Technetium-99m-Pyrophosphate Uptake as an Indicator of Myocardial Injury without Infarct

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Technetium-99m-pyrophosphate (PYP) is bound to calcium in necrotic myocardium and has been used clinically to evaluate myocardial infarction. Technetium-99m-PYP is also reported to accumulate in myocardium with unstable angina pectoris and it is speculated that severe ischemia with noninfarcted tissue may also increase uptake of ^{99m}Tc-PYP. In this paper, ^{99m}Tc-PYP uptake was determined in various models of myocardial ischemia of short duration to examine its applicability to the assessment of myocardial viability. **Methods:** In 23 open-chest dogs under anesthesia, models of ischemia-reperfusion of the left anterior descending artery (LAD) subjected to ischemia for 10, 30 or 60 min were produced. Wall motion was examined by echocardiography and myocardial blood flow was calculated using colored microspheres. Technetium-99m-PYP was injected after each ischemic intervention and reperfusion. **Results:** Technetium-99m-PYP showed 1.18 ± 0.009 in the uptake ratio (ischemic area/normal area) following 10-min ischemia (11 dogs). The uptake ratio following 30-min ischemia (8 dogs) showed a significantly higher increase than that following 10-min ischemia (4.09 ± 1.75; p <0.05), permitting in vivo and ex vivo imaging. After 60-min ischemia resulting in infarction (4 dogs), ^{99m}Tc-PYP uptake of the ischemic area showed an uptake ten times that of the normal area (transmural: 12.2 ± 2.9, epicardium: 7.5 ± 1.9, endcardium: 16.8 ± 4.1). **Conclusions:** These findings indicate that since ^{99m}Tc-PYP accumulates in injured myocardium, its concurrent use with blood flow imaging is useful for the assessment of severity of ischemia, injured area and myocardial viability.

Key Words: ischemia; technetium-99m-pyrophosphate; unstable angina

Since ^{99m}Tc-PYP is bound to calcium (hydroxyapatite crystals) in necrotic myocardium, it has been considered useful in the diagnosis and quantification of acute-phase myocardial infarction (1,2) and has been used in the clinical setting. Willerson et al. (3) and Jaffe et al. (4) reported that the uptake of ^{99m}Tc-PYP visualized diffuse, slightly positive findings in unstable angina. However, the results strongly suggested abnormal uptake of ^{99m}Tc-PYP in unstable AP to indicate myocardial necrosis (4). Their report was followed by several reports on the uptake of this agent in injured myocardial cells without infarction (5,6). Technetium-99m-PYP uptake early after reperfusion was reported to occur in experimental studies with dogs in myocardial cells that were viable but severely injured, possibly by excess cytosolic calcium (7). Alterations in cellular phospholipid metabolism are closely related to sarcolemmal membrane stability and increased Ca^{++} permeability, resulting in increased uptake of ^{99m}Tc-PYP (8). We have already reported that ^{99m}Tc-PYP uptake is closely related to myocardial stunning, and Ca^{++} antagonists inhibit stunning as well as increased uptake of ^{99m}Tc-PYP (9). Sarcolemmal Ca^{++} permeability is thus very likely to occur, resulting in increased uptake of ^{99m}Tc-PYP even after short duration of ischemia without infarct. We produced animal models of ischemia of different durations for determination of ^{99m}Tc-PYP uptake to evaluate its usefulness in the assessment of viable myocardium after ischemic insult.

METHODS

Mongrel dogs weighing 13–33 kg received an intramuscular injection of ketalar (2.5 mg/kg) for induction of anesthesia and an intravenous injection of nembutal (25 mg/kg) for its maintenance. For respiratory control, a tube inserted into the trachea was connected to a dual-phase control respirator (Harvard Apparatus, Southnatick, MA), through which 100% 2 l/min of oxygen was supplied. The fifth intercostal space was opened and the epicardium was immobilized in the form of a cradle and the LAD was separated. A Doppler flow meter was attached to the central segment or the periphery of the first diagonal branch for confirmation of blood flow, and an occluder for production of ischemia. A catheter was inserted into the left appendage of heart for the injection of colored microspheres for use in determining the absolute value of regional blood flow at each stage and for the injection of Evans blue dye before sacrifice for determination of the ischemic area.

A catheter to monitor blood pressure was introduced into one of the bilateral femoral arteries and another for blood sampling into the other. A catheter was introduced into the femoral vein for maintenance fluid infusion and the injection of a maintenance anesthetic. The ischemic period was 10 min (11 dogs; 10-min group), 30 min (8 dogs; 30-min group) or 60 min (4 dogs; 60-min group). Each ischemic period was followed by reperfusion for 5–15 min while confirming the blood flow using a Doppler flow meter to detect the onset of reactive hyperemia. Technetium-99m-
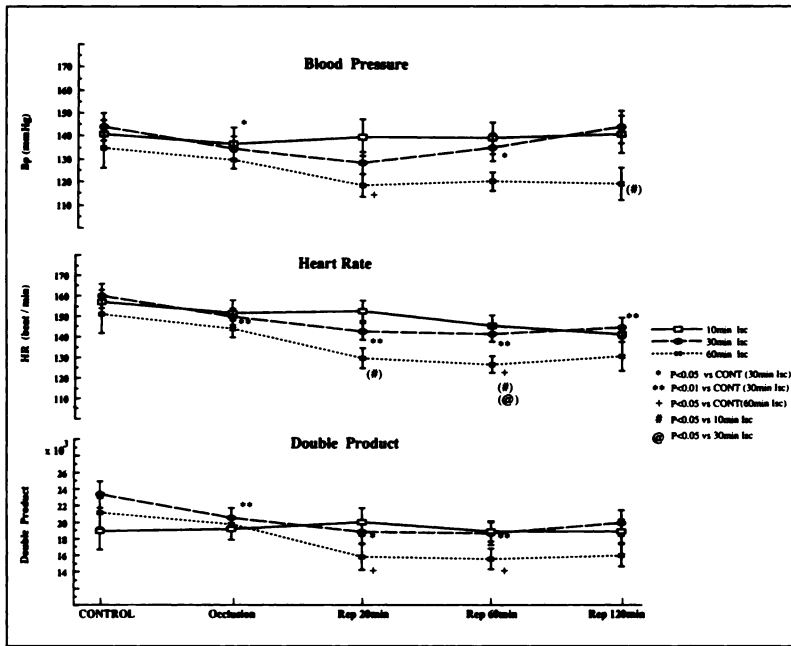
FIGURE 1. Blood pressure (BP), heart rate (HR) and double products (DP) during the experimental course are shown.

PYP was injected 20–30 min after reperfusion, and in vivo images of ^{99m}Tc-PYP were taken 2 hr later. Thallium-201 was injected, followed by in vivo imaging.

After Evans blue staining for confirmation of the ischemic size of at risk area, the animals were killed. The heart was cut into 3–4 slices in the direction of the short axis for ex vivo imaging with ^{99m}Tc-PYP and ^{201}Tl. Triphenyltetrazolium chloride (TTC) staining for detection of the area of myocardial infarction was carried out, and the ischemic and infarctional areas were traced. Ischemic areas were calculated as a percentage of the short cut surface on both sides of each slice and were averaged and multiplied by the weight of the slice. The total ischemic myocardial weight was summed up and expressed as a percentage of the left ventricular (LV) weight. The same method was applied to calculate the infarct size as a percentage of the LV weight. Twenty pieces (0.5–1 g) of the myocardium were obtained from the ischemic and normal areas for determination of tissue counts of ^{99m}Tc-PYP and ^{201}Tl, and we calculated the count ratio of the infarct or the ischemic areas to that of the normal areas.

Wall motion was measured by echocardiography (SVD-700; Shimazu, Kyoto, Japan). Wall motion and blood flow were evaluated at the time of control coronary occlusion 20, 60 and 120 min after reperfusion. Wall motion changes were recorded through the probe applied directly to the epicardial surface, and the endocardium was traced for analysis by the centerline method. Based on the chord index at the control time, the area of chord index at coronary occlusion was less than 2 s.d. from the mean of the normal dogs and was regarded as the ischemic area; subsequent changes in the chord index were examined.

Colored nonradioactive microspheres (E-Z Co Los Angeles, CA) were used to measure blood flow. At the time of control, coronary occlusion 20 and 120 min after reperfusion, about 2,000,000 microspheres/kg were injected into the catheter in the left appendage of the heart, and blood was simultaneously aspirated from the artery at a rate of 10 ml/min. The tissue and blood were routinely processed, and the number of microspheres was counted under a microscope. The absolute blood flow value was

obtained from the ratio of microspheres in the tissue to those in the blood.

All measured values were analyzed using Student's t-test, and serial changes using ANOVA. Values were expressed in terms of mean ± s.d., and p values of <0.05 were considered significant. Figures were expressed with mean ± s.e.e.

RESULTS

The heart rate, blood pressure and double products during the course are shown in Figure 1. Figure 2 shows the percent of size of area at risk and the percent of infarct size LV. The size of area at risk was 19.9% ± 4% after 10-min ischemia, 19.7% ± 2.9% after 30-min ischemia and 20.2% ± 5.1% after 60-min ischemia with no significant difference. No infarction was found after 10-min occlusion. As for 30-min ischemia, one dog was excluded because TTC
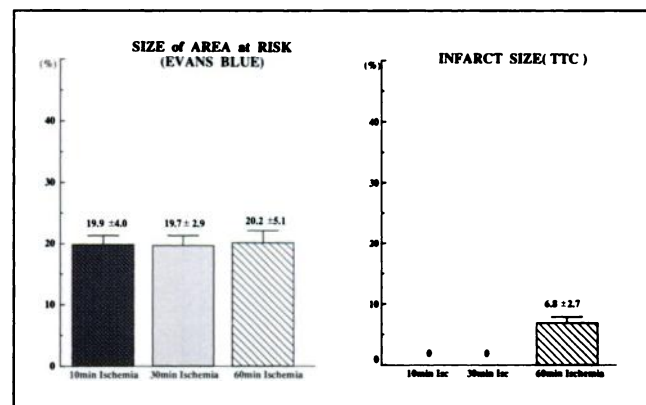

FIGURE 2. The size of area at risk defined with Evans blue (left) and the infarct size defined with TTC staining (right) are shown. Infarct was not detected in the group with 10-min and 30-min imaging of ischemia.
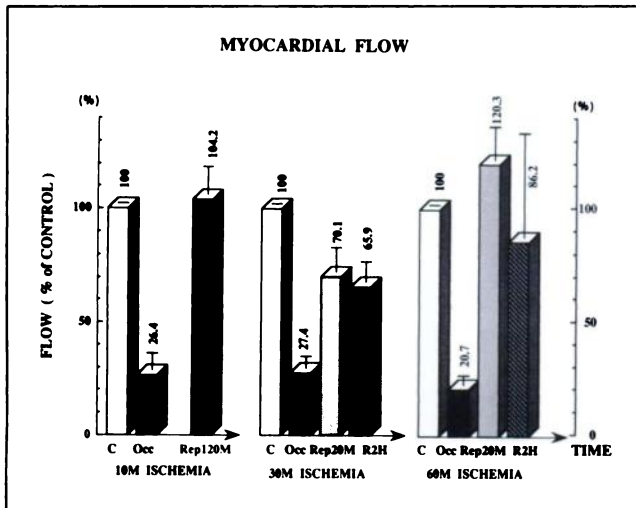
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FIGURE 3. Myocardial blood flow defined with colored microsphere in each stage (control, occlusion, 20 min and 120 min after reperfusion) is shown. Myocardial flow in 10-min ischemia after 20 min of reperfusion is not calculated or drawn here.

staining suspected small definite infarct in the ischemic area. Thus, in the group of 30-min ischemia, no dogs with definite infarct were included in this experiment. The infarct size after 60-min ischemia was 6.8% ± 2.7%.

Figure 3 shows changes in the RBF. When blood flow at the control time was regarded as 100%, blood flow during coronary occlusion was less than 30% in all ischemic protocols (10-min group: 26.4% ± 14.7%, 30-min group: 27.4% ± 13.9% and 60-min group: 20.7% ± 6.8%:NS). At 120 min after reperfusion, the blood flow recovered to the control level in the 10-min group, but it was 65.9% ± 23% in the 30-min group and 86.2% ± 46.2% in the 60-min group.

Figure 4 shows changes in regional wall motion. During coronary occlusion, the wall motion was less than 30% of that at the control time (10-min group: 21.7% ± 12.4%,
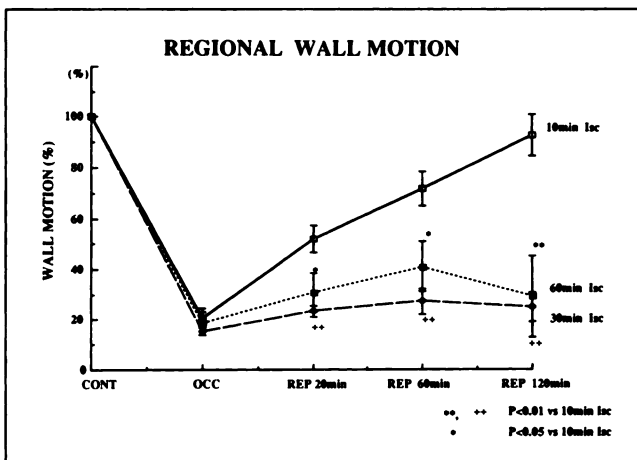

FIGURE 4. Sequential changes of regional wall motion were drawn in this figure. Compared to 10-min ischemia, dogs with 30- and 60-min ischemia showed significantly depressed recovery of wall motion after reperfusion. CONT-control, OCC-occlusion, REP-reperfusion.
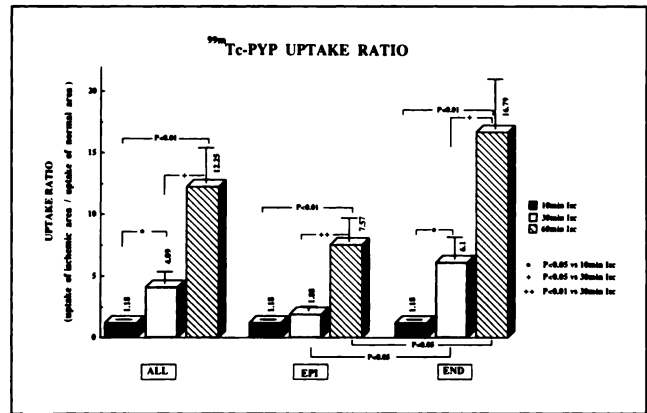

FIGURE 5. Technetium-99m-uptake ratio is shown in transmural (left), epicardial (middle) and endocardial (right) side. The number is the mean of each bar. ALL = transmural, EPI = epicardial, END = endocardial.

30-min group: 15.5% ± 3%, 60-min group: 18.5% ± 8.2%). After initiation of reperfusion, wall motion recovered to near the control level (93.2% ± 25.9%) 120 min later in the 10-min group. On the other hand, the value of wall motion in the 30-min and 60-min groups was significantly lower than that of the 10-min group at all time points, the value 2 hr after reperfusion was 25.1% ± 9.8% and 39.3% ± 28%, respectively.

Figure 5 shows the uptake ratio of ^{99m}Tc-PYP. The 60-min group developing infarction showed a significantly higher uptake ratio than that of the 10-min or 30-min ischemic groups. In 10-min ischemia, the uptake ratio compared to the normal area increased 1.18 ± 0.09. In 30-min ischemia, the uptake ratio was 4.09 ± 1.75, a value significantly higher than that of 10-min ischemia. At this time, the uptake ratio in the epicardium increased 1.88 ± 0.7, a value which was not significantly different from that of 10-min ischemia (1.18 ± 0.04). In the endocardium, the uptake ratio showed 6.1 ± 3.25, significantly higher than that of 10-min ischemia or that of the epicardium. In 30-min ischemia without infarction, imaging was already possible in vivo, as well as ex vivo (Fig. 5). In vivo imaging was possible when the uptake ratio of ^{99m}Tc-PYP was more than 2.1.

DISCUSSION

A report indicated that ^{99m}Tc-PYP is incorporated into infarcted myocardium, reflecting the Ca^{2+} level that has increased in necrotic myocardial cells, as does tritiated diphosphonated(^{3}H-EHDP), an imaging agent of the bone (2). This finding suggests that ^{99m}Tc-PYP is useful in quantitative and diagnostic tests of infarcted myocardium. It is now used for the diagnosis of acute phase myocardial infarction and quantification of the infarct size in the clinical setting. However, a clinical question about possible ^{99m}Tc-PYP uptake in angina pectoris, as well as infarction, has been raised.

Willerson et al. (3) observed positive findings in the planar image of ^{99m}Tc-PYP in seven of 101 patients without

infarction and reported that all seven patients had unstable angina pectoris. Abdulla et al. (5) examined 17 patients with chest pain using ^{99m}Tc-PYP and observed negative findings in the ten patients with stable angina and a significant uptake in all seven with unstable angina. They reported that this agent was a useful tracer for assessment of myocardial abnormalities in unstable angina pectoris. Jaffe et al. (4) compared the ^{99m}Tc-PYP images with plasma creatine kinase, MB isoenzyme (MB-CK) activity. Their report suggested that abnormal uptake of ^{99m}Tc-PYP images with unstable angina generally indicates myocardial necrosis (4). They report that only 8% could be classified as having positive scans without MB-CK elevation, however, the definition of unstable angina does not always mean active severe ischemia, and the ability of conventional gamma camera to detect ^{99m}Tc-PYP, especially at endocardial side, may limit the result.

Lessen et al. (6) obtained positive findings in 65 of 85 patients with unstable angina pectoris and speculated that ^{99m}Tc-PYP cannot be a predictor of prognosis, but is useful for the differential diagnosis of myocardial infarction (MI) and unstable angina pectoris. This imaging agent is also incorporated in injured myocardial cells other than infarction. To what extent does it reflect ischemia, cell injury or myocardial viability? Horner et al. (10) produced ischemia of 25, 40, 60 and 120 min in isolated rabbit hearts and examined changes in the ratio of uptake of ^{99m}Tc-PYP in each ischemia. They found the uptake ratio correlated well with functional and structural changes in the myocardium.

Bianco et al. (11) observed a significant increase in uptake after 40-min ischemia in a canine model of ischemia. In addition, like the infarction model (1), the uptake in the peripheral area of infarction with blood flow was markedly high, but in the central area of infarction with decreased or no blood flow, there was little or no increase in uptake. These findings suggest that ^{99m}Tc-PYP uptake reflects blood flow and viability. In a clinical study, Nishiyama et al. (12) reported that ^{99m}Tc-PYP as an MI imaging agent is taken up in the defect areas of ^{201}Tl. However, we previously found that there are overlapping images of ^{99m}Tc-PYP and ^{201}Tl in patients with AMI in whom acute phase reperfusion therapy (PTCR, PTCA) was successful, resulting in improvement in the blood flow (13).

Our previous study was based on the following questions: What trend does the uptake of ^{99m}Tc-PYP reflect in patients with unstable angina pectoris and those undergoing reperfusion in the acute phase of AMI? What is the severity of hemodynamic disorder when a significant uptake of this agent occurs? How does the uptake ratio change according to the duration of ischemia? In this study, we produced models of ischemia for 10 min (a duration shorter than the previous reports), 30 min and 60 min for examination of changes in the uptake of ^{99m}Tc-PYP, their relationships to the blood flow, cardiac function, and the mechanisms of these relationships. In 10-min ischemia, there was already a 1.18-fold increase in the uptake and the uptake ratio, more than 2.1 permitted imaging. In vivo

imaging was also highly feasible in 30-min ischemia without infarction. These findings suggest the applicability of ^{99m}Tc-PYP to the assessment of severity of ischemic injured area and viability of ischemic myocardium, even if wall motion is suppressed in the same fashion as infarct.

The precise mechanism of uptake for ^{99m}Tc-PYP, even after short ischemia, remains to be known. However, several reports suggest the uptake mechanism of ^{99m}Tc-PYP to be ischemic myocardium without infarct. Chien et al. (8) report membrane phospholipid metabolic alterations in ischemic canine myocardium. Phospholipid degradation, even without depleting total phospholiid content, results in a greater than 50% increase in rat Ca^{2+} efflux from vesicles preloaded by $Na^+:Ca^{2+}$ exchange. In addition to this finding, they reported that sarcolemmal vesicles isolated from ischemic myocardium after 3 hr of LAD occlusion have an increased Ca^{++} permeability and phosphatidyl choline depletion. Thus, phospholipid depletion during ischemia creates a Ca^{++} permeability defect resulting in increased intracellular Ca^{2+} and subsequent accumulation of ^{99m}Tc-PYP. Several reports indicate phospholipid metabolic disorders even after a short duration of ischemia (14,15), and reversal metabolic charge with Ca^{++} permeability disorder may be followed by ^{99m}Tc-PYP uptake. Nohara et al. (9) report the effect of diltiazem in enhancing the suppression of ^{99m}Tc-PYP in stunned myocardium without infarct. Thirty-minute ischemia followed by 2 hr of reperfusion showed 5.0 times higher uptake of ^{99m}Tc-PYP, however, pretreatment and post-treatment of diltiazem suppressed the uptake to 1.1 and 2.3, respectively, and improved stunning. These experimental data strongly support the Ca^{++} permeability change to be the cause of ^{99m}Tc-PYP uptake in ischemic myocardium without infarct, possibly after phospholipid metabolic alteration.

CONCLUSION

The uptake of ^{99m}Tc-PYP showed an increase in short ischemia, as well as infarction, permitting imaging even after 30-min ischemia without infarction. Ten-minute ischemia was enough to increase ^{99m}Tc-PYP uptake in areas of ischemia, however, it may not be sufficient for imaging by gamma camera. These findings indicate that ^{99m}Tc-PYP is also useful for the assessment of severity and area of ischemia and myocardial viability with the use of flow tracer. Precise mechanisms in the uptake of ^{99m}Tc-PYP, including membrane phospholipid and Ca^{++} metabolic disorder, remain unknown in short ischemia.

REFERENCES

1. Willerson JT, Buja LM, Stokely EM, et al. Infarct sizing in awake, unsedated dogs with acute anterior myocardial infarcts [Abstract]. J Nucl Med 1976;23:534.
2. Tofe AJ, Buja LM, Parkey RW, Bonte RJ, Willerson JT. Scitigraphy of canine myocardial infarcts: role of calcium and Tc-99m diphosphonate/pyrophosphate bone agents [Abstract]. J Nucl Med 1976;23:534.
3. Willerson JT, Parkey RW, Bonte FJ, Meyer SL, Atkins JM, Stokely EM. Technetium stanous pyrophosphate myocardial scintigrams in patients with chest pain of varying etiology. Circulation 1975;51:1046-1052.
4. Jaffe AS, Klein MS, Patel BR, Siegel BA, Roberts R. Abnormal technetium-

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99m pyrophospate images in unstable angina: ischemia versus infarction? *Am J Cardiol* 4:1035–1039, 1979.

5. Abdulla AM, Canedo MI, Cortez BC, Wilhelm SK. Detection of unstable angina by ^{99m}Tc-pyrophosphate myocardial scintigraphy. *Chest* 1976;69: 168–173.

6. Lessen J, Johanson BW, Nosslin B, Thorell J. Myocardial scintigraphy with ^{99m}Tc-pyrophosphate in patients with unstable angina pectoris. *Acta Med Scand* 1978;203:491–495.

7. Jansen DE, Corbett JR, Buja M, et al. Quantification of myocardial injury produced by temporary coronary artery occlusion and reflow with ^{99m}Tc-PYP. *Circulation* 1987;75:611–617.

8. Chien K, Reeres JP, Buja M, Bonte F, Parkey R, Willerson J. Temporal and topographic correlations with Tc-99m-ppi accumulation and an in vitro sarcoleminal Ca^{++} permeability defect. *Circ Res* 1981;48:711–719.

9. Nohara R, Kambara H, Okuda K, et al. Effect of diltiazem on stunned myocardium evaluated with ^{99m}Tc-PYP imaging in canine heart. *Jpn Circ J* 1992;262–271.

10. Horner WH, Alonso DR, Jacobstein JG. Ischemia and infarction in the isolated rabbit heart: a model for the evaluation of myocardial imaging agents. *J Nucl Med* 1980;21:523–528.

11. Bianco JA, Kemper AJ, Taylor A, Lazewatsky J, Tow DE, Khuri SF. Technetium-99m(Sn^{2+})pyrophosphate in ischemic and infarcted dog myocardium in early stages of acute coronary occlusion: histochemical and tissue-countings. *J Nucl Med* 1983;24:485–491.

12. Nishiyama H, Sodd V, Kahn VDB, et al. Comparison of positive and negative localization of MI in dogs [Abstract]. *J Nucl Med* 1976;23:534.

13. Hashimoto T, Kambara H, Fudo T, et al. Significance of technetium-99m/thallium-201 overlap on simultaneous dual-emission computed tomography in acute myocardial infarction. *Am J Cardiol* 1988;61:1181–1186.

14. Shaikh NA, Downar E. Time course of changes in porcine myocardial phospholipid levels during ischemia. *Circ Res* 1981;49:316–325.

15. Sobel BE, Corr PB, Goldstein RA, Wiktowski FX, Klein MS. Accumulation of lysophosphoslycerides with arrythmogenic properties in ischemic myocardium. *J Clin Invest* 1978;62:546–553.