Uptake Kinetics of Technetium-99m-Methoxyisobutylisonitrile and Thallium-201 in Adult Rat Heart Endothelial and Fibroblast-like Cells in Comparison to Myocytes

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Endothelial and fibroblast-like cells comprise 5%–10% of total cardiac cell volume and are more resistant to hypoxia than cardiomyocytes. However, their role in the uptake of radiotracers used for myocardial perfusion imaging has largely been ignored. Net uptake of ^{201}Tl and ^{99m}Tc-hexakis-2-methoxyisobutylisonitrile (^{99m}Tc-sestamibi) by cultured rat heart endothelial and fibroblast-like cells and quiescent myocytes was examined. Over a 30-min period, endothelial cells continuously accumulated ^{99m}Tc-sestamibi. Initial ^{201}Tl accumulation paralleled that of ^{99m}Tc-sestamibi; however, after 7 min ^{201}Tl accumulation plateaued whereas there was continued slow accumulation of ^{99m}Tc-sestamibi. Net uptake of the two radiotracers by fibroblast-like cells was similar to that of ^{201}Tl by endothelial cells. In the quiescent myocytes, there was an initial accumulation of both tracers with ^{201}Tl net uptake quickly reaching a plateau, whereas ^{99m}Tc-sestamibi accumulation continued throughout the 30-min period reaching a level 15-fold greater than that for ^{201}Tl. Myocyte uptake of ^{99m}Tc-sestamibi was approximately eight times greater than by the endothelial cells when expressed either as activity per mg protein or activity per cell volume. Although significant uptake differences exist among the three cell types, the fact that there is uptake by endothelial and fibroblast-like cells should be considered during image interpretation, particularly in situations in which the hypoxia resistant endothelial and fibroblast-like cells might be the remaining healthiest cell types.

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In the mammalian heart, endothelial cells and fibroblast-like cells comprise 5%–10% of the total heart cell volume and these cells are more resistant to the effects of ischemia and hypoxia than myocytes (*1–3*). Following myocardial infarction, there often continues to be a network of capillaries throughout the scar and the capillary endothelial cells have active Na-K pumps. Thus, under these circumstances, endothelial cells and fibroblast-like cells could take up a significant fraction of the injected radionuclides commonly used for myocardial perfusion imaging, ^{201}Tl and ^{99m}Tc-hexakis-2-methoxyisobutylisonitrile (^{99m}Tc-sestamibi). Furthermore, endothelial cells may be an important transport barrier, especially for ^{99m}Tc-sestamibi (*4*). Little quantitative information is available, however, regarding the uptake kinetics of these important tracers by endothelial and fibroblast-like cells. Therefore, the purpose of this study was to describe the net uptake of ^{201}Tl and ^{99m}Tc-sestamibi by isolated adult rat heart endothelial and fibroblast-like cells in comparison to uptake by isolated quiescent cardiomyocytes.

MATERIALS AND METHODS

Cell Isolation

Adult Rat Heart Endothelial Cells (RCEC). RCEC were isolated from 250–300-g Lewis rats using a modification of the method of Diglio as described previously (*5,6*). On the morning of the study, perfusion solutions were prepared by adding 5 ml 1.1 *M* glucose and 5 ml of 400 m*M* taurine, 100 µg streptomycin and 1 ml of 500,000 U/ml penicillin to 500 ml of modified Krebs-Ringer Buffer (MKRB) (115 m*M* NaCl, 2.6 m*M* KCl, 1.2 m*M* KH_2PO_4, 1.2 m*M* $MgSO_4$, 10 m*M* $NaHCO_3$, 10 m*M* 2-(4-(2-Hydroxy Ethyl)-1-Piper-azinyl)-Ethane-Sulfonic acid [HEPES]). The solution was placed in a (37°C) water bath and bubbled with Carbogen (95% O_2 and 5% CO_2).

The heart was removed under ether anesthesia and transferred immediately to a Petri dish containing an ice cold solution of MKRB. Fat was removed along with the pulmonary vessels and connective tissue and the heart attached to the perfusion apparatus where it was perfused with MKRB for 10 min at 10 ml/min. The epicardium was washed with 70% ETOH to fix the epicardial cells to prevent contamination of the RCEC cultures.

After a 10-min perfusion with enzyme-free MKRB, the per-

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fusate was switched to MKRB with collagenase (30 mg collagenase and 12.5 μl 100 mM Ca^{++} in 50 ml MKRB) and perfused for 25 min by recirculating the oxygenated MKRB. The recirculation was stopped and the perfusate switched to collagenase-free MKRB. Perfusion was continued until 50 ml of solution had been run through the heart. The perfusate then was collected in a sterile centrifuge tube. The collected solution was spun at 200 g for 5 min. The pelleted cells were resuspended in 10 ml RCEC working medium (400 ml M199 Earle's salt, 400 ml RPMI 1640, 10 ml 200 mM L-glutamine, 1 ml 5% gentamicin and 2.5 mg amphotericin B, 20,000 units heparin, 4.0 ml bovine brain extract, and 200 ml inactivated fetal calf serum), centrifuged, resuspended in culture medium and plated out on 3.5 cm diameter culture dishes precoated with fibronectin.

Three days after the initial isolation and plating, the cells were covered with growth medium (working medium minus amphotericin B) and allowed to grow to confluence at 37°C under humidified Carbogen gas. After the cells reached confluence, they were subcultured until enough plates were available for the radiotracer studies. Uptake studies were performed on cells having been passaged 3–5 times. Prior to studies, an aliquot of cells from one plate was removed and tested with antisera to Factor VIII antigen, monoclonal antibodies to rat lymphatic endothelial cells, and for evidence of acetylated low-density lipoprotein uptake to confirm they were pure endothelial cells that retained their functional characteristics (*6*).

Rat Cardiac Fibroblast-like Cells (RCFLC). RCFLC were isolated after removing the great vessels, atria, and as much connective tissue as possible from the heart described above. The heart was then transferred to a small, sterile petri dish, and minced very finely with a scalpel. The minced heart was placed in 20 ml of MKRB containing 5 μl of 100 mM Ca^{++}, 12 mg collagenase and 200 mg of fatty acid free albumin and incubated for 25 min at 37°C while bubbling with Carbogen. After the tissue was maximally dissociated, the solution was filtered through a 200-μm nylon filter and centrifuged at 67 g for 1.5 min at room temperature. The supernatant was transferred to a centrifuge tube to which 20 ml of RCEC working medium were added and the solution was centrifuged at 200 g for 5 min. This was repeated once. The pellet was resuspended in RCEC working medium and plated on 10-cm diameter petri dishes precoated with fibronectin. These cells were then cultured in the same manner as the RCEC and tested for absence of RCEC-like functional characteristics.

Adult Rat Heart Myocytes (RCMC). To isolate myocytes, the pellet produced during the first centrifugation during the RCFLC isolation (see above) was resuspended in 50 ml MKRB and 100 μl Ca^{++} (100 mM) and then centrifuged for 1.5 min at 67 g. This pellet was resuspended in 10 ml MKRB and 50 μl Ca^{++} (100 mM) and allowed to settle for 15 min. The pellet was resuspended a third time in 25 ml MKRB and 250 μl Ca^{++} (100 mM) and allowed to settle for 15 min. This pellet was resuspended in 5 ml of RCMC plating medium (190 ml M199 Earle's salt, 2 ml 200 mM L-glutamine, 2.3 ml 7.5% $NaHCO_3$, 2 ml 1 M HEPES, 20,000 units penicillin, 20 mg streptomycin, 8 ml inactivated fetal calf serum) and an aliquot of the suspension was obtained for determination of total number of cells and number of rod-shaped cardiomyocytes using a counting chamber. The suspension was diluted with plating medium to give approximately 10^5 myocytes/ml, which were plated out on 3.5-cm dishes precoated with RCMC plating medium. After allowing 4 hr for adherence, the medium was removed along with nonadherent cells by suction, covered with fresh medium and incubated overnight at 37°C in a humidified Carbogen atmosphere.

Study Protocol

Thallium and ^{99m}Tc-Sestamibi Kinetics. Basal Uptake: The culture medium was removed from 22 plates each of the RCEC, RCFLC, and RCMC, the plates were washed twice with Hank's Balanced Salt Solution (HBSS) and allowed to equilibrate for 15–30 min at 30–35°C. This solution was then replaced with 1 ml HBSS containing 2.66 ± 1.6 μCi ^{201}Tl and 120 ± 27 μCi ^{99m}Tc-sestamibi. Duplicate plates were incubated with radiotracers for each of the following times: 1, 3, 5, 7, 9, 11, 13, 15, 20 and 30 min. Ten seconds before removal of the radioactive solution a 100-μl aliquot was taken and saved for counting. The radiolabeled HBSS was removed from a plate, the plate rinsed twice with nonradioactive 4°C PBS, and the cells killed with 1 ml of 4°C, 0.11 M NaOH. The cells were mechanically loosened from the plate and transferred to a counting tube. To permit correction for nonspecific binding to the plates, duplicate plates that had been coated with the plating medium but without cells were treated in the same manner (control plates). To determine if non-metabolic or background binding to the cells occurred, duplicate plates containing cells were cooled to 4°C after which 1 ml of 4°C radioactive solution was added to the plates for 1 min and then removed. The plates were handled in an identical manner to the iced plates.

Data Analysis. All samples were counted in a gamma well counter with a 3-in. NaI detector. The initial counting parameters were a peak at 140 keV with a 20% window to determine ^{99m}Tc activity. After allowing two to four days for technetium decay, the samples were again counted using a peak of 70 keV with a 50% window for the ^{201}Tl. Along with standards for each radionuclide, a 1-ml aliquot of the radiolabeled medium was counted. All samples from each set of plates were decay corrected to the time of determination of specific activity of the standards solutions at the beginning of an experiment. After counting, the protein content of each sample was determined in duplicate using the Bradford method (*7*).

Data were corrected in two ways: (a) for nonspecific binding by subtracting the average activity on the control plates ($n = 2$) for an individual study from the activity on cell containing plates from that study and expressing the remaining activity as CPM/mg protein; and (b) for non-metabolic or background binding by subtracting the CPM on the control plates from the CPM on the iced plates and dividing the remaining activity by mg protein on the iced plates. This remaining activity represents a reasonable estimate of the non-metabolic binding of tracer to the cells, although some minimal residual metabolism may have remained at the temperature used, 4°C. The non-metabolic binding activity as determined above was then subtracted from the CPM/mg protein on each plate corrected for nonspecific binding as determined in (a). Activity on each cell plate was then expressed as a ratio of (CPM/mg protein)/(CPM/ml supernatant). Using this ratio assumes there is a constant but unknown relationship between mg protein and cell volume.

To determine if differences in cell size accounted for differences in uptake among the cell types (vide infra), the data was also expressed as (CPM/ml cell volume)/(CPM/ml supernatant). For myocytes, cell volume was assumed to be 0.0055 ml/mg protein based on an average of the value reported by Farmer et al. of 0.0050 ml/mg protein and unpublished work from our laboratory (0.0059 ml/mg protein) (*8*). Endothelial cell volume

was assumed to be 0.0108 ml/mg protein calculated from the data of Anversa et al. and from Simionescu and Simionescu (1, 9). Fibroblast-like cell volume was assumed to be equal to endothelial cell since the two cell lines histologically appear similar in size and no literature estimates of volume could be found.

Statistics. Corrected ^{201}Tl activity, both as (CPM/mg protein)/(CPM/ml supernatant) and (CPM/ml cell volume)/(CPM/ml supernatant), for all cell plates (n = 3–5) at each time point was compared to the ^{99m}Tc-sestamibi activity on the same plates using analysis of variance with the Scheffe procedure for multiple comparisons and a significance level of $p < 0.05$.

RESULTS

For endothelial cells, initial ^{201}Tl and ^{99m}Tc-sestamibi accumulation, corrected only for nonspecific binding on the control plates, was in parallel (Fig. 1). Subsequently, ^{201}Tl net accumulation plateaued, whereas there was continued uptake of ^{99m}Tc-sestamibi, such that a statistically significant difference existed at 30 min. (By simple paired t-testing, these differences were significant by 11 min.) This was true when the data were expressed in terms of (CPM/mg protein)/(CPM/ml supernatant) or when corrected for cell volume (Fig. 1). Subtraction of the non-metabolic uptake contribution to net accumulation did not change this relationship. As shown in Table 1, the difference between mean endothelial cell net accumulation corrected only for nonspecific binding and mean net accumulation corrected for non-metabolic uptake was very small for both ^{201}Tl and ^{99m}Tc-sestamibi.

The initial rate of ^{201}Tl net accumulation by the fibroblast-like cell (corrected only for nonspecific binding) was slower than by endothelial cells (Fig. 2). However, the net accumulation during the later time periods was similar to that for the endothelial cell. Technetium-99m-sestamibi accumulation (both rate and net) was markedly reduced in the fibroblast-like cell and not statistically different from ^{201}Tl. Correction for estimated cell volume did not change these observations. There was a small but statistically significant difference between ^{99m}Tc-sestamibi net accumulation corrected only for nonspecific binding compared to accumulation corrected for the non-metabolic component as shown in Table 1. This was not so for ^{201}Tl.

TABLE 1
Effect of Correction for Non-metabolic Uptake*

	Endothelial cell	Myocyte	Fibroblast-like cell
Thallium	0.0026 ± 0.01	0.0199 ± 0.01[†]	0.0176 ± 0.00
Sestamibi	0.0032 ± 0.02	0.0497 ± 0.09	0.0195 ± 0.00[†]

* Values shown are mean (± s.d.) differences between [(CPM/mg protein)/(CPM/ml supernatant)] for the samples corrected only for nonspecific binding (control plates) and after correction for non-metabolic binding (cells at 4°C).

[†] $p \leq 0.05$ for correction only for nonspecific binding versus correction for non-metabolic binding.

Net ^{201}Tl accumulation by quiescent myocytes was similar to that by endothelial cells (Fig. 3), whereas net ^{99m}Tc-sestamibi accumulation was progressive throughout the observation period reaching a 30-fold greater value by 30 min. Correction for non-metabolic accumulation or for myocyte volume did not change this relationship. In one study, uptake was observed through 60 min with accumulation continuing in an almost linear fashion (data not shown).

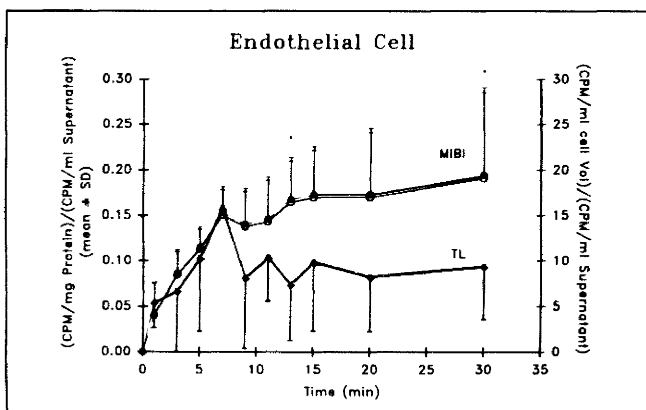

FIGURE 1. Radiotracer uptake by isolated, cultured endothelial cells is shown as a function of time after placement of tracer on the cell plate. There were three to five plates at each time point. Activity expressed in CPM/mg protein per CPM/ml of radiolabeled incubation medium is indicated by the solid symbols and related to the left vertical axis and [(CPM/ml cell vol)/(CPM/ml supernatant)] is indicated by the open symbols and related to the right vertical axis. * = $p \leq 0.05$.

DISCUSSION

Myocardial perfusion imaging with the gamma-emitting radionuclide, ^{201}Tl, is a clinically useful method for diagnosing coronary artery disease, evaluating the extent of coronary disease, or evaluating myocardial viability. This agent is delivered to and deposited in the myocardium in proportion to myocardial blood flow at low-to-intermedi-

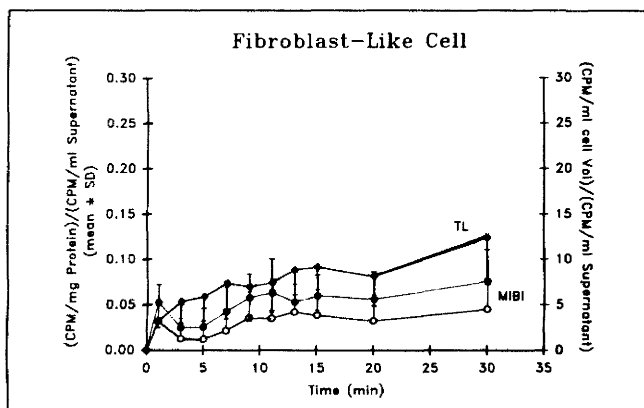

FIGURE 2. Fibroblast-like cell uptake of radiotracers is shown as a function of duration of incubation. Abbreviations and legend as in Figure 1.

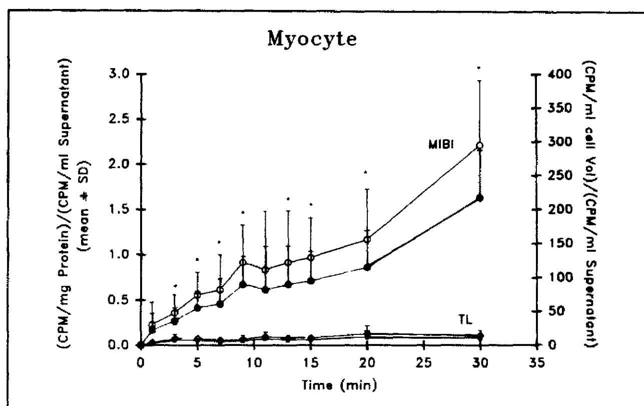

FIGURE 3. Uptake of radiotracers by isolated, quiescent myocytes expressed in as a function of duration of incubation. Abbreviations and legend as in Figure 1.

ate levels of flow when compared to microspheres (*10*). Numerous studies have suggested that ^{201}Tl behaves similarly to potassium relative to uptake and clearance from the myocardium with a large fraction (>60%) of uptake being a Na-K ATPase-dependent process (*11–13*). Uptake of another large fraction is thought to be ATP-dependent but not related to a monovalent ion gradient (*11*). Recently, these concepts have been challenged by Maublant et al. who have suggested that for thallium the uptake is largely independent of metabolism and that the in vivo deposition is largely a passive, flow-dependent phenomenon (*14*). Work by others using isolated perfused hearts and intact animals would support this position (*15,16*). However, no explanation is provided by Maublant as to the mechanism of uptake other than speculation that ^{201}Tl uptake is related to the metabolic functions responsible for maintaining cell membrane integrity and unrelated to mitochondrial metabolism. Neither the mechanism nor the kinetics of ^{201}Tl uptake in the cardiac endothelial or the fibroblast-like cell has been evaluated.

There are many ^{99m}Tc-isonitrile compounds that have been evaluated as potential myocardial perfusion imaging agents. These data have been reviewed recently (*17–19*). One of the most clinically promising is ^{99m}Tc-hexakis-2-methoxyisobutylisonitrile), an ether substituted analogue, referred to by several names such as ^{99m}Tc-sestamibi, RP-30, Cardiolite and others. The study of Maublant et al. in myocyte cultures suggest that its uptake and clearance is independent of cellular metabolism and was postulated to be related to lipophilicity (*14*). In this study, uptake was linear with time during the first 45 min and then demonstrated equilibrium conditions between 2–3 hr. Half-time for clearance from the cells was 28 min. However, in vivo studies have suggested that there is little washout over a 4-hr period (*20,21*). In another study of a slightly different compound (^{99m}Tc-carbomethoxyisopropylisonitrile, ^{99m}Tc-CPI), uptake was similar but washout much faster. The majority of ^{99m}Tc-CPI compound was found in the cell membrane fragment with only 9% in the mitochondria and 25% in the cytosol (*22*). When examined, no evidence of receptor binding was found (*22*). In a preliminary report, it was suggested that the ^{99m}Tc-sestamibi compound is bound to a cytosolic protein (molecular weight ~10,000 D) (*23*). In the study of Maublant, no significant change in kinetics was seen when the cells were exposed to agents that selectively affected various aspects of cell membrane transport. However, in a study by Piwnica-Worms et al., in which more severe conditions of transport inhibition were used, initial net uptake was not affected; however there was suppression at 60 min (*24*). The data from Chiu et al. strongly suggest that uptake is a function of the cell mitochondrial membrane potential (*25*). Thus, conflicting mechanisms of trapping are reported. Furthermore, all these studies have been done in either chick embryo myocytes, neonatal rat myocytes, or mouse fibroblasts that may have different characteristics of membrane kinetics and integrity compared to adult myocytes. Prior to our study, no data has been available relative to ^{99m}Tc-sestamibi kinetics in the endothelial cell or fibroblast-like cell.

Diglio et al. have reported a method for obtaining pure cultures of both endothelial cells and smooth muscle cells from the adult rat heart (*5*). These cultures can be maintained through multiple passages for an extended period of time without loss of functional or structural characteristics. The endothelial and fibroblast-like cells used in this study were isolated and cultured using a modification of the Diglio method that we have recently reported (*6*).

From our data, it is very evident that there are marked differences between myocytes and endothelial and fibroblast-like cells relative to the net accumulation of radiotracers commonly used for myocardial perfusion imaging as well as major differences between ^{201}Tl and ^{99m}Tc-sestamibi uptake. Net ^{201}Tl uptake by all three cell lines during the first 10 min of incubation with a subsequent equilibrium is not an unexpected finding given the potassium analogue characteristics of ^{201}Tl. The time to plateau for ^{201}Tl uptake was earlier than reported by other investigators but was a consistent finding for all our cell lines (*11, 12*). Unpublished data from a pilot study in our laboratory demonstrated the time to plateau for ^{201}Tl uptake by myocytes shifted from 10 to 20 min after onset of incubation when the cells were studied in the quiescent state and then stimulated at a rate of 280 times/min. This level of stimulation did not affect ^{99m}Tc-sestamibi uptake kinetics although measured oxygen consumption increased approximately 25%.

Technetium-99m-sestamibi accumulation was progressive over the 30-min incubation for both the endothelial cell and myocyte, but not for the fibroblast-like cell. This suggests a large apparent volume of distribution because of binding, or deposition in a compartment with high solubility (partition coefficient) for ^{99m}Tc-sestamibi or an active accumulation process. Additionally, myocyte accumulation of ^{99m}Tc-sestamibi was more than 8-fold greater than by endothelial cells. The recent finding of Chiu et al.

that ^{99m}Tc-sestamibi accumulation in mouse fibroblast cells is a function of the mitochondrial and plasma membrane potential could explain our results (*25*). If the accumulation is driven to a greater extent by the mitochondrial rather than by the plasma-membrane potential, then the myocyte, which has a much larger mitochondrial volume than the endothelial cell, would take up a much greater amount of ^{99m}Tc-sestamibi (*26*). Since both myocytes and endothelial cells presumedly have a much greater metabolic need than fibroblast-like cells, they should have a relatively larger mitochondrial to total cell volume ratio and thus greater total membrane potential to account for their greater ^{99m}Tc-sestamibi accumulation.

Our findings of significant net accumulation of ^{99m}Tc-sestamibi by endothelial cells also may explain in part the findings of Leppo and Meerdink. Using the multi-indicator dilution technique, they observed that the myocyte permeability-surface area product (PS_{pc}) for ^{99m}Tc-sestamibi was 50 times greater than that for the capillary wall (PS_c) (*4*). However, they did not account for the endothelial cell. If the endothelial cell avidity for ^{99m}Tc-sestamibi is high, as our data suggests, then there is little tracer reflux. Failure to account for endothelial uptake (either by virtue of a large volume of distribution or by chemical transformation and retention) forces the estimates of PS_{pc} to be artifactually too high. Nevertheless, the true PS_{pc} is likely to be relatively large given the magnitude of myocyte uptake we observed. Furthermore, if the endothelial cell is acting as a barrier to ^{99m}Tc-sestamibi ingress to the interstitial space and ultimately the myocyte, this could account in part for the relatively low extraction fraction observed by Leppo and Meerdink and by other investigators in spite of the large accumulation by isolated myocytes.

Endothelial uptake and trapping of ^{99m}Tc-sestamibi could also account in part for the reported higher uptake relative to microsphere measured flow in low-flow reactions of the myocardium (*27,28*). In addition, the postulated longer transit times in low-flow regions allowing greater net uptake and the relatively great resistance of the endothelial cell to ischemia would permit maintenance of the membrane potential and continued uptake even with reduced myocyte uptake.

The observation that there is often a small amount of residual activity seen in images of patients with prior myocardial infarction has been attributed to partial volume effect and scatter from activity in the blood pool. Since there are frequently large numbers of capillaries scattered throughout scar tissue, uptake of both ^{201}Tl and ^{99m}Tc-sestamibi by endothelial cells could account for a portion of this observed activity. In other clinical settings, such as during acute myocardial infarction or following thrombolytic therapy in which there might be relative sparing of the endothelial cells, uptake, which has been attributed to myocardial viability, might partially reflect radiotracer uptake by endothelial and to a lesser extent fibroblast-like cells.

In summary, we have shown that the endothelial cell takes up both ^{201}Tl and ^{99m}Tc-sestamibi, whereas there is minimal uptake by the fibroblast-like cell. Although the endothelial contribution to total cardiac radiotracer uptake must be small for anatomic reasons, it could have a greater role if it becomes a relatively large portion of metabolically active tissue as can occur in some ischemic/infarct states. Thus, endothelial uptake of ^{201}Tl and ^{99m}Tc-sestamibi should be considered in interpretation of myocardial perfusion images. The role of the endothelial cell as a transport barrier, especially for ^{99m}Tc-sestamibi, warrants further investigation.

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REFERENCES

1. Anversa P, Olivetti G, Melissari M, Loud AV. Stereological measurement of cellular and subcellular hypertrophy and hyperplasia in the papillary muscle of adult rat. *J Mol Cell Cardiol* 1980;12:781–795.
2. Mall G, Mattfeldt T, Rieger P, Volk B, Frolov VA. Morphometric analysis of the rabbit myocardium after chronic ethanol feeding-early capillary changes. *Basic Res Cardiol* 1982;77:57–67.
3. Buderus S, Siegmund B, Spahr R, Krutzfeldt A, Piper HM. Resistance of endothelial cells to anoxia/reoxygenation in isolated guinea pig hearts. *Am J Physiol* 1989;257:H448–H493.
4. Leppo JA, Meerdink DJ. Comparison of the myocardial uptake of a technetium-labeled isonitrile analogue and thallium. *Circ Res* 1989; 65:632–639.
5. Diglio CA, Grammas P, Giacomelli F, Wiener J. Rat heart-derived endothelial and smooth muscle cell cultures: isolation, cloning and characterization. *Tissue & Cell* 1988;20:477–492.
6. Linssen MCJG, Vork MM, de Jong YF, Glatz JFC, van der Vusse GJ. Fatty acid oxidation capacity and fatty acid-binding protein content of different cell types isolated from rat heart. *Mol Cell Biochem* 1990;98:19–25.
7. Bradford. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Chem* 1976;72:248–254.
8. Farmer BB, Mancina M, Williams ES, Watanabe AM. Isolation of calcium tolerant myocytes from adult rat heart: review of the literature and description of a method. *Life Science* 1983;33:1–18.
9. Simionescu M, Simionescu N. Isolation and characterization of endothelial cells from the heart microvasculature. *Microvasc Res* 1978;16:426–452.
10. Nielsen AP, Morris KG, Murdock R, Bruno FP, Cobb FR. Linear relationship between the distribution of thallium-201 and blood flow in ischemic and nonischemic myocardium during exercise. *Circulation* 1980;61:797–801.
11. Hunter DR, Haworth RA, Goknur AB, Hege JO, Berkoff HA. Control of thallium and sodium fluxes in isolated adult rat heart cells by anthopleurin-A, verapamil, and magnesium. *J Mol Cell Cardiol* 1986;18:1125–1132.
12. McCall D, Zimmer LJ, Katz AM. Kinetics of albumin exchange in cultured rat myocardial cells. *Circ Res* 1985;56:370–376.
13. Rauch B, Kubler W. Kinetics of cellular uptake of tracers used in myocar-

dial scintigraphy. *Basic Res Cardiol* 1985;80(suppl 1):65–68.
14. Maublant JC, Gachon P, Moins N. Hexakis (2-methoxy isobutylisonitrile) technetium-99m and thallium-201-chloride: uptake and release in cultured myocardial cells. *J Nucl Med* 1988;29:48–54.
15. Leppo JA, Macneil PB, Moring AF, Apstein CS. Separate effects of ischemia, hypoxia, and contractility on thallium-201 kinetics in rabbit myocardium. *J Nucl Med* 1986;27:66–74.
16. Okada RD, Pohost GM: The use of preintervention and postinterventon thallium imaging for assessing the early and late effects of experiment coronary arterial reperfusion in dogs. *Circulation* 1984;69:1153–1160.
17. Gerundini P, Maffioli L. Cationic complexes of technetium for myocardial imaging. *J Nucl Med* 1989;30:1415–1419.
18. Heo J, Hermann GA, Iskandrian AS, Askenase A, Segal BL. New myocardial perfusion imaging agents: description and applications. *Am Heart J* 1988;115:1111–1117.
19. Berman DS, ed. A symposium: technetium-99m myocardial perfusion imaging agents and their relation to thallium-201. *Am J Cardiol* 1990; 66:1E–96E.
20. Okada RD, Glover D, Gaffney T, Williams S. Myocardial kinetics of technetium-99m-hexakis-2-methoxy-2-methylpropyl isonitrile. *Circulation* 1988;77:491–498.
21. Stirner H, Buell U, Kleinhans E, Bares R, Gross W. Myocardial kinetics of ^{99m}Tc-hexakis-(2-methoxy-isobutyl-isonitrile) (HMIBI) in patients with coronary heart disease. A comparative study versus ^{201}Tl with SPECT. *Nucl Med Commun* 1988;9:15–23.
22. Piwnica-Worms D, Kronauge JF, Holman BL, Lister-James J, Davison A, Jones AG. Hexakis (carbomethoxyisopropylisonitrile) technetium (I), a new myocardial perfusion imaging agent: binding characteristics in cultured chick heart cells. *J Nucl Med* 1988;29:55–61.
23. Mousa SA, Maina M, Brown BA. Williams SJ. Studies on the mechanism of retention of RP-30 in the heart [Abstract]. *Nuklearmedizin* 1987;26:55.
24. Piwnica-Worms D, Kronauge JF, Delmon L, Holman BL, Marsh JD, Jones AG. Effect of metabolic inhibition on technetium-99m-MIBI kinetics in cultured chick myocardial cells. *J Nucl Med.* 1990;31:464–472.
25. Chiu ML, Kronauge JF, Piwnica-Worms D. Effect of mitochondrial and plasma-membrane potential on accumulation of hexakis (2-methoxyisobutylisonitrile) technetium (I) in cultured mouse fibroblasts. *J Nucl Med* 1990;31:1646–1653.
26. Chen LB. Mitochondrial membrane potential in living cells. *Ann Rev Cell Biol* 1988;4:155–181.
27. Li QS, Frank TL, Franceschi D, Wagner HN, Becker LC. Technetium-99m-methoxyisobutyl isonitrile (RP30) for quantification of myocardial ischemia and reperfusion in dogs. *J Nucl Med* 1988;29;1539–1548.
28. Canby RC, Silber S, Pohost GM. Relations of the myocardial imaging agents ^{99m}Tc-MIBI and ^{201}Tl to myocardial blood flow in a canine model of myocardial ischemic insult. *Circulation* 1990;81:289–296.