatter of each region identified previously was measured subsequently with tissue identified with reference to the photographs and drawings at intervals of 1, 2, 4, and 6 hours after occlusion. After the final measurement obtained 6 hours after coronary occlusion, colloidal carbon black (1 ml/kg) was injected manually into the left atrium over a period of 10 seconds, and the animal was killed. The distribution of colloidal carbon black administered under these conditions permits differentiation of the region of ischemic myocardium from surrounding nonischemic tissue even though some variation in perfusion pressure measured during injection may occur despite precautions to preclude it by injecting in a standardized fashion (Cotran et al., 1967; Kloner et al., 1974a). This technique was used to categorize regions as within a central ischemic zone or in a remote nonischemic zone. Only regions demarcated as clearly witin the ischemic region or distantly remote from the ischemic zone were used for analysis.

In group B of this series of experiments (nine dogs), the relationship between alterations in the ultrasonic backscatter and regional myocardial blood flow was characterized. Regional myocardial blood flow was measured with ^{141}Ce-labeled microspheres. Following measurements of the backscatter made prior to any intervention, a polyethylene catheter was placed in the left atrial appendage in preparation for subsequent injection of the microspheres. A second catheter was placed in the aorta via the left carotid artery and used to obtain reference samples for the calculation of myocardial blood flow. Following placement of these catheters, the left anterior descending coronary artery was ligated in two stages as described above. Two hours after coronary occlusion, the ultrasonic backscatter was measured in each region previously characterized prior to any intervention (i.e., at time zero). Thus, in these hearts, backscatter was measured at time zero and at 2 hours after ligation.

After these measurements had been obtained, 9 μm ^{141}Ce microspheres suspended in 1.5 ml of a solution of 10% dextran were injected intra-atrially. The microspheres were sonicated for 15 minutes prior to injection and viewed periodically with a microscope for verification of nonaggregation. A total of approximately 3 million microspheres were injected into the left atrium over 10 seconds and the catheter immediately flushed with 5 ml of saline. During the left atrial injection and for 3 minutes thereafter, arterial blood was withdrawn from the carotid catheter into a heparinized syringe at a constant rate (7.64 ml/min) with the use of a Harvard pump. The blood was then transferred into pre-weighed culture tubes prior to gamma counting. The heart was excised and a transmural biopsy was taken at each of the sites analyzed ultrasonically. Each biopsy was weighed and placed in a culture tube with 10% formalin. Radioactivity in blood and tissue was then assayed in a gamma counter with the center of the window set to the largest of the energy peaks of cerium, and the width of the window set to ±2 SD. (136–172 keV). Myocardial blood flow was calculated in units of ml/min per g (Heymann et al., 1977).

Experiments with Perfused Hearts

To permit analysis of serial interventions to elucidate the potential effect of changes in tissue water and changes of blood concentration upon the ultrasonic backscatter, a perfused heart preparation was employed. Healthy laboratory rabbits (2–3 kg) were killed by quick cervical dislocation. The hearts were excised immediately and suspended within 30 seconds in a modified Langendorff perfusion apparatus. Each heart was perfused with Krebs-Henseleit buffer equilibrated with 95% O_2 and 5% CO_2 through Silastic tubing.

In the first series of experiments (20 hearts) three conditions were employed: (1) Krebs-Henseleit (KH) buffer with 1% bovine serum albumin (BSA) was used as the perfusate for 30 minutes at a flow rate of 20 ml/min, or (2) KH buffer with 3% BSA was perfused for 30 minutes at a flow rate of 20 ml/min, or (3) KH buffer with 3% BSA and 0.3% hyaluronidase was used as the perfusate for 30 minutes at a flow rate of 20 ml/min. These conditions were selected because they permitted modification of cardiac fluid content, not because they closely simulate normoxic perfusion or ischemia in vivo. The flow rate of 20 ml/min with buffer provides adequate oxygenation, judged from persistence of stable ventricular performance and physiological rates and amplitudes of pressure development for at least 1 hour. After perfusion, each heart was removed from the perfusion apparatus, and the left ventricle was dissected and mounted on a Plex-

iglas frame which did not intersect the ultrasonic beam. The ultrasonic backscatter was measured in two to three regions of the left ventricle (each region was approximately 1.0 cm^2 in cross-section). After completion of the backscatter measurements, which required approximately 5 minutes, the heart was blotted dry, and 1.0 cm^2 biopsies were obtained from each region that had been analyzed ultrasonically. Each biopsy was subsequently analyzed for wet-to-dry weight ratio following drying of minced tissue to constant weight at 100°C for 5 days. It should be recognized, of course, that fluid shifts between intracellular and interstitial spaces, without net change in total fluid, would not be detected by this method.

In a second series of experiments performed with perfused hearts, interventions were performed to determine whether alteration in wet-to-dry weight ratio alone may account for changes in the measured ultrasonic backscatter or whether the presence of intravascular blood components may represent an additional contribution to these changes. Each heart was (1) mounted on a modified Langendorff apparatus and perfused for 30 seconds with Krebs-Henseleit buffer, 3% BSA, and 0.3% hyaluronidase (five rabbits) or (2) washed out by perfusion for 30 seconds with KH buffer, 3% BSA, and 0.3% hyaluronidase followed by perfusion with heparinized rabbit whole blood for 30 seconds at a flow rate of 7.5 ml/min (five rabbits). This flow rate provides adequate oxygenation with erythrocyte-containing medium to maintain stable ventricular performance for at least 1 hour with physiological left ventricular pressure and rates of pressure development. On completion of the brief interval of perfusion, each heart was removed from the perfusion apparatus, the utrasonic backscatter measured immediately, and the wet-to-dry weight ratio calculated. For comparison, backscatter measurements and wet-to-dry ratios were obtained from rabbit left ventricle (17 animals) of freshly obtained hearts under identical conditions.

Statistics

Data were analyzed using statistical methods described by Wallenstein et al. (1980). In particular, when comparisons were made among three or more groups a modified t-statistic was generated with appropriate critical values based on the Bonferroni procedure.

Results

In Figure 1, mean integrated backscatter is expressed as a function of the duration of ischemia in 22 ischemic regions and 23 nonischemic regions from the seven dogs studied in group A. The integrated backscater is presented as the percentage of control in Figure 1, with control defined as the average value of the integrated backscatter for all 45 sites prior to any intervention (i.e., at time zero).

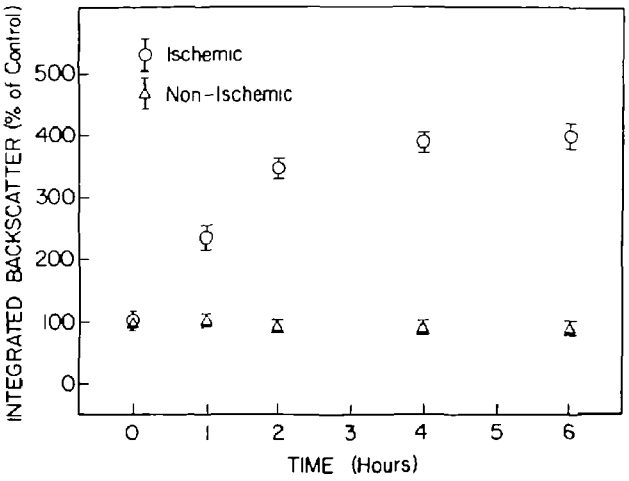

FIGURE 1 *Integrated backscatter of ischemic (○) and nonischemic (△) regions of canine myocardium as a function of time following coronary occlusion.*

The results presented in Figure 1 indicate that, at 1 hour after occlusion, the mean integrated backscatter from regions of ischemia was increased by 200% over that of control ($P < 0.01$). After 6 hours of ischemia, the mean integrated backscatter of ischemic regions was 400% greater than control ($P < 0.001$). In nonischemic areas the integrated backscatter measured under control conditions before coronary artery occlusion did not differ significantly from values obtained at any of the four intervals after ligation (1, 2, 4, and 6 hours).

Figure 2 illustrates alterations in the integrated backscatter of specific regions of myocardium associated with the development of ischemic injury. In this figure, the mean integrated backscatter is

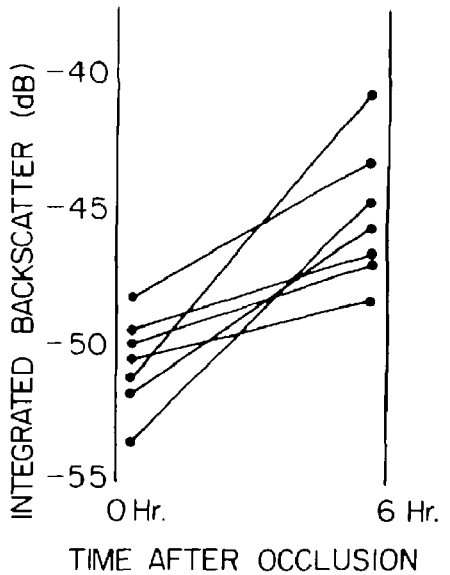

FIGURE 2 *The alteration in ultrasonic backscatter measured at time 0 and after 6 hours of ischemia for each animal studied in group A.*

presented for ischemic regions at two time intervals in each of the seven dogs of group A studied. The results at time zero correspond to measurements obtained prior to any intervention, and the results at 6 hours correspond to measurements obtained from the same sites after 6 hours of ischemia. Although variability is evident, the backscatter from ischemic regions was elevated relative to that from the nonischemic regions of the same heart for each heart studied.

The second series of experiments on dogs (group B) was designed to examine the relationship between alterations in the integrated backscatter and regional myocardial blood flow. Based on the results presented in Figure 1, these measurements were conducted after 2 hours of myocardial ischemia to ensure adequate ultrasonic differentiation of ischemic regions. In Figure 3, the flow distribution in all sites analyzed ultrasonically is illustrated in units of percentage of control, with control defined as the average flow from all nonischemic regions in each heart. Nonischemic regions were identified as those sites which were remote from the central ischemic zone. The results presented in Figure 3 show a clustering of points near flows of approximately 100% of control. There are also clusters near flows of 40% and 10% of control. Consequently, to define the relationship between myocardial blood flow and ultrasonic backscatter, the data from these experiments were classified into three groups corresponding to: (1) regions of low flow (less than 20% of control), (2) regions of moderate flow (greater than 20% but less than 60% of control), and (3) regions of relatively high flow (greater than 60% of control). Table 1 presents the mean and standard deviation of the absolute flow (in units of ml/min per g) in each of the three sets of sites.

The mean integrated backscatter was calculated for regions of the three types and expressed as percentage of control, with control defined as the mean integrated backscatter from a total of 77 sites

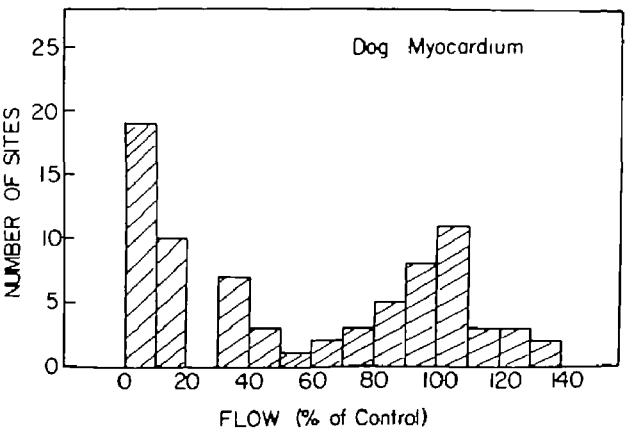

FIGURE 3 *Histogram of distribution of myocardial regions categorized according to myocardial blood flow determined from ^{141}Ce counts.*

TABLE 1 *Mean Myocardial Blood Flow in Normal, Moderately Ischemic, and Severely Ischemic Regions Defined by ^{141} Ce Microsphere Counts*

Severity of ischemia (blood flow as % of control)	Sites	Flow* (ml/min per g)
Flow < 20% of control	29	0.1 ± 0.1
20% < flow < 60%	11	0.5 ± 0.1
Flow > 60% of control	37	1.2 ± 0.3

Mean ± SD.

in nine dogs prior to any intervention. The integrated backscatter in regions of high flow (>60% control) was not significantly different from control (99 ± 9% (SD). Similarly, the integrated backscatter in regions of moderate flow (20 to 60% control) was not significantly different from control (100 ± 25%). In contrast, the integrated backscatter in regions of low flow (<20% control) was significantly increased over control (278 ± 36%) ($P < 0.001$).

The effects of myocardial water content upon the ultrasonic backscatter were evaluated in a perfused heart system with the use of perfusates with selected concentrations of albumin and hyaluronidase to induce fluid retention. Results are presented in Figure 4 with control defined on the basis of results from nonperfused, normal rabbit myocardium freshly obtained from 17 animals. Perfusion with modified Krebs-Henseleit buffer solution (KH) and 1% bovine serum albumin (BSA) for 30 minutes (nine hearts) resulted in a significant alteration in the wet-to-dry weight ratio compared to that in controls ($P < 0.01$), as illustrated in the bottom panel of Figure 4. In contrast, perfusion with a medium consisting of KH buffer, 3.0% BSA, and 0.3% hyaluronidase for 30 minutes resulted in an insignificant increase in the wet-to-dry weight ratio compared to control. The mean integrated backscatter from the hearts perfused with 1% albumin was significantly increased over that obtained from the hyaluronidase-perfused hearts ($P < 0.01$). To ensure that the change in backscatter was not related to the potential ultrasonic contrast effect of the increased albumin concentration in the hyaluronidase-perfused hearts, an additional control group was employed, comprised of hearts perfused with a medium consisting of KH buffer and 3.0% BSA. The results of backscatter measurements and measurements of the wet-to-dry weight ratio for this control group are presented in Table 2 and indicate no significant contrast effect upon backscatter associated with the increased albumin concentration of the hyaluronidase-perfused hearts. Consequently, the increased integrated backscatter from the 1% BSA-perfused hearts, compared to the hyaluronidase-perfused hearts, was a concomitant of the altered myocardial water content.

Although the increased backscatter noted in hearts perfused with 1% BSA compared to that in hearts of the group perfused with hyaluronidase appears to be related to myocardial water content,

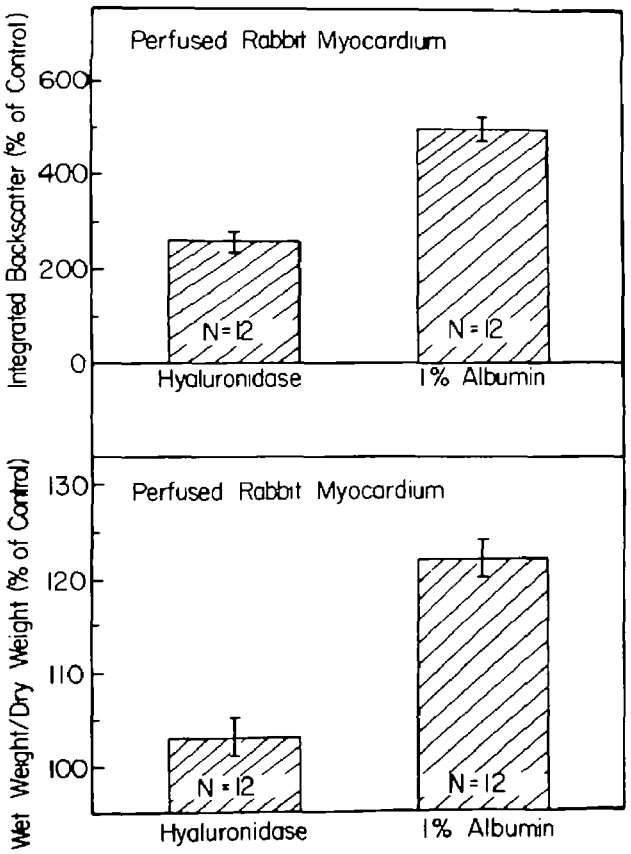

FIGURE 4 *Perfusion of myocardium with 1% albumin demonstrating a marked increase in backscatter compared to a moderate increase after hyaluronidase (top panel). Concomitant changes in myocardial water content demonstrating an increase with 1% albumin, but not with hyaluronidase (bottom panel).*

the integrated backscatter from the hyaluronidase-perfusated hearts was significantly greater than control (i.e., unperfused) even though myocardial water content was not altered significantly. To determine whether this increase in backscatter was related to the ultrasonic contrast effect of the buffer, additional perfusion experiments were conducted with results presented in Figure 5 and Table 2. Perfusion with KH buffer, 3.0% BSA, and 0.3% hyaluronidase for 30 seconds (an interval previously documented to remove the majority of intravascular blood elements) resulted in a significant increase in integrated backscatter compared to results in controls ($P < 0.01$). These results did not differ significantly from those obtained for hearts perfused for 30 minutes with KH buffer, 3.0% BSA, and 0.3% hyaluronidase. In contrast, hearts perfused for 30 seconds with KH buffer, 3.0% BSA, and 0.3% hyaluronidase followed by a 30-second perfusion with heparinized whole blood did not demonstrate increased integrated backscatter compared to that in controls. The wet-to-dry weight ratio for all three groups did not differ significantly from control values as illustrated in the bottom panel of Figure 5. Consequently, the increased backscatter of the hyaluronidase group appears to be directly related to the contrast effect of replacing blood with buffer.

Discussion

Results from the present study demonstrate that through analysis of quantitative, integrated, ultrasonic backscatter, ultrasonic analysis can be applied in reflection to characterize structural changes in myocardium indicative of acute myocardial ischemia. This approach to characterization of myocardial properties is adaptable for measurements in vivo in the open-chest dog.

A comparison of the mean backscatter from areas exhibiting different regional flow showed that the backscatter was significantly altered from control only in regions with flow less than 20% of control. This result suggests that there is no simple linear relationship between integrated backscatter and re-

TABLE 2 *Results of Ultrasonic Integrated Backscatter and Wet-to-Dry Weight Ratio among Perfused Rabbit Hearts*

Conditions	Rabbits	Sites	Integrated backscatter (% of control) (mean ± SE)	Wet weight: dry weight (expressed as % of control) (mean ± SE)
1% Albumin, 30-min perfusion	9	18	500 ± 25*	122 ± 2*
3% Albumin, 30-min perfusion	5	11	435 ± 20*	116 ± 2*
3% Albumin + 0.3% hyaluronidase, 30-min perfusion	6	12	260 ± 25*	103 ± 2
3% Albumin + 0.3% hyaluronidase, 30-sec perfusion	5	10	275 ± 25*	102 ± 2
Rabbit blood, 30-sec perfusion	5	10	100 ± 25	103 ± 2

* $P < 0.01$.

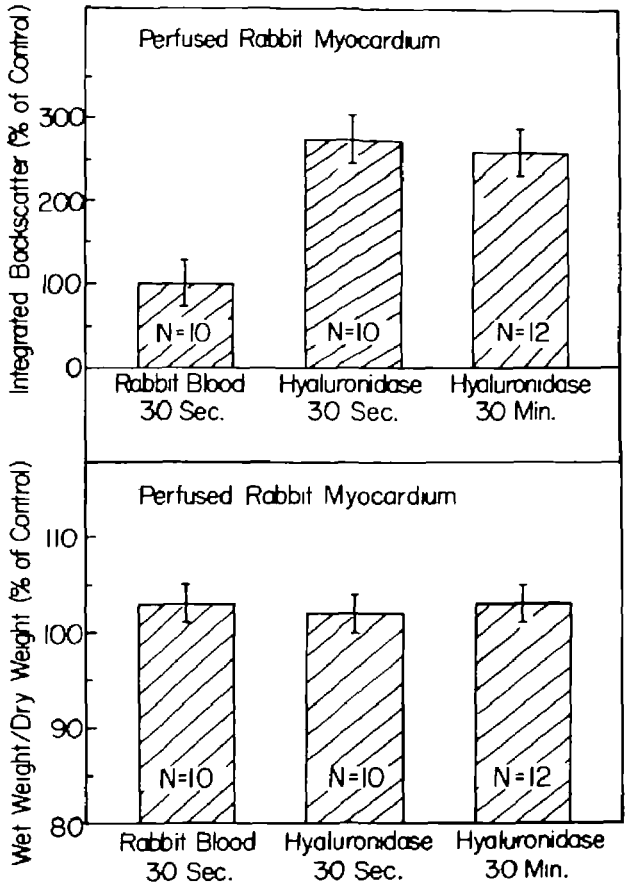

FIGURE 5 *Backscatter increased after perfusion with hyaluronidase alone, but normalized following replacement of perfusate with blood (top panel). These changes were secondary to contrast effect of blood and not associated with a change in myocardial water content (bottom panel).*

gional flow, a not surprising observation in view of the multiple determinants of backscatter, some of which have been identified already, such as collagen content of the tissue, formed blood cell elements, and fluid content (Mimbs et al., 1980).

Determinants potentially largely responsible for the alteration of ultrasonic properties of acutely ischemic myocardium include the development of interstitial and intracellular edema, changes in concentration of cytoplasmic proteins, differences in tissue volume between ischemic and nonischemic regions, and effects of changes in concentration of ultrasonic contrast agents such as blood, leukocyte infiltration, alterations in calcium content in ischemic tissue, and other factors. Characteristic histological changes occur only after several hours after coronary occlusion in the dog (Sommers and Jennings, 1964), but swollen organelles, mitochondrial densities, and myofibrillar relaxation are evident within 1 hour of persistent coronary occlusion (Kloner et al., 1974b).

Because myocardial fluid retention occurs early after coronary occlusion (Jennings et al., 1964; and Flear et al., 1976; Powell et al., 1977; Willerson et al., 1977), we elected to investigate the association of alteration in myocardial water content with changes in the ultrasonic backscatter. To test the hypothesis that an alteration in myocardial water content underlies a change in backscatter, a different experimental system—the perfused rabbit heart—was employed. The results presented in Figure 4 and Table 2 indicate that an increase in the wet-to-dry weight ratio of myocardium was accompanied by a significant increase in the ultrasonic backscatter. Because the method for alteration of the water content of myocardium employed in the present study was manipulation of the albumin and hyaluronidase concentrations in the medium, altered backscatter may have reflected a change in interstitial fluid tonicity. Additional studies would be required to determine whether intracellular and/or extracellular tonicity per se exert major influences independent of net water content of the tissue, although this seems somewhat unlikely. Additional experiments designed to elucidate the association between backscatter and myocardial water content in dogs in vivo could be performed by reperfusion induced by release of a coronary occluder and measurement of backscatter under both conditions before and after administration of mannitol by intravenous infusion.

To evaluate the potential effect of alterations in the concentrations of whole blood upon the ultrasonic backscatter, experiments were performed with perfused hearts. Data presented in Figure 5 demonstrated that the absence of intravascular blood elements (i.e., washout with Krebs-Henseleit buffer) resulted in an increase in ultrasonic backscatter independent of myocardial water content. When heparinized whole blood was immediately returned to hearts subjected initially to a 30-second washout with Krebs-Henseleit solution, the measured backscatter returned to a value similar to that in control, non-perfused hearts. Results of these experiments indicate that the ultrasonic backscatter from the heart changes with alterations in vascular content of whole blood. Since the contrast effect of whole blood is dependent primarily on the concentration of red blood cells, these results suggest that alterations in the concentration of red blood cells specifically may affect significantly the ultrasonic backscatter (Sigelman and Reid, 1973, and Shung, et al., 1976).

The method reported in this study has been demonstrated to be useful in characterizing myocardial properties by reflected ultrasound. Other approaches to quantitative ultrasonic characterization of tissues, such as measurement of echo signal amplitude, velocity, absorption, or attenuation alone have not been shown thus far to provide a reproducible method for quantitative characterization of tissue in vivo. However, additional developments [e.g., measurement of the ultrasonic attenuation using reflected signals (Kuc and Schwartz, 1979)] ultimately may permit the use of one or more

of these approaches. One of the inherent characteristics of the present method for measurement of ultrasonic backscatter is its potential applicability in a noninvasive clinical approach to quantitative characterization of myocardial properties. Success will depend ultimately on acquisition of data in closed-chest animals and human subjects with processing sufficiently robust to avoid distortion due to the chest wall. To this end, we are presently examining the utility of several potentially useful approaches, including the use of fast-transient recording to permit data storage for retrospective processing, broadband two-dimensional transducer arrays to obtain ultrasonic focusing throughout the entire myocardial segment of interest, a precise method to account for the ultrasonic properties of tissue interlocal to the transducer and the myocardial segment of interest, and variable gating for measurement of backscater within different segments of the same myocardial area (i.e., epicardial, endocardial, or intramyocardial).

Results from the present study demonstrate that measurement of the intrinsic backscatter properties of myocardium can be used to detect acute myocardial ischemia in vivo. The time course of altered backscatter has been defined for the initial 6 hours following coronary artery occlusion, with increased backscatter demonstrable as early as 1 hour after coronary ligation with a 400% increase in backscatter over control within 6 hours after ligation. With the use of a quantitative method for assessment of regional blood flow, we have shown that increased backscatter does not exhibit a simple linear relationship with flow. In fact, backscatter appears to be related directly to the cellular and structural concomitants of ischemia, since backscatter increased with increased water content and with decreased red blood cell content in perfused tissue. The applicability of the method developed to the clinical setting for noninvasive quantitative characterization of myocardial properties appears feasible, and this suggests that ultrasonic tissue characterization may prove to be useful for detecting and characterizing not only metabolic and structural consequences of myocardial ischemia, but changes indicative of other myopathic processes as well.

Acknowledgments

We appreciate the technical assistance provided by Delbert McGraw and preparation of the typescript by Joyce Kalayeh.

References

Cotran RS, Suter ER, Majno G (1967) The use of collidal carbon as a tracer for vascular injury. Vasc Dis **4**: 107–127

Fields S, Dunn F (1973) Correlation of echographic visualizability of tissue with biological composition and physiological state. J Acoust Soc Am **54**: 809–812

Flear CTG, Riemersma RA, Nandra A, Nandra G, Talbot R (1976) *In* Biochemistry and Pharmacology of Myocardial Hypertrophy, Hypoxia, and Infarction, vol 7, edited by P Harris, RI Bing, A Fleckenstein. Baltimore, University Park Press, pp 297–305

Heymann MA, Payne BD, Hoffman JIE, Rudolph AM (1977) Blood flow measurements with radionuclide-labeled particles. Progr Cardiovasc Dis **20**: 55–79

Jennings RB, Sommers HM, Kaltenbach JP, West JJ (1964) Electrolyte alterations in acute myocardial ischemic injury. Circ Res **14**: 260–269

Kloner RA, Ganote CE, Jennings RB (1974a) The "no-reflow" phenomenon after temporary coronary occlusion in the dog. J Clin Invest **54**: 1496–1508

Kloner RA, Ganote CE, Whalen DA, Jennings RB (1974b). Effect of a transient period of ischemia on myocardial cells. II. Fine structure during the first few minutes of reflow. Am J Path **74**: 399–414

Kuc R, Schwartz M (1979) Estimating the acoustic attenuation coefficient slope for liver from reflected ultrasound signals. IEEE Trans Sonics Ultrasonics **26**: 353–362

Linzer M (ed) (1976, 1977, 1978) Proceedings of the First, Second and Third International Symposiums on Ultrasonic Tissue Characterization (U.S. Department of Commerce, National Bureau of Standards, Washington, D.C.)

Mimbs JW, Yuhas DE, Miller JG, Weiss AN, Sobel BE (1977) Detection of myocardial infarction in vitro based on altered attenuation of ultrasound. Circ Res **41**: 192–197

Mimbs JW, O'Donnell M, Miller JG, Sobel BE (1979) Changes in ultrasonic attenuation indicative of early myocardial ischemic injury. Am J Physiol **236**: H340–H344

Mimbs JW, O'Donnell M, Bauwens D, Miller JG, Sobel BE (1980) The dependence of ultrasonic attenuation and backscatter on collagen content in dog and rabbit hearts. Circ Res **47**: 49–58

O'Donnell M, Bauwens D, Mimbs JW, Miller JG (1979) Broadband integrated backscatter: An approach to spatially localized tissue characterization in vivo. Proc IEEE Ultrasonics Symp **79**: CH1482-9/79, 175–178

Powell WJ Jr, Wittenberg J, Maturi RA, Dinsmore RE, Miller SW (1977) Detection of edema associated with myocardial ischemia by computerized tomography in isolated, arrested canine hearts. Circulation **55**: 99–108

Shung KK, Siegelmann RA, Reid JM (1976) Scattering of ultrasound by blood. IEEE Trans Biomed Eng **23**: 460–467

Shung KK, Reid JM (1977) Ultrasonic scattering from tissues. Proc IEEE Ultrasonics Symp **77**: CH1264-ISU, 230–233

Sigelman RA, Reid JM (1973) Analysis and measurement of ultrasound backscattering from an ensemble of scatterers excited by sinewave bursts. J Acoust Soc Am **53**: 1351–1355

Sommers HM, Jennings RB (1964) Experimental acute myocardial infarction. Lab Invest **13**: 1491–1503

Wallenstein S, Zucker CL, Fleiss JL (1980) Some statistical methods useful in circulation research. Circ Res **47**: 1–9

Willerson JT, Scales F, Mukherjee A, Platt M, Templeton GH, Fink GS, Buja LM (1977) Abnormal myocardial fluid retention as an early manifestation of ischemic injury. Am J Pathol **87**: 159–179