Effect of Graded Reductions of Coronary Pressure and Flow on Myocardial Metabolism and Performance: A Model of "Hibernating" Myocardium

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The term "hibernating" myocardium has been applied to chronic left ventricular dysfunction without angina or ischemic electrocardiographic changes in patients with coronary artery disease that is reversed by therapy that increases myocardial blood flow. To investigate the relation between coronary blood flow and ventricular function experimentally, graded reductions in coronary artery pressure were produced in isolated perfused rat hearts as contractile performance (peak systolic pressure and its first derivative [dP/dt]) and metabolic variables were measured using phosphorus-31 nuclear magnetic resonance (NMR) spectroscopy.

As coronary pressure and flow were reduced, significant reductions in myocardial oxygen consumption and contractile performance were observed, which returned to control levels when coronary artery pressure and flow were restored to baseline values. Two phases of metabolic abnormality were observed. With modest reductions in coronary perfusion, proportionate reductions in myocardial oxygen consumption and contractile behavior were accompanied by a slight reduction in creatine phosphate but no significant lactate production. With greater reductions in coronary artery pressure and flow, creatine phosphate decreased more, adenosine triphosphate levels and myocardial pHi decreased significantly and myocardial lactate production increased. The balanced reductions in myocardial contractility and oxygen consumption without metabolic abnormalities traditionally associated with "ischemia" observed in the first phase provides evidence in normal hearts for resetting of the myocardial contractile behavior and oxygen consumption in the presence of reduced coronary flow (that is, hibernating myocardium). The data suggest that reductions in adenosine diphosphate and the index of the reduced form of nicotinamide adenine dinucleotide (NADH) (lactate formation) do not explain the coupling between coronary artery pressure and flow and myocardial oxygen consumption as contractile performance decreases.

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Over the past 20 years, several groups of investigators (1–6) using inert gas techniques reported that regional and global myocardial tissue perfusion rates were reduced in patients with arteriographically significant coronary artery lesions. The reductions in coronary perfusion were accompanied by regional and global ventricular dysfunction; however, for reasons that were unexplained, the patients did not manifest angina pectoris, ischemic electrocardiographic (ECG) changes or release of lactate into the coronary sinus at rest. Studies (1–3) also indicated that restoration of normal coronary flow by revascularization in similar patients was associated with improved regional or global ventricular performance. It was postulated (1,5) that in response to chronic reductions in coronary perfusion, the heart adjusts its level of mechanical function downward, thereby limiting ischemia and infarction. The term "hibernating myocardium" was coined to describe these findings of chronic left ventricular dysfunction that is reversible by increasing coronary blood flow (7–9). Hibernating myocardium has been distinguished from "myocardial stunning," which is the prolonged postischemic dysfunction that occurs without necrosis of cardiocytes after single or repetitive brief episodes of coronary hypoperfusion (9). Because the usual signs of imbalance between myocardial oxygen supply and demand are often not present, the nature of the biochemical abnormality underlying the contractile dysfunction of the hibernating myocardium has not yet been explained.

The coupling between coronary blood flow and the contractile performance of the heart is incompletely understood. There is abundant evidence (10) that in animals and humans with a normal coronary circulation, the level of myocardial capillary blood flow is related linearly to the rate of myocardial oxygen consumption, which in turn is determined by the contractile behavior of cardiac muscle (primar-
ily heart rate, wall tension and contractility). Despite the primary role of the metabolic control of coronary blood flow, other evidence indicates that coronary perfusion can also determine the performance characteristics of the myocardium. Gregg (10-11) reported that increases in coronary artery perfusion pressure were associated with increased myocardial oxygen consumption and improved contractile performance, an occurrence that has been named the Gregg phenomenon (10-12). In analogous experiments using a variety of animal preparations and isolated perfused hearts, other workers (13-30) have shown that reductions in coronary artery perfusion were accompanied by a reduction and eventual loss of cardiac mechanical performance. Extreme reductions in coronary perfusion were also associated with reductions in high energy phosphorus-containing compounds in the myocardium and a rapid and extreme decrease in cardiac force generation (19-21, 26-28). Other experimental studies (23, 24, 27) indicated, however, that more modest reductions in coronary blood flow may also be associated with a decrease in contractile performance of the cardiac muscle, particularly in the subendocardium.

In the present study, phosphorus-31 nuclear magnetic resonance (NMR) spectroscopy was used to monitor myocardial high energy phosphate metabolites and pH in isolated perfused rat hearts whose contractile function was being measured. Graded reductions of coronary artery perfusion pressure were produced in the hearts as major determinants of myocardial oxygen consumption (end-diastolic pressure, afterload and heart rate) were controlled. The data were analyzed to ascertain whether alterations in the content of metabolites that influence the respiration of mitochondria (adenosine diphosphate [ADP], nicotinamide adenine dinucleotide [NADH], oxygen [O_2]) could explain coupling between the rates of coronary perfusion and myocardial oxygen consumption.

Methods

Experimental protocol. Rat hearts were isolated and perfused initially for 25 min at 66 mm Hg. Thereafter, baseline phosphorus-31 NMR spectroscopy acquisitions and measurements of coronary flow, lactate production and peak systolic pressure and the first derivative of pressure (dP/dt) were obtained over 10 min. Phosphorus-31 NMR spectroscopy acquisitions were obtained during the last 8 min of this 10 min period. Coronary flow and lactate production were determined from effluent samples collected in minutes 5 to 8, and peak systolic pressure and dP/dt were determined from recordings obtained between 3 and 5 min of this 10 min period. Thereafter, the height of the perfusion column was rapidly (2 to 3 s) decreased by 8 cm to the next perfusion pressure (58 mm Hg) and all measurements were repeated. This cycle was repeated using perfusion pressures of 51, 44, 36 and 29 mm Hg, after which the perfusion pressure was returned to the baseline level of 66 mm Hg and measurements were repeated a final time. Thus, a total of seven perfusion pressures were studied for each heart during a total experimental time of 70 min. At the end of the perfusion period, the heart was removed from the experimental apparatus, the atria and great vessels removed, the heart gently blotted and the whole heart wet weight determined. Selected hearts that were fixed and subjected to histologic examination revealed no evidence of myocardial necrosis.

Experimental preparation. All animal experiments were performed within guidelines for animal research established by the National Institutes of Health, using a protocol approved by the Columbia University Institutional Animal Care and Use Committee. Male Wistar rats (average weight 350 to 400 g) were used. Approximately 1 h before the induction of anesthesia, the rats were given an injection of heparin (1,000 U/kg intraperitoneally). They were anesthe-tized with ketamine (100 mg/ml) containing 4% acepromazine (1 to 2 ml/kg intramuscularly). After thoracotomy, the heart was quickly excised and arrested in iced isosmotic saline solution containing 20 mM potassium chloride (KCl). The heart was then rapidly cannulated and perfused at 66 mm Hg by way of the aorta with a nonrecirculating Langendorff perfusion apparatus. The oxygenated (95% oxygen/5% carbon dioxide) perfusate contained 118 mM sodium chloride, 6 mM KCl, 2.5 mM calcium chloride, 1.2 mM magnesium sulfate, 0.5 mM disodium ethylenediaminetetraacetate, 4 mM glucose, 25 mM sodium bicarbonate and 20 U insulin/liter and was maintained at 37°C.

After institution of retrograde flow, a compliant latex balloon was inserted through the left atrium into the left ventricle to measure myocardial function. The balloon was connected to a Statham-Gould transducer by a length of polyethylene tubing. Measurements were recorded on a physiologic recorder (model 7, Grass Instruments). Both peak systolic pressure and its first derivative (dP/dt) were continuously recorded after the volume in the balloon was adjusted to set the end-diastolic pressure to 5 mm Hg. Two platinum-tipped pacing electrodes were attached to the atria and connected by silver leads to agar-filled salt bridges (0.9% saline solution, 6% agarose in PE 240 tubing approximately 2 feet in length) connected to a Grass model S4 stimulus generator. The heart was paced at 5 Hz (300 beats/min) using a stimulus duration of 2 to 4 ms. A 20 mm glass NMR tube (Wilmad) was raised over the heart and attached to the perfusion apparatus. The top of the NMR tube was not sealed and was left open to room air. A suction line lowered to the bottom of this NMR tube collected any effluent as soon as it dripped off the heart. Timed collections of this effluent were used to measure coronary flow rate and myocardial lactate production. Effluent collected in this fashion could not be used to measure myocardial oxygen consumption because the perfusate equilibrates with room air, especially at low flow rates. Consequently, a separate series of matched experiments was performed outside of the spectrometer to determine the relation of perfusion pressure to oxygen consumption.
Phosphorus-31 NMR measurements. The perfused heart was studied in a Bruker wide-bore AM 300 spectrometer with a 20 mm phosphorus-31 probe. Acquisitions were obtained by summing transients (n = 264) over 8 min into a 4 K block after the application of a 30° pulse every 1.8 s. When these acquisition variables are used, significant saturation does not occur (31). Free induction decays were exponentially multiplied by a factor of 30. Fourier transformation does not occur (31). Free induction decays were zero when these acquisition variables were used. Significant saturation was studied in 3 Eruker wide-bore AM I/R conceptromerer 4 K block after the application of a 30° pulse every 1.8 s. Baseline values were estimated visually and peaks integrated blindly without regard to perfusion pressure by two investigators (A.M.K., A.C.W.) and averaged. The reproducibility of this technique has previously been reported (31). Areas obtained using hand integration in our laboratory also correlate well with computer integration using an objective computer baseline correction (finite automation and extrapolation) and curve fitting (Levenberg-Marquardt) routine (NMR, New Methods Research). All relative molar concentrations determined by NMR spectroscopy were normalized to 100%. The value at the initial control perfusion pressure (66 mm Hg).

Adenosine diphosphate (ADP) was calculated from the creatine kinase reaction:

\[
ADP + PCr + H^+ \rightarrow ATP + Cr,
\]

where \( PCr = \) phosphocreatine, \( H^+ = \) hydrogen ion; \( K_{eq} = \) the equilibrium constant; \( ATP = \) adenosine triphosphate and \( Cr = \) creatine.

For this reaction, ADP was determined by assuming that the hydrolysis of phosphocreatine resulted in the equimolar production of both creatine and inorganic phosphate (Pi) (32,33). This assumption allows for the substitution of Pi for Cr so that the equilibrium equation of this reaction can be solved for ADP in terms of NMR spectroscopy measurable compounds:

\[
[ADP] = \frac{[ATP][Pi]}{K_{eq}[H^+][PCr]}
\]

For this equation, \([H^+]\) was determined from pH assessed from the chemical shift difference between inorganic phosphate and phosphocreatine using the following relation (34):

\[
pH = 6.9 - \log \left( \frac{\delta - 5.805}{3.29 - \delta} \right)
\]

A value of \(1.66 \times 10^{-6}\) was used for the equilibrium constant \(K_{eq}\) (35). Initial concentrations of ATP, inorganic phosphate and phosphocreatine at the control perfusion pressure were assumed to be \(5.0 \times 10^{-3}\), \(1.7 \times 10^{-3}\) and \(1.7 \times 10^{-2}\) M, respectively (36). If the creatine kinase reaction remains in equilibrium and the value of \(K_{eq}\) remains constant during the experiment, changes in the calculated value of ADP should reflect true changes in myocardial ADP. Moreover, any errors in the initial value estimates of ATP, phosphocreatine, inorganic phosphate or \(K_{eq}\) would be reflected as a constant error in the calculated ADP value during each experimental period.

Lactate measurements. Myocardial lactate production was determined from collected coronary effluent. Lactate concentration in the effluent was determined using the end point production of the reduced form of nicotinamide adenine dinucleotide (NADH) in the reduction of lactate with nicotinamide adenine dinucleotide (NAD) in the presence of lactate dehydrogenase and hydrazine (Sigma kit 826-UV) (32). Lactate production was expressed as \(\mu M/min\cdot g\)-wet weight by normalizing the values for both coronary flow rate and heart wet weight.

Oxygen consumption measurements. Oxygen consumption was separately measured in the heart perfused outside of the spectrometer to confirm that reductions in coronary artery pressure were accompanied by a linear reduction in oxygen consumption (37,38). In these experiments, the heart was excised and perfused identically as previously described, except that coronary sinus drainage was obtained from a cannulated pulmonary artery and aortic perfusate was obtained from a "t" connection placed in the perfusion column just above the heart. Oxygen consumption was determined with the formula:

\[
O_2 \text{ Consumption (mmol/min} \cdot \text{g)} = \frac{[APO_2 - VPO_2] \times 10^{-3}}{[\text{Heart wet weight in g}]}
\]

where \(APO_2\) and \(VPO_2\) represent the partial pressure of oxygen in the aortic and coronary sinus (venous) perfusate, respectively (mm Hg) and \(2 \times 10^{-3}\) represents the solubility of oxygen (mmol O₂/mm Hg) at 37°C corrected for atmospheric pressure (39). In these experiments, the partial pressure of oxygen in the perfusate was measured in duplicate with use of an oximeter (model PHM71 Mk2, Radiometer Copenhagen).

Data analysis. Repeated measurements were compared by using a one-way analysis of variance with repetitions. Comparisons of mean values between measurements were made using Tukey's test. Values were considered significant at the \(p = 0.05\) level (40).

Results

Hemodynamic data. The functional response to stepwise reductions in perfusion pressure from 66 to 29 mm Hg is shown for a representative heart in Figure 1. The responses for all 15 hearts are summarized in Figure 2. For each reduction in coronary artery perfusion pressure, there was a significant decrease in peak systolic pressure, \(dP/dt\) and normalized coronary flow rate. Furthermore, when the perfusion pressure was returned to the baseline pressure of 66 mm Hg (after a total of 95 min of perfusion), all of these variables returned to baseline values.

Myocardial oxygen consumption. A linear relation between coronary artery perfusion pressure, myocardial func-
Lion and oxygen consumption in the isolated perfused rat heart has been demonstrated by other investigators (13, 14, 18). To validate this relation in our model, oxygen consumption was measured in two separate experiments performed outside of the spectrometer. Figure 3 demonstrates that with our perfused heart model, the same direct relation previously identified between coronary artery perfusion pressure, myocardial function, oxygen consumption and coronary flow was observed.
Figure 1. A representative stack plot of spectra from a perfused heart at each perfusion pressure level. Spectra were obtained over 8 min, starting 2 min after the perfusion pressure was changed. Each spectrum represents the Fourier transformation of the sum of 264 transients after exponential multiplication by a factor of 30. Labeled peaks include those of inorganic phosphate (Pi), phosphocreatine (PCr) and the three peaks of adenosine triphosphate (αATP, βATP, γATP). In this investigation, relative molar concentrations of these compounds were determined by integrating the area of the phosphocreatine, inorganic phosphate and βATP curves (see Methods). PPM = parts per million; TMP = thymidine monophosphate.

Metabolic data. Figure 4 depicts the simultaneously acquired phosphorus-31 NMR spectra (from the heart in Fig. 1) obtained during perfusion at each level of pressure. The plot demonstrates that for each reduction in perfusion pressure (from 66 to 29 mm Hg), there was a stepwise increase in inorganic phosphate and a stepwise decrease in phosphocreatine but little change in the beta-ATP peak. These changes returned to control values when the coronary perfusion pressure returned to the baseline pressure (66 mm Hg).

Figure 5 summarizes the changes in myocardial pH, ATP and the phosphocreatine/inorganic phosphate ratio as a function of the coronary artery perfusion pressure for all 15 hearts. No significant change in intracellular pH or ATP in the myocardium was noted until the perfusion pressure was reduced to 36 and 29 mm Hg, respectively. However, in response to each change in coronary perfusion pressure, there was a decrease in the phosphocreatine/inorganic phosphate ratio. These observations indicate that with reductions in coronary pressure and flow, important changes in ventricular function occur before the development of acidosis or depletion of ATP.

Because the di-protonated form of inorganic phosphate (H₂PO₄⁻) has been implicated as a possible modulator of myocardial contractility, H₂PO₄⁻ values were calculated from the phosphorus-31 NMR spectroscopy measurements of pH and inorganic phosphate (41–45). Because coronary artery pressure and flow were reduced, there were progressive increases in H₂PO₄⁻ (Fig. 6).

Figure 6. Calculated concentration of the di-protonated form of inorganic phosphate (H₂PO₄⁻) as a function of myocardial perfusion pressure. Concentrations of H₂PO₄⁻ were normalized to 100 for the initial perfusion pressure of 66 mm Hg for each heart. Remaining values were expressed relative to that initial value. H₂PO₄⁻ was calculated from phosphorus-31 NMR spectroscopy-determined pH and inorganic phosphate using the Henderson-Hasselbach relation and a pK₁ = -log of the dissociation constant) of H₂PO₄⁻ with HPO₄²⁻ of 6.79. This graph demonstrates a gradual increase in H₂PO₄⁻ as coronary artery perfusion pressure is decreased.
It has been reported previously (44.46–49) that increases in heart work and oxygen consumption were associated with increases in myocardial ADP. Thus, it was of interest to ascertain whether ADP, oxygen consumption and heart work were decreased by a reduction in coronary artery perfusion pressure. Figure 7 shows the ADP concentration as a function of perfusion pressure. Despite an almost fourfold reduction in oxygen consumption as perfusion pressure was lowered from 66 to 36 mm Hg (Fig. 3), there was no significant reduction in the concentration of ADP (Fig. 7). At the lowest perfusion pressure (29 mm Hg), ADP concentration increased.

Lactate production. It has been suggested (50–53) that changes in the mitochondrial NADH/NAD ratio may regulate myocardial oxygen consumption in the glucose-perfused heart. Mitochondrial NADH in intact perfused organs can be measured with surface fluorometry, but the technique is limited because glycolysis must be inhibited to suppress fluorescence from the cytoplasmic NADH pool and only the NADH in the top surface layer of cells can be examined (54). Kobayashi and Neely (53,55), using the isolated glucose-perfused rat heart, demonstrated that lactate production estimated from coronary sinus lactate concentration and flow correlates with the mitochondrial NADH/NAD ratio and oxygen consumption during periods of increased myocardial work. Although this approach is less direct than surface fluorometry for the determination of mitochondrial NADH, it has the advantage that the lactate production measurements are representative of the entire heart and not just the epicardial surface. Accordingly, we compared myocardial lactate production with coronary artery perfusion pressure in the same 15 hearts (Fig. 8). Lactate production remained at values not significantly different from control values as pressure was reduced from 66 to 36 mm Hg; myocardial lactate production increased, however, when the perfusion pressure was reduced to 29 mm Hg.

Discussion

A model of hibernating myocardium: two metabolic patterns. The results of these experiments in isolated perfused hearts in which heart rate and ventricular volume (preload and afterload) were controlled indicate that sustained and reversible reductions in ventricular contractility can be produced by reducing coronary artery pressure and flow. The induced depressions in cardiac contractile function were not associated with myocardial necrosis, but were associated with parallel reductions in myocardial oxygen consumption, which acted to reduce the tendency toward ischemia. Thus, the syndrome of hibernating myocardium was mimicked in vitro. Similar reductions in ventricular performance with induced graded reductions in coronary artery pressure and flow have also been observed (11–28,56) in isolated perfused guinea pig and ferret hearts, isolated blood-perfused rabbit hearts and septal preparations and intact dogs and pigs.

As will be discussed in more detail subsequently, the phosphorus-31 NMR data obtained simultaneously with the functional measurements indicate that myocardial “hibernation” may be associated with two metabolic patterns. With modest reductions in coronary artery pressure and flow, there is only minimal metabolic abnormality, no or slight reduction in creatine phosphate and no significant change in ATP, p'1 or lactate formation. With greater reductions in coronary artery pressure and flow, creatine phosphate decreases more. ATP levels and pH decline and there is an increase in lactate formation, consistent with enhanced glycolysis. These metabolic abnormalities are returned to control levels with restoration of coronary artery pressure and flow.

Relation between coronary artery pressure and flow and ventricular function. The linear relation between coronary artery perfusion pressure and flow is consistent with the lack of coronary autoregulation in the isolated perfused heart.
Similar decreases in ventricular performance with induced graded reductions in coronary artery pressure and flow have been observed in the isolated Langendorff perfused heart (13,14,18,19,26). Blood-perfused rabbit heart and dogs and pigs (17,22-25). In the studies of Vatner (23), subendocardial mechanical performance in dogs measured with ultrasonic crystals began to decrease with subendocardial blood flow reductions of only 10% and ceased with flow reductions >90%. In the present experiments, cardiac contractile performance decreased progressively with the first three reductions in coronary artery pressure and flow before there were any biochemical changes compatible with our current concepts of myocardial ischemia. Specifically, there was no significant reduction in the myocardial content of ATP and myocardial pH or increase in lactate production until the coronary perfusion pressure was reduced to 36 mm Hg.

Possible mechanism for contractile dysfunction. The mechanism responsible for the altered contractile performance during graded reductions in coronary artery pressure and flow has not been established definitively. Arnold et al. (14) proposed that alterations of coronary pressure, by changing distension in the coronary artery tree, induced proportional increases or decreases in myofibrillar sarcomere length, that could augment or diminish contraction by the Frank-Starling mechanism. Evidence for this "garden hose" effect has been marshalled (14-16). However, decreases in coronary artery flow have been paralleled by reductions in oxygen consumption in fibrillating and arrested perfused hearts (18,38,56,57). Other investigators observed parallel changes in cardiac performance when coronary artery flow was reduced or increased at constant perfusion pressure (46) or when myocardial oxygen delivery was increased or decreased at relatively constant levels of coronary perfusion by altering the hematocrit in isolated blood-perfused hearts (27). Recently, experiments (58,59) in the isolated perfused ferret heart showed that increases or decreases in coronary artery perfusion pressure and flow were accompanied by increases or decreases in developed systolic pressure and calcium transients within the cardiac myocytes. The alterations in calcium transients in those experiments (58,59) were dissociated from parallel alterations in end-diastolic volume, which argued against operation of the garden hose or Frank-Starling mechanism to explain the altered mechanical performance.

The metabolic data in the present study indicate that myocardial oxygen consumption and cardiac systolic performance decreased progressively with each reduction in coronary artery perfusion pressure and flow. However, the concentration of ATP did not decrease below control values until a perfusion pressure of 36 mm Hg was reached. Thus, a reduction in available ATP cannot explain the reduced contractility observed at levels between 66 and 36 mm Hg unless one speculates that there might have been a reduction in ATP concentration in a specific pool linked to the contractile mechanism. Although this hypothesis is possible, there is no definitive evidence in support of it (60).

Reductions in myocardial pH have been demonstrated (47,58) to interfere with the contractile function of cardiac myocytes, presumably by inhibiting the interaction between calcium ions and troponin-C of the actin-myosin complex. Myocardial pH estimated from the phosphorus-31 NMR spectroscopy measurements did not decrease significantly in the present experiments until the coronary perfusion pressure was reduced to 36 mm Hg, the level at which lactate production by the heart began to increase significantly. Thus, the progressive decreases in contractile performance observed with reductions in coronary artery perfusion pressure from 66 to 36 mm Hg cannot be attributed to intracellular acidosis.

The di-protonated form of inorganic phosphate (H₂PO₄⁻) has been shown (41-45) to inhibit contractility in fatigued skeletal muscle, superfused cardiac fibers and hypoxic or tetanized isolated perfused rat and ferret hearts. In the present study in glucose and bicarbonate-perfused rat hearts, there was a gradual increase in cardiac H₂PO₄⁻ as coronary perfusion pressure was reduced. This finding raises the possibility that H₂PO₄⁻ may play a role in regulating myocardial contractility in this model. Against this suggestion, however, are data by Kitakaze et al. (59), who found that there was no change in phosphorus metabolites as contractile performance decreased in response to comparably reduced in coronary artery perfusion pressure in crystalloid-perfused ferret hearts. Additionally, Marshall (27) studied high energy phosphates in a blood-perfused rabbit heart model and did not find changes in creatine phosphate or ATP (and by inference H₂PO₄⁻) during initial reductions in coronary flow and pressure that were associated with reduced contractile performance.

Sequence of metabolic changes. Because phosphorus-31 NMR spectroscopy can potentially be applied to the study of patients with reduced coronary perfusion due to coronary atherosclerosis, the present experiments were also designed to compare different metabolic and functional indexes as coronary artery perfusion and flow were decreased in a controlled fashion. The data indicate that as coronary artery pressure and flow were reduced, the first observable change was in systolic performance, followed by a decrease in the phosphocreatine/inorganic phosphate ratio. With further reductions in coronary flow, there was a progressive decrease in this ratio in parallel with reduced ventricular performance. Reductions in ATP and myocardial pH occurred only at very low perfusion pressures (36 and 29 mm Hg) in association with the onset of significant increases in myocardial lactate production. Thus, in this setting, myocardial lactate production (in comparison with the phosphocreatine/inorganic phosphate ratio) was an insensitive indicator of the interaction between coronary artery blood flow and the oxidative metabolism of the heart.

Coupling between cardiac work and metabolism during alterations of coronary artery pressure and flow. In the present experiments, the rate of myocardial oxygen consumption decreased linearly along with contractile perfor-
Before the level of 36 mm Hg was reached, ATP production by way of oxidative pathways was closely coupled to the contractile work of the heart. The mechanism responsible for the regulation of oxidative metabolism during alterations of cardiac work remains unclear and is the subject of extensive research (46). The net reaction for mitochondrial respiration is as follows:

\[
\text{NADH} + 3 \text{ADP} + 3 \text{Pi} + 1/2 \text{O}_2 + \text{H}^+ \rightarrow \text{NAD}^+ + 3 \text{ATP} + \text{H}_2\text{O}.
\]

The concentrations of ADP and NADH and oxygen availability have been suggested (61-66) as potential regulators of the rate of oxidative metabolism in heart muscle. Accordingly, indexes of two of these substances were evaluated as myocardial oxygen consumption decreased during the initial reductions in coronary perfusion.

**ATP hydrolysis products.** Chance and Williams and their coworkers (32,33,61) originated the concept of feedback control of mitochondrial respiration by ATP hydrolysis products generated during cardiac work. They obtained data showing stimulation of mitochondrial respiration in vitro by ADP or the cytosolic phosphorylation potential or [ATP]/[ADP] (inorganic phosphate) ratio, or both, during transition from state 4 (resting) to state 3 (active) mitochondrial respiration when neither oxygen nor carbon substrates were limiting. Using conventional biochemical techniques, Geisen and Kammermeier (62) observed a direct relation between the phosphorylation potential and oxygen consumption in perfused rat hearts during increases in cardiac work. Similarly, using phosphorus-31 NMR spectroscopy, others (49,61) observed an increase in calculated ADP as cardiac work and oxygen consumption increased in both feline and canine experimental preparations. However, in the present experiments, the calculated content of ADP in the myocardium was unchanged and then increased (rather than decreased) as cardiac contractile work and myocardial oxygen consumption decreased with reduced coronary artery perfusion pressure and flow. This finding is not consistent with the concept that ADP controlled respiration in this model (46).

Other workers (63-65) also reported data that do not support the hypothesis that ADP or the cytosolic phosphorylation potential controls respiration in the intact myocardium. In studies of isolated rat hearts perfused with glucose and insulin, Urgubil et al. (66) found that the myocardial concentration of ADP was not related to myocardial work. Clark and Willis (26), using a slightly different protocol in isolated rat hearts, observed that there was an inverse relation between the cytosolic phosphorylation potential and coronary flow at low rates (2 to 4 m/min) but no relation of ADP to flow at higher flow rates. In the study of isolated blood-perfused rabbit hearts by Marschall (27), there were no changes in creatine phosphate or ATP during the initial reductions in coronary artery flow and myocardial oxygen consumption, a finding that suggests that significant alterations in ADP are not responsible for the reduced oxygen consumption. Lastly, in a study of graded coronary occlusion in an open chest porcine model, Schaefer et al. (28) found a linear relation between the phosphocreatine/inorganic phosphate ratio (but not ATP) and endocardial blood flow (microspheres). Because this ratio is proportional to [ADP], these results also suggest that ADP did not decrease as coronary flow decreased.

**NADH:NAD-lactate formation.** Other investigators (46,56,67) have suggested that the redox state of the mitochondria may be involved in the regulation of mitochondrial respiration. Studies (50,63-65) in perfused hearts and isolated mitochondria indicated that increases in mitochondrial NADH might increase ATP production without changes in ADP or inorganic phosphate. It was also suggested (46) that increases in mitochondrial calcium ions might activate mitochondrial pyruvate dehydrogenase, NAD-linked isocitrate dehydrogenase and alpha-ketoglutarate dehydrogenase, leading to enhanced production of mitochondrial NADH with consequent stimulation of respiration. In the present experiments, lactate production was used as an index to reflect the level of NADH. As myocardial oxygen consumption and work decreased with the first three reductions in coronary pressure flow, there was no change in lactate production (or ATP). This suggests that a decrease in the steady state mitochondrial NADH did not occur. This conclusion is consistent with results of Moravec et al. (68), who measured mitochondrial NADH using surface fluorescence in glucose-perfused rat hearts. When aortic pressure was reduced from 70 to 40 mm Hg, there was only a transient reduction in NADH fluorescence, which returned to normal within 1 min. Biochemical measurements in hearts perfused at both of these pressure levels revealed no differences between values at 70 and those at 40 mm Hg. Also, in the study of Marshall (27) discussed previously, lactate concentrations in heart muscle did not decrease as coronary flow and oxygen consumption were reduced. Thus, the accumulated data do not support the hypothesis that reductions in NADH in the heart muscle accounted for the decrease in myocardial oxygen consumption that was observed as coronary perfusion was reduced.

**Oxygen delivery.** Other investigators (46,69-71) have postulated that oxygen delivery to the mitochondria may regulate mitochondrial respiration. Experimental support (72) for this hypothesis is controversial because of methodologic difficulties in optical measurements of the interaction between oxygen and mitochondrial enzymes in the heart. With each reduction in coronary artery perfusion pressure and flow in the present experiments, there was a decrease in mitochondrial oxygen consumption with no change in ATP (until the lowest levels of pressure, when evidence for anaerobic glycolysis appeared). Because the estimates of NADH, ADP, inorganic phosphate and hydrogen ions either did not decrease or increased, this suggests the net reaction...
for mitochondrial respiration) that the reduced rate of myocardial oxygen consumption may have been mediated by a decrease in oxygen delivery or sensing at the mitochondria. Although the present experiments raise the possibility by exclusion, they do not provide direct evidence that reduced oxygen delivery or sensing accounted for the reductions in myocardial oxygen consumption that paralleled the decreases in cardiac work. Using surface fluorometry, Opre (73) showed that this preparation is not oxygen deficient even at low levels of perfusion pressure. In addition, the perfusate was well oxygenated and venous oxygen tensions were >100 mm Hg even at the lowest perfusion pressure. The maximal mitochondrial constant (Km) for oxygen in vitro is 1 mm Hg and the theoretical maximal pressure gradient for oxygen across capillary membranes is about 25 mm Hg (74). This makes it unlikely that there was insufficient delivery of oxygen to the mitochondria. Nevertheless, several investigators (74–76) suggested that oxygen sensing may occur in situ even at values >1 mm Hg for the Km of oxygen.

Conclusions. Coronary artery pressure was reduced in progressive increments in isolated rat hearts as contractile performance (peak systolic pressure and dP/dt) and metabolic variables were measured by phosphorus-31 NMR spectroscopy. As coronary artery pressure and flow decreased, significant reductions in contractile performance and myocardial oxygen consumption were observed before any decrease in ATP and myocardial pH or significant lactate formation. The balanced reductions in ventricular performance and oxygen consumption without traditional metabolic markers of ischemia provide a model for hibernating myocardium. With further reductions in coronary artery pressure and flow, ATP and pH decreased and lactate production increased. Myocardial creatine phosphate decreased progressively and inorganic phosphate increased significantly as ventricular performance declined. Contractility and metabolic abnormalities returned to control values when coronary artery pressure and flow returned to the initial values. Analysis of the data suggests that the coupling of myocardial contractility and oxygen consumption in this setting was not mediated by ADP, pH or NADH but might be related to oxygen delivery to the tissue.

References
KELLER ET AL.
A MODEL OF "HIBERNATING" MYOCARDIUM


