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Hydrogen peroxide contrast–enhanced two-dimensional echocardiography: real-time in vivo delineation of regional myocardial perfusion

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ABSTRACT Intravascular injection of hydrogen peroxide (HP) produces oxygen microbubbles suitable for echocardiographic contrast enhancement. This study evaluates HP contrast–enhanced two-dimensional echocardiography (2DE) during acute coronary occlusion in a canine model by comparison with perfusion indicated by antemortem monastral blue (MB) staining, postmortem triphenyltetrazolium chloride (TTC) infarct sizing, and abnormal wall motion (AWM). Injections of a fresh mixture of 1 to 2 ml 0.3% HP and 1 ml blood were made in 10 closed-chest dogs 6 hr after coronary ligation and short-axis 2DE images were made at four anatomic levels. Optimal preinjection 2DE settings were determined by interaction with an on-line, color-coded, videodensitometer system. The circumferential extent of 2DE contrast defect (ECD) was strongly predictive of the pathologic extent of malperfusion (MB = 0.94 ECD + 4.7%, SEE = 7.7%, r = .93) and of infarction (TTC = 0.84 ECD + 5%, SEE = 9.4%, r = .89). Moreover, ECD was superior to wall motion abnormality in predicting the extent of both malperfusion and infarction (MB = 0.85 AWM + 0.5%, SEE = 13.1%, r = .78; TTC = 0.77 AWM − 0.2%, SEE = 13.5%, r = .75). No adverse hemodynamic effects were seen after HP injection. This study validates the use of HP contrast–enhanced 2DE as a readily repeatable, accurate, in vivo real-time method of quantifying the extent of abnormal regional myocardial perfusion during acute myocardial infarction. Available data indicate that further study is warranted to determine if myocardial contrast adequate to define regional perfusion can be obtained safely by this method in man.
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RECENT CLINICAL STUDIES in which two-dimensional echocardiography was used in patients with acute myocardial infarction have implied potential widespread applicability for this technique. Comparison of the extent of wall motion abnormality measured by thallium perfusion defects[1] or at postmortem examination[2] have suggested that infarct size and wall motion abnormality are closely related. Carefully controlled animal studies, however, have cast doubt on the reliability of such estimates. Lieberman et al.,[3] using an open-chest model, reported that at 48 hr the transmural extent of infarction bore a nonlinear relationship to wall motion abnormality, with almost all areas that had greater than 20% infarction showing 100% dysfunction. Nieminen et al.,[4] using a closed-chest model, showed that the extent of abnormally contracting myocardium, when viewed 2 hr after ligation, markedly overestimated the extent of infarction. Although this relationship was statistically significant and improved with time, correlation and estimating error for infarct size remained relatively poor at 48 hr.[4]

The problem of relating the extent of abnormal wall motion to the extent of an underlying abnormality in perfusion is circumvented with the use of contrast agents that allow direct imaging of the altered perfusion rather than its functional consequence. This more direct approach has led to a resurgence of interest in the application of echocardiographic contrast techniques to the problem of myocardial perfusion imaging. Preliminary reports have demonstrated that the echoreflective properties of entrapped microbubbles, such as those produced by indocyanine dye, saline, or "micro-

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balloons,''[5, 6] if delivered in sufficient quantities by intracoronary injection, result in contrast enhancement of perfused myocardium. More recently, Armstrong et al.[7] accurately defined regions of abnormal perfusion in a single short-axis echocardiographic section obtained in an open-chest model after the supra-aortic injection of specially prepared gelatin-encapsulated microbubbles as a contrast-enhancing agent.[7]

The injection of a mixture of hydrogen peroxide and blood results in the generation of oxygen microbubbles. Preliminary studies in our laboratory and in others in which open-chest models were used[8, 9] suggest that these microbubbles are suitable for use as a myocardial contrast-enhancing agent.

The purpose of this study was to determine whether the echocardiographic myocardial contrast produced by a dilute hydrogen peroxide and blood mixture could define an in vivo zone of low flow that correlates with the extent of reduced perfusion determined by a dye technique, histochemical evidence of infarction, and the extent of wall motion abnormality 6 hr after coronary ligation in a closed-chest canine model.

Methods

After preliminary imaging, which showed adequate two-dimensional echocardiographic cardiac epicardial and endocardial target definition, 10 mongrel dogs* were anesthetized with 20 mg/kg iv pentobarbital, intubated, and placed on a Harvard respirator. The left femoral artery and vein were cannulated for arterial pressure measurement and fluid administration. A standard left thoracotomy was performed. The heart was exposed and a pericardial sling was constructed. The left atrium was cannulated and the line was exteriorized through a puncture wound. The left anterior descending artery distal to the first diagonal (six dogs) or the circumflex artery distal to the first obtuse marginal (four dogs) was dissected free and, 5 min after intravenous injection of 50 mg lidocaine, was doubly ligated with No. 2 silk sutures. Thirty minutes after occlusion the pericardial sling was released, a chest tube was exteriorized and attached to suction, and the chest was closed.

Echocardiographic contrast injection and recording. Five hours after occlusion, the right femoral artery was cannulated and a No. 8F pigtail catheter (USCI) was advanced under fluoroscopic guidance to a level just above the aortic cusps. The catheter was attached to bulb-flush extension tubing connected to a pressure bag containing 1000 cc normal saline and a constant drip was maintained. To prevent clotting of the catheter side holes, 2000 U sodium heparin was added to the flush solution and the same amount was given intravenously.

The left ventricle was imaged with a Varian 3000 two-dimensional ultrasonograph with a 2.25 mHz phased-array transducer interfaced with a commercial color videodensitometer (Colorado Video) that could be adjusted to display the two-dimensional image in color according to a predetermined scale based on voltage present on a pixel-by-pixel basis at the output phosphor of a red/green/blue color monitor.[10] In preliminary experiments, we determined that perfusion of myocardium with H_2O_2 microbubbles in normal animals was associated with an increase in echo reflectance equivalent to an increase in voltage of 0.08 to 0.20 mV/pixel when a mean baseline level of 0.20 mV/pixel was present. The increase in reflectance, which becomes maximal 10 sec after injection, can be observed to decrease initially in the subepicardial layer after approximately 30 to 45 sec and may be present in the subendocardial layer up to 5 min after injection. Therefore, before each injection, gain and depth settings were adjusted so that an image obtained homogeneously displayed 0.20 to 0.28 mV/pixel at epicardial and endocardial targets when the animal was in end-expiration.

Preliminary experiments revealed that the optimal increase in myocardial reflectance was obtained with a fresh mixture of 1 to 2 ml 0.3% hydrogen peroxide and 1 ml blood rapidly flushed into the ascending aorta. Larger doses of hydrogen peroxide resulted in profound microbubble generation in myocardium nearer to the transducer and this prevented sound energy from reaching deeper structures and lower doses gave insufficient contrast. For the initial injection, 2.0 ml of 0.3% H_2O_2 was drawn into a 10 ml syringe, followed by the drawing of 1 ml heparinized blood. The mixture was agitated for 5 sec and slowly injected into distal portions of the catheter flush tubing and then flushed into the aorta with 10 to 15 ml saline by rapid compression of the flush bulb. If the initial images showed evidence of shadowing, the dose of H_2O_2 was adjusted downward in 0.5 ml decrements. A series of three to six injections was given to each animal at 10 min intervals. Echocardiographic images obtained at four levels during injection (mitral valve, high papillary muscle, low papillary muscle, and apex) were recorded for future analysis with a Panasonic 3260 reel-to-reel color recorder on ½ inch Scotch 361 videotape.

Data tapes were played back and the series of injections were observed in real time. Tracings of single frames occurring at end-diastole (between the P and R waves of the accompanying electrocardiographic signal) for each level were made on an acetate overlay applied to the monitor screen. Care was taken to record all tracings between 10 and 60 sec after injection, during end-expiration, on a frame in which peak echocardiographic signals were present across the perfusion defect.

Tissue and histochemical staining. After completion of the series of contrast injections (6 to 6½ hr after occlusion) the dogs' chests were reopened. Monastral blue (MB) pigment (0.5 ml/kg) was injected via the left atrial line as previously described.[11] The blue dye circulates and stains tissues receiving flow; zones of severely depressed flow remain unstained. Twenty seconds after injection, the animals were killed with KCl solution and their hearts were removed. The left ventricle was washed in iced saline, dissected free from the right ventricle and atria, and weighed. It was then quick-frozen with liquid refrigerant (Freon). Sequential 5 mm slices were made in a plane parallel to the atrioventricular groove, beginning at the apex, with a commercial meat slicer. Between 10 and 14 slices were obtained from each ventricle.

The pattern of MB staining on the basal side of each slice was traced on an acetate overlay and the slices photographed with a camera that allowed 1:1 reproduction (Polaroid). Approximately 15 min after the animals were killed, the slices were immersed in a triphenyltetrazolium chloride (TTC) stain at 37° C for 15 min according to the method of Lie et al.[13] They were then dipped in buffered formalin for 15 sec to enhance contrast and were traced and photographed as above. Several previous studies have shown that the TTC technique is reliable in identifying zones of infarction.[14–16]

*Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School, the West Roxbury VA Medical Center's Animal Studies Subcommittee, and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal-Resources, National Research Council (DHEW publication No. [NIH] 78-23, revised 1978).

Wall motion analysis. Two-dimensional left ventricular wall motion was analyzed independently by three observers at four levels in each animal (apex, low papillary muscles, high papillary muscle, and mitral valve) by marking the area of abnormality on an appropriate schematic form. Wall motion abnormalities were expressed as percentage of circumference involved

$$\left(\frac{\text{Degrees of abnormality}}{360 \text{ degrees}} \times 100 = \%\ \text{Circumference abnormal}\right)$$

When all observers agreed within 5% on a mean value for a given level, the mean value was accepted. When variation was greater than 5% (7/40 levels), images were reviewed as a group and percent contraction abnormality was determined by consensus.

Data analysis. The mean circumferential extent of abnormality was determined for each echocardiographic, MB, and TTC section as described in detail in figure 1. Values for the extent of abnormality obtained from the slices of left ventricle were averaged for the following regions: the apical region, designated as the distal-most slices that did not contain papillary muscle invaginations (two to three 5 mm slices); the upper and lower papillary muscle regions, made up of equal numbers of the slices containing papillary muscle (three to four slices per region); and the mitral valve region, designated as all sections above the papillary muscles. Data from the most distal apical slice, which did not contain any cavities, and any proximal slice that contained mitral valve ring or epicardial fat, were not used in calculations. The actual slices of anatomic regions were then compared with their respective echocardiographic images.

Data obtained on examination of the slices and echocardiographic data were also analyzed to determine percentage of area that was abnormal. With a commercial light-pen system (Irex Cardio 80) areas of abnormal staining or contrast enhancement were determined for each slice of left ventricle. The slices were ordered as above and the percentage of area was defined as the sum of the area of abnormality for all pertinent slices divided by the total area of those slices. Corresponding regions from echocardiographic and tissue sections were then compared.

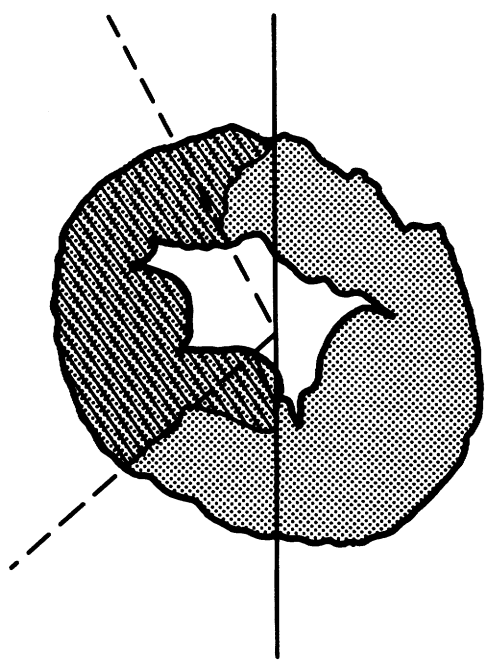

FIGURE 1. A schematic representation of a slice of the left ventricle illustrating the analysis of the circumferential extent of infarction or malperfusion. The area of abnormality is shown by hatching. The center of mass of the cross-sectional slice was determined by superimposing a circle with a known center of mass over the epicardial border. Radii were drawn to encompass the maximal (solid line) and the minimal (broken line) extents of the abnormality and the subtended angles were determined. Percent circumferential extent = (maximal angle + minimal angle/2 × 36) × 100.

Data were tabulated and correlated and estimating errors were determined with a standard desk-top calculator (Litton Monroe 325 Scientist). All group data are expressed as mean ± SD.

Interobserver and intraobserver variability. Twelve echocardiographic levels were selected for analysis at random, with at least one level selected from the echocardiogram of each animal. The course of injection was observed again by a second observer and the appropriate level was traced. The mean difference in the observed circumferential extent of echocardiographic contrast defect (ECD) between observers was 5.4 ± 5.6%, with a correlation coefficient of .94. The mean difference between the initial observation and a second observation performed by the first observer 4 weeks later was 3.4 ± 2.3%, with a correlation coefficient of .99.

Similar values for reproducibility were obtained by the area method (interobserver, 5.3 ± 4.4%, r = .97; intraobserver, 3.2 ± 1.9%, r = .99).

Results

Hemodynamic effects of hydrogen peroxide contrast injection. A total of 42 supra-aortic injections were made in the 10 animals at 5.9 ± 0.4 hr after coronary ligation. Mean heart rate was 124 + 33 beats/min and did not change with injection. Mean left atrial pressure was 8.4 ± 2.5 mm Hg and was also unchanged during the course of injection. Average preinjection mean arterial pressure was 98.3 ± 12.7 mm Hg and fell to 94.6 ± 12.6 mm Hg at 30 sec, with a return to preinjection values by 75 sec after injection. Mean arterial pressure did not change with 32 of 42 injections. The maximal fall in pressure observed with any injection, 18 mm Hg, returned to baseline by 60 sec after injection. There was no alteration in ambient ventricular ectopic activity in the 10 min after injection in any animal.

The use of the circumferential and area methods of analysis resulted in identical conclusions. Raw data for each method of analysis are presented in table 1, as are regression equations and correlations found by the area method. Results of data analysis by the circumferential method will be presented in detail below.

Histochemical and MB staining. Mean left ventricular weight was 128 ± 29 g (range 86 to 167). In one animal, the MB staining method was insufficient to accurately define the region of normal perfusion. In the remaining nine animals the circumferential extent of absent MB staining ranged from 0 to 80% of any given section. The circumferential extent of absent TTC staining ranged from 0 to 78%. Six hours after ligation the correlation between results with these two methods

was high (TTC = 0.93 MB − 0.3%, SEE = 4.7%, r = .97; figure 2); 93 ± 4% of the area of malperfusion as determined by the MB method was also determined by the TTC method to be infarcted. For the ventricle as a whole, when calculated by the area method, a mean area of 28.7 ± 7.3% was malperfused and an area of 23.4 ± 9.0% was infarcted ($p < .01$).

Relationship of in vivo ECD to MB and histochemical staining. Two-dimensional echocardiograms obtained during supra-aortic hydrogen peroxide injection showed homogeneous enhancement of normal myocardium. Regions not showing enhancement were well

TABLE 1
Individual data obtained with area and circumferential methods

Ligation site	Dog No.	Level	ECD		MB		TTC		WMA
			% Area	% Circ	% Area	% Circ	% Area	% Circ	(% circ)
LCx	1	MV	20	20	26	27	8	25	57
		HP	16	24	17	18	10	17	30
		LP	24	31	24	25	13	24	26
		AP	21	26	21	27	9	20	51
	2	MV	32	37	40	44	36	42	26
		HP	37	35	38	36	31	36	42
		LP	42	41	44	42	35	37	43
		AP	41	38	44	47	41	42	45
	3	MV	31	32	17	24	3	8	18
		HP	25	34	19	25	12	22	30
		LP	19	29	24	32	15	28	37
		AP	18	20	8	26	8	18	49
	4	MV	20	21	19	29	13	30	37
		HP	22	21	12	20	9	20	30
		LP	19	22	24	26	20	31	34
		AP	25	25	36	43	25	36	40
LAD	1	MV	0	0	1	2	1	2	0
		HP	15	19	22	24	19	23	59
		LP	21	27	46	49	42	46	35
		AP	74	75	60	71	59	61	59
	2	MV	0	0	0	0	0	0	0
		HP	7	13	9	8	6	9	27
		LP	35	39	40	48	30	34	41
		AP	62	69	80	79	79	79	57
	3	MV	0	0	0	0	0	0	0
		HP	25	25	34	34	27	33	35
		LP	44	45	52	51	43	46	54
		AP	79	81	69	68	62	63	69
	4	MV	0	0	0	0	0	0	29
		HP	20	20	22	27	25	32	42
		LP	24	29	34	44	39	45	52
		AP	42	45	59	60	51	54	64
	5	MV	5	6	6	8	5	7	0
		HP	21	28	22	24	28	22	51
		LP	52	59	58	57	41	65	64
		AP	74	76	66	72	62	62	72
	6	MV	11	12	—	—	0	0	47
		HP	21	21	—	—	21	33	49
		LP	32	33	—	—	40	44	68
		AP	78	78	—	—	55	58	75

Regression equations, correlations, and SEEs for the area method are: TTC = 0.94 MB − 3.2%, r = 0.96, SEE = 5.4%; TTC = 0.86 ECD + 0.9%, r = 0.88, SEE = 9.7%; MB = 0.95 ECD + 3.7%, r = 0.92, SEE = 8.4%. Variability was: interobserver = 5.3 ± 4.4%, r = 0.97; intraobserver = 3.2 ± 1.9%, r = 0.99. The circumferential method is presented in detail in the text and the accompanying figures.

WMA = wall motion abnormality; % Circ = percent circumference involved; % Area = percent area involved, LAD = left anterior descending coronary artery; LCx = Left circumflex coronary artery; MV = mitral valve; HP = high papillary muscle; LP = low papillary muscle; AP = apical level.

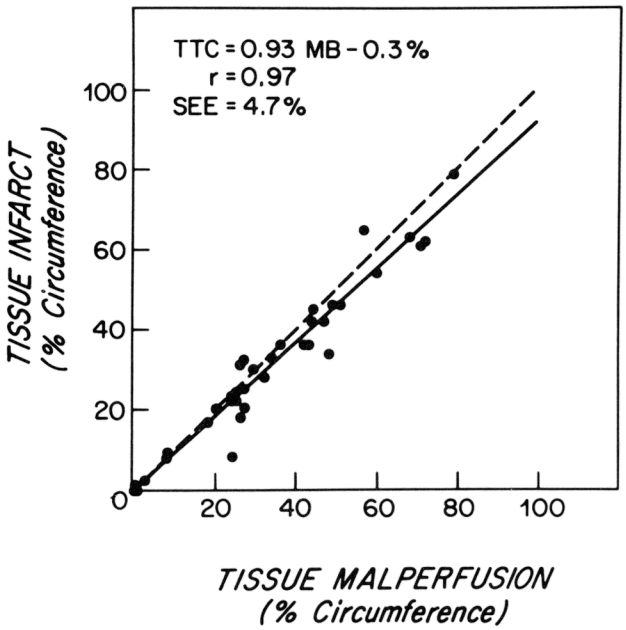

FIGURE 2. Relationship of circumferential extent of malperfusion and infarction 6 hr after coronary ligation. The regression line is shown by the solid line and the line of identity by the dashed line. The extent of infarction, determined by the absence of TTC staining, was 93 ± 4% (mean ± SD) of the extent of myocardial malperfusion as indicated by the supravital MB dye.

defined and were located within an area of abnormal wall motion (figure 3). The ECD ranged from 0 to 78% of the circumferential extent of any given echocardiographic section. ECD was strongly predictive of the pathologic extent of both malperfusion determined by MB staining and infarction determined by TTC staining (MB = 0.94 ECD + 4.7%, SEE = 7.7%, r = .93; TTC = 0.84 ECD + 5%, SEE = 9.4%, r = .89; figure 4). Results with the ECD method related slightly more closely to those with the MB than the TTC staining techniques. This is most apparent when the relationships are examined on a level-by-level basis (table 2). Correlation coefficients ranged from .68 to .95 for ECD vs MB as opposed to .45 to .88 for ECD vs TTC.

Relationship of abnormal wall motion to measures of perfusion and infarction. There was a significant linear correlation between abnormal wall motion (% circumference) and tissue malperfusion determined by MB staining (r = .78, figure 5, *A*), between abnormal wall motion and infarct size (r = .75, figure 5, *B*), and between abnormal wall motion and ECD method results (r = .73, figure 6). However, the extent of abnormal wall motion tended to overpredict the extent of infarction and malperfusion as indicated by the MB

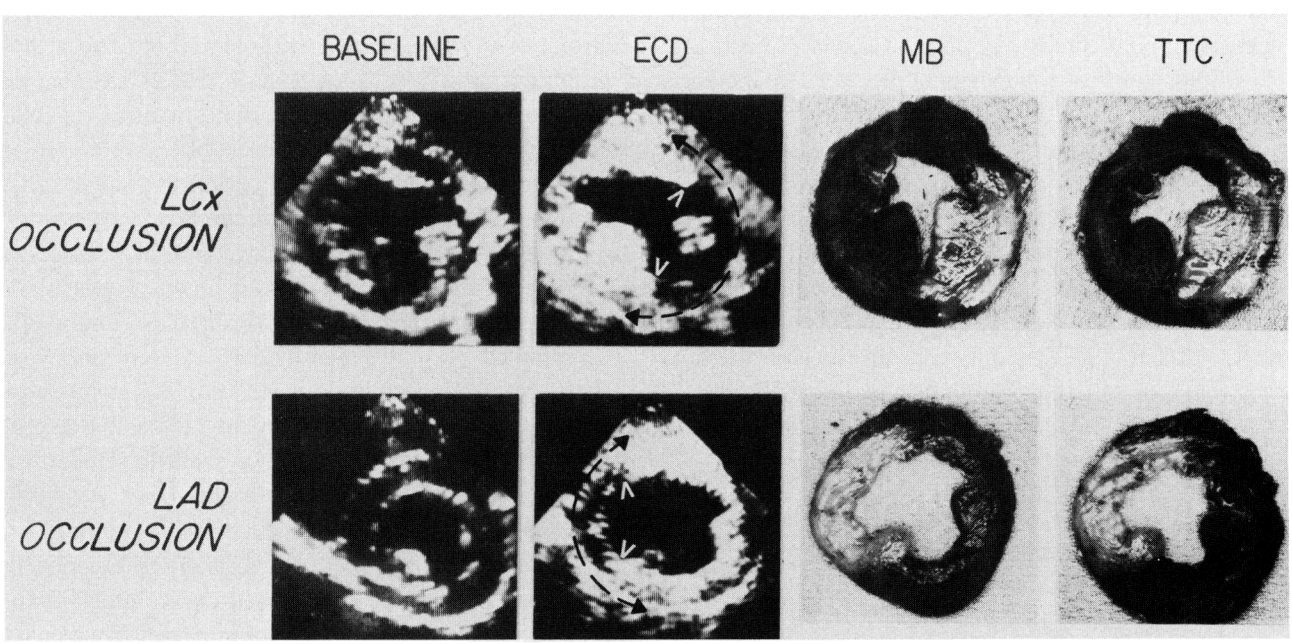

FIGURE 3. Examples of two-dimensional echocardiographic images and their corresponding left ventricle slices. *Top,* After left circumflex (LCx) coronary artery ligation, a short-axis image of the midpapillary muscle level was obtained before injection of contrast agent (baseline). After injection of the H_2O_2/blood mixture, the myocardial area with normal perfusion lit up while the ECD remained dark (white arrows). Abnormal wall motion was seen in real time, which is designated by the black dashed line between black arrows. To determine the area at risk, MB dye was injected in the left atriums of the dogs before they were killed. The area with abnormal myocardial perfusion was not impregnated with dye (light area). After incubating the slices in TTC for 15 min at 37° C, the area of infarction remained unstained (light area). *Bottom,* Similar sequence after left anterior descending (LAD) coronary ligation. Examples are from the middle to low papillary muscle levels. All sections are oriented as follows:

Septum ⟷ Posterior
Anterior ⟷ Lateral

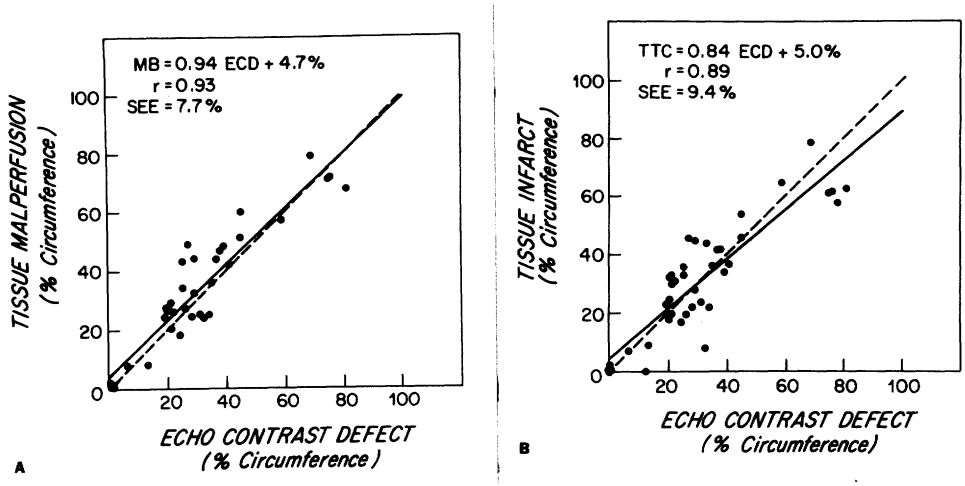

FIGURE 4. *A*, Relationship of circumferential extent of malperfusion (MB) and ECD 6 hr after ligation. Regression line is shown by solid line and line of identity is dashed line. *B*, Relationship of circumferential extent of infarction (TTC) and ECD 6 hr after coronary ligation. Regression line is shown by solid line and line of identity is dashed line.

and ECD methods. This overprediction was most marked when the abnormally perfused area was relatively small, i.e., less than 40% of the circumference. Results for larger regions were more closely related.

Discussion

This study shows that the extent of regional myocardial malperfusion as demonstrated by MB staining is accurately predicted in vivo by the extent of echocardiographic contrast enhancement produced by the aortic root injection of a small volume of a new contrast agent, a hydrogen peroxide and blood mixture. Close correlation between area of risk, as demonstrated by MB staining, and area of infarction, as shown by TTC staining, has previously been demonstrated after 6 hr of occlusion without a myocardial salvaging intervention.[17, 18] Thus, the extent of infarction also correlated well with that of ECD in our series in which no attempt was made at salvage. The extent of the ECD was a considerably more accurate indicator of the state of regional myocardial perfusion and infarction than was wall motion in our series. The overestimation of ischemic region size by wall motion analysis in our series was similar to that previously reported.[4]

TABLE 2
Correlation of extent of abnormality at each level

	MV		HP		LP		AP	
	r	SEE (%)	r	SEE (%)	r	SEE (%)	r	SEE (%)
MB vs TTC	.95	5.0	.96	2.3	.86	6.4	.99	3.0
ECD vs TTC	.79	9.4	.45	7.7	.68	8.7	.88	9.5
ECD vs MB	.95	4.9	.68	6.1	.72	8.0	.92	7.7

The correlation is higher and the estimating error is smaller for percent circumferential extent of abnormality at all levels for ECD vs MB than for ECD vs TTC, indicating that ECD corresponds to malperfusion rather than infarction.

MV = mitral valve level; HP = high papillary muscle level; LP = low papillary muscle level; AP = apical level.

DeMaria et al.[6] have demonstrated that intracoronary injection of solutions containing microbubbles results in increased echocardiographic contrast in normally perfused myocardium. More recently, Armstrong et al.[7] have shown the potential of echocardiographic contrast enhancement in the assessment of regional myocardial perfusion abnormalities. In their study gelatin-encapsulated microbubbles were used as a contrast agent and a single echocardiographic cross section was obtained during circumflex occlusion by direct application of the echocardiographic transducer to the right ventricular free wall in an open-chest model. Contrast enhancement, as measured by commercial light meter, correctly identified 48 of 51 octants with more than 50% normal flow and 19 of 21 octants with reduced flow, as determined by the radioactive microsphere technique. No attempt was made to standardize injection volume, gain, depth, or gray-scale settings in this study.

In our study the clinical situation was more closely simulated by the use of a closed-chest model. Standardization of gain and depth settings was achieved by interactive use of an on-line color-scale videodensitometer. Since respiration alters the depth of the echocardiographic target and hence its reflectance, all measurements were taken at full expiration. This careful preliminary setting of baseline values allowed easy detection of contrast borders during playback and obviated the need for subtraction of initial from final values to determine contrast enhancement.

In this study we used a new echocardiographic con-

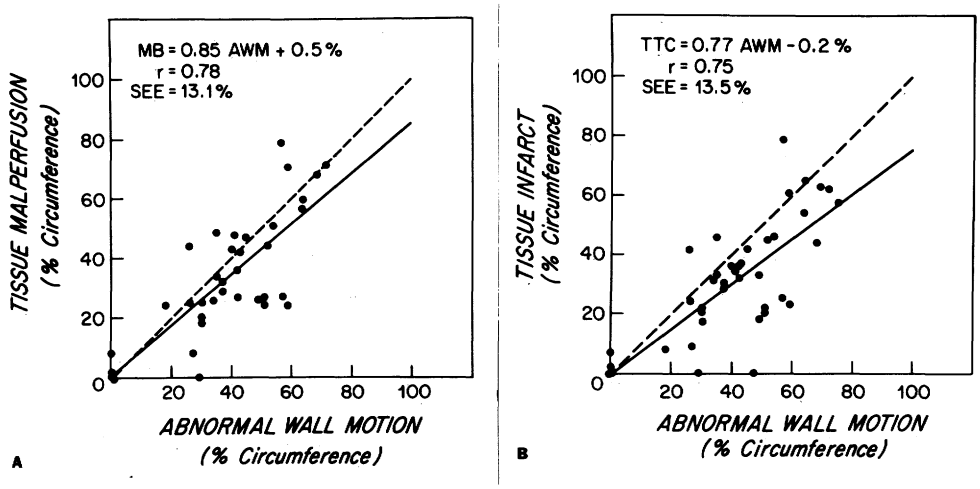

FIGURE 5. *A*, Relationship of the circumferential extent of malperfusion (MB) and abnormal wall motion (AWM) 6 hr after ligation. Regression line is shown by solid line and line of identity is dashed line. *B*, Relationship of circumferential extent of infarction (TTC) and abnormal wall motion (AWM) 6 hr after ligation. Regression line is shown by solid line and line of identity is dashed line.

trast agent of dilute hydrogen peroxide and blood. This mixture results in the generation of oxygen microbubbles by leukocyte enzymes. Gross et al.[8] and Armstrong et al.,[9] in preliminary reports, have commented on the associated intense echocardiographic contrast effect produced in the myocardium by hydrogen peroxide–associated microbubble generation. In their study Armstrong et al. achieved results in defining area of infarction similar to those in our study. They showed that the size of echo contrast defect measured at a single cross-sectional level corresponds well to tissue infarction at the same level measured by nitro blue tetrazolium staining after killing of the animal model.

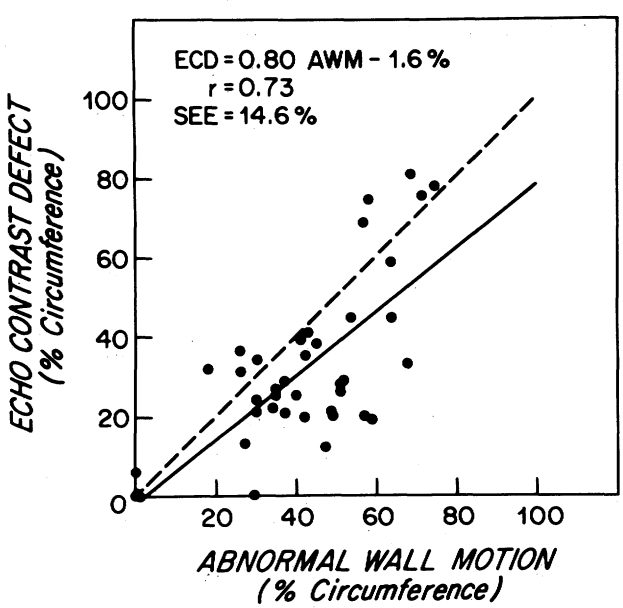

FIGURE 6. Relationship of circumferential extent of ECD and abnormal wall motion (AWM). Regression line is shown by solid line and line of identity is dashed line.

The intense echocardiographic contrast engendered by the mixture of hydrogen peroxide and blood obviates the need for intracoronary contrast injection. Supra-aortic injection allows the entire coronary circulation to be observed after one injection, it is easier to perform than right and left coronary catheterization, and it avoids the opacification of the left ventricle associated with left atrial injection.

Although the precise rheologic mechanisms of supra-aortic microbubble injection are not known, the hydrogen peroxide and blood injection we used resulted in homogeneous contrast enhancement throughout all levels of the myocardium. No attempt was made to quantitate regional flows other than by comparison with antemortem MB staining. Our experience suggests that absolute quantification of regional flow by this technique may prove difficult because of the problem of echocardiographic shadowing of the deeper myocardium by the intense echoes generated in the more superficial myocardial layers. When viewed in playback with the videodensitometer engaged, an unpredictable gradient in contrast enhancement was observed. Myocardium located nearer was more greatly enhanced than that located further from the transducer, despite identical baseline values. No additional information regarding the presence of a perfusion defect was observed with the densitometer engaged during playback and, as its use causes some degradation of image quality, no attempt was made to quantify perfusion defect size by this method.

Myocardial perfusion imaging with hydrogen peroxide is applicable at present to the laboratory investigation of short- and long-term alterations in regional myocardial blood flow. No important effect was seen

on hemodynamics or cardiac irritability after as many as six injections of the dilute hydrogen peroxide and blood mixture. This method is potentially applicable to the clinical study of acute and evolving myocardial infarction in man if the small amounts of hydrogen peroxide given prove to be safe.

The limiting toxicity of intravascular hydrogen peroxide injection is related to the potential for the generation of oxygen macroemboli in addition to the microemboli necessary for ultrasonic contrast. The size and rate of formation of oxygen bubbles produced by hydrogen peroxide injection is dependent on the amount, dilution, and rate of the solution injected. Gross et al.[8] noted marked intravascular foaming and ventricular fibrillation when a 3.0% solution was injected into the left coronary artery of a dog model, but doses in the range used in our study were tolerated without difficulty in their experiments and in those of Armstrong et al.,[9] who also gave supra-aortic injections to dogs. In work aimed at further assessing the potential toxicity of cerebral macroembolization, Gaffney* gave serial left atrial injections of 1, 2, 5, and 10 ml of a 0.3% H_2O_2 solution to five conscious dogs instrumented over the long term. No signs of altered mental status or mental disturbance were seen.

Hydrogen peroxide has been injected intravascularly with safety in man. Gaffney et al.[19] used intravenous peroxide injection in a dose similar to the one we used as a right heart contrast agent in 36 patients without sequellae. Wang et al.[20] have reported their experience with 100 patients, 21 of whom had cyanotic congenital heart disease, after the intravenous injection of doses of hydrogen peroxide 1.5 to 5 times larger than those we used. Six of their patients developed "transient dizziness" at the time of injection, but none of the patients developed any signs of mental disturbance or ischemia. Mallams et al.[21] infused a far larger dose, 250 ml of a 0.06% to 0.48% hydrogen peroxide solution, into the carotid artery or abdominal aorta over 40 to 60 min as an adjunct to radiation therapy in 102 patients being treated for malignancies. In their patients they reported occasional local arterial spasm and several transient ischemic attacks in the carotid artery series "if the system is overloaded and capillary bubbling appears." All ischemic attacks were reversed without residua.

Meltzer et al.[22] have determined that a total volume of less than 50 mm^3 of microbubbles is necessary to produce striking echocardiographic contrast. Since oxygen microbubbles produced by hydrogen peroxide injection are rapidly diluted and reabsorbed by the bloodstream, further careful studies are indicated to determine whether adequate myocardial contrast can be obtained in man at doses that do not cause macroembolization.

To our knowledge the use of hydrogen peroxide contrast echocardiography is unique in that it can produce a real-time in vivo measure of myocardial perfusion. The only other in vivo methods of regional myocardial perfusion assessment are found in nuclear medicine. Compared with thallium perfusion imaging and radioactive gas washout methods, contrast echocardiography would appear to have significant advantages in ease of performance, resolution,[23] and repeatability of measurement. All equipment necessary is portable and readily available. Multiple assessments can be made as often as every 5 to 10 min over several hours to several days. The area at risk in an acute myocardial infarction can be rapidly assessed and observed over time. Studies in our laboratory done by this method are currently under way to further assess its value in experiments with interventions designed to reduce infarct size.

Hydrogen peroxide contrast–enhanced two-dimensional echocardiography allows repeated in vivo delineation of zones of decreased myocardial blood flow and these correlate more closely than the extent of wall motion abnormality with the extent of myocardial malperfusion demonstrated by MB staining. The technique was easy to perform and was not accompanied by significant hemodynamic alterations in an animal model. Available data suggest that this technique may be applicable to studies of evolving myocardial infarction in man.

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