

Uptake of Technetium-99m-Teboroxime in Cultured Myocardial Cells: Comparison with Thallium-201 and Technetium-99m-Sestamibi

Jean C. Maublant, Nicole Moins, Pierre Gachon, Monique Renoux, Zheng Zhang and Annie Veyre

Division of Nuclear Medicine, Centre Jean Perrin and INSERM U195 and U71, Faculty of Medicine, Université d'Auvergne, 63011 Clermont-Ferrand, France

The effects of metabolic inhibition on the uptake of ^{99m}Tc -teboroxime were assessed in cultured myocardial cells and compared with ^{201}Tl and ^{99m}Tc -sestamibi. Metabolic impairment was induced by cyanide (CN), a blocker of the mitochondrial respiratory chain, iodoacetate (IAA), an inhibitor of the glycolytic pathway, and ouabain, an inhibitor of $\text{Na}^+\text{-K}^+$ sarcolemmal ATPase. Cellular viability was appreciated by the trypan blue exclusion method. The effects of low temperature and of cellular death resulting from osmotic lysis were also assessed. Net cellular uptake of the radiotracers and the amount of proteins in the culture dishes were measured. All experiments were performed in parallel with control conditions and the results were expressed relatively to the control values. Teboroxime uptake was clearly decreased at low temperature ($29.6\% \pm 2.2\%$ at 0°C , $p < 0.001$), while metabolic inhibition or osmotic lysis had no definite effect. The uptake of ^{201}Tl and sestamibi was severely diminished in the presence of a mixture of 5 mM CN and 0.1 mM IAA, but ^{201}Tl was less resistant than sestamibi ($13.7\% \pm 0.3\%$ and $73.5\% \pm 3.3\%$, respectively, after 1 hr of preincubation, $p < 0.001$ for both). Uptake of both tracers was very low in the presence of dead cells ($12.1\% \pm 1.3\%$ for ^{201}Tl and $4.1\% \pm 0.2\%$ for sestamibi, $p < 0.001$ for both). Ouabain had a detrimental effect only on ^{201}Tl uptake at doses higher than 100 μM . Of these three currently available coronary blood flow imaging agents, teboroxime shows the lowest sensitivity to metabolic impairment.

J Nucl Med 1993;34:255-259

Initially aimed at the scintigraphic imaging of myocardial blood flow, ^{201}Tl , ^{99m}Tc -methoxyisobutylisonitrile (sestamibi) (1) and ^{99m}Tc -teboroxime (2) are now widely investigated for the detection of myocardial viability. Such capabilities, however, imply conflicting properties. The ideal agent for blood flow imaging should be passively extracted like microspheres by the myocardium while viability is a reflection of metabolic integrity, regardless of

the level of tissular blood perfusion. It has been demonstrated that the myocardial uptake of the three agents is indeed grossly proportional to the local coronary blood flow in situations of acute and irreversible ischemia (3,4) although this figure could be different in reversible ischemia (5-7). It has also been clearly shown that energy-dependent mechanisms are involved in the cellular uptake of ^{201}Tl and sestamibi (8). We have formerly reported on the kinetics of teboroxime in basal conditions (9) as well as on the effect of some conditions of metabolic inhibition and of variations of the extracellular pH on the uptake of ^{201}Tl and sestamibi in cultured cells (11). Using the same model, we assessed the effect of mild to strong metabolic inhibition on the uptake of teboroxime in comparison with the uptake of ^{201}Tl and sestamibi. A lack of effect of metabolic impairment on teboroxime uptake would indicate unique capabilities for coronary blood flow imaging, especially in the postischemic situations during which a mismatch can be observed between tissue blood flow and metabolic activity.

MATERIALS AND METHODS

Myocardial Cell Cultures

The cultures were prepared according to the technique of Harary (10) with few modifications, as previously described (8). Briefly, the cells were harvested from the ventricles of 2-4-day-old Sprague-Dawley rats by repeated cycles of trypsinization (trypsin at 0.2% concentration) and centrifuged at 900 g for 5 min. The pellets were resuspended in a growth medium (Ham F-10), complemented with 14 mM NaHCO_3 , 1 mM CaCl_2 , 100 U/ml of penicillin, 100 $\mu\text{g/ml}$ of streptomycin and 10% fetal calf serum. The suspension was then placed in a culture dish. To reduce the proportion of nonmuscular cells, the differential attachment technique was applied (11). This is based on the fact that it takes longer for the myocytes than the nonmuscular cells such as fibroblast-like cells to attach to the walls of the culture dish. Therefore all the cells that had attached to the culture dish during the first 3-hr incubation period were discarded. The final plating of the supernatant was done in 35 mm diameter culture dishes ($65,000$ cells/ cm^2) with 2 ml of nutrient medium per dish. By 3 days, confluent monolayers with at least 80% of synchronously beating ventricular myocytes were obtained and used for the experiments. From the beginning of the preparation, the

Received Aug. 28, 1991; revision accepted Sept. 30, 1992. For correspondence and reprints contact: Jean C. Maublant, MD, PhD, Division of Nuclear Medicine, Centre Jean Perrin, 63011 Clermont-Ferrand, France.

cultures were kept at 37°C in a water-saturated atmosphere (5% CO₂, 95% air).

Tracer Preparations

A 370 kBq (10 μ Ci)/ml ²⁰¹Tl solution was obtained by dilution of a 37 MBq/ml preparation of thallos chloride (CIS Bio International, Gif-sur-Yvette, France). Technetium-99m-sestamibi (Cardiolite, E.I. du Pont de Nemours, N. Billerica, MA) was formed from a lyophilized kit containing tetrakis (2-methoxy isobutyl isonitrile) copper(I) tetrafluoroborate, stannous chloride and cysteine in a citrate buffer and mannitol matrix (Du Pont de Nemours, N. Billerica, MA). When mixed with 3 GBq of ^{99m}Tc-sodium pertechnetate eluted from a commercial technetium generator (CIS Bio International, Gif-sur-Yvette, France) and after 15 min of heating at 100°C in boiling water, a hexakis cationic complex was formed. The solution was diluted to 370 kBq/ml. Reversed-phase thin-layer chromatography with aluminium oxide plates in ethanol showed that the purity of the preparation was always higher than 90%.

Lyophilized teboroxime (Cardiotec, Bristol-Meyers-Squibb Diagnostics, New Brunswick, NJ) was reconstituted with 3 GBq of ^{99m}Tc-sodium pertechnetate eluted from a commercial technetium generator (CIS Bio International, Gif-sur-Yvette, France). Technetium-99m-labeled teboroxime was obtained after 15 min of heating at 100°C in a boiling water bath. The radiochemical purity was determined using Whatman 31 ET chromatography strips developed into a 0.9% NaCl solution for determination of the fraction of noncomplexed ^{99m}Tc and into a 50/50 (vol/vol) 0.9% NaCl/acetone solution for determination of the percentage of reduced/hydrolysed ^{99m}Tc-teboroxime. These values were lower than 10%.

Tracer Net Uptake Measurements

Thirty minutes before the experiment, the incubation medium was replaced by 2 ml of serum-free medium, pH 7.4. The cells were then incubated for 20 min with 37 kBq of ²⁰¹Tl-chloride, ^{99m}Tc-sestamibi or ^{99m}Tc-teboroxime. The dishes then were rapidly washed three times with cold saline. The cells were dissolved in a water solution with 1% sodium dodecylsulfate and 10 mM sodium borate and transferred into a test tube. The activity was measured in a sodium iodide gamma well-counter (1 min per sample). The amount of proteins (range 200–300 μ g) was measured by the method of Lowry (12) using bovine serum albumin as standard.

It has been verified that the amount of tracer that binds to the lateral walls of the plastic culture dishes is negligible for the three

tracers when the cells have developed for more than 2 days, which was always the case in the present study.

Inhibition of Cellular Metabolism

Different metabolic inhibitors have been tested, namely, sodium cyanide (CN) 5 mM, an inhibitor of the respiratory chain (13); sodium iodoacetate 0.1 mM (IAA), an inhibitor of glycolysis (14); and ouabain, an inhibitor of the Na⁺-K⁺ ATPase (15) at doses ranging between 10 μ M and 1 mM. CN and IAA were added to the incubation medium at times ranging between 20 and 240 min before the tracer was added. Cellular viability was visually by the trypan blue dye exclusion technique. Ouabain was always added 15 min before the tracer.

Fixation in Dead Cells

To define the role of passive mechanisms of cellular and sarcolemmal fixation, the tracers were incubated in preparations of dead cells. In a series, the cells were submitted to osmotic lysis by a slow wash with distilled water followed by a new wash with the incubation medium. The success of the lytic membrane disruption was appreciated by the dosage (DGKC method at 25°C) of lactate dehydrogenase (LDH) in the dish. The cells were then incubated for 20 min with 37 kBq of tracer added into 2 ml of the usual incubation medium. In another series, cellular death was obtained by preincubating the dishes at 10 or 0°C for 10 min.

Presentation of Results and Statistics

Cellular net uptake was expressed in percent of the dose added to the culture dish. In control conditions, net cellular uptake was in the 3%–5% range for ²⁰¹Tl and sestamibi and 15%–20% for teboroxime. Because of variations of these values between different experiments, likely due to variations in the number of cells present in the dishes, the net uptake was expressed in percent of the control values obtained the same day using the same batch of cells and the same tracer preparation.

Values are given as mean \pm s.d. Comparisons were undertaken with Student's t-test for unpaired data. A p value lower than 0.05 was considered statistically significant.

RESULTS

Table 1 shows that 20 min of pretreatment by the mixture of 5 mM NaCN and 0.1 mM IAA rapidly decreased ²⁰¹Tl net uptake, but not sestamibi or teboroxime net uptake. With 1 hr or more of preincubation, both ²⁰¹Tl and sestamibi net uptakes were severely impaired, but at a lesser extent for sestamibi than ²⁰¹Tl. Teboroxime net

TABLE 1
Effects of Preincubation Time in the Presence of 0.1 mM Iodoacetate and 5 mM Cyanide on Net Cellular Uptake of ²⁰¹Tl, ^{99m}Tc-Sestamibi and ^{99m}Tc-Teboroxime and on the Amount of Proteins Left in the Culture Dishes

Time (in min)	Net cell uptake (% controls)			Proteins (% controls)
	²⁰¹ Tl	^{99m} Tc-sestamibi	^{99m} Tc-teboroxime	
0 (controls)	100.0 \pm 2.2 (5)	100.0 \pm 4.6 (5)	100.0 \pm 1.7 (3)	100.0 \pm 2.6 (5)
20	40.6 \pm 2.8 (6)*	96.7 \pm 6.1 (5)	99.5 \pm 2.3 (4)	95.2 \pm 5.4 (11)
60	13.7 \pm 0.3 (6)*	73.5 \pm 3.3 (5)*	97.5 \pm 1.5 (4)	85.4 \pm 5.9 (11)*
120	3.6 \pm 0.1 (6)*	12.3 \pm 2.3 (5)*	96.8 \pm 2.2 (4)	65.0 \pm 8.6 (11)*
240	2.1 \pm 0.5 (6)*	12.8 \pm 1.1 (5)*	80.4 \pm 1.5 (4)*	54.6 \pm 7.2 (11)*

* p < 0.001 when compared with controls.

Incubation time for each tracer was 20 min. Data are expressed as mean \pm s.d. (number of samples).

TABLE 2
Effects of a 15 min Preincubation with Ouabain on the Net Cellular Uptake of ²⁰¹Tl, ^{99m}Tc-Sestamibi and ^{99m}Tc-Teboroxime and on the Amount of Proteins Left in the Culture Dishes

Ouabain	Net cell uptake (% controls)			Proteins (% controls)
	²⁰¹ Tl	^{99m} Tc-Sestamibi	^{99m} Tc-Teboroxime	
0 (controls)	100.0 ± 4.8 (5)	100.0 ± 9.6 (5)	100.0 ± 5.6 (5)	100.0 ± 4.5 (5)
10 μM	105.8 ± 26.2 (10)	125.1 ± 22.2 (6)*	88.9 ± 2.2 (5)*	99.8 ± 13.1 (6)
100 μM	67.7 ± 4.9 (7)†	116.7 ± 17.5 (5)	84.6 ± 1.0 (5)†	103.1 ± 5.3 (5)
500 μM	42.3 ± 7.3 (20)†	128.1 ± 18.4 (5)*	79.0 ± 0.9 (5)†	100.2 ± 3.2 (5)
1 mM	36.5 ± 3.9 (7)†	140.2 ± 13.2 (10)†	80.7 ± 3.1 (10)†	99.6 ± 3.2 (5)

* p < 0.05; † p < 0.001 when compared with controls.
Incubation time for each tracer was 20 min. Data are expressed as mean ± s.d. (number of samples).

uptake only dropped 20% following 4 hr of inhibition. The protein content started to decrease after 1 hr of incubation with the inhibitors, but the relative loss was always lower than the relative decrease of ²⁰¹Tl and sestamibi uptake, and higher than the relative decrease of teboroxime uptake. Estimation of cellular viability by the trypan blue exclusion method showed no effect after 1 hr of inhibition, then a decline of 50% after 2 hr and a decline of 75% after 4 hr.

The net uptake response with various concentrations of ouabain is shown in Table 2. There was no effect on ²⁰¹Tl uptake with the 10 μM dose, but a significant decrease appeared with the 0.1 mM dose. Uptake of MIBI was never decreased by ouabain, but was moderately increased at high doses. Teboroxime uptake was slightly decreased at all concentrations.

The effect of low temperatures on the incubation medium is shown in Table 3. All tracers demonstrated decreased uptake in such conditions. Uptake after osmotic lysis is shown in Figure 1. Thallium-201 and sestamibi were severely impaired, while teboroxime uptake remained nearly normal, although the amount of proteins was decreased by 14%.

DISCUSSION

Effects of Metabolic Inhibition

The results of this study confirm that both ²⁰¹Tl and sestamibi accumulation are significantly affected by met-

abolic perturbations, but that ²⁰¹Tl responds to milder injury. They also demonstrate that teboroxime net uptake is insensitive to severe metabolic impairment, and in that sense, it behaves like an almost ideal blood flow imaging agent. However, low temperature severely lowered teboroxime uptake. Since low temperature is known to slow metabolism and increase membrane viscosity (decrease membrane fluidity), it is likely that teboroxime uptake is not mediated by an active phenomenon, but is due to diffusion through or in the cellular membrane. The small effect of a significant loss of proteins induced by cell death resulting from either osmotic lysis or metabolic inhibition over the degree of net uptake of teboroxime could be an indication about its site of cellular fixation. In the event of cell death, the cytoplasm is washed away while most of the membranes remain bound to the walls of the dish. Therefore, a fraction of the protein content is lost with the cytoplasm, while the remnant is kept within the membranes. Since teboroxime uptake is only moderately decreased even when the protein content is dramatically reduced, it seems to indicate indirectly that teboroxime binds to the cell membranes or to the hydrophobic regions of membrane proteins. The role of ouabain, which induces a moderate but constant inhibitory effect at all tested concentrations, remains to be elucidated.

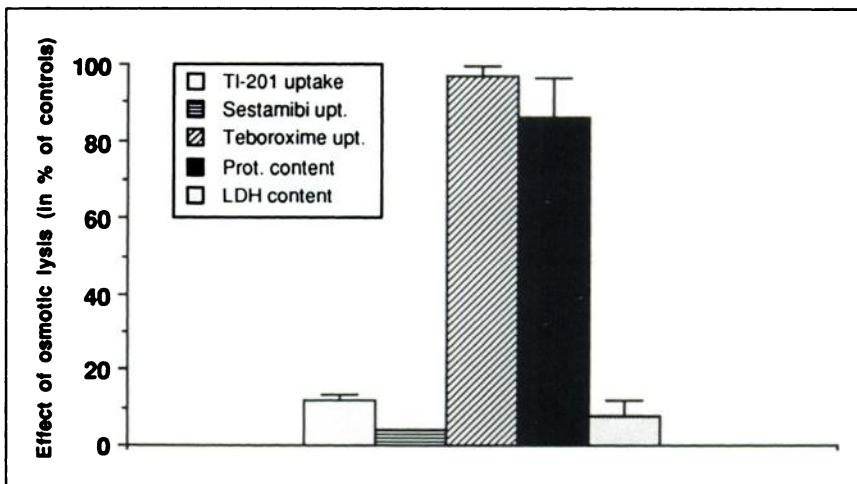
Previous experimental studies of the biological behavior of teboroxime focused primarily on the measurement of its myocardial extraction and washout as a function of

TABLE 3
Effects of Low Temperatures on Net Cellular Uptake of ²⁰¹Tl, ^{99m}Tc-Sestamibi and ^{99m}Tc-Teboroxime and on the Amount of Proteins Present in the Culture Dishes

Temperature	Net cell uptake (% controls)			Proteins (% controls)
	²⁰¹ Tl	^{99m} Tc-Sestamibi	^{99m} Tc-Teboroxime	
37°C	100.0 ± 4.2 (5)	100.0 ± 4.1 (5)	100.0 ± 2.4 (4)	100.0 ± 8.6 (4)
10°C	45.5 ± 13.5 (5)*	6.1 ± 4.2 (4)*	na	93.6 ± 4.9 (4)
0°C	7.4 ± 0.4 (5)*	2.3 ± 0.7 (4)*	29.6 ± 2.2 (4)*	96.1 ± 9.8 (4)

* p < 0.001 when compared with 37°C.
Incubation time for each tracer was 20 min. Data are expressed as mean ± s.d. (number of samples).

FIGURE 1. Effect of osmotic lysis on the cellular accumulation of ^{201}Tl , $^{99\text{m}}\text{Tc}$ -sestamibi and $^{99\text{m}}\text{Tc}$ -teboroxime following 20 min of incubation with the tracer and on the protein (prot.) and lactate dehydrogenase (LDH) contents in the culture dishes. Values are normalized to matching controls. Bars denote standard deviation.



coronary blood flow in whole hearts (16,17). However, it is important to understand the role of metabolism independently of blood flow delivery since these two parameters can be dissociated, especially in the postischemic situations. Our results suggest that teboroxime uptake should always faithfully reflect blood flow, which is not the case for ^{201}Tl and sestamibi.

As a potassium analog (18), ^{201}Tl enters the cell mostly through the Na^+/K^+ ATPase, as shown by McCall et al. (19), and is therefore under the dependence of oxidative metabolism. The inhibitory effect of ouabain appears at dose levels similar to those reported to be efficient for radioactive rubidium and potassium in the same model of cultured cardiac cells of newborn rats (20). By using a nearly similar model, Delano et al. observed an inhibition of ^{201}Tl uptake by 0.15 mM of ouabain and no effect on sestamibi uptake (21). However, we have found that around 35% of the ^{201}Tl uptake by the cells is not inhibited by ouabain, which suggests the presence of another mechanism of cellular accumulation.

The detrimental consequences of a metabolic inhibition during oxidative phosphorylation or glycolysis on the uptake of ^{201}Tl and sestamibi have been observed by several authors in various experimental settings. The effect of hypoxia on cellular ^{201}Tl uptake independent of blood flow was first demonstrated by Friedman et al. on cultured chick myocardial cells (22). By using cultured fetal mouse heart in anoxic conditions, Ingwall et al. (23) found that ^{201}Tl is more sensitive than sestamibi for indicating cellular viability. Following cellular damage induced by CN or sarcolemmal membrane damage induced by detergent Triton X-100 in isolated rat hearts, Beanlands et al. (24) found a significantly reduced peak accumulation, a marked reduction in accumulation rates and an increase in clearance rates of sestamibi with both treatments even after correction for variations in regional blood flow distribution. Kronauge et al. (25) observed in cultured chick heart cells that sestamibi uptake is reduced by 35% in the presence of rotenone, an inhibitor of oxidative phosphoryl-

ation. In the same model, depolarization of the mitochondrial membrane potential by the protonophore carbonyl cyanide *m*-chlorophenylhydrazone also decreases sestamibi uptake (26), while hyperpolarization by nigericin shows the opposite effect (27). Recently, it has been demonstrated that nearly 90% of sestamibi activity is associated with mitochondria in an energy-dependent manner (28).

Previously, we reported that ^{201}Tl kinetics, but not sestamibi kinetics, are modified by metabolic inhibition (8). The time of exposure to metabolic inhibition in the related experiments did not exceed 20 min. On the contrary, in the present study it appears that significant effects are shown for longer periods of metabolic inhibition. We had also reported that ouabain does not affect ^{201}Tl uptake, but only the 10 μM dose had been tested. When using higher ouabain concentrations, a clear inhibitory effect is now demonstrated.

An increase of sestamibi uptake induced by high doses of ouabain has also been reported by Piwnicka-Worms et al. (26) in cultured chick myocardial cells. These authors suggest that ouabain induces a loss of cell volume resulting in a partial depolarization of the plasma membrane potential and, consequently, hyperpolarization across the inner mitochondrial membrane.

Limitations of the Cultured Cells Model

Results obtained in this model of isolated cells in culture do not necessarily apply to clinical situations. Apart from the species differences, this experimental model lacks an interstitial compartment. Also, the tracer concentration in the incubation medium remains nearly constant during all measurements. This is due to the larger extracellular volume compared to the intracellular volume (2 ml versus about 1 μl), while the blood tracer concentration in humans rapidly decreases following the bolus injection. Another major difference is the composition of the medium and of blood, in particular, the higher concentration of proteins in blood.

The nonspecific binding of the tracers to the dish appears to be negligible. In the absence of myocardial

cells, the percentage of total activity that binds to the walls of the dish reaches 3% with sestamibi and 2% with teboroxime.

Metabolic impairment was produced by incubating the cells with oxidative phosphorylation and glycolysis blockers. The efficiency of this procedure has been demonstrated to closely simulate conditions of ischemia (29,30). The possible consequences of trypsinization on cellular viability seem negligible, since after 2–3 days in culture the mother cells have divided several times (31).

CONCLUSION

Teboroxime uptake in cultured myocardial cells is largely independent of the metabolic status of the cells, which is a unique behavior when compared with ^{201}Tl and sestamibi. This tracer should be particularly suitable as a myocardial blood flow imaging agent in situations of discrepancy between coronary blood flow and metabolic activity of the myocardial tissue, as in the posts ischemic phase.

ACKNOWLEDGMENTS

This work was partly supported by a grant from the Bristol-Myers Squibb Pharmaceutical Research Institute, formerly the Squibb Institute for Medical Research, New Brunswick, NJ, and by the Institut National pour la Santé et la Recherche Médicale, Paris, France.

REFERENCES

1. Jones AG, Davison A, Abrams MJ, et al. Biological studies of a new class of technetium complexes: the hexakis (alkylisonitrile) technetium (I) cations. *Int J Nucl Med Biol* 1984;11:225–234.
2. Narra RK, Nunn AD, Kuzynski BL, Feld T, Wedeking P, Eckelman WC. A neutral technetium-99m complex for myocardial imaging. *J Nucl Med* 1989;30:1830–1837.
3. Okada RD, Glover D, Gaffney T, Williams S. Myocardial kinetics of technetium-99m-hexakis-2-methoxy-2-methylpropyl-isonitrile. *Circulation* 1988;77:491–498.
4. Canby RC, Silber S, Pohost GM. Relations of the myocardial imaging agents $^{99\text{m}}\text{Tc-MIBI}$ and ^{201}Tl to myocardial blood flow in a canine model of myocardial ischemic insult. *Circulation* 1990;81:289–296.
5. Mousa SA, Cooney JM, Williams SJ. Regional myocardial distribution of RP-30 in animal models of myocardial ischemia and reperfusion [Abstract]. *J Nucl Med* 1987;28:620.
6. Sinusas AJ, Weber KA, Bergin JD, et al. Correlation of myocardial uptake of technetium-99m-methoxy-isobutyl-isonitrile with regional flow during coronary occlusion and reperfusion [Abstract]. *J Nucl Med* 1989;30:756.
7. Meerdink DJ, Leppo JA. Myocardial transport of hexakis (2-methoxyisobutylisonitrile) and thallium before and after coronary reperfusion. *Circ Res* 1990;66:1738–1746.
8. Maublant JC, Gachon P, Moins N. Hexakis(2-methoxyisobutyl-isonitrile) technetium-99m and thallium-201 chloride: uptake and release in cultured myocardial cells. *J Nucl Med* 1988;29:48–54.
9. Maublant JC, Moins N, Gachon P. Uptake and release of two new Tc-99m-labeled myocardial blood flow imaging agents in cultured cardiac

- cells. *Eur J Nucl Med* 1989;15:180–182.
10. Harary I, Farley B. In vitro studies on single beating rat heart cells. *Exp Cell Res* 1963;29:451–465.
11. Blondel B, Roijen I, Cheneval JP. Heart cells in culture: a simple method for increasing the proportion of myoblasts. *Exper* 1970;27:356–358.
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265–275.
13. Doorey AJ, Barry WH. The effects of inhibition of oxidative phosphorylation and glycolysis on contractility and high-energy phosphate content in cultured chick heart cells. *Circ Res* 1983;53:192–201.
14. Buja LM, Hagler HK, Parsons D, Chien K, Reynolds RC, Willerson JT. Alterations of ultrastructure and elemental composition in cultured neonatal rat cardiac myocytes after metabolic inhibition with iodoacetic acid. *Lab Invest* 1985;53:397–412.
15. McCall D. Cation exchange and glycoside binding in cultured rat heart cells. *Am J Physiol* 1979;236:C87–C95.
16. Marshall RC, Leidholdt EM, Zhang DA, Barnett CA. The effect of flow on technetium-99m-teboroxime (SQ30217) and thallium-201 extraction and retention in rabbit heart. *J Nucl Med* 1991;32:1979–1988.
17. Rumsey WL, Rosenpire KC, Nunn AD. Myocardial extraction of teboroxime: effects of teboroxime interaction with blood. *J Nucl Med* 1992;33:94–101.
18. Gehring PJ, Hammond PB. The interrelationship between thallium and potassium in animals. *J Pharmacol Exp Ther* 1967;155:187–201.
19. McCall D, Zimmer LJ, Katz AM. Kinetics of thallium exchange in cultured rat myocardial cells. *Circ Res* 1985;56:370–376.
20. Werdan K, Wagenknecht B, Zwissler B, Brown L, Krawietz W, Erdmann E. Cardiac glycoside receptors in cultured heart cells. II. Characterization of a high affinity and a low affinity binding site in heart muscle cells from neonatal rats. *Biochem Pharmacol* 1984;33:1873–1886.
21. Delano ML, Sands H, Gallagher BM. Transport of $^{42}\text{K}^+$, $^{201}\text{Tl}^+$ and $[^{99\text{m}}\text{Tc}(\text{DMPE})_2\cdot\text{Cl}_2]^+$ by neonatal rat myocyte cultures. *Biochem Pharmacol* 1985;18:3377–3380.
22. Friedman BJ, Beihn R, Friedman JP. The effect of hypoxia on thallium kinetics in cultured chick myocardial cells. *J Nucl Med* 1987;28:1453–1460.
23. Ingwall JS, Reis IG, Kramer MF, Newell JB, Boucher CA. Technetium-99m-MIBI versus ^{201}Tl accumulation during myocardial injury: metabolic determinants [Abstract]. *Circulation* 1989;80(suppl II):II-619.
24. Beanlands RSB, Dawood F, Wen WH, et al. Are the kinetics of technetium-99m-methoxyisobutyl-isonitrile affected by cell metabolism and viability? *Circulation* 1990;82:1802–1814.
25. Kronauge JF, Piwnica-Worms D, Holman BL, Marsh JD, Davison A, Jones AG. Effects of metabolic inhibitors on Tc-MIBI uptake into cultured chick cells [Abstract]. *J Nucl Med* 1988;29:820.
26. Piwnica-Worms D, Kronauge JF, Chiu ML. Uptake and retention of hexakis (2-methoxyisobutylisonitrile) technetium (I) in cultured chick myocardial cells. *Circulation* 1990;82:1826–1838.
27. Chiu ML, Kronauge JF, Piwnica-Worms D. Effect of mitochondrial and plasma membrane potentials on accumulation of hexakis (2-methoxyisobutylisonitrile) technetium (I) in cultured mouse fibroblasts. *J Nucl Med* 1990;31:1646–1653.
28. Carvalho PA, Chiu ML, Kronauge JF, et al. Subcellular distribution and analysis of technetium-99m-MIBI in isolated perfused rat hearts. *J Nucl Med* 1992;33:1516–1521.
29. Higgins TJC, Bailey PJ. The effects of cyanide and iodoacetate intoxication and ischemia on enzyme release from the perfused rat heart. *Biochim Biophys Acta* 1983;762:67–75.
30. Van der Laarse A, Altona IC, van Dijkman PRM, Zoet TCM, de Vries J, Lelkens RRM. Metabolic blocker-induced cell damage in rat cardiac tissue: comparison of three models currently used: the isolated heart, heart cell cultures and isolated myocytes. *Res Commun Chem Pathol Pharmacol* 1984;43:43–54.
31. Perissel B, Charbonné F, Moalic JM, Malet P. Initial stages of trypsinized cell culture of cardiac myoblasts: ultrastructural data. *J Mol Cell Cardiol* 1980;12:63–75.