Correlation Between [5-3H]Glucose and [U-14C]Deoxyglucose as Markers of Glycolysis in Reperfused Myocardium

A. James Liedtke, Britta Renstrom, and Stephen H. Nellis

Studies were conducted in extracorporeally perfused, intact, working pig hearts to determine whether, in heart muscle, trace-labeled deoxyglucose serves as an accurate marker of glycolytic flux in reperfusion after exposures to mild to moderate regional ischemia. In the main study, two groups of hearts were compared, as distinguished by levels of glucose in the whole-blood perfusate (euglycemic hearts [group I], blood glucose of 7.4±0.2 μmol/ml, n=7; hyperglycemic hearts [group II], blood glucose of 12.9±0.5 μmol/ml, n=8). Both groups were subjected to a 60% reduction in anterior descending coronary flow for 30 minutes followed by reperfusion for 40 minutes. Modest and comparable regional mechanical stunning during reflow was noted in both groups. Glucose utilization, as estimated from the release of H2O from the steady-state infusion of [5-3H]glucose during aerobic perfusion, was modest but during reperfusion was noted to increase significantly above aerobic values in each of the two groups, with a doubling of rates in group I hearts compared with group I hearts (p<0.041 or p<0.090). Net lactate extraction was comparable in reflow in both groups, suggesting in this specific instance a preferential enhancement of glucose oxidation in hyperglycemic group II hearts. Shifts in accumulation of tissue radioactivity of [U-14C]2-deoxyglucose in reperfused myocardium were not able to track these trends. The variability of 14C-labeled radioactivity among animals was marked and essentially masked any ability to discern trends in glycolysis as described by tritiated glucose between the aerobic and reperfusion intervals. When the data were arrayed by linear regression analysis, the slopes derived from 14C-labeled deoxyglucose were either discordant or insensitive to those described by 3H-labeled glucose. Tissue glycogen levels were slow to recover in early reflow and at end reperfusion were still significantly depressed from aerobic levels. The present data indicate that coronary reperfusion and hyperglycemia have influence in determining glycolytic flux in myocardium. Labeled deoxyglucose, considered solely as a marker of exogenous glucose utilization, appears to be an insensitive agent in describing these events at conditions of relatively low glucose flux. (Circulation Research 1992;71:689–700)

Key Words • glycolgen • glycolysis • reflow • euglycemia • hyperglycemia • myocardial ischemia

Glycolysis in heart muscle is regulated at several key transport and enzymatic reactions and in aerobic myocardium is responsive to changes in insulin, other hormones, the availability of glucose and alternate substrates in the perfusate, and left ventricular work.1,2 Glycolysis is accelerated in anoxia and in all but the most profound states of myocardial ischemia.3–6 During reperfusion after ischemia, consensus regarding glucose utilization is less settled. Glycolytic flux varies widely and may be delayed, as reported in rat hearts,7 or increased, as described in dog hearts,8,9 in which most glucose is processed nonoxidatively. Glucose oxidation measured directly, particularly in the presence of excess fatty acids in coronary perfusate, is markedly depressed in reperfusion.10,11 Conversely, in viable, but jeopardized, reperfused myocardium, as characterized by positron emission tomography (PET), the rate of glucose metabolism12 estimated from uptake and hexokinase-mediated phosphorylation of [18F]2-deoxy-2-fluoro-D-glucose (FDG) appears uniformly increased. This discordant relation between metabolic activity and perfusion, termed the flow/metabolic mismatch, has been observed in several clinical populations examined by PET at reperfusion intervals of hours, days, or months after a cardiac event.13–18 Its interpretation remains controversial. Camici et al19 have postulated that this mismatch may represent an adaptation to a situation of persistent hypoperfusion resulting in a chronic stimulation of glycolysis secondary to mild tissue hypoxia. We have questioned whether sufficient energy could be derived to maintain cell viability from this substrate source alone, even in the presence of stunned or hibernating contractile dysfunction, and have shown10,20,21 along with others8,11 that fatty acids, rather than carbohydrates, are preferred in short-term reperfusion protocols. Questions about whether FDG is a valid marker of glycolysis have also been raised.
Modeling of FDG as a glucose analogue for glycolysis in myocardium comes largely from work in brain metabolism, where glycogen synthesis is essentially nil. This is not true in heart muscle, particularly during reflow, and expressions termed “myocardial glucose metabolism,”\(^{22}\) “myocardial metabolic rate for glucose,”\(^{7,12}\) and “regional myocardial glucose utilization”\(^{24}\) were developed in part to recognize that FDG flux rates are unable to distinguish or separate glycogen synthesis from glycolysis. The present studies were designed to further detail the sensitivity of deoxyglucose as a marker of exogenous glucose utilization. Experiments in intact pig hearts were performed to vary glycolysis during reperfusion. We tested the hypothesis that labeled deoxyglucose is an accurate marker of glucose utilization per se in reperfused myocardium.

### Materials and Methods

Fifteen adolescent swine with an average weight of 49.1 ± 0.81 kg were studied in the main protocol. Six additional swine (average weight, 44.0 ± 0.6 kg) were studied in separate protocols used to test the various methods of measurement and the experimental approach. Animals in the main study were divided into two groups: euglycemic swine (group I, \(n=7\)) and hyperglycemic swine (group II, \(n=8\)). All animals were anesthetized with pentobarbital sodium (35 mg/kg i.v.) and intubated with controlled positive ventilation using oxygen-supplemented room air. The preparation and instrumentation used in this study have been described elsewhere\(^ {10,20,21,25,26}\) and will only be briefly reviewed here. The coronary arteries were perfused separately via an arterioarterial shunt connected extracorporeally. Blood was withdrawn from a femoral artery and returned via three low-flow perfusion pumps to the proximally cannulated right, main left, and left anterior descending (LAD) coronary arteries. Included in the LAD perfusion circuit was a 50-ml mixing chamber and an access port to infuse indocyanine green. The LAD circuit also included another port for infusing labeled substrates, [5-\(^3\)H]glucose, and [U-\(^14\)C]deoxyglucose; this port was connected to a special section within the perfusion circuit (volume, 8 ml) consisting of alternate segments of small (\(\frac{1}{4}\)-in. i.d.) and large (\(\frac{3}{8}\)-in. i.d.) bore tubing. This section was used for mixing the tracer with whole-blood perfusate. A venous cannula was inserted anteriorly into the great cardiac vein, which, together with another arterial port, was used to sample for blood gases and metabolites.

Aerobic flows were adjusted so that coronary perfusion pressures approximated arterial pressures and the venous oxygen saturation was maintained at approximately 40%. Left ventricular pressure and its maximal first derivative were measured by a high-fidelity manometer-tipped pressure device (Millar Instruments, Houston, Tex.) placed into the left ventricular chamber. Segment shortening was measured by ultrasonic crystals placed at midmyocardial depth in the LAD perfusion system and used to characterize regional contractility. In each of the fifteen animals, the LAD flow was maintained at aerobic conditions for the first 40 minutes of perfusion, reduced abruptly by 60% for the next 30 minutes, and then returned acutely to preschematic levels for the final 40 minutes of reperfusion. Right coronary and left circumflex arteries were held at aerobic flows throughout the studies. To set a condition of near-constant myocardial oxygen demand in all hearts, blood volumes were replenished throughout the studies with 6% dextran in saline to maintain systemic pressures at approximately 100 mm Hg. In the main study, radioactive [5-\(^3\)H]glucose (84 µCi per animal at infusion rates of 2.31 \times 10^4 dpm/min) and [U-\(^14\)C]deoxyglucose (46 µCi per animal at 1.27 \times 10^4 dpm/min) were infused into the LAD coronary circulation during aerobic perfusion and reperfusion. By using the stated specific activity of 304 µCi/µmol for the latter compound, this calculated to a total concentration per animal of 150 nmol, which is well below the dose that inhibits glycolysis. To further lessen pharmacological loading, infusions were discontinued during the interval of ischemia. In the eight supplemented animals, hyperglycemia was initiated by giving a 50 g oral dose of dextrose before surgery and instrumentation. Serum glucose levels were maintained at approximately 200 mg/dl, with infusion of dextrose as necessary. In the seven euglycemic pigs, serum glucose levels were maintained at approximately 100 mg/dl.

Indocyanine green indicator was administered (0.33 mg/dl) to estimate venous cross contamination and dilution in the LAD circulation. This dilution factor (K) was used in the calculation of \(^3\)H production from labeled exogenous glucose according to the following formula:

\[
\text{Glucose utilization (µmol \cdot hr}^{-1} \cdot \text{g dry wt}^{-1}) = \frac{[}\Delta\text{H}_2\text{O} \times Q_{\text{LAD}} \times 60 \times (1 - Ht)]}{[K \times \text{ASA} \times \text{LAD dry wt}]}
\]

where \(\Delta\text{H}_2\text{O}\) is the difference between venous and arterial \(^3\)H water (dpm/ml), \(Q_{\text{LAD}}\) is the flow in the LAD bed (ml/min), \(Ht\) is the hematocrit, and ASA (arterial specific activity of glucose [dpm/µmol]) is the ratio of \((A_O \times B)/(C_O \times Q_{\text{LAD}})\), where \(A_O\) is the concentration of infused [5-\(^3\)H]glucose (dpm/ml), B is the infusion rate of labeled glucose (ml/min), and \(C_O\) is the arterial glucose concentration (µmol/ml). \(^3\)H water production has been previously used to estimate rates of glycolysis from exogenous glucose.\(^ {3-5}\) Measurements were made during aerobic perfusion and reperfusion.

An independent estimate of anaerobic glycolysis was also obtained from net lactate extraction/production values (µmol \cdot hr}^{-1} \cdot \text{g dry wt}^{-1}) sampled from the coronary arterial perfusate and coronary effluent and calculated according to the following expression:

\[
\text{Lactate extraction or production} = \frac{[V_L - A_L] \times Q_{\text{LAD}} \times 60)}{[K \times \text{LAD dry wt}]}
\]

where \(V_L\) is the lactate concentration in coronary venous effluent (µmol/ml) and \(A_L\) is the lactate concentration in coronary arterial perfusate (µmol/ml). Lactate concentration was determined enzymatically.\(^ {27}\)

Tissue biopsies were also taken from the LAD bed during the aerobic and reperfusion intervals at 10-minute intervals and used to describe the accumulation of labeled 2-deoxyglucose in myocardium. Duplicate biopsies were taken at three time points (85, 100, 110 minutes) of perfusion. To obtain an expression of glycolysis from exogenous substrate utilization, termed the glycolytic index, using the kinetics of 2-deoxyglucose, deoxyglucose accumulation was estimated as follows:
Deoxyglucose accumulation \((\mu\text{mol} \cdot \text{hr}^{-1} \cdot \text{g dry wt}^{-1}) = (S \times C_T \times 60)/(DG \times 0.67)\)

where \(S\) (slope) is the increase in tissue radioactivity of isotopic accumulation per minute (dpm/g dry wt), \(DG\) is the arterial concentration of labeled deoxyglucose (dpm/ml), and 0.67 is the lump constant for DG kinetics as determined by Gambhir et al., who derived this factor to account for the differences in transport and phosphorylation of \(^{18}\text{FDG}\) and glucose measured over 12–55 minutes of sampling. The slope \(S\) was calculated in two ways. As a first approach, \(S\) was expressed as

\[ S = \Delta D / \Delta t \]

where \(\Delta D\) is the difference in average tissue radioactivity of 2-deoxyglucose between time points (dpm/g dry wt) and \(\Delta t\) is the time in minutes between biopsy acquisitions. During reperfusion in these studies, \(\Delta D\) was calculated only between 85 and 100 minutes of perfusion and between 100 and 110 minutes of perfusion when duplicate tissue samples were obtained.

By a second method, \(S\) was derived from a formula using a least-squares fit of the 2-deoxyglucose tissue data as expressed by

\[ TA = S \times t + TA_0 \]

where \(TA\) is the tissue radioactivity (dpm/g dry wt) at time \(t\) (minutes) and \(TA_0\) is the extrapolated tissue radioactivity at zero perfusion time (dpm/g dry wt). For the purpose of this calculation, it was assumed that the deoxyglucose accumulation as a fraction of its arterial concentration correlated directly with rates of glycolysis as a fraction of the arterial glucose concentration. Since the tissue samples in these experiments were obtained well after the equilibrium of tracer compound between interstitial and intracellular spaces (range of half-times, 1.5–4.0 minutes) as reported by Krivokapich et al., the slope function expressed above is that of a Patlak analysis for trapped tracers.

In the main studies, metabolic and mechanical data were obtained at 10-minute intervals in both glucose groups throughout the perfusion trials. Regional myocardial oxygen consumption (mmol \(\cdot\) hr\(^{-1} \cdot\) g dry wt\(^{-1}\)) was obtained along with measurements of serum fatty acids (\(\mu\text{mol/ml}\)) and glucose (\(\mu\text{mol/ml}\)). At the conclusion of each study, tissue samples from both left circumflex and LAD beds were collected, frozen, and stored at \(-70^\circ\text{C}\) for further analysis. The two beds were separated by visual inspection after injection of India ink through the LAD cannula. The LAD perfusion bed was dissected free, and both beds were weighed separately. The LAD weight was used to normalize the metabolic data as indicated above.

**Analysis of Samples**

Blood samples from the coronary artery and vein were collected every 10 minutes and centrifuged at 1,500g for 10 minutes at 4°C to obtain plasma that was counted for total radioactivity. Separation of tritium-labeled water from \(^3\text{H}\)-glucose in plasma was achieved by using a Dowex-1 borate column according to Rogut, Neely, and colleagues. Blood samples from the femoral artery were likewise centrifuged at 1,500g for 10 minutes, and the plasma was counted for total radioactivity. Biopsies from the LAD bed were also obtained at 10, 20, and 40 minutes during the aerobic period and at 80, 85, 90, 100, and 110 minutes during the reperfusion interval, and duplicate biopsies were taken at selected time points during reflow. Biopsies were performed using a TRU-CUT disposable biopsy needle (11.4-cm cannula and 20-mm specimen notch). The biopsies were weighed before being digested in Soluene 350 and counted for radioactivity. The tissue content of nonphosphorylated deoxyglucose and deoxyglucose phosphate was determined by separating the two compounds on a Dowex-2 Cl\(^-\) column according to Detwiler. Nonphosphorylated deoxyglucose was removed from the column using 5 mM Tris base, and deoxyglucose phosphate was eluted with 0.2 M HCl. The two fractions of deoxyglucose were counted, and the percent distribution was calculated. Nonphosphorylated deoxyglucose was described also by its extracellular and intracellular constituents. The extracellular concentration was calculated by multiplying the extracellular space, previously determined using labeled sorbitol, by the perfusate (plasma) concentration of nonphosphorylated deoxyglucose. This product was subtracted from the measured nonphosphorylated deoxyglucose to obtain the intracellular concentration. Glycogen was isolated from tissue and estimated enzymatically according to Bradley and Kaslow. A portion of this isolated glycogen was also counted for radioactivity. In separate studies in additional hearts (see below), myocardium was also processed to measure glucose-6-phosphate.

**Protocols**

Studies were performed in intact, extracorporeally perfused, working swine hearts. The purpose in the main studies was to characterize glucose utilization during early reperfusion at conditions of euglycemia and hyperglycemia and to evaluate deoxyglucose as a suitable marker in describing glucose utilization. In all animals of each glucose group \((n = 7\) and 8 for the euglycemic and hyperglycemic groups, respectively), the LAD flow was reduced by 60% for 30 minutes to obtain mild to moderate ischemia and then returned to aerobic levels for the last 40 minutes of reperfusion. Serum glucose was monitored throughout the studies. Biopsies were taken at approximately 10-minute intervals (see above) during aerobic perfusion and reperfusion. Mechanical and metabolic data were collected every 10 minutes throughout the studies. All metabolic data in the LAD bed were normalized by dry weight of the LAD myocardium. Statistical comparisons of data were made within groups by using paired Student's \(t\) tests and between groups by using nonpaired Student's \(t\) tests. Group comparisons of function were made using all data contained within each of the three perfusion intervals, i.e., aerobic, ischemic, and reperfusion periods. Statistical significance was defined by two-tailed probability values. Except where indicated, data are reported as mean±SEM.

By using the same methods and statistical review, separate studies were also conducted in six extracorporeally perfused hearts to further examine details of the experimental approach or measurements and to describe possible mechanisms of the results of the main study. To further clarify methodological particulars, in three hearts LAD flow was again reduced by 60% for 30...
minutes and reperfused afterwards for another 40 minutes. Multiple biopsies were obtained at 35 minutes of perfusion (aerobic interval), 80 minutes of perfusion (early reperfusion), and 110 minutes of perfusion (end reperfusion). In one set of biopsy material acquired over the three sampling times, extracellular and intracellular nonphosphorylated deoxyglucose was estimated to describe the delivery of substrate during steady-state infusions of labeled deoxyglucose. From a second set of biopsy material, tissue glycogen was measured as was the amount of [5-3H]glucose incorporated into glycogen stores over time. From the third set of biopsies, tissue glucose-6-phosphate concentrations were determined.

Three other animals were prepared to investigate the tissue distribution of labeled deoxyglucose. The validity of this marker in describing glucose utilization is dependent not only on its characteristics of tissue accumulation as it relates to glucose metabolism but also on the comparability of radioactivity among tissue samples at a given metabolic state. Uniformity of label expressed either as homogeneity or heterogeneity of 2-deoxyglucose deposition was examined. Labeled deoxyglucose was infused in two hearts perfused at aerobic flows for only 40 minutes and in one heart rendered ischemic (60% decrease in LAD flow) for 30 minutes and reperfused for 30 minutes. Ten small biopsies (0.072±0.006 g dry wt) were taken and analyzed for radioactivity in each of the aerobic hearts at 40 minutes of end perfusion. Similarly, 10 biopsies (0.117±0.003 g dry wt) and one larger core biopsy (28.2 g dry wt) were taken from the LAD bed at 100 minutes of perfusion (30 minutes of reperfusion) in the ischemia/reflow heart. The large biopsy was frozen in liquid nitrogen, powdered, and mixed as a homogenate. Ten additional small samples (0.135±0.012 g dry wt) were taken from this homogenate and processed as described in the analysis of samples section. The purpose of measuring radioactivity in the 10 small biopsies was to define the standard deviation of tissue heterogeneity for 2-deoxyglucose deposition as a function of sampling site, size, and flow delivery. The purpose of acquiring similar measurements from samples of homogenate from the large biopsy was to establish the error of the measurement.

Results

Main Protocol

As previously stated, the two animal study groups were distinguished by different blood glucose concentrations. Levels in the euglycemic (group I) animals were 7.4±0.2 μmol/ml, and levels in the hyperglycemic (group II) animals averaged 12.9±0.5 μmol/ml (p<0.001). Serum fatty acids in both groups averaged 0.42±0.04 μmol/ml (p=NS between groups). Controlled variables for the experiments are shown in Figure 1. Combined coronary flows (Figure 1A) in the aerobically perfused circumflex and right coronary systems were 9.9±0.5 ml·min⁻¹·g dry wt⁻¹ in group I and 10.9±0.5 ml·min⁻¹·g dry wt⁻¹ in group II (p=NS between groups). LAD flows were also comparable (p=NS between groups) for the aerobic, ischemic, and reflow intervals and averaged 5.5±0.2, 2.2±0.1, and 5.5±0.2 ml·min⁻¹·g dry wt⁻¹, respectively. In an effort to fix oxygen demand in these studies, hearts in both groups were intermittently volume-loaded to maintain left ventricular pressures at a near constant level (Figure 1B). Peak systolic pressures throughout the perfusion trials averaged 102±2 mm Hg (p=NS between groups).

Dependent variables reflecting regional mechanical and metabolic functions in response to variations in LAD flow are shown in Figure 2. As described by percent systolic shortening, modest regional stunning (Figure 2A) was noted in both groups when comparing preischemic with reperfusion values (group I: from 97±3% to 74±8%, p<0.05; group II: from 100±2% to 80±9%, p<0.05). Myocardial oxygen consumption (Figure 2B) directly followed these shifts in regional motion but with a trend toward greater recovery in the reflow interval (group I: from 1.14±0.07 to 0.95±0.09 mmol·hr⁻¹·g dry wt⁻¹, p<0.01; group II: from 1.01±0.04 to 0.95±0.05 mmol·hr⁻¹·g dry wt⁻¹, p=NS between intervals).

We have previously characterized rates of oxidation for fatty acids and glucose using this model system and indirectly estimated rates of total glycolysis in ischemia and reperfusion. In this study, exogenous
glucose utilization was measured directly during reperfusion using [5-3H]glucose (Figure 3A) and demonstrated a significant increase when compared with low-level aerobic values (group I: from 6.5±3.5 to 18.4±5.7 μmol·hr⁻¹·g dry wt⁻¹, p<0.023; group II: from 6.0±7.9 to 40.1±7.4 μmol·hr⁻¹·g dry wt⁻¹, p<0.001). Differences were also evident between groups during reflow (group II>group I, p<0.041), suggesting an enhancement of glycolysis based on the availability of excess glucose in the coronary perfusate.

Net lactate extraction and production values were also obtained (Figure 3B). Lactate extraction was observed in both groups during aerobic and reperfusion intervals (group I: from −21.6±4.2 to −2.4±5.4 μmol·hr⁻¹·g dry wt⁻¹, p<0.002; group II: from −27.0±8.4 to −14.4±6.0 μmol·hr⁻¹·g dry wt⁻¹, p=NS between intervals). During the ischemic interval, there was the anticipated conversion to net lactate production in both groups. No statistical differences between groups were noted for any of the perfusion intervals. The disparity between increased glucose utilization in group II hearts and the absence of increased lactate production during reperfusion may indicate greater glucose oxidation in this group (as discussed later, there was no difference in tissue glycogen levels at end reflow between groups).

A main purpose of the protocol was to evaluate the sensitivity of [U-14C]2-deoxyglucose as a marker in estimating glucose utilization. Biopsy data of the radiotracer buildup of this tracer in myocardium are shown in Figure 4. Accumulation proceeded in a linear fashion during the aerobic period, similar to the data recently published for 18FDG in aerobic rat hearts. This trend was temporarily interrupted during early reflow (80, 85, and 90 minutes of perfusion), perhaps because of heterogeneity of flow distribution in early recovery or attenuation of the glucose transport mechanisms secondary to sarcolemmal dysfunction after ischemia. Thereafter, in reperfusion, accumulation again proceeded linearly.

These data were then used to calculate a glycolytic index derived from exogenous substrate in both heart groups (Figure 5). As a first approach, calculations were made to describe a slope function using ΔD/Δt on the data displayed in Figure 4 (see "Materials and Methods"). There was marked variation of radioactivity among animals in both the euglycemic and hyperglycemic groups, particularly during reperfusion, which es-
By guest on January 8, 2018 http://circres.ahajournals.org/ Downloaded from

sententially masked any ability to discern trends in glycolysis between the aerobic and reperfusion intervals (group I: from 63.9±26.6 to 38.6±19.1 μmol · hr⁻¹ · g dry wt⁻¹, p=NS; group II: from 96.8±51.6 to 92.9±53.8 μmol · hr⁻¹ · g dry wt⁻¹, p=NS). This is in contrast to the trends shown for [5-²H]glucose in Figure 3A. The deoxyglucose marker similarly was unable to discriminate trends between groups during reperfusion as described by the tritiated glucose data.

As a second analytic approach, the tissue accumulation data of Figure 4 for the aerobic and reperfusion intervals were characterized by a least-squares array (Figure 6A). By using this method and all data points for each perfusion interval, the glycolytic index for the aerobic perfusion and reperfusion was from 62.4±24.5 to 68.1±16.9 μmol · hr⁻¹ · g dry wt⁻¹ for group I hearts and from 97.0±44.6 to 71.0±34.8 μmol · hr⁻¹ · g dry wt⁻¹ for group II hearts. There were no statistical differences between values in aerobic perfusion and reperfusion within groups nor between groups at either perfusion interval. Moreover, if one reexpresses the deoxyglucose data as a ratio of one perfusion state to the other and compares the changes with those of the tritium-labeled method (Figures 3 and 6B), a discordant pattern between markers is demonstrated that was statistically significant (p<0.054) in euglycemic hearts (p=NS in group II hearts). Thus, by least-squares fit, 2-deoxyglucose was again unable to adequately describe the shifts in glycolysis estimated by the tritium-labeled glucose method.

On inspection of the two groups in Figure 4, it is noted that the data points for 80 and 85 minutes visually appear nonlinear with respect to the remainder of the reperfusion data (this is not as evident when looking at the individual animal responses). A modified approach to the least-squares calculations would be to remove...
by [U-14C]deoxyglucose (panel A) and [3-3H]glucose (panel B) recalculated from Figure 6, in which data points for perfusion times of 80 and 85 minutes have been deleted (see text).

FIGURE 7 Graphs showing rates of glucose utilization for euglycemic (group I) and hyperglycemic (group II) hearts estimated by [U-14C]deoxyglucose (panel A) and [3-3H]glucose (panel B) recalculated from Figure 6, in which data points for perfusion times of 80 and 85 minutes have been deleted (see text).

these two time points from both the labeled deoxyglucose and labeled glucose data and reanalyze the relations (Figures 7A and 7B). Rates of glycolysis by [3-3H]glucose estimates for the aerobic and reperfusion intervals were from 6.5±3.5 to 15.1±5.9 μmol·hr⁻¹·g dry wt⁻¹ for group I hearts (p<0.056) and from 6.0±7.9 to 38.0±10.5 μmol·hr⁻¹·g dry wt⁻¹ for group II hearts (p<0.016). Glycolysis tended again to be higher in group II versus group I hearts during reperfusion (p<0.090). The glycolytic index described by [U-14C]2-deoxyglucose for the two perfusion intervals was from 62.4±24.5 to 91.0±28.4 μmol·hr⁻¹·g dry wt⁻¹ for group I hearts (p=NS) and from 97.0±44.6 to 158.9±86.9 μmol·hr⁻¹·g dry wt⁻¹ for group II hearts (p=NS). There was no statistical difference between groups using this marker nor between markers using the revised data.

Nonphosphorylated and phosphorylated forms of deoxyglucose were measured in myocardium at end reperfusion in both groups (Table 1). The nonphosphorylated form (=17%) most likely represents labeled substrate delivered by constant infusion and awaiting phosphorylation by the rate-limiting reaction regulated by hexokinase. Krivokapich et al28 reported similar findings. The predominant constituent in both groups was phosphorylated deoxyglucose (=83%).

Table 1 lists the tissue concentrations of glycogen in LAD- and circumflex-perfused myocardium. There were no differences between groups, but within groups the glycogen content in reperfused hearts was 37–40% below that in adjacent aerobic tissue. This diminution is similar to or greater than those incremental decreases previously reported in pig hearts rendered moderately ischemic90–92 and suggests that net glycogen resynthesis is not as rapid or predominant in pigs as in other species.29 The presence of some resynthesis, albeit incomplete, is suggested by the distribution of [3-3H]glucose in glycogen tissue biopsies. In reperfused LAD myocardium at end reperfusion, 11.5±2.4 μmol/g dry wt glycogen stores contained labeled glucose in euglycemic hearts, and 12.2±2.2 μmol/g dry wt glycogen stores contained label in hyperglycemic hearts.

Protocols Dealing With Experimental Approach

A total of six additional animals were studied, three of which were used to describe the relation between extracellular and intracellular [U-14C]2-deoxyglucose over the course of the perfusion trial. The protocol for the three intact extracorporeally perfused pig hearts was identical to that of the main study: 40 minutes of aerobic perfusion, 30 minutes of ischemia, and 40 minutes of reperfusion. Excess glucose was provided for the coronary perfusate (11.1±1.5 μmol/ml), similar to group II hearts in the main study. LAD flow rates for the three perfusion intervals were 5.2±0.2 ml·min⁻¹·g dry wt⁻¹ (aerobic perfusion), 2.1±0.6 ml·min⁻¹·g dry wt⁻¹ (ischemia), and 5.2±0.2 ml·min⁻¹·g dry wt⁻¹ (reperfusion). [U-14C]2-Deoxyglucose (42.8±0.6 μCi per animal at an infusion rate of 1.18×10⁶ dpm/min and at a specific activity of 320 μCi/μmol) was administered into the coronary perfusate as a constant infusion beginning at 0 minutes of perfusion. Sequential myocardial biopsies (average weight, 0.39±0.3 g dry wt) in triplicate were obtained at 35 minutes (aerobic perfusion) and 80 minutes (early reperfusion) of perfusion, and a large biopsy core (average weight, 32.9±1.8 g dry wt) was sampled at 110 minutes (end reperfusion). From one set

| Table 1. Biopsy Data for Intracellular Metabolites in Group I and Group II Swine Hearts |
|--------------------------------|----------------|-----------------|
| **Biopsies from LAD myocardium** | **Group I** | **Group II** | **p** |
| Deoxyglucose (%) | 13.4±3.1 | 21.1±5.4 | NS |
| Deoxyglucose-PO₄ (%) | 86.6±3.1 | 78.9±5.4 | NS |
| **p** | <0.001 | <0.003 |
| **Biopsies from LAD+LCF myocardium** | | | |
| LAD glycogen (μmol/g dry wt) | 155±18 | 172±15 | NS |
| LCF glycogen (μmol/g dry wt) | 256±10 | 274±21 | NS |
| **p** | <0.001 | <0.001 |

Group I, euglycemic hearts; group II, hyperglycemic hearts; p*, statistical comparisons between groups; LAD, left anterior descending coronary artery; p, statistical comparisons within groups; LCF, left circumflex coronary artery; NS, not significant. Values are mean±SEM.

Phosphorylated deoxyglucose is entirely intracellular in location. Unphosphorylated deoxyglucose has both intracellular and extracellular components. Intracellular deoxyglucose is estimated by subtracting the calculated extracellular concentration from the measured total deoxyglucose. Extracellular deoxyglucose is calculated by multiplying the perfusate (plasma) concentration of deoxyglucose times the extracellular space as determined by [³H]sorbitol,33
of biopsy material, the deoxyglucose data were derived (Table 2). Extracellular concentration of the nonphosphorylated precursor pool of deoxyglucose varied little over time, as predicted for a constant infusion administration of substrate. The 9.2% increase from 80 to 110 minutes was statistically significant (p<0.051). Intracellular concentration of nonphosphorylated substrate showed more variability (p=NS), and the increased levels at 80 minutes may reflect the variation of substrate delivery in early reperfusion previously observed in Figure 4.

A second set of biopsies was used to measure tissue glycogen levels over the course of perfusion and the incorporation of [5-3H]glucose into glycogen stores (Table 2). The purpose of these experiments was to determine the fraction of glucose uptake processed to glycogen synthesis in reperfusion and to estimate the error in calculating exogenous glucose utilization from \(^{3}\text{H}_{2}\text{O}\) production. [\(^{3}\text{H}\)]Glucose was administered as in the main study into the coronary perfusate (84.8±6.5 \(\mu\text{Ci}\) per animal at an infusion rate of 2.33±10\(^{4}\) dpm/min) beginning at 0 minutes of perfusion time. Myocardial glycogen levels at 35 minutes of perfusion (aerobic values) were comparable to circumflex tissue values reported in Table 1, whereas reperfusion values were less than those of the main study (again emphasizing the limited net resynthesis that occurs early on after ischemia). Uptake of \(^{3}\text{H}\) into glycogen during aerobic and early reperfusion intervals was virtually absent in two of the three hearts examined and only occurred at the end-reperfusion sampling time in all hearts. This translates into a rate of 886±329 dpm \(\cdot\) min\(^{-1}\) \(\cdot\) g dry wt\(^{-1}\) uptake of label from exogenous substrate into glycogen resynthesis from 80 to 110 minutes of perfusion. If one compares this with the rate of glucose utilization at comparable times (group II data, Figure 3A, corrected into dpm \(\cdot\) min\(^{-1}\) \(\cdot\) g dry wt\(^{-1}\)) reported in the main study (3,377±527 dpm \(\cdot\) min\(^{-1}\) \(\cdot\) g dry wt\(^{-1}\)), one notes as a “worst case scenario” that only 15% of counts from exogenous glucose was diverted to glycogen in late reflow. The remainder of counts was directly processed through glycolysis. This small diversion to glycogen, which would not all reenter the glycolytic cascade, can be ignored for practical purposes in calculating glycolytic flux, and in our opinion the specific activity of glucose in perfusate is a reasonable choice to use in such determinations.

A third set of biopsies was sampled to measure myocardial levels of glucose-6-phosphate at 35, 80, and 110 minutes of perfusion; these were 63±18, 37±3, and 10±1 nmoI/g dry wt, respectively. The downward trend in reflow no doubt reflects the accelerated rate of glycolytic flux, which siphons off this intermediate toward more distal products.

In the final three animals (two with aerobic perfusion and one with ischemia/reperfusion), distribution of labeled deoxyglucose in myocardium was evaluated as a potential explanation for the insensitivity of the marker in describing glucose utilization. In the two aerobic hearts, glucose was varied in the coronary perfusate: low (7.6±0.4 \(\mu\text{mol}\) /ml) glucose in one heart and high (14.2±1.2 \(\mu\text{mol}\) /ml) glucose administered with 200 microcounts/ml exogenous insulin in the other heart. Both hearts were extracorporeally perfused at aerobic flows only (LAD flow: 5.6 ml \(\cdot\) min\(^{-1}\) \(\cdot\) g dry wt\(^{-1}\) for low glucose and 6.6 ml \(\cdot\) min\(^{-1}\) \(\cdot\) g dry wt\(^{-1}\) for high glucose plus insulin; left circumflex plus right coronary artery combined flows: 10.0 ml \(\cdot\) min\(^{-1}\) \(\cdot\) g dry wt\(^{-1}\) for low glucose and 10.5 ml \(\cdot\) min\(^{-1}\) \(\cdot\) g dry wt\(^{-1}\) for high glucose plus insulin) for 40 minutes. Mechanical work was comparable in both hearts; i.e., left ventricular pressure was 121±3 and 117±2 mm Hg in low glucose and in high glucose plus insulin hearts, respectively. Each heart received dual tracers: 81 \(\mu\text{Ci}\) [5-3H]glucose and 45 \(\mu\text{Ci}\) [U-\(^{14}\text{C}\)]deoxyglucose for low glucose and 89 \(\mu\text{Ci}\) [5-3H]glucose and 45 \(\mu\text{Ci}\) [U-\(^{14}\text{C}\)]deoxyglucose for high glucose plus insulin. Glucose utilization estimated by \(^{3}\text{H}\)glucose was 6.1±5.1 and 33.5±13.0 \(\mu\text{mol}\) \(\cdot\) hr\(^{-1}\) \(\cdot\) g dry wt\(^{-1}\) in low glucose and in high glucose plus insulin hearts, respectively.

### Table 2. Biopsy Data From Swine Hearts for Deoxyglucose, Myocardial Glycogen, and [5-3H]Glucose Incorporation Into Myocardial Glycogen

<table>
<thead>
<tr>
<th>Perfusion (minutes)</th>
<th>35</th>
<th>80</th>
<th>110</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>[U-(^{14}\text{C})]2-Deoxyglucose values</strong>&lt;br&gt;(nmol/g dry wt)&lt;br&gt;Myocardial space</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extracellular</td>
<td>0.081±0.004</td>
<td>0.076±0.004</td>
<td>0.083±0.004</td>
</tr>
<tr>
<td>Intracellular</td>
<td>0.008±0.003</td>
<td>0.043±0.009</td>
<td>0.025±0.018</td>
</tr>
<tr>
<td><strong>Myocardial glycogen stores</strong>&lt;br&gt;(µmol/g dry wt)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pig 1</td>
<td>293.9</td>
<td>126.7</td>
<td>133.5</td>
</tr>
<tr>
<td>Pig 2</td>
<td>270.6</td>
<td>125.9</td>
<td>82.1</td>
</tr>
<tr>
<td>Pig 3</td>
<td>243.9</td>
<td>78.7</td>
<td>75.0</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>270±14</td>
<td>110±16</td>
<td>97±18</td>
</tr>
<tr>
<td><strong>[5-3H]Glucose incorporation into myocardial glycogen</strong>&lt;br&gt;(dpm/g dry wt)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pig 1</td>
<td>24,619</td>
<td>20,942</td>
<td>57,825</td>
</tr>
<tr>
<td>Pig 2</td>
<td>...</td>
<td>...</td>
<td>4,264</td>
</tr>
<tr>
<td>Pig 3</td>
<td>...</td>
<td>...</td>
<td>11,589</td>
</tr>
</tbody>
</table>
The dispersal of tissue radioactivity at aerobic perfusion was obtained from 10 biopsies scattered throughout the LAD perfusion bed taken at 40 minutes of end perfusion. Variation in distribution of radioactivity was large (74,595±12,027 dpm/g dry wt [mean±SEM] for low glucose and 166,934±18,668 dpm/g dry wt for high glucose plus insulin), but directionally, the means were in agreement with the trends observed by the [3H]glucose method; however, the standard deviation expressed as a percent of the mean (51% and 35%, respectively) reflected a large heterogeneity of dispersal. To further evaluate whether this dispersal was due to flow delivery to spatially separate myocardium within a common perfusion bed or some other property of the isotope, similar experiments were conducted in an ischemic/reflow animal. As in the main studies, LAD flow was reduced by 60% for 30 minutes and reperfused for 30 minutes. Left ventricular pressure was 84±2 mm Hg; perfusate glucose was augmented to 11.4±1.0 μmol/ml. [U-13C6]Deoxyglucose (34 μCi) was delivered at 1.08×104 dpm/min. Radioactivity was measured at 100 minutes (end reperfusion) in 10 small biopsies scattered throughout the LAD perfusion bed and from 10 samples of homogenate acquired from a large central core of LAD tissue. The latter was obtained to gain an estimate of the error in the measurement. Variation of radioactivity was again large for the biopsies (149,453±6,842 dpm/g dry wt) and small for the homogenate (134,545±2,038 dpm/g dry wt); the standard deviation as a percent of the mean was 14.5% and 4.8%, respectively, and was statistically different between biopsies and homogenate (p<0.05). These results indicate that the large dispersal of counts occurred both at conditions of aerobic perfusion and reperfusion and could not be explained by error in the measurement.

As a final exercise, the 14.5% heterogeneity of counts obtained above was used to estimate the contribution of spatial scatter on the data of the main study displayed in Figures 5 and 6. This variance (SD) among biopsies was used to construct new SEMs (heavy error bars in Figures 8 and 9), assuming that tissue heterogeneity as a function of biopsy site and small tissue size was the only explanation for the variance of data in the euglycemic and hyperglycemic heart groups.

Slopes of 2-deoxyglucose accumulation during reflow were again calculated only between the time points where duplicate samples were obtained. For these calculations, the standard deviation of the difference between the average of the samples taken at the two time points was designated as equal to the standard deviation of the individual tissue samples. Thus, the standard deviation in the slope (SD slope) is given by

\[ SD_{\text{slope}} = SD_{\text{sample}} / \Delta t \]

where SD sample is 14.5% of the average counts for the time period. The slopes during aerobic perfusions were obtained from single biopsies, and in this case the predicted standard deviation is

\[ SD_{\text{slope}} = \frac{SD_{\text{sample}} \times \sqrt{2}}{\Delta t} \]

The standard deviation of the slope for both perfusion intervals was then converted to the standard deviation of the glycolytic index (SD index) by the expression

\[ SD_{\text{index}} = SD_{\text{slope}} \times \frac{C_G \times 60}{DG \times 0.67} \]

Finally, the estimated standard error (SEM) was calculated from the standard deviation by the expression:

\[ SEM = SD / \sqrt{n} \]

These estimated SEMs are depicted in Figure 8 along with the actual SEM (heavy versus light error bars, respectively). As noted, the heterogeneity in tissue counts as a function of biopsy sites and tissue sample size at most time points accounted for only a small fraction of the variation in the 2-deoxyglucose measurements describing the glycolytic index. This suggests the large scatter in the data was due to some other inherent characteristic of the marker.

To further resolve this scatter problem, the tissue accumulation data of deoxyglucose for aerobic and reperfusion intervals shown in Figure 6A were reanalyzed using a statistical algorithm. This program calculated 500 different theoretical sets of tissue biopsies. Each set consisted of the same number of samples per time point as the number of biopsies actually acquired. The numbers representing tissue radioactivity at a given time were produced, assuming a normal distribution with a standard deviation of 14.5% and a mean radioactivity equal to that actually measured. Slopes were calculated from the 500 example sets, and the standard deviation was calculated. These SD slope values were converted to SEM index values as described previously and are shown in Figure 9 by the heavy error bars. Here again, the estimated variation in the data accounted for by the biopsy technique (heavy bars) was only a portion of the total variation (light error bars) in measurements.
observed in both animal groups, the latter of which more reflected scatter that was due to some other property of deoxyglucose among animals.

**Discussion**

The purpose of the present studies was to describe rates of glucose utilization in heart muscle estimated by the exogenous steady-state administration of [5-3H]glucose and to correlate this with a comparable estimate derived from equilibrium labeling with [U-14C]2-deoxyglucose. Glucose utilization was characterized at two different conditions of coronary flow (aerobic perfusion and reperfusion after reversible ischemia) in euaglycemic and hyperglycemic hearts. By using the tritium-labeled glucose technique, it was noted that glucose utilization during aerobic flows at conditions of mixed substrate availability in coronary perfusate was relatively low in both heart groups. In euaglycemic hearts, a 2.8-fold increase in glucose utilization was described. Hyperglycemia as an independent determinant of carbohydrate metabolism affected another twofold increase in glycyls during reflow. Net lactate extraction occurred during reperfusion in both groups, suggesting greater glucose oxidation in hyperglycemic hearts. Glycogen resynthesis in both groups was incomplete by the end of the reperfusion interval at 110 minutes, but the late accumulation of tritium label in the glycogen stores implied that resynthesis had begun. Deoxyglucose as a tissue marker of glucose utilization in this study failed to distinguish the trends prescribed by reflow or elevated blood glucose as described by [5-3H]glucose and was insensitive to either perturbation.


The animal model and extracorporeal perfusion system together with the analytic methods of measuring metabolic rates of substrate utilization and tissue concentrations of metabolites have been described elsewhere, and we were not essentially modified in this report. As in a previous report, we calculated glucose utilization from tritium-labeled glucose by using the specific activity of [5-3H]glucose in perfusate rather than intracellular glucose-6-phosphate. In the addendum studies reported in "Protocols Dealing With Experimental Approach," it was determined that the error of such calculations was small. This strategy emphasizes the contribution of exogenous substrate rather than glycogen and better simulates the kinetics of deoxyglucose in clinical PET studies. The only new method used in the present study was a sequential procurement of biopsies to sample myocardium for radioactivity of 2-deoxyglucose throughout the course of aerobic perfusion and reperfusion. The isotope was delivered as a steady-state infusion, and the precursor pool of substrate was comparatively constant over time (Table 2). For the purposes of calculation, the lump constant for 18FDG was used. Switching from the fluorine to carbon label effected no major shift in this constant in brain tissue. The constant is similarly unaffected by varying aerobic coronary flows or metabolic performance in myocardium as long as glucose availability is not exhausted.

Since our main goal was to evaluate the sensitivity of the deoxyglucose isotope as a tissue marker of glycysis, which in turn might prove useful in analyzing historical data derived from PET, it was important to determine the variation of the measurements determined by biopsy assay. The duplicate biopsies acquired at 85, 100, and 110 minutes provided an estimate of tissue heterogeneity at several points of perfusion. To calculate the standard deviation of these biopsies, the difference in radioactivity in the two biopsies was determined by subtracting the counts in the first biopsy from those in the second biopsy. The standard deviation of this difference was then calculated for each time point and included for the data from both heart groups. For data with a normal distribution, the standard deviation of a difference is equal to the standard deviation of individual samples multiplied by √2. The standard deviation as estimated from the duplicate biopsies was 9.0% at 85 minutes, 13.2% at 100 minutes, and 12.3% at 110 minutes of perfusion. These numbers agree favorably with those of the multiple tissue biopsies obtained from the ischemic/reflow heart in the separate studies. The counts contained in the samples from the homogenized tissue as an estimate of the error in the measurement of deoxyglucose were much closer in agreement than those from the tissue biopsies. Thus, the variability found in the biopsy measurements of radioactivity most likely originated from the heterogeneity of deoxyglucose deposition tissue and was not due to errors in measurement, biopsy technique, size of sample, or flow delivery. The error bars (heavy bars in Figures 8 and 9) per se appeared within agreement of the measurements over the range of responses recently reported in studies with FDG. Interestingly, this cumulative error estimate did not completely explain the scatter of the data shown in Figure 6A.

Evidence from several sources and experimental conditions has shown that glucose in the presence of mixed substrate availability is a relatively minor source of carbon for oxidative metabolism, produces only about 30% of acetyl coenzyme A for use by the citric acid cycle, and has inherent regulatory constraints that prevent sufficient glycolytic flux to support high rates of.
oxidative metabolism. In the fasted, aerobic, rested state, approximately 60–70% of the glucose extracted by the normal myocardium is stored as glycogen. Conversely, in states of anaerobiosis, glycolytic flux is increased at least threefold in hypoxia as well as all but the most dense or prolonged exposures to ischemia. This is felt to be due to the combined effects of coronary venous washout in hypoxia and the increased activity of many of the enzymes at key regulatory sites of the glycolytic pathway in both hypoxia and ischemia. Guth et al. confirmed the nonoxidative fate of this acceleration in glycolysis when they described in graded ischemia that the enhanced uptake of exogenous glucose was largely converted to lactate (16-fold increase) and released. It has long been argued that this increase in glycolysis yielded ATP, which, although quantitatively insufficient to sustain contraction, was spatially situated in the cytosol to better support several functions of cell maintenance.

The profiles of carbohydrate metabolism in short- or long-term reperfusion after myocardial ischemia differ in the literature. Myears et al. reported, in reperfused dog hearts after 1 hour of regional coronary occlusion and using intracoronary infusions of [1-14C]glucose to describe glucose utilization, a 273% increase in glycolysis above aerobic values, only 25% of which proceeded to oxidative metabolism. Schwaiger et al. observed, in reperfused dog hearts at 24 hours after a 3-hour coronary occlusion, a 286% increase in glucose uptake, the majority of which went to anaerobic glycolysis. Conversely, Mochizuki and Neely noted no overshoot in glycolysis in isolated in vitro rat hearts perfused with glucose and insulin during reflow. Our data support previous findings in dog hearts and show a moderate increase in glycolysis after recovery from ischemia. This flux rate was, however, insufficient to cause lactate production. We had previously shown, using an ischemia/reperfusion protocol in a similar pig heart model, that glucose as a substrate was not preferentially used in reperfusion and that glucose oxidation was presumably suppressed by that of fatty acid oxidation.

The influence of hyperglycemia was reviewed in this study because as an intervention it has been commonly used in clinical PET imaging studies to enhance uptake of deoxyglucose in patients with unstable angina or after myocardial infarction. In normal hearts from healthy subjects at aerobic conditions, hyperglycemia is noted to enhance both extraction and oxidation of exogenous glucose as well as glycogen storage. It is this increase in glucose uptake, possibly via increased release of endogenous insulin, that improves the imaging resolution of PET clinical evaluations. The influence of excess glucose in ischemic hearts has long been debated and contested, particularly as a therapeutic strategy, with no clear consensus. We observed in earlier experiments in pig hearts rendered globally ischemic (−492% below control values) that hyperglycemia increased glucose uptake/consumption but did not significantly modify glycolytic flux, energy production, or mechanical performance. More recently, Runnman et al. reported an improvement in cardiac function in hypoxic rabbit hearts affected by excess exogenous glucose in perfusate that was associated with enhanced glucose utilization measured by [2-13C]glucose and a sparing of endogenous glycogenolysis. Since lactate production was not affected in their data, one interpretation might be that hyperglycemia directed more glucose to residual oxidative metabolism. In the present study of ischemia and reperfusion, glycolysis was also further accelerated by hyperglycemia, and by inference from the lactate data, glucose oxidation was increased as in hypoxic hearts. Unfortunately and in contrast to the data of Lopaschuk et al., this did not predict a favorable restoration of mechanical function.

A main objective of this study was to evaluate the sensitivity of tissue accumulation of [1-14C]deoxyglucose as an accurate marker of glycogenolysis and to quantitatively compare it with [6-13C]deoxyglucose. Disappointingly, this carbon-labeled tracer was not able to characterize either the influence of reperfusion or hyperglycemia on glycolysis. This lack of sensitivity of deoxyglucose as a marker of glycolysis is helpful in a negative sense in interpreting the previously reported flow/metabolic mismatch data using PET. Whatever flow-dependent metabolic perturbation that this tissue tracer technique is representing, the signal does not appear to be tightly coupled with that of an accepted independent measure of glycolysis, at least when glycolytic rates are moderately low. Buxton and Schelbert recently reported, moreover, that the increased glycolysis described by 18FDG appeared to be the result of activity in the remote normal zones of myocardium rather than the posts ischemic reperfused myocardium. In our judgment, until these several issues of marker sensitivity are resolved, it would be premature to interpret these deoxyglucose data as a means to understand bioenergetics or substrate preference in jeopardized ischemic or reperfused myocardium.

Acknowledgments

We would like to thank S.K. Rasmussen, D.K. Paulson, E. Scarbrough, and C.R. Kidd for their technical assistance.

References


34. Bradley DC, Kaslow HR: Radiometric assays for glyceral, glucose, and glycogen. Anal Biochem 1989;180:11–16


Correlation between [5-3H]glucose and [U-14C]deoxyglucose as markers of glycolysis in reperfused myocardium.
A J Liedtke, B Renstrom and S H Nellis

Circ Res. 1992;71:689-700
doi: 10.1161/01.RES.71.3.689

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/71/3/689

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/