Cardiac failure appears rapidly during severe hypoxia and precedes a substantial reduction in adenosine triphosphate content. Reduced adenosine triphosphate turnover, in the presence of nearly normal content, may be the metabolic basis for contractile failure during hypoxia. To measure both the myocardial content and the turnover rates of high-energy phosphate compounds during hypoxia, we performed $^{31}$P-nuclear magnetic resonance studies by placing a surface coil directly over the left ventricle in intubated rats that were instrumented for hemodynamic measurements and ventilated with either 21, 10, or 8% $O_2$. Normoxia produced a hemodynamic and metabolic steady state for 4 hours and hypoxia for at least 60 minutes. Under normoxic ventilation ($n = 10$, mean ± SD), the arterial $P_{O_2}$ was $96 ± 14$, pH $7.38 ± 0.11$, and systolic blood pressure $96 ± 8$ mm Hg; under hypoxic ventilation with 10% $O_2$ ($n = 5$), the arterial $P_{O_2}$ was $57 ± 10$, pH $7.39 ± 0.09$, and systolic pressure $68 ± 10$; and under hypoxic ventilation with 8% $O_2$ ($n = 5$), the $P_{O_2}$ was $52 ± 7$, pH $7.37 ± 0.04$, and systolic pressure $51 ± 4$. Hypoxic ventilation with 10 or 8% $O_2$ decreased the creatine phosphate content from $51.4 ± 5.4$ umol/g dry wt to $39.3 ± 5.4$ and $45.6 ± 4.1$ and depressed adenosine triphosphate slightly from $25.0 \mu$mol/g dry wt to $21.8 ± 2.1$ and $21.9 ± 1.0$, respectively. High-energy phosphate turnover, measured as flux through the creatine kinase reaction, decreased from $22.7 ± 6.7 \mu$mol/g dry wt/sec during normoxic ventilation to $13.7 ± 3.6$ and $15.9 ± 2.6$ during ventilation with 10 and 8% $O_2$, respectively. Thus, the decreased turnover of high-energy phosphate compounds, not their tissue contents, may be the metabolic basis for contractile failure during hypoxia. (Circulation Research 1987;60:871–878)

The energetic basis for cardiac failure during hypoxia is still uncertain. Several studies have found that adenosine triphosphate (ATP) depletion, intracellular acidosis, and changes in the calcium transient do not match the magnitude or the time course of contractile failure during oxygen deprivation.

The myocardium relies on oxidative metabolism to generate most of the ATP for cardiac contraction. But during hypoxia, ATP levels fall only slightly while contraction fails rapidly and severely. The discrepancy between ATP levels and contractility during hypoxia has been attributed to decreases in either energy availability or utilization. Pool et al. observed that myocardial high-energy phosphate levels were preserved during hypoxic heart failure in the dog and suggested that hypoxia may decrease the availability of ATP in a specific cellular compartment (e.g., myofibrillar) important for contraction. Kammermeier et al. calculated a decrease in the free energy for ATP hydrolysis during hypoxia and consequently proposed that decreased energy availability produces hypoxic cardiac failure, but this was not confirmed by Matthews et al. who concluded that cardiac contraction is directly sensitive to $P_{O_2}$. Hypoxia may inhibit the utilization of available ATP at the myofilament by producing intracellular acidosis. Although investigators have found decreased sensitivity of the myofilament to calcium at pH < 7.0, the role of intracellular acidosis in inhibiting the utilization of available ATP remains unclear. Most studies have reported that hypoxia, in contrast to ischemia, produces only small changes in intracellular pH that do not match the magnitude or the time course of the change in contractility. Direct calcium measurements suggest that the availability of calcium at the myofilament is not significantly inhibited by hypoxia. Some have proposed that the inotropic state of the myocardium is directly responsive to $P_{O_2}$.

The purpose of this study was to test the hypothesis that hypoxia may depress high-energy phosphate turnover but produce only small changes in high-energy phosphate content. To test this hypothesis, we used $^{31}$P-NMR techniques to measure high-energy phosphate content and flux through the creatine kinase reaction in the hypoxic myocardium of the living rat. Previous studies have suggested that the creatine kinase reaction may couple ATP availability to ATP utilization under normoxic conditions (reviewed in Bessman and Geiger), but the reaction has not been evaluated during hypoxic contractile failure. In this study, we estimated the turnover rates of creatine phosphate (CrP) and ATP directly in the heart of intubated and catheterized rats to obtain simultaneous hemodynamic and metabolic measurements during hypoxia. We ob-
served a marked decrease in the flux through the creatine kinase reaction during hypoxic contractile failure. The results suggest that oxygen deprivation impairs mitochondrial oxidative phosphorylation and, thus, limits the transfer of ATP via the reaction catalyzed by creatine kinase.

**Materials and Methods**

**Open-Chest Preparation**

Each male Sprague-Dawley rat, weighing 300–400 g, was anesthetized initially with 2% halothane vaporized (Fluotec Vaporizer, Cyprane Limited, Keighley, England) and delivered by constant flow (Harvard Small Animal Respirator) through a nasal cone. The rat was intubated through a tracheostomy with 1.5-cm length of polyethylene tubing (1.14 mm i.d., 1.57 mm o.d., Clay-Adams, Parsippany, N.J.) connected to Tygon tubing (3.2 mm i.d., 6.4 mm o.d.) that was attached to the respirator. The respirator was adjusted to deliver a tidal volume of 10 ml/kg 60 times per minute. A transverse thoracic incision was made just inferior to ligated internal thoracic arteries and extended laterally to the mid-axillary line and inferiorly to the costal margin. Estimated blood loss for each of the 10 preparations used for this study was less than 2.0 ml and was replaced through a right internal jugular cannula with an equal volume of 145 mM NaCl that contained 10 U/ml heparin. After surgery, the halothane dose was decreased to 1.25% for the remainder of the study to avoid hypoxia-aggravated halothane toxicity. The body temperature of the rat was maintained by running water at 35° C through 2 loops of Tygon tubing placed against its body. When ventilated with room air, the preparation showed hemodynamic and metabolic stability for more than 4 hours with <10% change in heart rate, blood pressure, or myocardial ATP or CrP content. At the end of the study, halothane was increased to 4% to produce lethal cardiovascular collapse. (Figure 1 shows rat preparation.)

**Hemodynamic Measurements**

The left common carotid artery was cannulated with a 2.0-cm length of polyethylene tubing (0.73 mm i.d., 0.98 mm o.d.) connected to a 120-cm length of Tygon Microbore Tubing (1.27 mm i.d., 2.34 mm o.d., Norton Plastics, Akron, Ohio). The tubing was connected to a Statham P23dB pressure transducer (Oxnard, Calif.) for continuous measurement of central aortic pressure and heart rate on a Hewlett-Packard 7754B recorder and 8805D pressure amplifier. The transducer system allowed pressure recordings to be made with high accuracy: damped natural frequency $N_d = 51 \pm 7$ Hz, damping coefficient $D = 0.50 \pm 0.12$, and undamped natural frequency $N = 67 \pm 8$ Hz for 4 determinations.13

**Hypoxia**

For simultaneous hemodynamic and NMR measurements, 5 rats were ventilated with room air and with 10% $O_2$ (balance nitrogen); a second group of 5 rats was ventilated with room air and then with 8% $O_2$. Each ventilation period lasted 60 minutes. For arterial blood gas measurements (Corning 168 pH/Blood Gas Analyzer, Medfield, Mass.), samples of left ventricular blood were obtained after 60 minutes of ventilation with room air, 10%, or 8% $O_2$ in 4 rats. We chose to perform the hypoxic ventilation with 10 or 8% $O_2$ because other studies showed that rats deteriorated hemodynamically in <60 minutes when <8% $O_2$ is used (data not shown).

$^3$P-NMR Measurements

The rat was placed in an aluminum probe with the apex of the left ventricle loosely positioned against a Parafilm-covered, 1.4-cm, 2-turn surface coil. Intercostal muscle was retracted more than 1 cm from the coil. The probe was placed in the bore of an Oxford Instruments 8.45T magnet (Oxford, England) interfaced with a Nicolet NT360 spectrometer (Madison, Wis.). The magnetic field was shimmed by maximizing the signal intensity for $^23$Na at 95.24 MHz. $^3$P-NMR spectra were recorded by signal-averaging 32 scans obtained after a 15-μsec broadband pulse (equivalent to a 90° pulse) and a 12.5-second interpulse delay.

Studies with phantoms showed that the surface coil obtained 50% of the signal from the superficial volume.
2 mm deep and that 75% of the signal arose from a volume 4 mm deep. The contribution of skeletal muscle signal to the spectra was calculated as less than 5%. \(^{31}P\)-NMR spectra were also obtained from a preparation within 10 minutes after excision of the heart. Only signals for 2,3-diphosphoglycerate (2,3 DPG) and ATP in a ratio of 25:1 (characteristic of blood) could be detected. No skeletal muscle CrP signal was detected from the cardiectomized rats at a time when the ischemic skeletal muscle CrP and ATP contents were >90% of their control values (data not shown).

Magnetization transfer was observed when each broadband pulse was preceded by a low-power, narrowband pulse at the resonance frequency of \(^{31}P\)ATP for 0 to 4.8 seconds. Separate studies showed that the narrowband pulse directly attenuated the CrP magnetization by less than 5% when the carrier frequency was placed 350 Hz downfield from the resonance for creatine phosphate.

Magnetization areas were measured with the Nicolet Integration Program. During ventilation with 21% O\(_2\), myocardial ATP was set to 25 \(\mu\)mol/g dry wt after subtracting the estimated red cell ATP that contributed to the \((\beta\text{-P})\)ATP resonance area. (An average of 12.8% of the total observable ATP was attributed to red cells.) The myocardial ATP during hypoxic ventilation was normalized to the normoxic value. The ATP content of red cells was determined from the ratio of 2,3 DPG:ATP measured in blood drawn from the left ventricle at the end of the study. Under the NMR conditions for this study, the ratio of 2,3 DPG:ATP in red cells was 22.7 ± 3.4 \((n = 6)\).

Metabolic stability of the preparation was assessed by obtaining unsaturated \(^{31}P\)-NMR spectra for measurements of steady-state ATP and CrP before and after the 40-minute magnetization transfer experiment. NMR measurements were made only during physiologic and metabolic steady state defined by less than a 10% change in heart rate, blood pressure, and steady-state levels of ATP and CrP.

**Tissue Creatine Content and Creatine Kinase Activity**

At the end of each study, 5–10 mg of myocardial tissue were homogenized in 0.1 M K\(_2\)HPO\(_4\), pH 7.4, with 1 mM ethyleneglycol-bis-(\(\beta\)-aminoethyl ether)\(N, N'\) tetraacetate and 1 mM \(\beta\)-mercaptoethanol at 4° C for a final tissue concentration 5 mg/ml. Total creatine content was measured in these homogenates by using the method of Kammermeier.\(^{15}\) After the addition of 1% Triton X-100 to a final concentration of 0.1%, total creatine kinase activity was determined by the method of Rosalki.\(^{16}\) Creatine kinase isozyme distribution was assessed electrophoretically by the method of Hall and DeLuca.\(^{17}\)

**Statistical Analysis**

The T1 for CrP and the rate constant for the creatine kinase reaction were obtained from the parameters for the single-exponential function between magnetization area and saturation time with variance-weighted non-linear regression analysis.\(^{18,19}\) Differences among blood gas measurements for the three ventilation conditions were compared by using the Multiple Range Test of Newman-Keuls only if a significant effect was proved by a two-way analysis of variance.\(^{20}\) Differences for the rate-pressure products, myocardial CrP and ATP, rate constants, T1 values, and chemical flux values among the three ventilation conditions were determined by using paired and unpaired Student’s t tests where appropriate.\(^{20}\) All calculations and regression analyses were performed with the Research and Statistics Management Package (Bolt, Beranek and Newman, Cambridge, Mass.; VAX 11/780 Computer, Digital Equipment Corporation, Maynard, Mass.). All data are presented as mean ± SD.

**Results**

**Preparation Stability**

During ventilation with room air and 1.25% halothane, the 10 rats had a systolic blood pressure of 96 ± 8 mm Hg and heart rate of 316 ± 16 beats/min (Table 1). Hypoxic ventilation depressed systolic blood pressure and, to a lesser degree, heart rate. Ventilation with 10 or 8% O\(_2\), depressed blood pressure to 68 ± 10 and 51 ± 4 mm Hg, respectively.

Ventilation with either 21, 10, or 8% O\(_2\) produced conditions that were hemodynamically and metabolically stable for at least 60 minutes (Figure 2). Heart rate increased slightly and systolic pressure decreased slightly during ventilation with 10% O\(_2\). These insignificant changes offset each other and produced an unvarying rate-pressure product during mild hypoxia. After each ventilation period, the ATP and CrP contents varied by less than 10% from the values at the beginning of the period. In other studies, ventilation of anesthetized rats with room air for 4 hours produced less than a 10% decrease in blood pressure, heart rate, or myocardial ATP and CrP contents.

The hemodynamic and metabolic effects of hypoxic ventilation with 10% O\(_2\) were fully reversible. In 3 of 5 rats, the gas mixture was switched to 21% O\(_2\) after hypoxic ventilation; the systolic blood pressure rose to 100 ± 5 mm Hg, the ATP content increased from 21.5 ± 1.2 to 25.0 \(\mu\)mol/g dry wt, and the CrP content recovered from 33.2 ± 0.7 to 47.3 ± 5.5 \(\mu\)mol/g dry wt to match the values under normoxic ventilation (Table 1).

Arterial blood pH was normal under each ventilation condition (Table 2). Decreasing the inspired O\(_2\) from 21 to 10% produced a decrease in both P\(_O_2\), and the hemoglobin saturation. Decreasing the O\(_2\) further to 8% produced only a trend toward a decrease in P\(_O_2\) and saturation.

**Magnetization Transfer Studies**

Examples of \(^{31}P\)-NMR spectra from each ventilation period are presented in Figures 3 and 4. The unsaturated, fully relaxed spectra (the first spectrum in each stack) show peaks for 2,3 DPG, CrP, and the 3 phosphorus atoms of ATP. A signal for inorganic phosphate could not be discriminated from the 2,3 DPG...
Table 1. NMR and Hemodynamic Measurements at 21, 10, and 8% O2

<table>
<thead>
<tr>
<th>%O2</th>
<th>SBP</th>
<th>HR</th>
<th>ATP</th>
<th>CrP</th>
<th>k</th>
<th>Flux</th>
<th>Tl</th>
<th>[ADP]</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>99±4</td>
<td>316±15</td>
<td>25.0</td>
<td>50.6±6.5</td>
<td>0.42±0.11</td>
<td>21.6±8.0</td>
<td>2.9±0.4</td>
<td>35±21</td>
</tr>
<tr>
<td>10</td>
<td>68±10</td>
<td>306±27</td>
<td>21.9±1.0</td>
<td>45.6±4.1</td>
<td>0.13±0.05</td>
<td>5.9±2.6</td>
<td>2.6±0.3</td>
<td>53±20</td>
</tr>
<tr>
<td>8</td>
<td>92±11</td>
<td>315±15</td>
<td>25.0</td>
<td>52.2±4.3</td>
<td>0.45±0.09</td>
<td>23.9±5.3</td>
<td>2.8±0.4</td>
<td>46±9</td>
</tr>
</tbody>
</table>

Simultaneous 3P-NMR and hemodynamic measurements were made in anesthetized rats. Five rats were ventilated with room air and with 10% O2 (Group I); five rats were ventilated with room air and then with 8% O2 (Group II).

Systolic blood pressure (SBP, mm Hg) and heart rate (HR, min⁻¹) were measured from a central aortic catheter. ATP and creatine phosphate contents (CrP, µmol/g dry wt), k (sec⁻¹), flux via the creatine kinase reaction (µmol/g dry wt/sec), and Tl for CrP (seconds) were measured with 31P-NMR techniques. [ADP] was estimated from creatine kinase equilibrium²¹ and from NMR and biochemical analysis of metabolite content (Table 3).

*p<0.05 between 21 and 10% O2 (within Group I) or between 21 and 8% O2 (within Group II) by paired t test (n=5).

tp<0.05 between 10 and 8% O2 (between Groups I and II) by unpaired t test. No statistical differences exist between the two groups during room air ventilation.

peaks, thereby precluding measurement of myocardial intracellular pH.

After selective saturation of the resonance for [γ-P]ATP for 0.3 to 4.8 seconds, we observed evidence of high-energy phosphate exchange between CrP and ATP via the creatine kinase reaction. Under normoxic ventilation (Figures 3A and 4A), transfer of magnetically visible phosphate from CrP to ATP occurred at a moderate rate, as shown by the rate of disappearance of signal at CrP. During ventilation with 10% O2 (Figure 4B), the rate of disappearance of magnetically visible CrP decreased substantially, and during ventilation with 8% O2, the rate decreased even further, suggesting that transfer via creatine kinase had also decreased to a greater degree during severe hypoxia.

Although hypoxia produced severe hypotension, the high-energy phosphate content was only slightly decreased (Figure 5A). Thus, high-energy phosphate content correlates poorly with cardiac performance during hypoxia. On the other hand, the kinetic measurements show that cardiac performance is related much more closely to the rate constant and flux values for the creatine kinase reaction in the direction of ATP synthesis during hypoxia (Figures 5B and 5C).

Analyzing the results by ventilation groups (Table 1), we observed that ventilation with 10% O2 decreased both ATP and CrP contents compared with room air ventilation. The ATP and CrP contents were indistinguishable for rats ventilated with either 10 or 8% O2, reflecting the small differences in arterial blood Po2 and hemoglobin oxygen saturation between the two groups (Table 2). However, ventilation with 8% O2 did enhance the severity of hypotension, of slowing of the heart rate, and of decreases in rate constant and flux values for the creatine kinase reaction as compared with ventilation with 10% O2.

Biochemical Analysis

Estimates for free cytosolic [ADP] were made from the creatine kinase equilibrium constant²¹ and from

Table 2. Blood Gas Measurements

<table>
<thead>
<tr>
<th>%O2</th>
<th>pH</th>
<th>P02</th>
<th>Pco2</th>
<th>O2 Sat</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>7.38±0.11</td>
<td>96±14</td>
<td>33±6</td>
<td>97±1</td>
</tr>
<tr>
<td>10</td>
<td>7.39±0.09</td>
<td>57±10</td>
<td>37±9</td>
<td>83±8</td>
</tr>
<tr>
<td>8</td>
<td>7.37±0.04</td>
<td>52±7</td>
<td>34±6</td>
<td>78±10</td>
</tr>
</tbody>
</table>

Arterial blood was drawn directly from the left ventricle during ventilation with room air, 10 and 8% O2 for measurement of pH, P02, Pco2, and hemoglobin oxygen saturation (O2 sat, %).

*p<0.05 between adjacent groups (n=4).
A21%0xygen
pOj=92
pCOj=36
pH=7.37
SBP=260
HR=320

FIGURE 3. Hypoxia (10% O2). 31P-NMR and hemodynamic
recordings were made during ventilation with room air (A) or
10% O2 (B) in the same rat. Magnetization transfer experiments
were performed by saturating the [γ-P]ATP resonance for 0 to
4.8 seconds and measuring the rate of disappearance of creatine
phosphate magnetization. 2,3-DPG, 2,3-diphosphoglycerate; CrP, creatine phosphate; γ, α, and β, respective phosphorus atoms of ATP; SBP, systolic blood pressure (mm Hg); HR, heart rate (per minute).

A21%0xygen
pOj=53
pCOj=34
pH=7.36
SBP=285

FIGURE 4. Severe hypoxia (8% O2). Simultaneous hemody-
namic and 31P-NMR measurements were made in the same rat
during ventilation with room air (A) or 8% O2 (B). Abbrevia-
tions are the same as for Figure 3.

FIGURE 5. Physiologic chemistry of creatine kinase during hypoxia. The relations among ATP and creatine phosphate content (μmol/g dry wt) (A), and the rate constants (B), and flux values (μmol/g dry wt/sec) (C) for creatine kinase are plotted as a function of rate-pressure product for all three ventilation conditions. The y intercepts for k vs. RPP and flux vs. RPP are not different from zero: k, −0.069 + 0.072; flux, −6.9 + 4.1.

estimates of the concentration of the substrates for the reaction based on intracellular water content of 0.65 ml/g tissue. Hypoxic ventilation with 10 or 8% O2 increased the estimate for [ADP] compared with room air ventilation, which is consistent with the decrease in high-energy phosphate content.

Hypoxic ventilation with 10 or 8% O2 produced no change in myocardial creatine kinase activity, isozyme distribution, or total creatine content (Table 3).

FIGURE 5. Physiologic chemistry of creatine kinase during hypoxia. The relations among ATP and creatine phosphate content (μmol/g dry wt) (A), and the rate constants (B), and flux values (μmol/g dry wt/sec) (C) for creatine kinase are plotted as a function of rate-pressure product for all three ventilation conditions. The y intercepts for k vs. RPP and flux vs. RPP are not different from zero: k, −0.069 + 0.072; flux, −6.9 + 4.1.

estimates of the concentration of the substrates for the reaction based on intracellular water content of 0.65 ml/g tissue. Hypoxic ventilation with 10 or 8% O2 increased the estimate for [ADP] compared with room air ventilation, which is consistent with the decrease in high-energy phosphate content.

Hypoxic ventilation with 10 or 8% O2 produced no change in myocardial creatine kinase activity, isozyme distribution, or total creatine content (Table 3).

Discussion

We observed that hypoxia depressed cardiac performance severely in the living rat but decreased only slightly the myocardial contents of ATP and CrP. Magnetization transfer measurements showed that hypoxia decreased flux through the creatine kinase reac-

Table 3. Biochemical Measurements of Creatine Kinase Activity, Mitochondrial Isozyme Activity, and Total Tissue Creatine

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine kinase</td>
<td>7.0 ± 0.1</td>
<td>7.7 ± 0.1</td>
<td>7.0 ± 0.9</td>
</tr>
<tr>
<td>% Mito</td>
<td>31 ± 4</td>
<td>26 ± 4</td>
<td>29 ± 6</td>
</tr>
<tr>
<td>Total creatine</td>
<td>78 ± 8</td>
<td>74 ± 7</td>
<td>78 ± 23</td>
</tr>
</tbody>
</table>

Creatine kinase activity (IU/mg protein), % of total activity comprised by mitochondrial isozyme (% mito), and total tissue creatine (μmol/g dry wt) were measured by standard spectrophotometric and electrophoretic techniques in all hearts after NMR and hemodynamic measurements. These were compared with results from 5 control hearts that were not subjected to hypoxia. Rats in Group I were ventilated with room air and 10% O2; those in Group II were ventilated with room air and then 8% O2; control rats breathed only room air before sacrifice. No statistical differences exist among the three groups.
tion, demonstrating that cardiac performance matches the turnover, not the contents, of high-energy phosphate compounds during hypoxia. The discrepancy between cardiac performance and the myocardial content of ATP observed here is consistent with observations made by many other investigators. We observed that hypoxia (8% O₂) depressed cardiac performance by about 50%, while ATP content fell by only 13%. A similar discrepancy between high-energy phosphate content and cardiac performance has been seen in a variety of preparations during both hypoxia 2-3 and ischemia. 9,22,23 Measuring ATP and CrP contents provides only indirect evidence for defining the balance between high-energy phosphate synthesis and utilization. The depressed ATP and CrP contents only imply that hypoxia transiently impairs high-energy phosphate synthesis to a greater degree than it does utilization. A new steady state is then reached in which synthesis of ATP is matched to utilization, albeit at lower levels of high-energy phosphate compounds.

More importantly, we observed directly that turnover rates for the high-energy phosphate compounds through the creatine kinase reaction matched cardiac performance during hypoxia. Ventilation with 10% O₂ reduced the rate-pressure product by 34% and flux through the creatine kinase reaction by 37%. Ventilation with 8% O₂ depressed the rate-pressure product by 49% and flux by 75%.

The [31P-NMR measurements for this study required that steady state be maintained for at least 60 minutes. Because of this requirement, the blood-perfused heart in the intubated rat has advantages over the isolated, buffer-perfused heart. During ventilation of the rat with room air, we observed no significant change in hemodynamic or metabolic measurements for as long as 4 hours. In contrast, buffer-perfused hearts, gassed with 95% O₂, remain stable for about 90 minutes. 19 During hypoxic ventilation with 10 or 8% O₂, the rats showed stable, though depressed, metabolic and hemodynamic measurements for at least 60 minutes. Although ventilation with 8% O₂ produced greater cardiac depression than 10% O₂, we observed no differences in arterial P₅₀, hemoglobin oxygen saturation, or myocardial ATP and CrP contents between the two ventilation conditions. Like Pool et al., 2 we observed progressive hemodynamic deterioration when rats were ventilated with <8% O₂.

Although the metabolic and hemodynamic effects of graded hypoxia may be studied in the living rat with greater cardiac stability, certain disadvantages do exist. One of these is the inability to measure myocardial oxygen consumption. In the working heart, oxygen consumption is related to the rate-pressure product 24,25. Accordingly, we used the rate-pressure product as a physiologic index of the rate of ATP synthesis and utilization. Another limitation is the inability to distinguish consistently the inorganic phosphate resonance peak, chemical shift of which provides estimates for intracellular pH. 9 Investigating intracellular pH is potentially important for two reasons: the creatine kinase reaction is pH-dependent, and intracellular acidosis may impair the sensitivity of the myofilament to calcium and alter the utilization of ATP. The following discussion provides evidence that the potential pH effects do not alter the conclusions of the study.

The creatine kinase reaction (EC 2.7.3.2) is pH-dependent:

\[ \text{Mg} \cdot \text{ADP} + \text{H}^+ + \text{CrP} \Rightarrow \text{Mg} \cdot \text{ATP} + \text{crea} \]

We studied the reaction in the direction of ATP synthesis to avoid the complication of other ATP reactions that produce underestimates for the reverse direction of the creatine kinase reaction with the method of magnetization transfer. 19 The magnetization transfer analysis simplifies the study of the forward direction of the reaction from a third-order process involving ADP, H⁺, and CrP to a first-order reaction involving CrP alone. Consequently, the pseudo-first-order rate constant, k, calculated from magnetization transfer, can be expressed in terms of the true rate constant, k', where k = k' [ADP][H⁺]. If the true rate constant, k', does not change, the observed decreases in the pseudo-first-order rate constant, k, during hypoxia must be attributed to decreases in [ADP] or [H⁺]. In this study, we calculated an increase in the estimate for [ADP] during hypoxia. During oxygen deprivation, net ATP hydrolysis and other reactions would most likely increase 2,6,26 or maintain 9 intracellular [H⁺]. Thus, the decreases in the pseudo-first-order rate constant observed in this study, opposite in direction to changes in calculated [ADP] and expected [H⁺], must reflect actual decreases in the true rate constant for the reaction during hypoxia.

The extent of intracellular acidosis that occurs during hypoxia has been measured by other investigators. In the isolated rabbit septum, Cobbe and Poole-Wilson 9 observed a decrease in tissue pH of 0.52 pH unit after 60 minutes of complete anoxia. On the other hand, LaManna et al. 8 observed that intracellular pH decreased in the isolated toad ventricle strip by only 0.11 pH unit after 30 minutes of hypoxia (~0% O₂) by using the light absorption of the pH dye neutral red and cytochrome c. Using [31P-NMR to measure intracellular pH in the isolated rat heart during hypoxia (~0% O₂), Matthews et al. 5 observed a decrease of 0.19 pH unit. Jacobus et al. 6 found no change in intracellular pH, as determined by [31P-NMR, in the isolated rabbit heart perfused under mildly hypoxic conditions with 65% O₂ compared with 95% O₂. The effect of pH on the creatine kinase reaction has been measured in solutions of MM creatine kinase, the dominant isozyme in heart. Cook et al. 6 found no significant change in the initial reaction velocity for Mg·ADP phosphorylation and only about a 15% depression in the velocity for creatine phosphorylation when the pH was decreased from 7.2 to 6.5 at 35°C. Thus, the creatine kinase reaction in vitro shows only mild sensitivity to pH over the range that may be expected during hypoxia.

The effect of [H⁺] on contractile force has been studied by a number of investigators. Fabiato and Fabiato 6 observed that tension development by skinned
cardiac fibers fell by approximately 10% for every 0.1 decrease in pH over the range of 7.0 to 6.6. This is consistent with the 50% decrease in tension developed by rat trabeculae that accompanied a decrease in pH from 7.35 to 6.8 observed by Ricciardi et al. Since the calcium transient is unaffected by hypoxia, the actual role of acidosis in hypoxic contractile still remains in dispute because the time course and magnitude of hypoxic contractile failure is not matched by measurements of intracellular acidosis. In the isolated ventricular strip, LaManna et al. reported that intracellular pH began to decrease 4.5 minutes after the first observed decrease in peak isometric twitch tension during hypoxia. Matthews et al. observed that hypoxia produced contractile failure in the isolated rat heart that preceded the development of intracellular acidosis. In a related study, Jacobus et al. convincingly demonstrated that contractile failure during ischemia, a condition that produces more severe intracellular acidosis than hypoxia, could not be attributed solely to decreases in intracellular pH.

In this study, direct measurement of myocardial intracellular pH would have provided useful information. We observed that blood pH did not fall during hypoxia. In view of the research discussed above, it is likely that intracellular pH was only mildly decreased; acidosis alone would not account for contractile failure, and decreases in pH did not inhibit flux through the creatine kinase reaction in this study.

The role of inorganic phosphate accumulation in early hypoxic failure has been investigated by Kusuoka et al. in the isolated ferret heart. These investigators found that the average decline in maximal calcium-activated pressure was 1.7% per 1 μmol/g dry wt increase in inorganic phosphate concentration. We could not measure inorganic phosphate accumulation directly during hypoxia because of the presence of the lines for 2,3 DPG. However, an estimate for inorganic phosphate accumulation during hypoxia can be obtained from measuring the decrease in total high-energy phosphate content. If the reduction of 10–15 μmol/g dry wt in total high-energy phosphate during hypoxia is matched by a reciprocal increase in inorganic phosphate, the expected reduction in maximal calcium-activated pressure would be about 20%. During hypoxia, systolic pressure decreased 31 and 45% during ventilation with 10 and 8% O₂, respectively, suggesting that other mechanisms contribute to contractile failure during hypoxia.

In summary, the results presented here are consistent with the model of coupled reactions in the myocardium where the utilization ATP is driven by oxidative phosphorylation at the mitochondrion and the myofibrillar ATPase at the sarcomere. During hypoxia, the decreased availability of oxygen for reduction at the terminal reaction of electron transport may lead to a decreased transmembrane proton motive force for new ADP phosphorylation. This decreases the export of ATP from the mitochondrion via the ATP-ADP translocase and also decreases the rate of the creatine kinase reaction catalyzed by the mitochondrial iso-
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