Effects of Treatment with Pyruvate and Tromethamine in Experimental Myocardial Ischemia

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SUMMARY  Failure of glycolysis to increase sufficiently to supply optimal levels of energy production in ischemic heart muscle is due in part to the cumulative restraints of acidosis on rate-limiting enzymes, particularly glyceraldehyde-3-phosphate dehydrogenase. In an effort to modify this inhibition and salvage jeopardized myocardium, treatment with excess levels of pyruvate and tromethamine (Tris), designed to buffer intracellular hydrogen ion accumulations and improve the oxidation-reduction ratio, NAD+/NADH, was tested in 59 swine hearts in two separate preparations of global and regional ischemia. Global ischemia, per se, caused hemodynamic deterioration and shortened survival time (44.3 ± 3.1 minutes). Myocardial oxygen consumption, fatty acid oxidation, and glucose uptake were all significantly (P < 0.001) reduced as were estimates of glycolysis and tissue stores of creatine phosphate and ATP (P < 0.01). Although treatment with Tris alone was inconclusive, administrations of pyruvate (40-50 mM) buffered with Tris (added directly into the coronary perfusate) effected an improvement in mechanical function and a significant prolongation in survival time (56.9 ± 2.6 minutes, P < 0.01). Glycogenolysis was enhanced and levels of key glycolytic intermediates were reduced, suggesting an acceleration of glycolytic flux. Excess levels of pyruvate (1.52 ± 0.48 μmol/ml of coronary perfusate) provided added substrate for oxidation and led to a greater than 5-fold increase in rates of pyruvate decarboxylation as compared to untreated ischemic hearts. Combined effects of pyruvate oxidation and accelerated glycolysis favorably preserved tissue stores of high energy phosphates. In a model of regional myocardial ischemia, pyruvate and Tris therapy, administered systemically, significantly decreased both the size (P < 0.025) and severity (P < 0.001) of ischemic injury as estimated by open-chest epicardial S-T segment mapping techniques. These data suggest that metabolic manipulations may provide another mode of therapy for the preservation of ischemic myocardium. Treatment with pyruvate and Tris represents one such approach and was shown to reduce ischemic injury, prolong survival, and improve mechanical and metabolic functions.

CARDIAC MUSCLE is capable of using a variety of metabolic fuels, with the two most preferred substrates being fatty acids and glucose. Rates of utilization and selection of any one substrate are dependent on a number of interrelated factors including the concentration of that substrate in plasma, the availability of alternate competitive substrates, oxygen delivery to the myocardium, the mechanical activity of the heart, and the plasma levels of certain hormones. In well oxygenated hearts, fatty acid is the preferred substrate with energy production resulting from both β-oxidation and the tricarboxylic acid cycle. This is prevented in myocardial ischemia, and fatty acid metabolism is significantly modified with a buildup of long chain acyl-CoA groups, appreciable reduction in acetyl-CoA synthesis, presumably from abnormalities of the carnitine translocase system in the mitochondrial membrane, and a marked decrease in ATP production.

It has long been hoped that to fill this void in anaerobic energy production, carbohydrate metabolism might in some way be stimulated and accelerated. Unfortunately, from reported rates of glycogen breakdown and glucose uptake during studies on sustained oxygen deprivation in dog and rat hearts, only 10-15% of the energy needs of mammalian hearts can be met through anaerobic glycolysis. The critical enzymatic step in ischemic inhibition of glycolysis appears to be at glyceraldehyde-3-phosphate dehydrogenase, in part from restraints induced by intracellular accumulations of hydrogen ion and NADH. One approach to relieve this inhibition would be to increase the intracellular NAD+/NADH ratio or provide excess buffer, or both. Such an alteration in oxidation-reduction products is accomplished through the conversion of pyruvate to lactate by lactate dehydrogenase. The present studies were designed to test whether this biochemical maneuver offers real advantages in protecting ischemic myocardium. Two major questions were addressed: (1) Do treatments with pyruvate and tromethamine (Tris) buffer favorably improve glucose metabolism and energy production in the ischemic myocardium? (2) Will the metabolic sequelae which result effect practical benefits in modifying the extent of ischemic injury in heart muscle? Metabolic experiments were conducted in a previously described intact, globally ischemic, working swine heart preparation. Estimates of changes in ischemic injury were performed on a modified version of a regionally ischemic heart model using repetitive multiple-site epicardial ST-T segment mapping during reversible coronary occlusions.

Methods
Fifty-nine swine of either sex, weighing 26.5-59.6 kg (average, 37.8 kg), were studied by either of two protocols...
after anesthesia with pentobarbital (35 mg/kg, iv) and the establishment of controlled positive-pressure ventilation using 100% oxygen. Each animal’s arterial pH, Po2, Pco2, and CO2-combining power were determined frequently throughout the studies to ensure adequacy of ventilation and acid-base balance. Experiments involving both global and regional myocardial ischemia were conducted.

The global ischemia preparation in the working swine heart has been previously described, including the methods and types of metabolic and mechanical parameters available for measurement. A pump-regulated right heart bypass circuit was established in the open-chest anesthetized swine both to control systemic cardiac output and use the right ventricle as a reservoir for coronary effluent. Total coronary perfusion to the left and right coronary arteries was controlled independently through a separate closed-loop perfusion circuit regulated by two Sam s low flow perfusion pumps. The coronary perfusate, drained from the right ventricular reservoir, consisted of the animal’s own whole blood obtained by exchange transfusion with dextran, which then was supplemented with additional substrates and hormone and continuously reoxygenated by a Bentley blood oxygenator (O2-CO2 mixture, 97:3%) prior to perfusion. Substrate augmentations to the coronary perfusate included [U-14C]palmitate (New England Nuclear) complexed to bovine albumin, fraction V (4 g/100 ml), to give an average initial free fatty acid concentration of 0.7 mM; excess glucose to provide an initial loop concentration of 31.3 mM; and regular zinc insulin, 0.025 U/ml of perfusate. Labeled palmitate was used to estimate free fatty acid consumption, extra glucose to avoid substrate depletion during the course of perfusion, and insulin to ensure rapid intracellular transport of glucose into the myocardium. Hemodynamic and metabolic parameters for each heart were determined at 10-minute intervals for a maximum possible 60 minutes of coronary perfusion trial. Measurements included heart rate (controlled by an atrial pacemaker on demand mode), coronary flow (summed from the flows of the two coronary perfusion pumps), cardiac index (determined from the flow rate of the right heart bypass perfusion pump and normalized by animal weight in kilograms), aortic and left ventricular pressures, myocardial oxygen consumption, uptake and oxidation of free fatty acids by the myocardium, and rates of glucose consumption, using methods and calculations previously described.

All hemodynamic data were recorded on an eight-channel Mark 200 Brush recorder. At the conclusion of each study, either at the completion of the 60-minute perfusion or following the development of ventricular fibrillation from ischemia-induced mechanical failure, transmural sections of ventricular myocardium were immediately removed and frozen between blocks of aluminum that were being cooled in liquid nitrogen. The frozen tissue later was powdered and samples were obtained for determination of tissue glycogen. Additional samples of powder were extracted in a 6% perchloric acid and the buffered supernatant fraction was used to determine tissue concentrations of creatine phosphat e, ATP, ADP, AMP, lactate, pyruvate, glucose 6-phosphate, fructose 1,6-diphosphate, triosephosphates and α-glycerol phosphate. Creatine phosphate and ATP were assayed by the hexokinase glucose-6-phosphate dehydrogenase procedure. ADP and AMP were determined after the method of Adam; tissue lactate, glucose 6-phosphate, and α-glycerol phosphate by the procedure of Hohorst; pyruvate after the methods of Bucher et al.; and fructose 1,6-diphosphate and triosephosphates (glyceraldehyde 3-phosphate and dihydroxyacetone phosphate) from the methods of Bucher and Hohorst.

The protocol for evaluating metabolism during whole heart ischemia consisted of comparing three separate groups of hearts provided with similar levels of substrate and hormone and rendered similarly ischemic. Normal coronary perfusion in all hearts was maintained for 20 minutes and then critically restricted over the subsequent 10-minute period. The initial 20-minute period of control perfusion was designed to permit adequate mixing of the various ingredients in the coronary central loop volume and to allow for equilibration of intracellular and extracellular 14C-palmitate, together with achievement of steady state production rates of 14CO2 from oxidation of 14C-palmitate. A gradual, rather than a precipitous, reduction in coronary flow was necessary because in this preparation a sudden drop in coronary flow of large magnitude resulted almost invariably in the development of ventricular fibrillation. In the three groups of ischemic hearts studied, nine were untreated, 12 received pyruvate (40-50 mM) buffered in Tris, and to seven only Tris (50 mM) was administered. The treated groups received therapy as a constant infusion selectively into the coronary perfusate between 15 and 30 minutes of perfusion so that drug delivery was completed by the time of onset of absolute ischemia. Tissue samples for metabolites from ischemic myocardium in the three groups were compared with data obtained from a fourth group of nine untreated hearts provided with similar levels of substrates and hormone which were perfused for 60 minutes at normal rates of coronary flow.

To further examine the metabolism of pyruvate during global ischemia, a separate series of studies was conducted on 10 swine hearts provided with similar perfusate concentrations of fatty acid (unlabeled), glucose, and insulin. Five hearts were untreated; five received pyruvate and Tris treatment in the previously described dosage; and tracer amounts (60 µC) of [U-14C]pyruvate (New England Nuclear) were administered selectively into the coronary perfusate of all hearts. Arterial and venous samples of coronary perfusate blood were collected and centrifuged, and a sample of serum was used for determining radioactivity due to 14C-pyruvate. To remove any labeled CO2, the sample of serum was first treated with 0.1 ml of 2 N HCl, allowed to stand for 45 minutes, then neutralized with 0.1 ml of 2 N NaOH before counting. Total pyruvate content of serum was estimated by the methods of Bucher et al.

The rate of pyruvate oxidation was determined by measuring 14CO2 production from labeled pyruvate. Separate arterial and venous blood samples were collected under heptane. Whole blood (1 ml) was transferred to a 25-ml Erlenmeyer flask containing 1.0 ml of 12 N H2SO4 in the bottom of the flask and 0.4 ml of Hyamine hydroxide in a plastic central bucket suspended from the stopper. After mixing the blood and acid, the samples were allowed to

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stand for 2 hours before the buckets were removed, placed in
10 ml of toluene scintillator, and counted in a Beckman 150
liquid scintillation counter. Corrections for quenching
were made by the channels-ratio method. Pyruvate oxidation
was calculated as: μmol pyruvate/hour per g = venous-
arterial 14CO2 (counts/min per ml) × coronary flow (ml/
hour)1/2 [arterial + venous specific activity of 14C-pyruvate
(counts/min per μmol of pyruvate) × dry weight of heart
g). All metabolic data were expressed per gram of dry
weight of ventricular myocardium.

A second protocol was developed to evaluate the effects
of pyruvate plus Tris buffer and Tris buffer alone on changing
the extent of regional myocardial ischemia. Methods for
estimating size of ischemic injury in a model of reversible
corony occlusions were adapted from the techniques of
Maroko et al.14-22 and modified for use in anesthetized swine.
In 12 animals, hearts were exposed through bilateral
thoracotomy and suspended in a pericardial cradle. To
measure aortic pressure, a Teflon tubing catheter was passed
retrograde into the central aorta from a femoral artery and
connected to a Statham P23Db pressure transducer. A high
fidelity, manometer-tipped pressure device (Statham model
P866) also was passed to the left ventricle from a carotid
artery. Pressure signals from the device were differentiated
by a direct-coupled, solid state, constant input impedance
circuit on the Mark 200 Brush recorder (zero phase shift
from 0-90 Hz) and isovolumic estimates of contractility,
i.e., dp/dtmax, and dp/dt normalized for total developed left
ventricular pressure (Vp m)22 were determined.

For each heart the left anterior descending coronary
artery or one of its major diagonal branches was dissected
free from adjacent epicardial tissue so that a nonserrated
bulldog vascular clamp could be placed periodically to effect
temporary 15-minute coronary occlusions. Ten to twelve
sites distant from, near to, and within the area of ischemia
were chosen for epicardial unipolar electrocardiographic
monitoring with a saline-wetted, cotton wick exploring
electrode. Hemodynamic and electrocardiographic measure-
ments were recorded for each animal prior to and follow-
ing 15 minutes of coronary occlusion, either with or
without systemically administered iv therapy (pyruvate, 400
mm, buffered in Tris, nine animals; Tris alone, 400 mM, three
animals). Recovery periods between coronary occlusions
extended for at least 30 minutes between occlusions. Ther-
apies were randomized with untreated ischemic trials and
begun at a constant infusion (100 mmol of either pyruvate or
Tris in 250 ml of 0.9 % saline infused at 7.5 ml/min) before
ischemia and were continued for the entirety of the coronary
occlusion trials. Electrocardiographic data were standard-
ized (1 mV = 1 mm S-T segment elevation) and analyzed
(significant myocardial injury defined by S-T segment
elevations exceeding 2 mV) as previously described.14-22
Arterial blood samples during control, ischemia, and
ischemia plus treatment trials were obtained for analysis of
blood concentrations of glucose,21 pyruvate,21 and insulin.28

Metabolic, mechanical, and electrophysiological mea-
surements in either protocol were compared by paired or
unpaired Student's t-tests with statistical significance
defined for probability values less than 5%.

Results

EFFECTS OF PYRUVATE AND TRIS THERAPY ON
GLOBALLY ISCHEMIC HEARTS

Comparisons of hemodynamic and metabolic data for the
two groups of working swine hearts, one untreated and the
second treated with pyruvate and Tris, are illustrated in
Figures 1 and 2. The data are displayed as a function of time
during the course of myocardial perfusion both preceding
and following critical restrictions in total coronary flow.
Heart rates and cardiac output were similar between the two
groups at comparable time periods. Ischemia (coronary flow
reduced from 5.0 to 2.4 ml/min per g dry weight of
ventricular myocardium) in untreated hearts induced a
gradual decline in left ventricular pressure development
(Fig. 1) despite adjustments in systemic cardiac output (34.4

\[
\text{FIGURE 1 Hemodynamic performance during global heart is-
chemia. At comparable restrictions in total coronary blood flow,
treated hearts with buffered pyruvate have much greater preserva-
} 
\text{tion in mechanical function, as shown by the significant differences
in left ventricular (LV) end-diastolic pressure. Survival time for
} 
\text{treated hearts was 12.6 minutes longer than for untreated ischemic
hearts.}
\]
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Several metabolic responses to global heart ischemia. Myocardial oxygen consumption, glucose uptake, and fatty acid oxidation declined progressively in both groups as a function of global ischemia. Changes in myocardial oxygen consumption appeared terminally more pronounced in untreated hearts, whereas depressions in glucose uptake and 14CO2 production from labeled palmitate were greater in treated hearts.

Estimates of glycolysis were made by comparing the tissue levels of glycolytic intermediates in ischemic hearts to values for a separate control group of nine hearts perfused at normal coronary flows for 60 minutes (Fig. 3). Despite differences in mean perfusion times between the two groups, the ischemic data in general conformed to previous studies regarding ischemic sequelae in heart muscle.2,9,10,19 Glycogenolysis was accelerated during ischemia, as reflected by the respective levels of tissue glycogen (230.0 µmol/g in control hearts vs. 184.8 µmol/g in ischemic hearts, P < 0.01). Inhibition at glyceraldehyde-3-phosphate dehydrogenase produced the expected buildup of tissue glucose-6-phosphate (1,274.8 vs. 1,008.3 µmol/g, P < 0.005), fructose 1,6-diphosphate (204.5 vs. 59.9 µmol/g, P < 0.001), α-glycerol phosphate (6,878.6 vs. 427.1 µmol/g, P < 0.001), and combined trioses (dihydroxyacetone phosphate and glyceraldehyde-3-phosphate, 362.0 vs. 52.4 µmol/g, P < 0.001) while reducing myocardial concentrations of pyruvate (189.1 vs. 310.3 µmol/g, P < 0.001). Further inhibitions at pyruvate dehydrogenase led to buildup of

![Figure 2](http://circres.ahajournals.org/)

**Figure 2** Several metabolic responses to global heart ischemia. Myocardial oxygen consumption, glucose uptake, and fatty acid oxidation declined progressively in both groups as a function of global ischemia. Changes in myocardial oxygen consumption appeared terminally more pronounced in untreated hearts, whereas depressions in glucose uptake and 14CO2 production from labeled palmitate were greater in treated hearts.

![Figure 3](http://circres.ahajournals.org/)

**Figure 3** Tissue concentrations of glycolytic intermediates. As compared with untreated nonischemic hearts (shown to the left in each panel), ischemia significantly reduced glycogen but elevated all other metabolites. Treatment with buffered pyruvate significantly reduced all tissue levels of glycolytic intermediates with the exception of lactate, suggesting acceleration in both glycogenolysis and glycolysis.
tissue lactate (62.3 vs. 22.4 μmol/g, P < 0.001) in ischemic hearts.

As a result of these diminutions in fatty acid and carbohydrate utilization, energy production by ischemic myocardium was significantly reduced (Fig. 4). Compared with tissue stores of metabolites in nonischemic hearts, both creatine phosphate and ATP levels were significantly reduced (22.8 ± 48.8 μmol/g, P < 0.001, and 16.4 vs. 21.6 μmol/g, P < 0.01, respectively) while adenosine intermediates (ADP and AMP) were increased.

Addition of pyruvate and Tris to the coronary perfusate (pyruvate concentration increased from 0.11 ± 0.03 to 1.52 ± 0.48 μmol/ml) caused several significant changes in mechanical and metabolic functions of other hearts rendered globally ischemic. At comparable levels of heart rate, cardiac output, and restrictions in total coronary flow, the treated group of hearts, as opposed to the untreated ischemic group, evinced much less compromise of left ventricular hemodynamic performance (similar declines in left ventricular pressure development with adjustments in systemic cardiac output from 31.6 ml/min per kg at 20 minutes of perfusion to 37.3 ml/min per kg terminally, but a rise of only 4 mm Hg in left ventricular end-diastolic pressure) (Fig. 1). Group survival time was significantly prolonged over that of untreated hearts (56.9 ± 2.6 minutes, P < 0.01) and ventricular fibrillation occurred in only two of 12 hearts.

Metabolically (Fig. 2), myocardial oxygen consumption by the treated group remained somewhat higher than in untreated ischemic hearts, declining only by 20% (0.70 mmol/hour per g at 20 minutes of perfusion to 0.56 mmol/hour per g terminally). Cell uptake of exogenous glucose fell by 76.6% (612.2 μmol/hour per g at 20 minutes of perfusion to 143.1 μmol/hour per g terminally); this value was greater than for the untreated group and presumably reflected the effects of a longer exposure to global ischemia. Declines in cellular uptake of free fatty acids (-38.9%) and intracellular fatty acid oxidation (-77.4%) by treated hearts also were observed. However, the reductions in fatty acid oxidation were statistically greater (P < 0.05) in treated hearts and appeared to antecede the onset of absolute global ischemia, corresponding more in timing with the initiation of therapy. Such a temporal relationship suggested that pyruvate may be acting as a preferred substrate for oxidation and thereby may diminish fatty acid oxidation through competitive mechanisms at the level of the citric acid cycle.

To test this hypothesis, a separate study was conducted on 10 additional swine hearts provided with similar levels of fatty acid, glucose, and insulin. Five hearts were untreated, five were treated with pyruvate and Tris (50 mM each) administered in the coronary perfusate over the 15-minute interval preceding the induction of absolute ischemia, and all received tracer quantities (60 μCi) of [U-14C]pyruvate to estimate rates of oxidation (Fig. 5). Because of the previously demonstrated differences in survival times between treated and untreated hearts, only the first 20 minutes of absolute ischemia were studied (one untreated heart failed to survive this study period). At comparable levels of coronary flow restriction, similar declines in ventricular pressure development and myocardial oxygen consumption were observed for the two groups, whereas left ventricular end-diastolic pressure again rose to higher values in untreated hearts. In hearts treated with high concentrations of pyruvate (1.19 ± 0.29 vs. 0.13 ± 0.03 μmol/ml in the untreated group), pyruvate oxidation increased more than 5-fold both during the preischemic perfusion and over the 20-minute exposure to whole heart ischemia. This sug-
gested that a larger fraction of the limited available oxygen was used to oxidize pyruvate.

Rates of intracellular glycolysis for the original groups of hearts are shown in Figure 3. Compared to untreated ischemic hearts, addition of pyruvate and Tris resulted in significant decreases in all glycolytic intermediates. Despite differences in group mean survival times, these trends suggested improved function of glyceraldehyde-3-phosphate dehydrogenase in treated hearts, with subsequent increased glycolysis. Glycogenolysis also was greater. Although the treated hearts were exposed to much greater levels of pyruvate over the course of perfusion, there was not an appreciable further buildup of tissue lactate as compared to untreated ischemic hearts. Data for high energy phosphates (Fig. 4) in the treated group were no different than in untreated hearts despite a longer exposure by 12.6 minutes to global ischemia. This suggested enhanced energy production in treated hearts, in part contributed to both by glycolysis and utilization and oxidation of pyruvate.

Tris alone did not appear to be protective (Table 1). Although group mean survival time was slightly improved for the nine hearts thus treated (49.9 ± 5.1 minutes), it was not statistically different from that of the untreated ischemic group. Mechanical failure was pronounced and metabolic dysfunctions were similar to those of untreated hearts. Tissue glycolytic intermediates also were comparable to findings for untreated rather than pyruvate and Tris-treated hearts (glucose 6-phosphate, 1,151.1 μmol/g; glycogen, 172.1 μmol/g), as were tissue stores of adenosine products (Table 1).

**EFFECTS OF PYRUVATE AND TRIS THERAPY ON REGIONALLY ISCHEMIC HEARTS**

To evaluate whether the metabolic advantages described above applied to conditions of regional cardiac ischemia, separate studies were conducted on 12 swine using the

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**Table 1** Hemodynamic and Metabolic Data from Swine Hearts Treated with Tromethamine (Tris)

<table>
<thead>
<tr>
<th></th>
<th>Coronary flow (ml/min/g)</th>
<th>Aortic pressure (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>MVO₂ (μmol/h/g)</th>
<th>Glucose uptake (μmol/h/g)</th>
<th>FFA oxidation (*¹⁴CO₂ production) (μmol/h/g)</th>
<th>CP (μmol/g)</th>
<th>ATP (μmol/g)</th>
<th>AMP (μmol/g)</th>
<th>ADP (μmol/g)</th>
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<tbody>
<tr>
<td><strong>Preischemia</strong></td>
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<td></td>
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<tr>
<td>Mean</td>
<td>5.35</td>
<td>86.3</td>
<td>6.0</td>
<td>0.72</td>
<td>496</td>
<td>18.4</td>
<td>29.6</td>
<td>15.2</td>
<td>0.38</td>
<td>2.87</td>
</tr>
<tr>
<td>SEM</td>
<td>±0.50</td>
<td>±2.1</td>
<td>±1.1</td>
<td>±0.03</td>
<td>±96</td>
<td>±4.5</td>
<td>±2.1</td>
<td>±1.0</td>
<td>±0.08</td>
<td>±0.08</td>
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<tr>
<td>Δ</td>
<td>-58.1</td>
<td>-9.6</td>
<td>+383.3</td>
<td>-38.9</td>
<td>-37.5</td>
<td>-46.2</td>
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<tr>
<td><strong>Ischemia</strong></td>
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<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>+0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
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<tr>
<td>P*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<td>NS</td>
<td>NS</td>
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<tr>
<td>P***</td>
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<td>&lt;0.001</td>
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LVEDP = left ventricular end-diastolic pressure; MVO₂ = myocardial oxygen consumption; FFA = free fatty acid; CP = creatine phosphate. NS = nonsignificance between statistical comparisons.

Preischemic data were taken at 20 minutes of perfusion; ischemic data were taken terminally at the group mean survival time of 50.0 ± 5.1 minutes. P values represent intragroup statistical comparisons between data at 20 minutes of perfusion and terminally at 50.0 minutes of perfusion. P* values represent intergroup comparisons of terminal data between the Tris-treated hearts and untreated ischemic hearts. P** values are between Tris-treated hearts and untreated nonischemic hearts. NS denotes nonsignificance between statistical comparisons.

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**Table 2** Hemodynamic Data before and during Regional Coronary Artery Occlusions in Swine Heart Preparation

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Heart rate (beats/min)</th>
<th>Aortic mean pressure (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>LV dp/dt/dp (mm Hg/sec/mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pyruvate and Tris</strong></td>
<td>(n = 12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>97.7</td>
<td>97.6</td>
<td>94.0</td>
<td>96.2</td>
</tr>
<tr>
<td>SEM</td>
<td>9.3</td>
<td>8.7</td>
<td>8.2</td>
<td>3.7</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P*</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td><strong>Tris</strong> (n = 5)</td>
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<tr>
<td>Mean</td>
<td>98.5</td>
<td>97.0</td>
<td>90.3</td>
<td>94.2</td>
</tr>
<tr>
<td>SEM</td>
<td>8.5</td>
<td>9.8</td>
<td>7.9</td>
<td>4.8</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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</table>

Ocl. = coronary artery occlusion (15 minutes); LVEDP = left ventricular end-diastolic pressure.

P represents paired Student's t-test comparisons between nonischemic control periods and the respective occlusion trials with and without treatment (control data shown here are a composite of the two separate control periods). P* refers to the comparisons between the occlusion trials with and without therapy. NS denotes nonsignificance.
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open-chest epicardial S-T mapping methods of Maroko et al. The size of the area of ischemic injury to the heart that resulted from transient 15-minute occlusions of the left anterior descending coronary artery or one of its major diagonal branches was considered small and caused no significant changes in any of the measured hemodynamic parameters during either drug trial (Table 2). Treatment with pyruvate and Tris, or Tris alone, was administered systemically as a constant iv infusion over 30 minutes in a volume of 250 ml of saline. Such preloading tended to slow the heart rate and raise left ventricular and aortic mean pressures. Left ventricular contractility remained unaltered. As compared to untreated occlusion trials (Fig. 6), pyruvate and Tris caused both a decrease in the severity and size of ischemic injury as evidenced by the significant reductions in both the total sum of S-T elevations and the number of epicardial sites with S-T elevations greater than 2 mV (P < 0.001 and P < 0.025, respectively, between collations). These benefits were not the result of pyruvate-stimulated hepatic gluconeogenesis (Table 3) which might have acted secondarily to salvage ischemic heart muscle by increasing blood levels of glucose and insulin. These data suggested, rather, an efficacious interaction of pyruvate and Tris with the heart. Tris therapy alone was ineffective in preserving jeopardized myocardium (Fig. 6).

Discussion

The search for methods to salvage and protect ischemic myocardium has been extensive, particularly over the past one to two decades, and many new therapeutic advances have been made. Such therapies may be categorized according to their mechanisms of action, and all serve to act either separately or in combination to increase the oxygen supply to ischemic tissue, decrease the oxygen demands of cardiac muscle, alter substrate metabolism by the heart and thereby maximize energy production, and/or attenuate the inflammatory response initiated by dying and infarcted heart tissue which might adversely effect viable adjacent areas. Representative examples of interventions which fall under one or another of these headings and which have been shown experimentally or clinically to have therapeutic efficacy include: coronary artery reperfusion or its clinical analogue, saphenous vein aortocoronary bypass grafting, nitroglycerin, and intraaortic balloon counterpulsation, all of which act by improving oxygen delivery through enhanced coronary flow; nitroprusside and other vasodilators, β-blocking agents, and digitalis in the failing heart, which act by lessening the hemodynamic determinants of myocardial oxygen consumption; hyaluronidase, which is said to deliver essential nutrients to ischemic tissue through extracellular diffusion; and steroids, other hormones, and hyperosmotic agents which act by attenuating or lessening several components of inflammation.

Attempts to modify substrate utilization by ischemic heart muscle through metabolic manipulations have in the past centered mainly on treatments involving the infusions of solutions containing excess glucose, insulin, and potas-

<table>
<thead>
<tr>
<th>Table 3 Changes in Blood Levels of Pyruvate, Glucose, and Insulin during Regional Ischemia with and without Therapy</th>
</tr>
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<tbody>
<tr>
<td>Pyruvate (μmol/ml) Glucose (mg/100 ml) Insulin (μU/ml)</td>
</tr>
<tr>
<td>Control Occl. Occl. + therapy Control Occl. Occl. + therapy Control Occl. Occl. + therapy</td>
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<tr>
<td>Mean 0.45 0.44 2.07 132.4 131.4 131.4 7.20 6.74 8.00</td>
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<tr>
<td>SEM 0.12 0.12 0.40 22.5 19.4 16.3 1.35 0.95 1.08</td>
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<td>P NS &lt;0.001 NS NS NS NS NS</td>
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Abbreviations and statistical comparisons are identical to those in Table 2.
PYRUVATE AND TRIS IN MYOCARDIAL ISCHEMIA/Liedtke et al.

As first reported in 1961, \(^6\) success with this therapy was explained by an array of theoretical speculations, most important of which was the potential enhancement of energy production through accelerated anaerobic glycolysis.\(^7\) Unfortunately, large clinical series confirming these early reports have not as yet been forthcoming, and both the results and hypotheses used to support early claims have in one way or another since been challenged.\(^8\) - 12

Chief among the criticisms raised has been the clearly documented finding that glycolytic flux is not accelerated but rather critically impaired during myocardial ischemia due to inhibitory influences on several glycolytic enzymes. Although the enzymatic capacity of the glycolytic pathway in mammalian hearts theoretically is capable of supplying sufficient energy for normal hemodynamic function (as in reptilian hearts), in a practical sense only 10-15% of total energy requirements are met.\(^13\) - 24

Of the key rate-controlling glycolytic enzymes, i.e., hexokinase, phosphofructokinase, glyceroldehyde-3-phosphate dehydrogenase, pyruvate kinase, and pyruvate dehydrogenase, glyceroldehyde-3-phosphate dehydrogenase activity appears most restrained by the products of cardiac ischemia.\(^10\) - 11

The purpose of the present study was to attempt to remove this inhibition and improve glucose metabolism in two separate models of myocardial ischemia. Buffered solutions of pyruvate collectively reduced the severity of ischemic injury, improved hemodynamic performance, and prolonged survival. These changes were associated metabolically with accelerated glycolysis and enhanced decarboxylation from pyruvate which resulted in continued production of high energy phosphates even during progressive ischemia. Buffering, per se, appeared inadequate to explain these findings because Tris therapy alone, in either protocol, was ineffective in preserving myocardial functions. Final judgments regarding Tris, however, will require further studies on the exact dose-response relationships for this agent and on the ease with which Tris enters cells. It may be that Tris undergoes significant delay in transport across the cell membrane which obviates its use in a setting of heart muscle ischemia but which may be circumvented by other buffering agents.

In addition to the effects on glycolytic flux, presumably from favorable adjustments in NAD\(^+\)/NADH ratios, treatment with buffered pyruvate solutions improved oxidative phosphorylation from pyruvate through the tricarboxylic acid cycle. Evans et al.\(^25\) first showed the competitive relationship of pyruvate and free fatty acids for available oxygen in nonischemic rat hearts, and this interaction also appears to hold for ischemic hearts. By presumed mass action effects, pyruvate in excessive doses was oxidized preferentially to fatty acids. Myocardial oxygen consumption was somewhat higher in treated hearts, possibly as a result of this excess available substrate. Pyruvate has many advantages as a substrate in that it is readily metabolized by heart muscle and must undergo only a minimum number of preliminary reactions prior to entry into the citric acid cycle. By its irreversible combination with coenzyme A to form acetyl-CoA, pyruvate in high concentrations may also benefit mitochondrial function. \(^1\) - 2 Oxidation of fatty acids is critically impaired by myocardial ischemia with a consequent buildup of long chain fatty acyl-CoA derivatives in the mitochondria. These latter compounds are known to inhibit a number of essential metabolic pathways including translocation of adenine nucleotides across the inner mitochondrial membrane. This, in turn, may be detrimental to residual myocardial energy production. Conversion of pyruvate to acetyl-CoA may lower coenzyme A levels that otherwise would be used for fatty acyl-CoA formation and thus benefit mitochondrial membrane function. Future studies using activators of pyruvate dehydrogenase, such as dichloroacetate,\(^10\) may prove to exert even greater effects in promoting oxidation of pyruvate.

Interestingly, lactate levels in heart muscle were no higher in swine hearts treated with pyruvate-Tris than in untreated ischemic hearts. Several explanations may be operative. First, the lack of increase in tissue lactate levels in treated hearts does not in itself indicate that increased lactate formation had not occurred, because excess lactate may have escaped from the myocardium under the conditions of Tris buffering. Second, a preferentially greater amount of pyruvate may have been processed by pyruvate dehydrogenase to acetyl-CoA and subsequent oxidation as suggested by the labeled decarboxylation data, rather than by lactate dehydrogenase to lactate. Furthermore, previous data indicate that pyruvate is only incompletely incorporated intracellularly in heart muscle in that the concentration of pyruvate in the perfusion medium always exceeds that in heart muscle water.\(^3\) Thus, the amount of substrate actually participating in intracellular metabolism would be less than that suggested by the concentrations present in the coronary perfusate. Nevertheless, lactate, per se, in increased levels does inhibit glycolysis\(^4\) and therefore detracts from the goals of pyruvate therapy. A better choice for testing in future trials might be acetate, which could be utilized directly by the tricarboxylic acid cycle without inhibiting anaerobic glycolysis.

The ischemic model used in the present study was developed in swine because of their close similarities to man with respect to cardiac anatomy, ventricular performance, and anatomic distribution of and perfusion by major coronary arteries.\(^4\) - 5 The principle features of the globally ischemic preparation include a working heart with an intact circulation and innervation, complete control of flows to the systemic and coronary circulations, a whole blood coronary perfusate in which concentrations of oxygen, hormones, and substrates to the myocardium can be adjusted, and an opportunity to make correlative measurements of several hemodynamic and metabolic parameters. We recognize for this model that the global pattern of oxygen deprivation is not strictly analogous to that encountered in ischemic settings in humans except in cases of severe multivessel coronary artery disease. The global model was designed primarily to facilitate measurement of metabolic rates. Previous studies have documented that, in regional ischemia, a variety of coronary flow patterns and populations of normal and ischemic cells exist in the area of tissue supplied by the occluded artery.\(^4\) - 5 Because of this heterogeneous distribution of normal, ischemic, and irreversibly damaged cells, it is virtually impossible to accurately sample and measure only those metabolic changes occurring in
ischemic tissue and to correlate these with mechanical events as a function of restrictions in a coronary flow. A global pattern of oxygen deprivation was used therefore to allow a more accurate assessment of ischemic metabolism as it relates to mechanical performance, and to correlate these findings with regulation of coronary flow.

To allow for a broader and more complete interpretation of results, the agents under consideration also were tested in a modified version of a regional ischemic heart preparation. The mode of delivery for pyruvate and Tris to the myocardium was systemic rather than selective. At comparable rates of delivery buffered pyruvate, but not Tris alone, again reduced the extent and severity of myocardial ischemia. Consideration was given to possible indirect mechanisms which might explain this effect, but none was found. Assuming that pyruvate is completely dissociated and can exert an osmotic pressure, the predicted increase in serum osmolarity, based on the rise in serum pyruvate concentration (2.07 μmol/ml), is only twice this number in milliosmoles and well below the therapeutic range noted for such hyperosmotic agents as mannitol. Initiation of hepatic gluconeogenesis by pyruvate also was tested, since subsequent release of glucose, and particularly insulin, into the periphery might secondarily improve the ischemic myocardium through the hormone's inhibitory action on lipolysis. However, no such simulation and release was found (Table 3). These data thus support findings from the globally ischemic model, indicating that treatment with pyruvate acts primarily through its metabolic influences on the ischemic heart.

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