Effect of Coronary Blood Flow on Glycolytic Flux and Intracellular pH in Isolated Rat Hearts

By James R. Neely, Jeffrey T. Whitmer, and Michael J. Rovetto

ABSTRACT

The rate of coronary blood flow was varied in isolated working rat heart preparations to determine its influence on the rate of glucose utilization, tissue high-energy phosphates, and intracellular pH. A 60% reduction in coronary blood flow resulted in a 30% reduction in oxygen consumption, an accelerated rate of glucose utilization, lower tissue levels of high-energy phosphates, and higher tissue levels of lactate and H+. Ventricular performance deteriorated as reflected by a decrease in heart rate and peak systolic pressure. Further reductions in coronary blood flow resulted in inhibition of glycolysis, a greater decrease in tissue levels of high-energy phosphates, and higher tissue levels of both lactate and H+. These changes in glycolytic flux, tissue metabolites, and ventricular performance were proportional to the degree of restriction in coronary blood flow. The importance of coronary blood flow and washout of the interstitial space in the maintenance of accelerated glycolytic flux in oxygen-deficient hearts is emphasized. It is concluded that acceleration of ATP production from glycolysis can occur only in the marginally ischemic tissue in the peripheral area of tissue supplied by an occluded artery. The central area of tissue which receives a low rate of coronary blood flow will have a reduced rate of ATP production due to both a lack of oxygen and an inhibition of glycolysis.

The harmful effects of a restriction of coronary blood flow that leads to a reduction in mechanical function, cell death, and myocardial infarction may result from lack of washout of the interstitial space as from lower rates of delivery of oxygen and other substrates to the tissue (1–3). Alterations in mechanical and metabolic functions of myoccardial cells are generally reversible up to 20 minutes following restriction of coronary blood flow, but irreversible damage and cell death occur between 20 and 60 minutes (2–7). The time of onset of cell death may be directly proportional to the restriction in coronary blood flow. Several studies (7–14) have emphasized the heterogeneity of blood flow in and around an infarcting region. Flow ranges from near zero in the center of the infarcting zone to normal or above normal in the surrounding tissue. Irreversible cell damage would, therefore, be expected to occur more rapidly in the region of tissue receiving the least amount of coronary blood flow.

The role of coronary blood flow in maintaining a suitable intracellular environment in addition to providing the oxygen supply has been emphasized recently in studies comparing the effects of anoxia with those of ischemia (15–17). Ischemia has been shown to be more detrimental to cellular function and metabolism than is anoxia in which coronary blood flow is maintained at normal rates. Anoxia accelerates glycolysis in cardiac muscle (18–19), and the rate of glucose utilization is increased by a regional reduction in coronary blood flow (20, 21). In contrast, glycolysis is reduced in the working rat heart when coronary blood flow is severely restricted to the whole heart (17). Also, a 60% reduction in blood flow to the whole heart in a working pig heart preparation results in reduced rates of glucose consumption (22). Part of this discrepancy in results may be due to an incomplete description of the effects of coronary blood flow on the metabolic and functional activity of the heart. In the regional ischemic models, glucose utilization by a heterogeneous population of tissue receiving a variety of coronary blood flows and performing different levels of mechanical work has been measured (20, 21). Therefore, glycolysis may have been accelerated in the normal or mildly ischemic tissue and inhibited in the more severely ischemic cells. In the global ischemic models, only the control and one condition of ischemia have been studied (17). Therefore, the purpose of the present work was to study the relationship between coronary blood flow...
and glycolysis over a wide range of coronary flow rates.

**Methods**

Hearts were removed from 250-350-g male Sprague-Dawley rats and perfused in a working heart apparatus as described previously (23). Ischemia was induced by placing a one-way valve in the aortic outflow tract which prevented retrograde perfusion of the coronary arteries during diastole and reduced coronary blood flow by about 60% (5). Systolic pressure development, heart rate, and coronary blood flow for hearts perfused by this method are shown in Figure 1. In hearts which were electrically paced at 230 beats/min following a 60% reduction in coronary blood flow, ventricular failure resulted within 10 minutes; this phenomenon in turn caused a further reduction in coronary blood flow to 10% of control rates after 30 minutes. If the hearts were not paced, ventricular failure was manifest as a smaller reduction in peak systolic pressure development and a greater decrease in heart rate. Without pacing, peak systolic pressure development declined by about 20% and heart rate decreased from about 250 to 180 beats/min during 30 minutes of reduced coronary blood flow. In these hearts, the rate of coronary blood flow remained at about 40% of the control rate for up to 1 hour of perfusion. About 10% of the hearts that were not paced developed pressure failure similar to that in the paced hearts, and flow deteriorated. These hearts were not included in the study. Intermediate flow rates between 10 and 40% of control could be maintained in electrically paced hearts by providing different levels of aortic perfusion pressure after ventricular failure had occurred. Anoxia was induced by perfusing the hearts with buffer equilibrated with 96% N2-4% CO2 gas mixture, pH 7.35. Following failure of the ventricle, coronary blood flow was maintained at control rates by providing a hydrostatic perfusion pressure of 60 mm Hg. After 10 minutes of anoxic perfusion, coronary blood flow was reduced to either 2 or 5 ml/min by use of a mechanical pump connected to the aortic outflow tract, and perfusion at these lower rates was continued for 30 minutes.

The basic perfusate was Krebs-Henseleit-bicarbonate buffer containing 11 mM glucose and, where indicated, 1.2 mM palmitate bound to 3% albumin. When fatty acids were used in the perfusate, the fatty acid-albumin complex was prepared as described previously (24).

The rate of glucose utilization was determined by measuring the production of 14CO2 from uniformly labeled 14 C-glucose as described earlier (25). Oxygen consumption was calculated from the arterial-venous difference in oxygen tension (Po2) and the rate of coronary blood flow. The rate of glucose oxidation was determined by measuring 14CO2 production from uniformly labeled 14C-glucose.

Intracellular pH was calculated from the distribution of 14C-5,5-dimethyl-2,4-oxazolidinedione (DMO) (26); 50 mg/100 ml of nonlabeled DMO was included in the buffer as a carrier. Also, 1H-n-sorbitol and 50 mg/100 ml of nonlabeled sorbitol were included in the buffer for measurement of the extracellular space. Preliminary experiments were conducted to determine the time necessary for final equilibration of the DMO. These studies indicated that the distribution of DMO within the tissue was complete within 3 minutes. The intracellular pH calculated by this procedure was 6.70 + 0.13, 6.91 + 0.08, 6.99 + 0.07, 7.04 + 0.02, 7.02 + 0.03, and 7.02 + 0.01 after 1.0, 1.5, 3, 10, 18, and 30 minutes of perfusion, respectively. In subsequent experiments, intracellular pH was measured after at least 10 minutes of exposure to DMO.

Hearts used for lactate, adenosine triphosphate (ATP), and creatine phosphate analysis were rapidly frozen by clamping them with aluminum tongs cooled in liquid nitrogen. The frozen tissue was powdered and extracted in 6% perchloric acid, and the neutralized
extract was used to determine tissue levels of metabolites. Lactate was assayed by the lactate dehydrogenase procedure, and ATP and creatine phosphate were assayed by the hexokinase, glucose-6-P-dehydrogenase procedure as described in Bergmeyer (27).

**Results**

**EFFECTS OF CORONARY BLOOD FLOW ON GLYCOLYTIC FLUX AND GLUCOSE OXIDATION**

Figure 2 shows the rate of glucose utilization as a function of perfusion time at three different rates of coronary blood flow. In the control hearts, coronary blood flow averaged about 15 ml/min and was maintained throughout the perfusion period. In these hearts, the rate of exogenous glucose utilization was about 4.5 μmoles/g dry tissue min⁻¹. When the rate of coronary blood flow was reduced and allowed to decline as ventricular failure occurred, glycolysis was inhibited. The rate of utilization was not limited by glucose availability (17) but represented inhibition within the glycolytic pathway. However, if coronary blood flow was maintained at about 2 ml/min, utilization of exogenous glucose was accelerated to above the control rate after a lag period of about 10 minutes. When the rate of coronary blood flow was maintained at about 5 ml/min, glycolysis was increased even further. At these flows, the glycolytic rate was the same whether the hearts were electrically paced or not. The apparent lag in the increased rate of exogenous glucose utilization probably reflects a rapid breakdown of glycogen and dilution of the specific activity of the 3H-glucose-6-P pool. The tissue content of glycogen was essentially depleted during the first 16 minutes of perfusion under ischemic conditions (17). Glycogen levels decreased rapidly in the first 4-8 minutes of ischemic perfusion and then at a slower rate. These data indicated that glucose from glycogen was preferentially utilized as a substrate for glycolysis and that acceleration of exogenous glucose utilization occurred only after the tissue stores of glycogen were essentially depleted.

The effect of ischemia on glucose utilization in hearts perfused with a combination of glucose and fatty acid is illustrated in Figure 3. The control rate of utilization in these hearts was somewhat lower than that in hearts perfused with glucose as the only exogenous substrate (compare Figs. 2 and 3), illustrating the well-known inhibitory effect of fatty acid oxidation on glycolysis in aerobic hearts. When coronary blood flow was reduced, the steady-state rate of glucose utilization was essentially the same as that found in ischemic hearts perfused with glucose as the only substrate. In addition, the rate of utilization was roughly proportional to coronary blood flow in the ischemic hearts.

The rate of oxygen consumption in control hearts averaged about 30 μmoles/g dry weight min⁻¹ (Fig. 4). This rate decreased by about 30% when coro-
Coronary blood flow was reduced to 5 ml/min, and at lower flows oxygen consumption decreased in direct proportion to the reduction in coronary blood flow and oxygen delivery. When the rate of coronary blood flow was held at any of the intermediate levels, the rate of oxygen consumption decreased immediately, but the reduced rate was maintained for at least 30 minutes of perfusion. Oxidation of glucose averaged about 2 µmoles/min in the control hearts (Fig. 5). In ischemic hearts receiving 5 ml/min of flow, glucose oxidation was reduced by about 50% initially, but subsequently increased to above the control rate. This reduction in the first 10 minutes of perfusion correlates with the lag in acceleration of \( ^3 \text{H}_2\text{O} \) production from exogenous glucose (Fig. 2) and probably also represents dilution of the specific activity of the glucose-6-P pool by breakdown of glycogen. Oxidation of glucose accounted for about 40% of the total oxygen consumed in control hearts, and presumably oxidation of endogenous lipid accounted for the remainder. In the ischemic hearts, oxidation of exogenous glucose accounted for about 90% of the total oxygen consumed after 20 minutes of perfusion.

Although the rate of glycolysis was accelerated by ischemia when coronary blood flow was maintained at either 2 or 6 ml/min, the maximum rate observed in ischemic hearts was only about 60% of that found in anaerobic hearts. The rate of glucose utilization increased to about 14 µmoles/g dry tissue min\(^{-1}\) in anoxic hearts when coronary blood flow was maintained at control levels (Fig. 6). However, when coronary blood flow was reduced, the rate of glycolysis declined to about the same extent as it did in oxygenated hearts receiving a comparable coronary blood flow. These data indicate that simple oxygen deficiency with maintenance of coronary blood flow accelerated glycolysis but that reduced coronary blood flow inhibited this source of ATP.

**Effects of Coronary Blood Flow on Tissue Levels of High-Energy Phosphates, Lactate, and \( ^{14} \text{CO}_2 \)**

Tissue levels of high-energy phosphates were low in both anoxic and ischemic hearts, but the levels of ATP and creatine phosphate decreased in proportion to the restriction in coronary blood flow and oxygen delivery in hearts perfused with oxygenated perfusate (Table 1). The levels of creatine phosphate were somewhat higher than those of ATP in control hearts, especially when palmitate was present, but they appeared to be more sensitive to changes in coronary blood flow. In anoxic hearts, the levels of creatine phosphate were low regardless of coronary blood flow, but the levels of ATP were maintained somewhat at the higher flow rates. Tissue levels of lactate were slightly higher in aerobic hearts perfused with palmitate when coro-
Effects of reducing coronary blood flow in anoxic hearts on the rate of glucose utilization. The hearts received a 10-minute preliminary perfusion prior to starting anoxic perfusion with perfusate containing 11 mM glucose and bubbled with a 95% N\textsubscript{2}-5% CO\textsubscript{2} gas mixture. The rate of flow in some of the anoxic hearts was maintained at the control rate by providing a 60-mm Hg hydrostatic perfusion pressure. Flow was reduced to the lower rates by decreasing the hydrostatic perfusion pressure. Rates of glucose utilization were determined after 10 minutes of anoxic perfusion at each coronary blood flow.

Coronary blood flow was maintained at control levels. In the ischemic hearts, however, lactate accumulated in proportion to the restriction in flow regardless of the substrates present. Anoxia elevated the level of lactate at control flow rates, but restriction of flow in these hearts resulted in accumulation of lactate to levels similar to those found in oxygenated hearts.

A decrease in cellular pH is generally thought to be associated with an accumulation of metabolic products such as lactate and CO\textsubscript{2}. In the present study, changes in intracellular pH in relationship to the restriction in coronary blood flow and oxygen availability were estimated by the DMO procedure (Table 2). Calculation of intracellular pH from the tissue distribution of DMO is based on the theory that weak acids penetrate the cell membrane in the protonated form and that the distribution of acid across the membrane depends on the concentration gradient of H\textsuperscript{+} (26). Therefore, accumulation of DMO in the intracellular space will depend on the pH in the extracellular compartment, and this pH must be known before intracellular pH can be calculated. Obviously, the pH in the interstitial space adjacent to the myocardial cells should be used in the calculation, but this pH is impossible to obtain. In the present study, the extracellular pH used for the calculation was the pH measured in the coronary venous perfusate. The arterial perfusate pH was maintained at 7.35. The decrease in pH measured in the coronary, effluent as the perfusate passed through the vascular bed would be expected to represent only the direction of change in interstitial pH and not the absolute change. Coronary effluent pH may be higher than the true interstitial pH which would cause an overestimation of intracellular pH. Therefore, the changes in both extracellular and intracellular pH, reported in Table 2, are minimum changes.

### Table 1: Effects of Coronary Blood Flow on High-Energy Phosphates and Lactate in Aerobic and Anoxic Hearts

<table>
<thead>
<tr>
<th>Coronary blood flow (ml/min)</th>
<th>Substrate</th>
<th>ATP (moles/g dry wt)</th>
<th>Creatine phosphate (moles/g dry wt)</th>
<th>Lactate (moles/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic Hearts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Glucose</td>
<td>22 ± 0.8</td>
<td>23 ± 0.2</td>
<td>3 ± 0.9</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>16 ± 0.8</td>
<td>14 ± 0.5</td>
<td>18 ± 3.0</td>
</tr>
<tr>
<td>2.6</td>
<td></td>
<td>15 ± 1.5</td>
<td>10 ± 1.3</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>10 ± 0.6</td>
<td>3 ± 0.7</td>
<td>56 ± 6</td>
</tr>
<tr>
<td>13</td>
<td>Glucose and palmitate</td>
<td>20 ± 0.7</td>
<td>26 ± 1</td>
<td>9 ± 1.8</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>20 ± 1.6</td>
<td>14 ± 1.4</td>
<td>27 ± 2.9</td>
</tr>
<tr>
<td>0.6</td>
<td></td>
<td>11 ± 0.4</td>
<td>4 ± 0.3</td>
<td>54 ± 2.0</td>
</tr>
<tr>
<td>Anoxic Hearts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Glucose</td>
<td>12 ± 2</td>
<td>5 ± 1.0</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>12 ± 2</td>
<td>5 ± 1.0</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>6.3 ± 1.2</td>
<td>2.3 ± 0.7</td>
<td>41 ± 7</td>
</tr>
</tbody>
</table>

Hearts were perfused for 20 minutes with coronary blood flow maintained at the levels indicated in the table. The perfusate was bubbled with a 95% O\textsubscript{2}-5% CO\textsubscript{2} gas mixture for aerobic hearts and a 95% N\textsubscript{2}-5% CO\textsubscript{2} gas mixture for anoxic hearts; the perfusate contained 11 mM glucose in both experiments. When present, palmitate (1.0 mM) was bound to 3% bovine serum albumin. Each value represents the mean ± SE for six to eight hearts.

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TABLE 2

Effect of Coronary Blood Flow and Oxygen Supply on Tissue pH

<table>
<thead>
<tr>
<th>Coronary blood flow (ml/min)</th>
<th>Perfusion time (minutes)</th>
<th>Coronary effluent pH</th>
<th>Intracellular pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control Hearts</td>
<td>Ischemic Hearts without Maintained Flow</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>7.25 ± 0.03</td>
<td>7.04 ± 0.02</td>
</tr>
<tr>
<td>15</td>
<td>35</td>
<td>7.25 ± 0.01</td>
<td>6.87 ± 0.02</td>
</tr>
<tr>
<td>15</td>
<td>4</td>
<td>7.25 ± 0.03</td>
<td>6.84 ± 0.02</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td>7.05 ± 0.03</td>
<td>6.83 ± 0.02</td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td>6.87 ± 0.02</td>
<td>6.83 ± 0.02</td>
</tr>
<tr>
<td>1</td>
<td>26</td>
<td>6.84 ± 0.02</td>
<td>6.83 ± 0.02</td>
</tr>
<tr>
<td>1</td>
<td>36</td>
<td>6.79 ± 0.02</td>
<td>6.80 ± 0.02</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>6.84 ± 0.02</td>
<td>6.83 ± 0.02</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>7.01 ± 0.03</td>
<td>6.97 ± 0.04</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>7.02 ± 0.02</td>
<td>6.94 ± 0.03</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>7.27 ± 0.02</td>
<td>7.01 ± 0.01</td>
</tr>
</tbody>
</table>

Hearts were perfused for the times indicated with perfusate containing 11 mM glucose and bubbled with a 95% O2-5% CO2 gas mixture for control and ischemic hearts and a 95% N2-5% CO2 gas mixture for anoxic hearts. In one group of ischemic hearts, perfusion was continued for 36 minutes, and coronary blood flow was allowed to decline as ventricular failure occurred corresponding to the electrically paced hearts in Figure 1 (ischemic without maintained flow). In the ischemic hearts with maintained flow, ischemia was induced and flow was maintained at either 6 or 2 ml/min for 20 minutes. The pH of the coronary effluent was the same throughout the 20 minutes, and only the last values are shown. Each value represents the mean ± SE for 6-12 hearts.

In control hearts, perfusate pH decreased from 7.35 to 7.25 on one passage through the heart. In ischemic hearts, the effluent pH generally decreased in proportion to the restriction in coronary blood flow whether flow was maintained constant for 20 minutes or allowed to decline as ventricular failure occurred. Similarly, intracellular pH declined in proportion to the restriction in flow. The decline in extracellular pH was larger than that in intracellular pH at all flow rates studied, indicating that the intracellular space is better buffered than the extracellular space in hearts perfused with Krebs-Henseleit-bicarbonate buffer. When the extracellular pH was below 7.0, the pH of both spaces was equal. This observation held whether the extracellular pH was decreased by ischemia or adjusted with various buffers in aerobic control hearts (data not shown). In contrast to ischemia, anoxia caused only a small decrease in pH in the first 2 minutes of perfusion, and the pH had returned to control levels after 30 minutes.

Discussion

Since occlusion of a coronary artery results in a heterogeneous pattern of blood flow in and around the area of tissue supplied by the occluded artery (8), it was of interest to study the rates of energy metabolism that might be expected in the peripheral central areas of the ischemic tissue. One would expect the rate of oxygen consumption and ATP production from oxidative metabolism to be proportional to coronary blood flow and to also occur in a heterogeneous pattern. Anaerobic production of ATP normally represents less than 5% of the total, and even under conditions of hypoxia or anoxia acceleration of glycolysis can produce only about 20% of the ATP required by aerobic tissue (28-30). In the present study, aerobic hearts produced about 190 μmoles ATP/g min⁻¹ (Table 3). If an equivalent amount of ATP were to be produced from glycolysis, the rate would have to be about 90

TABLE 3

Effects of Coronary Blood Flow on ATP Production from Glycolysis and Oxidative Metabolism in Perfused Rat Hearts

<table>
<thead>
<tr>
<th>Condition</th>
<th>Coronary blood flow (ml/g min⁻¹)</th>
<th>Total ATP produced (μmoles/g min⁻¹)</th>
<th>ATP from Glycolysis</th>
<th>Oxidative metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>189</td>
<td>9</td>
<td>180</td>
</tr>
<tr>
<td>Ischemic</td>
<td>4.7</td>
<td>138</td>
<td>18</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>70</td>
<td>18</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>31</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>Anoxic</td>
<td>13</td>
<td>34</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>8</td>
<td>13</td>
<td>0</td>
</tr>
</tbody>
</table>

The perfusate contained 11 mM glucose. Glucose oxidation was estimated by measuring ¹⁴CO₂ production from U-¹⁴C-glucose. Rates of ATP production were calculated after 20 minutes of perfusion under the conditions indicated. Total ATP production was calculated from glycolytic flux and oxygen consumption, assuming that two ATP molecules are generated per glucose molecule from glycolysis and a P-O ratio of 3.
μmoles glucose/g min⁻¹ or about 20 times the control rate. The most rapid rates of glycolysis occur under anoxic conditions when glycogen is rapidly broken down (17, 28, 31). However, tissue glycogen is essentially depleted within 4 minutes (31), and the steady-state rate of glycolysis from exogenous glucose represents only about 20% of that required for normal aerobic production of ATP (Table 3). A reduction in coronary blood flow of 60% accelerated glycolytic production of ATP (about 18 μmoles ATP/g min⁻¹), but this rate was less than 10% of the normal aerobic rate. Oxidative metabolism using the residual amount of oxygen consumption (20 μmoles O₂/g min⁻¹) could account for another 120 μmoles of ATP, leaving the tissue energy deficient by about 50 μmoles ATP/g min⁻¹. This deficit resulted in a decreased energy expenditure as indicated by a slower heart rate and a small reduction in peak systolic pressure.

With further reductions in coronary blood flow, oxygen consumption declined proportionally. The rate of glycolysis was inhibited, tissue levels of ATP and creatine phosphate declined in proportion to the restriction in flow, and ventricular failure was more severe. At the lowest rates of coronary blood flow studied, total ATP production from both oxidative and glycolytic processes averaged about 30 μmoles/g min⁻¹, only about 15% of the normal aerobic rate. It, therefore, appears that the central area of tissue supplied by an occluded artery does not receive enough coronary blood flow to maintain even the low anaerobic rate of ATP production.

From these studies, it appears that maximum stimulation of glycolysis is able to supplement energy production from oxidative sources to near the normal rate in the peripheral area of infarcting tissue where coronary blood flow is adequate to supply about 85% of the normal oxygen consumed. As flow decreases from this level, glycolysis, even if it is maximally stimulated, is not capable of making up the deficit between oxidative ATP production and the normal ATP requirements. To complicate this lack of energy production, glycolysis becomes progressively inhibited as coronary blood flow is decreased.

Inhibition of glycolysis in ischemic hearts was associated with increased tissue levels of lactate and a lowering of both the extracellular and the intracellular pH. The importance of coronary blood flow in maintaining accelerated glycolytic rates is emphasized by the observations that in anoxic hearts with normal tissue pH (after a slight transient decrease in the first 2 minutes of perfusion) tissue lactate did not accumulate to anything like the same extent as it did in ischemic hearts and glycolysis proceeded at a rapid rate. Reducing coronary blood flow in the anoxic hearts inhibited glycolysis. It can be concluded from these observations that washout of the interstitial space with removal of lactate and maintenance of cellular pH are important factors in maintaining accelerated glycolytic flux and that lack of washout of the interstitial space complicates energy production in ischemic tissue. Calculated intracellular pH using DMO as a marker may represent minimum values since the interstitial pH cannot be measured directly, but the procedure appears to be useful for estimating relative pH changes. The decrease in coronary effluent and tissue pH measured in ischemic hearts in the present study agrees with the decrease (about 0.6 units) in interstitial pH measured with microelectrodes following ligation of a coronary artery (23).

Isolated rat hearts perfused with Krebs-Henseleit-bicarbonate buffer have coronary blood flows ranging from 10 to 15 ml/g wet tissue. This rate of flow is much higher than that which has been observed in hearts from large animals perfused with blood. Estimates of in vivo coronary flow rates in the rat are not available, but it can be assumed that, since the rat heart beats at about three times the rate of large animal hearts and develops about the same peak systolic pressure, energy requirements and, therefore, coronary blood flow will be higher per gram of tissue in the rat heart and may approach 3.0 ml/g wet weight as compared with 1 ml/g in larger animals. Thus, a 60% reduction in the flow rate in isolated rat hearts was at least twice the in vivo flow rate. However, this in vitro flow rate was insufficient to provide adequate oxygen and to prevent lactate accumulation, decreased pH, and ventricular failure, indicating that an ischemic condition was induced. Since coronary flow rates less than 2 ml/g wet weight min⁻¹ in the rat heart resulted in glycolytic inhibition, it is unlikely that glycolysis can be stimulated in a blood-perfused heart in which coronary blood flow is decreased sufficiently to limit oxygen supply. In this regard, a 50 or 60% reduction in coronary blood flow in the swine heart (normal coronary flow about 1 ml/g wet weight min⁻¹) results in inhibition of glycolysis, a large increase in tissue lactate, and ventricular failure (22). Therefore, maximum stimulation of glycolysis is unlikely in larger blood-perfused hearts even in the peripheral area of infarcting tissue. The increased glucose utilization observed with regional ischemia in larger in situ hearts (20, 21) is more likely due to increased...
metabolism in the nonischemic tissue. Both the rate of coronary blood flow and the contractile force have been reported to be increased in tissue immediately adjacent to the area served by an occluded vessel (9, 33).

Cardiac glycogen appears to be utilized very rapidly in anoxic (31) and arrested hearts receiving no coronary blood flow (34–36). In anoxic hearts, rapid utilization of glycogen results from activation of phosphorylase b by increased tissue levels of adenosine monophosphate (AMP) and inorganic phosphate and decreased levels of ATP and glucose-6-P (31). In addition phosphorylase b conversion to phosphorylase a occurs (31, 34, 35). The rate of glycogenolysis, although greatly accelerated, appears to occur at a slower rate in ischemic than in anoxic hearts (17, 36). In potassium-arrested hearts, the rate of glycogenolysis appears to decrease as tissue lactate accumulates (35). In the isolated heart, acceleration of glycogenolysis under ischemic conditions may result from increased levels of inorganic phosphate due to breakdown of creatine phosphate and increased levels of 5'-AMP (5, 17).

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