Effects of Acidosis and Ischemia on Contractility and Intracellular pH of Rat Heart

CHARLES STEENBERGEN, GILBERT DELEEUW, TERRELL RICH, AND JOHN R. WILLIAMSON

SUMMARY The effects of respiratory and metabolic acidosis on myocardial contractility and energy production have been investigated in the perfused rat heart. Respiratory acidosis, produced by increasing the PCO₂, caused an 80% inhibition of pressure development at pH 6.7. When artificial buffers (plus HCl) were used in place of bicarbonate and CO₂, only a 30% inhibition of pressure development was observed at pH 6.7. Respiratory acidosis produced a greater intracellular acidosis than artificial buffer acidosis at the same extracellular pH. We conclude that both intracellular and extracellular H⁺ impair myocardial function but by separate mechanisms. Intracellular acidosis per se was shown to have little effect on the balance between energy production and energy utilization, and energy stores were relatively well maintained under these conditions. The contribution of intracellular acidosis to ischemic heart failure was examined using an ischemia model in which the coronary flow was decreased during diastole. Consequent restricted oxygen delivery produced a pattern of heterogeneous oxygenation. A fall in effluent pH was concomitant with the decline in myocardial performance, and the intracellular pH fell as the extracellular space became more acidic. The data suggest that the fall of intracellular and extracellular pH were the principal determinants of the decline of pressure development in the tissue as a whole during the early stages of ischemia. We conclude that mechanical function is depressed in ischemia not only in anoxic regions of the heart but also in adjacent aerobic regions because of the pH change.

ALTHOUGH it is well documented that decreased oxygen delivery and accumulation of metabolic end products are components of ischemia, the relative contribution of acidosis and hypoxia to ischemic heart failure has not been properly delineated. Myocardial performance is impaired by either anoxia, produced by equilibrating the perfusate with nitrogen instead of oxygen while maintaining the coronary flow, or by acidosis, produced by adding HCl or increasing the PCO₂ in the perfusate.

Previous experiments have demonstrated depression of myocardial contractility by acidosis, but the relative importance of intracellular and extracellular pH has not been unequivocally determined. Initially, it was suggested that extracellular pH was the principal determinant of cardiac work, but more recently the importance of intracellular pH has been recognized. A role for intracellular pH was based on the assumption that there is a greater decline in intracellular pH during respiratory acidosis, produced by increasing the PCO₂, than during metabolic acidosis, produced by decreasing the bicarbonate concentration. This difference is considered to be due to the high permeability of the sarcolemma to CO₂ and its relatively low permeability to protons or bicarbonate. Because metabolic acidosis causes a smaller negative inotropic effect than respiratory acidosis at the same extracellular pH, it was concluded that intracellular pH is an important determinant of myocardial contractility. However, direct measurements of intracellular pH in perfused or in situ rat hearts indicate that the pH gradient between the extracellular and intracellular spaces is more a function of the extracellular pH than of the PCO₂. Therefore, the relative importance of extracellular vs. intracellular pH in determining contractile function remains unresolved.

A more precise separation between the effects of extracellular and intracellular pH can be made by varying one while maintaining the other constant. To change the extracellular pH without significantly altering the intracellular pH, zwitterionic artificial buffers can be used to replace the CO₂-HCO₃⁻ buffering system. It has been demonstrated that artificial buffer acidosis results in a less severe decline in cardiac work than respiratory acidosis at the same extracellular pH, but no intracellular pH measurements were reported.

Myocardial contractility is depressed in anoxia despite high tissue ATP levels. It has been shown that the slow inward calcium current is decreased and it has been suggested that this may result from decreased cyclic AMP levels, but the precise mechanism for the decrease in pressure development during anoxia is unknown. However, it seems likely that the mechanism must involve feedback from the energy balance within the cell. ATP synthesis is severely impaired by the lack of oxidative phosphorylation. Although glycolytic flux has been shown to increase in anoxia, it is insufficient to maintain energy production at its aerobic level and creatine phosphate reserves are depleted. Ultimately, cardiac work is severely reduced until energy utilization equals energy production.

The relative significance of acidosis and hypoxia in causing the initial decrease in cardiac work in ischemia is not known. It has been reported for dog heart that high energy phosphate compounds are depleted before the extracellular pH is significantly lowered and this finding was interpreted as showing that decreased ATP availabili-
ity is the main determinant of cardiac work in ischemia.\textsuperscript{25} Other investigators have stressed the importance of acidosis.\textsuperscript{14, 26, 27} It also has been shown that the Pco\textsubscript{2} in the ischemic myocardium is elevated\textsuperscript{28} and that intracellular pH is decreased,\textsuperscript{29} but this has not been correlated with contractility changes.

The present study is primarily concerned with investigating the effect of intracellular and extracellular acidosis on myocardial performance and the relative contribution of acidosis to the development of ischemic heart failure. Intracellular pH has been measured and the contribution of extracellular and intracellular pH to contractility changes has been quantitated for the isolated, perfused heart. Furthermore, results from a model of whole tissue ischemia, similar to that described by Neely et al.,\textsuperscript{30} suggest that the decline in pressure development in ischemia results from a decline in pH of both intracellular and effluent fluid.

**Methods**

**EXPERIMENTAL**

**Heart Perfusion**

Hearts were excised from 300 to 400-g male Sprague-Dawley rats, and the aorta was cannulated as quickly as possible so that coronary flow would be interrupted only briefly. The pulmonary artery and left atrium were subsequently cannulated, and left atrial filling was commenced from a reservoir placed 12 cm above the left atrium. In most of the experiments, hearts were perfused using the closed aorta preparation. This was achieved by clamping the aortic outflow and thus diverting the entire left ventricular output through the coronary vessels.\textsuperscript{14} Elasticity was provided by a 3-ml air space in line with the aortic cannula. Left ventricular pressure, aortic pressure, effluent pH and O\textsubscript{2} tension, and coronary flow rate were measured as previously described.\textsuperscript{31}

Pyridine nucleotide fluorescence was measured with a three-way light pipe which allowed for simultaneous measurements of pyridine nucleotide fluorescence and reflected 366 nm excitation light, as described by Chance et al.\textsuperscript{32} Changes of reflected light were indicative of tissue swelling or misplacement of the light pipe from the heart. Furthermore, it also has been shown that the Pco\textsubscript{2} in the ischemic myocardium is elevated and that intracellular pH is decreased, but this has not been correlated with contractility changes.

Intracellular pH was determined by the DMO (dimethyl-2,4-oxizolidine dione) distribution method as described by Waddell and Bates.\textsuperscript{34} [\textsuperscript{3}H]-Sorbitol, [\textsuperscript{14}C]-DMO, and 0.2 mm carrier DMO were added to the experimental perfusate. Immediately after a heart was frozen, perfusate was collected to determine the extracellular \textsuperscript{3}H and \textsuperscript{14}C counts per unit volume. The total tissue water content was obtained from the difference between the wet and dry weights. Samples of the perchloric acid extracts of the lyophilized hearts and the perfusates were mixed with Handifluor and counted in three-channel liquid scintillation counter which automatically corrected for quenching. The tissue \textsuperscript{3}H dpm were used to determine the total extracellular volume, assuming that the sorbitol does not penetrate the plasma membrane, and the intracellular volume was calculated by difference from the
total tissue water. The intracellular pH was calculated using the following formulas:

\[ pH_i = pK_a + \log(\alpha(V_e + 1)C_i - V_r) - 1 \]
\[ \alpha = 1 + K_a/\left[H^+\right]_p \]

where the \( pK_a \) of DMO is 6.13, \( V_r \) is the ratio of extracellular volume to intracellular volume, \( C_i \) is the ratio of tissue \( ^{14}C \) dpm per unit volume of tissue water to perfusate \( ^{14}C \) dpm per unit volume, and \([H^+]_p\) is the perfusate hydrogen ion concentration.

**Results**

**EFFECTS OF ACIDOSIS ON MYOCARDIAL CONTRACTILITY**

The effect of respiratory acidosis and artificial buffer acidosis on left ventricular pressure development is shown in Figure 1. The experiments were performed using the closed aorta preparation, which minimizes mixing artifacts. When an abrupt change in perfusate pH from 7.4 to 6.6 was achieved by switching from the control left atrial perfusate reservoir to a second reservoir containing the same buffer equilibrated at a lower pH, there was a rapid fall in pressure development immediately after the pH change. The decline in pressure development was essentially complete in less than 1 minute regardless of which buffer was used. However, with respiratory acidosis, the left ventricular pressure declined by 80%, but with artificial buffer acidosis, it fell by only 20 to 40%. The change in left ventricular pressure was quantitated as a percent of the pressure that was developed by the same buffer when the perfusate was at pH 7.4, at a constant left atrial filling pressure. This method of computing the effect of acidosis on myocardial performance gave reproducible results irrespective of the initial control left ventricular pressure.

Figure 2 further demonstrates the difference between the effects of respiratory acidosis and artificial buffer acidosis on left ventricular pressure development. The pH of effluent fluid from the pulmonary artery was measured and was assumed to be indicative of the extracellular pH. The data were obtained by perfusing the heart with pH 7.4 buffer until the mechanical indices were stable, then rapidly switching to a second left atrial reservoir containing the same buffer equilibrated at a lower pH. The hearts were perfused under the experimental conditions for 1 minute and then returned to the control conditions. The mechanical indices were again allowed to stabilize before the next pH transition. When the pH had fallen to 7.2, the myocardial response to respiratory acidosis was already significantly different from the response to artificial buffer acidosis, and this difference became even greater as the pH declined until it reached a maximum at pH 6.7-6.8. At pH 6.7, respiratory acidosis produced on 80% decline in pressure development, whereas artificial buffer acidosis caused only a 20-40% decline. The effect of artificial buffer acidosis on cardiac work was essentially independent of the type of artificial buffer, and it was also independent of the concentration of artificial buffer in the range from 1 to 25 mM (unpublished data from this laboratory).

**Figure 1** Kinetics of the fall of contractility during a pH transition. Hearts were perfused using the closed aorta preparation. At the time indicated, the arterial pH was rapidly changed from 7.4 to 6.6 by switching left atrial filling to a second reservoir containing the same buffer equilibrated at pH 6.6. When the perfusate contained 25 mM HCO$_3^-$, acidosis was produced by increasing the PCO$_2$ resulting in a respiratory acidosis. When the bicarbonate was replaced with 10 mM Tris, 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), or 10 mM 2-N-morpholinopropanesulfonic acid (MOPS), acidosis was produced by adding HCl in the absence of CO$_2$ resulting in an artificial buffer acidosis.

To determine whether artificial buffers have a protective effect on intracellular pH in the presence of CO$_2$, pressure development was studied as a function of pH with 10 mM MES added to the bicarbonate buffer. The pH was lowered by increasing the PCO$_2$. The results were virtually identical to that obtained under the same condi-
a change to respiratory acidosis with bicarbonate-CO$_2$ gave equivalent changes in pressure development. In both buffer at the same arterial pH, using separate left atrial perfusion at pH 7.4 with bicarbonate buffer to artificial

2 is the major
tions without MES. This indicates that Pco$_2$ is the major determinant of cardiac work and that MES has no inherent inotropic activity or intracellular buffering capacity.

Figure 3 shows several abrupt transitions from control perfusion at pH 7.4 with bicarbonate buffer to artificial buffer acidosis at several different pH values, followed by a change to respiratory acidosis with bicarbonate-CO$_2$ buffer at the same arterial pH, using separate left atrial reservoirs. Results are expressed as a percentage of the left ventricular pressure obtained from perfusion with bicarbonate buffer at pH 7.4 at a constant left atrial filling pressure. The reciprocal transitions from the bicarbonate control at pH 7.4 to respiratory acidosis followed by artificial buffer acidosis at the same extracellular pH gave equivalent changes in pressure development. In both cases, at an extracellular pH of 7.0, artificial buffer acidosis produced only about a 20% decline in pressure development whereas respiratory acidosis resulted in about a 60% decline. These data demonstrate that factors other than changes of extracellular pH are important determinants of cardiac work.

**RELATIONSHIP BETWEEN INTRACELLULAR pH AND EXTRACELLULAR pH**

Measurements of intracellular pH revealed that the relationship between extracellular and intracellular pH was significantly different when hearts were perfused with artificial buffers or with bicarbonate buffer. All intracellular pH determinations were made after 3 minutes of perfusion under the experimental conditions, using the DMO distribution method. Table 1 demonstrates that at an extracellular pH of about 7.3, the difference between extracellular and intracellular pH was 0.28 pH unit in bicarbonate buffer but only 0.08 pH unit in artificial buffer, in this case 25 mM MES. As the extracellular pH was lowered to 6.7 in respiratory acidosis, the difference between intracellular pH and extracellular pH essentially disappeared while, in artificial buffer acidosis, the intracellular pH was 0.31 pH unit higher than the extracellular pH. In metabolic acidosis (decrease of bicarbonate concentration), at an extracellular pH of 6.65, the intracellular pH was 6.89, which is higher than that observed in respiratory acidosis but less than in artificial buffer acidosis. Figure 4 shows the linear regression lines describing intracellular pH changes accompanying respiratory acidosis and artificial buffer acidosis. In the range from pH 7.4 to pH 6.6, a fall in extracellular pH of 0.1 pH unit produced a decline in intracellular pH of 0.05 pH unit in respiratory acidosis but of only 0.026 pH unit in artificial buffer acidosis. These data indicate that the intracellular pH is more responsive to changes of the extracellular pH during respiratory acidosis than during artificial buffer acidosis.

**ENERGY PRODUCTION DURING ACIDOSIS**

The decreased contractile activity of the heart induced by acidosis indicates a decline in energy utilization. Consequently a concomitant decrease of energy production

<table>
<thead>
<tr>
<th>Table 1 Effect of Respiratory, Metabolic, and Artificial Buffer Acidosis on the Relationship between Intracellular and Extracellular pH in Perfused Rat Hearts</th>
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<tbody>
<tr>
<td>Perfusion condition</td>
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<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Bicarbonate control</td>
</tr>
<tr>
<td>Respiratory acidosis</td>
</tr>
<tr>
<td>Respiratory acidosis</td>
</tr>
<tr>
<td>Metabolic acidosis</td>
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<tr>
<td>Artificial buffer, control</td>
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<tr>
<td>Artificial buffer, acidosis</td>
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</tbody>
</table>

Hearts were perfused for 15 minutes with Krebs-Henseleit buffer for the bicarbonate control, respiratory acidosis, and metabolic acidosis or with 35 mM 2-N-morpholinoethane sulfonic acid (MES) for the artificial buffer control and artificial buffer acidosis, each containing 5 mM glucose and insulin (1 x 10$^{-5}$ unit/ml) at pH 7.4, using the closed aorta preparation. At time zero, left atrial filling was switched to a second reservoir and the hearts were perfused for an additional 3 minutes. Hearts were frozen with tongs precooled in liquid nitrogen, lyophilized, and extracted with perchloric acid. $^3$H-Sorbitol was used to calculate the extracellular space, and the $^4$C-dimethyl-2,4-oxazolidinedione (DMO) distribution between extracellular and intracellular spaces was used to calculate the intracellular pH.

Values shown are means ± SEM of five to ten hearts.

CrP + Cr = 66.8 ± 1.4 µmol/g dry weight.
must occur as a new steady state balance between ATP production and utilization is achieved. The changes in high energy phosphate levels which accompany respiratory acidosis, metabolic acidosis, and artificial buffer acidosis are shown in Table 1. Values were measured after 3 minutes of perfusion under the appropriate experimental conditions. In respiratory acidosis at an extracellular pH of 6.75, the creatine phosphate-creatine ratio was decreased by 20% from the control conditions. In artificial buffer, lowering the extracellular pH from 7.24 to 6.70 increased the creatine phosphate-creatine ratio by 16%. On the other hand, metabolic acidosis resulted in little change in high energy phosphate levels compared with the bicarbonate controls. Thus, in contrast with previous results, the present data show no precipitous fall in high energy phosphate levels with acidosis. With artificial buffer acidosis, the fall of contractile activity was associated with an increase of intracellular steady state energy levels, as observed for decreased work at a constant extracellular pH. With respiratory acidosis, energy levels decreased, which suggests a specific inhibitory effect of intracellular H ions on energy production.

EFFECT OF ISCHEMIA ON MYOCARDIAL FUNCTION

Respiratory acidosis below pH 6.7 frequently led to the development of ischemia as reported in more detail elsewhere. This was manifest after the pH transition had already been completed by a further fall in coronary flow rate and left ventricular pressure. In addition, pyridine nucleotides became extensively reduced, as shown in Figure 5. This effect provided the most sensitive and reliable index of tissue hypoxia with respiratory acidosis. In this particular heart, ischemia developed about 1 minute after the pH transition. The decline in coronary flow from 4.6 ml/min to 0.7 ml/min indicated increased vascular resistance, since the coronary perfusion pressure was unchanged. The increase in vascular resistance could be most easily appreciated after retrograde pumping was initiated. The aortic pressure that was required to provide a coronary flow of 4.2 ml/min after the onset of ischemia was much greater than the aortic pressure immediately following the pH change when the coronary flow rate was 4.6 ml/min. As shown in Figure 5, retrograde pumping to restore the coronary flow rate caused a reoxidation of the pyridine nucleotides but failed to improve contractile performance.

Table 2 describes the effects of hypoxia, respiratory acidosis, and changes of coronary flow rate on intracellular pH and high energy phosphate and lactate levels. Ischemic respiratory acidosis resulted in a greater than 6-fold increase in tissue lactate and a 72% decline in the creatine phosphate-creatine ratio compared with control hearts. The intracellular pH was 0.1 pH unit lower than the extracellular pH while, in nonschismic respiratory acidosis, the intracellular pH and the extracellular pH were essentially equal. Thus ischemic respiratory acidosis was associated with a greater intracellular acidosis than nonschismic respiratory acidosis. Anoxia, with coronary flow maintained by retrograde aortic pumping, lowered the ATP/ADP ratio by 57% and the creatine phosphate-creatine ratio by 92%. Lactate levels increased to nearly 13 μmol/g dry weight, compared with 16.5 μmol/g dry weight in ischemic respiratory acidosis. Respiratory acidosis in anoxia had very minimal further effects on high energy phosphate and lactate levels compared with anoxic controls. The pH gradient between the intracellular and extracellular spaces was less in acidotic anoxia than in ischemic respiratory acidosis.

The relationship between ischemia, extracellular pH, and intracellular pH was investigated further by perfusing hearts with a one-way check valve above the aortic cannula. When the bypass around the check valve was

![Figure 4](http://circres.ahajournals.org/)

**FIGURE 4** Correlation between intracellular pH and coronary effluent pH during respiratory acidosis and artificial buffer acidosis. Intracellular pH determinations were made by the dimethyl-2,4-oxizolidine dione (DMO) distribution method. Respiratory acidosis was produced by increasing the PCO2 in 25 mM bicarbonate buffer. Artificial buffer acidosis resulted from the addition of HCl to perfusate containing 25 mM MES in place of the bicarbonate buffer.

![Figure 5](http://circres.ahajournals.org/)

**FIGURE 5** The effect of a rapid pH transition on myocardial function. The closed aorta preparation was used for this experiment. On the left is an anoxic calibration of pyridine nucleotide fluorescence. On the right is a rapid pH transition in which the arterial pH was changed from 7.4 to 6.65 at the point indicated by switching left atrial filling to a second reservoir containing bicarbonate buffer equilibrated at pH 6.65 by increasing the PCO2. After several minutes, retrograde aortic pumping was begun with buffer at the same pH to increase the coronary flow. LVP = left ventricular pressure; dp/dt = the first derivative of left ventricular pressure.
clamped, there was an immediate fall in the diastolic aortic pressure, and hence, a decline in the coronary flow (Fig. 6). Left ventricular pressure, systolic aortic pressure, coronary flow, and coronary effluent pH subsequently decreased gradually for several minutes and then fell more rapidly with the onset of severe heart failure. The more rapid decline in myocardial function was accompanied by a reduction of the pyridine nucleotides. When the coronary flow was increased with retrograde aortic pumping, the pyridine nucleotides returned to their control level. When the bypass was reopened and retrograde pumping was stopped, the heart resumed normal function, indicating full reversibility of the change in mechanical activity.

Figure 7 illustrates the effects of a 25%, 50%, and 75% decline in left ventricular pressure on coronary effluent pH, cardiac output through the one-way valve, and extent of pyridine nucleotide reduction. There was no immediate change of cardiac output upon clamping the bypass, but after about 1 minute, a sharp fall occurred. By the time the left ventricular pressure had declined to 75% of its control value, the pressure was insufficient to produce ejection of fluid through the one-way valve and the heart behaved as a closed aorta preparation with the entire cardiac output escaping through the coronary circulation. Intracellular pH measurements were made after the systolic aortic pressure had fallen to 75%, 50%, and 25% of its preischemic level. The extracellular pH was estimated from the coronary effluent pH. This causes some error, particularly at low coronary flow rates; hence, values calculated for the intracellular pH with severe ischemia are less reliable than other values. As shown in Figure 7, when the bypass was opened after the left ventricular pressure had fallen, the coronary effluent pH fell transiently before returning to its control level. This represented washout of the more acidic fluid in the dead space, and the lowest pH which was recorded during this washout was used as the extracellular pH at the time when the bypass was opened.

The effect of ischemia on the relationship between extracellular pH and intracellular pH as compared with respiratory acidosis is depicted in Figure 8. The solid line is the linear regression line for respiratory acidosis taken from Figure 4, and the open circles joined by the dashed line represent values for ischemic hearts. When the extracellular pH was greater than 7.1, the relationship between intracellular and extracellular pH was essentially the same as in respiratory acidosis. However, as the extracellular pH fell below 7.1, the intracellular pH declined further in ischemia than in respiratory acidosis for a given decrease in extracellular pH.

The correlation between extracellular pH and pressure development in graded ischemia, shown in Table 3, is similar to the data presented in Figure 2 for respiratory acidosis. In both instances, a 25% decline in pressure development occurred at pH 7.2 and a 50% decline occurred at pH 7.1. However, at pH 6.9, respiratory

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>pH&lt;sub&gt;E&lt;/sub&gt;</th>
<th>pH&lt;sub&gt;i&lt;/sub&gt;</th>
<th>ΔpH</th>
<th>Lactate (µmol/g dry wt)</th>
<th>CrP</th>
<th>ATP/ADP</th>
<th>CrP/Cr</th>
</tr>
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<tbody>
<tr>
<td>Bicarbonate control</td>
<td>7.33 ± 0.01</td>
<td>7.05 ± 0.02</td>
<td>0.28 ± 0.02</td>
<td>2.5 ± 0.4</td>
<td>29.7 ± 0.9</td>
<td>6.5</td>
<td>0.76</td>
</tr>
<tr>
<td>Increased P&lt;sub&gt;CO&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;, nonischemic</td>
<td>6.75 ± 0.03</td>
<td>6.76 ± 0.03</td>
<td>-0.01 ± 0.02</td>
<td>4.5 ± 0.5</td>
<td>26.0 ± 1.3</td>
<td>5.7</td>
<td>0.61</td>
</tr>
<tr>
<td>Increased P&lt;sub&gt;CO&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;, ischemic</td>
<td>6.79 ± 0.02</td>
<td>6.69 ± 0.04</td>
<td>0.10 ± 0.03</td>
<td>16.5 ± 1.9</td>
<td>10.9 ± 1.3</td>
<td>2.4</td>
<td>0.21</td>
</tr>
<tr>
<td>Anoxic</td>
<td>7.21 ± 0.03</td>
<td>6.93 ± 0.03</td>
<td>0.28 ± 0.03</td>
<td>12.9 ± 0.8</td>
<td>3.5 ± 0.6</td>
<td>2.8</td>
<td>0.06</td>
</tr>
<tr>
<td>Increased P&lt;sub&gt;CO&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;, anoxic</td>
<td>6.77 ± 0.02</td>
<td>6.73 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>11.4 ± 0.9</td>
<td>2.4 ± 0.1</td>
<td>2.9</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Hearts were perfused as described in Table 1. In the nonischemic, acidotic hearts and in the anoxic hearts, coronary flow was maintained by retrograde aortic pumping whenever it fell below 7 ml/min. pH<sub>E</sub> refers to effluent pH. pH<sub>i</sub> refers to intracellular pH.

Values shown are means ± SEM of four to ten hearts.

ATP + ADP = 24.2 ± 0.6 µmol/g dry weight. CrP + Cr = 65.6 ± 1.3 µmol/g dry weight.
Acidosis resulted in a 70% decrease in left ventricular pressure while ischemia resulted in a 75% decrease. Concomitant with the decreased tension development, decreased effluent pH, and decreased intracellular pH, ischemia caused lactate accumulation and a significant fall in the ATP/ADP and creatine phosphate-creatine ratios. Even when the systolic aortic pressure had fallen by only 7%, there was already a 35% decline in the ATP/ADP ratio, a 49% decline in the creatine phosphate-creatine ratio, and a nearly 3-fold increase in tissue lactate. By the time the systolic aortic pressure had fallen to 25% of its preischemic level, tissue lactate had increased over 10-fold, the ATP/ADP ratio was 35% of the control value, and the creatine phosphate-creatine ratio was only 13% of the control value. Thus, as ischemia became more severe, there was a progressive depletion of high energy phosphate intermediates, which correlated with an increase of tissue NADH content.

**Discussion**

**EFFECT OF INTRACELLULAR pH AND EXTRACELLULAR pH ON CONTRACTILITY**

Previous work has indicated that respiratory acidosis caused a greater decline in pressure development than metabolic acidosis, presumably due to a difference in intracellular pH. The present study permits a dissociation between the effects of intracellular and extracellular acidosis and gives steady state intracellular pH values that permit quantitation of the effect of extracellular and intracellular pH on pressure development. The intracellular pH determinations were made 3 minutes after the contractility change. However, since pressure development was stable during this time, these intracellular pH values should be pertinent. The results indicate that, in the rat heart, intracellular acidosis caused a greater decline in myocardial function than was produced by extracellular acidosis. A fall in extracellular pH of 0.5 pH unit without a significant change in intracellular pH resulted in only a 25% decrease in left ventricular pressure. However, when the extracellular pH fell by 0.5 pH unit and the intracellular pH fell by 0.25 pH unit, there was an 80% decline in left ventricular pressure. The present data are in agreement with the previous assumption that the PCO$_2$ is the major determinant of intracellular pH, but a small H$^+$ flux across the plasma membrane seems likely, since the intracellular pH in metabolic acidosis was lower than the intracellular pH in the bicarbonate control even though the PCO$_2$ was the same in both conditions.

Previous studies have indicated that the fall in pressure development as a consequence of acidosis can be reversed.
by increasing the extracellular Ca\(^{2+}\) concentration. In artificial buffer acidosis, the increase in extracellular Ca\(^{2+}\) concentration required to raise the left ventricular pressure to its control level was significantly less than in respiratory acidosis at any given extracellular pH. Thus it seems likely that the interaction between H\(^{+}\) and Ca\(^{2+}\) mediates the response of myocardial contractility to acidosis.

It has been previously suggested that H\(^{+}\) may interfere directly with Ca\(^{2+}\) entry into the myocardial cells. This may occur because of competition between H\(^{+}\) and Ca\(^{2+}\) for superficial binding sites on the sarcolemma. Voltage clamp experiments on frog atrial fibers have shown that artificial buffer acidosis decreases the slow inward calcium current that occurs during the plateau phase of the action potential. Likewise, metabolic acidosis in embryonic chick ventricles depresses the amplitude and duration of the slow calcium current. Even though it is generally agreed that the Ca\(^{2+}\) influx during the plateau phase of the action potential provides a significant amount of the Ca\(^{2+}\) required for contraction, the source and relative contribution of the remainder is unclear.

On the basis of voltage clamp experiments, Morad and Goldman suggest that about half of the contraction-producing Ca\(^{2+}\) comes from Ca\(^{2+}\) influx during the plateau phase of the action potential with the remainder from release of Ca\(^{2+}\) from internal stores. Fabiato and Fabiato have demonstrated, with skinned fibers, a calcium-dependent calcium release mechanism in rat ventricular muscle in which the amplitude of the contractions was increased as the free Ca\(^{2+}\) was increased from a pCa of 7.4 to 5.5. Thus a decrease in Ca\(^{2+}\) influx would be expected to decrease the contraction-producing Ca\(^{2+}\) pool directly and to diminish secondarily the release of Ca\(^{2+}\) from internal stores.

A second model for excitation-contraction coupling has been proposed by Langer in which there are both electrogenic and nonelectrogenic components of Ca\(^{2+}\) influx across the sarcolemma but no significant release of internal calcium. Only the electrogenic Ca\(^{2+}\) influx would be seen by the voltage clamp technique. It can be postulated that if extracellular H\(^{+}\) competes with Ca\(^{2+}\) for superficial sarcolemmal binding sites, then both the electrogenic and nonelectrogenic Ca\(^{2+}\) influxes would be affected.

Even though there is evidence for a direct interaction between extracellular H\(^{+}\) and Ca\(^{2+}\), it is also possible that extracellular acidosis and/or intracellular acidosis decrease the contraction-producing Ca\(^{2+}\) pool by altering ionic conductances of the sarcolemma. Thus if the height and duration of the plateau phase of the action potential were shortened in acidosis, there would also be a less Ca\(^{2+}\) influx. However, it appears that changes in the duration of the action potential during acidosis have less effect than the decrease in the slow inward calcium current.

Competition between H\(^{+}\) and Ca\(^{2+}\) for binding sites on troponin has also been previously postulated. This mechanism would account for the effect of intracellular acidosis on pressure development by inhibiting the actin-myosin interaction. Experimental evidence for this proposal at present appears contradictory. Initially it was thought that increasing the pH from 6.5 to 8.0 caused a significant increase in the affinity of troponin for Ca\(^{2+}\), but more recent data from the same laboratory, obtained with improved methodology, suggest that pH changes over this range had no effect on Ca\(^{2+}\) binding to troponin. Nevertheless, it is evident that competitive interaction between H\(^{+}\) and Ca\(^{2+}\) can be demonstrated with glycol-extracted muscle fibers.

**EFFECT OF ACIDOSIS ON ENERGY UTILIZATION AND PRODUCTION**

Although the detailed mechanism of H\(^{+}\) ion interactions at specific Ca\(^{2+}\)-binding sites on membranes in cardiac tissue has not yet been fully elucidated, it appears that changes of energy metabolism are essentially secondary to changes of contractile activity. This conclusion is supported by data of Williamson et al. which showed that, during the initial fall of left ventricular pressure with respiratory acidosis, creatine phosphate levels and the ATP/ADP ratio increased. Likewise, data in the present paper show a small increase of the creatine phosphate-creatine ratio with artificial buffer acidosis in the steady state. Energy production and energy utilization fall in

**TABLE 3 Effects of Graded Ischemia in Perfused Rat Hearts**

| Systolic aortic pressure (% of preischemic level) | pH\(_{e}\) | pH\(_{i}\) | ΔpH | Lactate (μmol/g dry wt) | CrP (μmol/g dry wt) | NADH (μmol/g dry wt) | ATP/ADP | CrP/Cr |
|---|---|---|---|---|---|---|---|---|---|
| 100 | 7.33 | 7.05 ± 0.02 | 0.28 ± 0.02 | 2.4 ± 0.4 | 29.7 ± 0.9 | 0.27 ± 0.06 | 6.5 ± 0.76 |
| 90 | 7.26 | 7.05 ± 0.03 | 0.23 ± 0.03 | 7.1 ± 1.0 | 19.8 ± 0.1 | 0.82 ± 0.09 | 4.2 ± 0.39 |
| 75 | 7.20 | 6.99 ± 0.03 | 0.21 ± 0.03 | 17.4 ± 2.3 | 15.0 ± 1.0 | 1.32 ± 0.05 | 3.1 ± 0.27 |
| 50 | 7.08 | 6.90 ± 0.02 | 0.18 ± 0.02 | 24.5 ± 2.6 | 9.1 ± 0.3 | 1.44 ± 0.11 | 2.7 ± 0.15 |
| 25 | 6.90 | 6.72 ± 0.01 | 0.18 ± 0.02 | 26.0 ± 3.3 | 6.6 ± 1.4 | 1.91 ± 0.13 | 2.3 ± 0.10 |

Hearts were perfused for 15 minutes in Krebs-Henseleit buffer containing 5 mM glucose and insulin (1 × 10\(^{-5}\) unit/ml) at pH 7.4 on the apparatus which had a one-way valve in the aortic outflow tract and a bypass around the valve. At time zero, the bypass was clamped. Hearts were frozen with tongs precooled in liquid nitrogen after the systolic aortic pressure fell to the desired percent of its preischemic level. The preischemic systolic aortic pressure was defined as the systolic aortic pressure immediately after the bypass was clamped. Control hearts were perfused using the closed aorta preparation. The frozen tissue was lyophilized and then extracted with perchloric acid. ATP + ADP = 24.5 ± 0.6 μmol/g dry weight; CrP + Cr = 70.6 ± 0.7 μmol/g dry weight.

Values shown are means ± SEM of three to ten hearts.

ATP + ADP = 24.5 ± 0.6 μmol/g dry weight; CrP + Cr = 70.6 ± 0.7 μmol/g dry weight.
parallel as cardiac work decreases, and when the decrease of intracellular pH is small, the metabolic changes and regulatory factors which coordinate these processes are similar to those observed for a cardiac work transition at a constant arterial pH of 7.4. The slight decline in ATP/ADP and creatine phosphate-creatine ratios in nonischemic respiratory acidosis may be due to the direct inhibitory effect of H+ on phosphofructokinase and citrate synthase. During respiratory acidosis with glucose as sole exogenous substrate, there is an oxidation of pyridine nucleotides, suggesting a relative substrate deficiency. Addition of acetate or octanoate during nonischemic respiratory acidosis produced an increase of the high energy phosphate levels and an increase in the state of reduction of the pyridine nucleotides without improvement of contractile activity. The conclusion is reached, therefore, that in nonischemic respiratory acidosis energy production does not limit myocardial performance. However, with severe intracellular acidosis, there is a marked increase of vascular resistance, which diminishes oxygen delivery to regions of the heart and results in heterogenous areas of anoxia. This phenomenon accounts for the large decrease of energy levels observed in the whole heart with severe respiratory acidosis of pH 6.6 to 6.7.

CONTRIBUTION OF ACIDOSIS TO ISCHEMIC FAILURE

The present study has delineated the effects of extracellular and intracellular acidosis on myocardial performance. It is well known that acidosis is a prominent component of ischemia, but the relative contribution of acidosis to the decline in pressure development observed in ischemia has not been well documented. Hypoxia is another important component of ischemia, and it has been shown that hypoxia results in decreased cardiac work. Therefore, either acidosis or oxygen availability could be the principal determinant of cardiac work in ischemia.

In the model of ischemia used in the present study, the fall in pressure development paralleled the decline in effluent pH. It can be inferred that the fall in pH was a major determinant of cardiac work because the extracellular pH associated with a given decline in pressure development was essentially the same in the early stages of ischemia (systolic aortic pressure ≥ 50% of its preischemic level) as in respiratory acidosis.

Concomitant with the development of acidosis in ischemia was the appearance of small anoxic zones in the myocardium as recorded by flash photographs of pyridine nucleotide fluorescence. These essentially nonfunctioning areas might be expected to contribute to the fall in pressure development. However, if the anoxic zones made a significant contribution, then pressure development would be expected to be depressed to a greater extent than observed for a given fall of extracellular pH (compare Table 3 and Figure 2). It is probable that the initial fall in cardiac work in this ischemia model can be explained on the basis of respiratory acidosis, and even though the anoxic zones probably contribute to depressed contractile activity, they appear to play a less significant role. As the ischemic process became more severe, the anoxic zones became more prominent and probably contribute substantially to the further decline in pressure development.

The levels of high energy phosphates, lactate, and NADH must be interpreted in light of the heterogeneity of tissue oxygenation. The fall in tissue ATP and creatine phosphate levels represent the sum of anoxic tissue with very low levels and normoxic tissue with higher levels. Likewise, the lactate levels represent a mixture of anoxic, ischemic areas with massive lactate accumulation and well perfused areas with low levels. Thus the tissue levels of the various intermediates can only be interpreted as showing that, as ischemia became more severe, the population of anoxic cells increased relative to the normoxic ones.

The significance of the data presented in this paper is, first, that intracellular pH is quantitatively a more important determinant of myocardial contractility than extracellular pH. Second, intracellular acidosis does not severely impair ATP production unless it is severe enough to cause intense vasoconstriction and anoxic areas. Third, the decline in pressure development during the early stages of ischemia results from intracellular and extracellular acidosis, not from a decrease in energy levels.

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