Brief Review

Myocardial Contractile Function During Ischemia and Hypoxia

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Introduction

Scope of Review

Myocardial ischemia is one of the most common causes of serious illness and early death in developed societies. Furthermore, a variety of surgical procedures necessitate limited periods of ischemia. The reduced blood flow, which characterizes ischemia, deprives the heart of substrates and allows metabolites to accumulate. These changes lead to decreased tension production and to the appearance of arrhythmias. For these reasons, the effects of ischemia on myocardial cell function are the subject of great interest and active investigation. In the past decade, it has become possible to measure intracellular concentrations of many of the crucial ions and metabolites within the myocardial cell. The way in which these intracellular constituents modify contractile function has also become clearer from studies of their effects on the isolated components of the cardiac cell.

In this review, we consider how the application of these new experimental techniques has contributed to our understanding of myocardial contractile function during ischemia and hypoxia; electrophysiological aspects are discussed only in so far as they affect contraction. Arrhythmias are not considered. Many earlier reviews overlap parts of this review and are recommended for the reader who requires more background information. Metabolic aspects have been reviewed by Neely and Morgan and by Gibbs. Electrophysiological aspects have been considered by Carmeliet and by Janse and Kleber. Ionic changes and more particularly the role of calcium have been described by Nayler et al., Poole-Wilson, and Jennings and Steenbergen.

The first section of the review describes briefly the main changes in mechanical performance of a heart subjected to global ischemia. The second and third sections describe the major metabolic and ionic changes that occur during ischemia and hypoxia and consider how these changes are brought about. In the final three sections the metabolic and ionic changes that may underlie the altered mechanical performance observed during ischemia are considered.

Mechanical Changes During Ischemia

The effects of ischemia on pressure development by the heart are now well recognized. When a beating heart is subjected to global ischemia the developed pressure declines rapidly over several minutes (acute ischemic failure). After 10–20 minutes developed pressure is small or absent and a gradual rise in diastolic pressure occurs (ischemic contracture). After about 1 hour of complete ischemia, histological and biochemical evidence of cell damage becomes apparent (e.g., contraction bands, swollen mitochondria, and leakage of intracellular enzymes into the extracellular space). If perfusion of the heart is restarted before or during the early part of the ischemic contracture, recovery of developed pressure is virtually complete. However, if reperfusion is started when the contracture is well established, then developed pressure fails to recover and cell damage is accelerated, leading to massive enzyme release (reperfusion damage).

Ischemia vs. Hypoxia

Ischemia is difficult to study experimentally because it is not possible to apply drugs to the heart or to change the extracellular ionic composition during ischemia. In addition, for preparations of cardiac tissue that have no blood supply, e.g., isolated cells, there is no direct experimental equivalent to ischemia. For these reasons, many studies have maintained perfusion of the tissue but removed all or most of the O2 (anoxia or hypoxia) or used agents that inhibit oxidative phosphorylation, e.g., cyanide (CN). However, ischemia involves both inadequate supply of substrates, notably oxygen and glucose, and inadequate removal of products of metabolism, notably lactate, H+ and K+.

In an attempt to mimic ischemia more closely, hypoxia has frequently been combined with procedures that inhibit anaerobic glycolysis. Under these conditions, all the main mechanical features of ischemia can be observed. Thus, in anoxia, when glycolysis can continue, the developed pressure of a heart falls to about one-third of normal but can then continue at this level for at least 30 minutes. However, in anoxia, after glycolysis has been prevented, the developed pressure declines rapidly and completely, a response similar to that observed in ischemia. It has also been shown that an hypoxic contracture and a reoxygenation paradox can be elicited in perfused preparations subjected to hypoxia with glycolysis prevented and...
have properties comparable to the equivalent phenomena in ischemia. The similarity between mechanical responses in ischemia and in hypoxia with glycolytic inhibition suggests that it is the prevention of adenosine triphosphate (ATP) production and/or its metabolic consequences that lead to the mechanical features of ischemia; accumulation of the products of metabolism no doubt modifies the mechanical response but does not seem to be essential for their occurrence. Thus, throughout this review, while the aim is to explain the cellular changes during ischemia and their consequences for myocardial contraction, there will be extensive use of observations made in perfused tissues but with oxidative phosphorylation and anaerobic glycolysis blocked.

Metabolic Consequences of Ischemia and Hypoxia
Changes in Phosphorus-Containing Metabolites
Both ischemia and hypoxia reduce the oxygen supply to the myocardial cells and reduce the rate of ATP production by oxidative phosphorylation. If there were no other compensatory mechanisms, the concentration of ATP would fall at a rate that is the difference between the rate of production and consumption of ATP. In fact, however, [ATP] remains relatively constant during the first few minutes of hypoxia while phosphocreatine concentration ([PCr]) falls and inorganic phosphate concentration ([Pi]) rises.13,15-17 These changes can be understood with the help of the model shown in Figure 1, which is a development of that described by Carlson and Wilkie.18 This model shows

![Graph showing changes in phosphorus metabolites](http://circres.ahajournals.org/)

**Figure 1.** Model of concentrations of PCr, ATP, ADP, AMP, and Pi in the presence of creatine kinase and myokinase as ATP is hydrolyzed. The thermodynamic affinity (free energy change) of ATP hydrolysis is also shown. The following reactions were assumed to occur:

\[ \text{ATP} \rightarrow \text{ADP} + \text{Pi} \quad (1) \]

\[ \text{PCr} + \text{ADP} \rightleftharpoons \text{ATP} + \text{Cr} \quad (2) \]

\[ 2\text{ADP} \rightleftharpoons \text{ATP} + \text{AMP} \quad (3) \]

Starting concentrations were [ATP] = 7 mM, [PCr] = 25 mM, [ADP] = [AMP] = [Cr] = [Pi] = 0. This model is described by the following equations that were solved for various values of [Pi].

\[ \text{Total adenine} = 7 \text{mM} = [\text{ATP}] + [\text{ADP}] + [\text{AMP}] \quad (4) \]

\[ \text{Total creatine} = 25 \text{mM} = [\text{PCr}] + [\text{Cr}] \quad (5) \]

\[ \text{Total phosphate} = 46 \text{mM} = [\text{PCr}] + [\text{Pi}] + 3[\text{ATP}] + 2[\text{ADP}] + [\text{AMP}] \quad (6) \]

\[ \frac{[\text{ATP}][\text{Cr}]}{[\text{ADP}][\text{PCr}]} = 200 \quad (7) \]

\[ \frac{[\text{ADP}][\text{PCr}]}{[\text{ADP}]^2} = 1 \quad (8) \]

Equilibrium constants for the reaction catalyzed by creatine kinase (7) and myokinase (8) were taken from Lawson and Veech.21 Hydrolysis of ATP was regarded as irreversible. The reactions were assumed to occur at pH 7.0, and changes in pH were ignored. The 5 equations (4-8) can be reduced to a cubic equation that was solved iteratively. The results are similar (over the relevant range) to those of Carlson and Wilkie18 but very different from those of Kubler and Katz.8 No details of the method of calculation are given in Kubler and Katz and we do not know the reason for the discrepancy.

A_{ATP} was calculated using the equation given in the text (page 156), with \(-\Delta G_{\text{p}} = 30 \text{kJ/mol.} \) The arrow represents the approximate concentration of P metabolites in aerobic perfused hearts at low or moderate workloads.

Note that, as plotted, the abscissa has units of moles of Pi hydrolyzed. However, during a period when there is net consumption of ATP in excess of production, the figure can be used to indicate the way in which P metabolites will change as a function of time.
how the concentrations of ATP, PCr, Pi, adenosine diphosphate (ADP), and adenosine monophosphate (AMP) will change as ATP is hydrolyzed, assuming that creatine kinase and myokinase are present in sufficient concentrations to maintain the reactions catalyzed by them at equilibrium. 19,20

Under normal aerobic working conditions, P-metabolites should be similar to those near the left of the diagram as indicated by the arrow, i.e., [Cr] 2–3 times greater than [ATP], [Pi] less than [ATP], and [ADP] very low. 19 (Note that total ADP is much higher than myoplasmic [ADP] because of binding to myosin; see discussion in Dawson and Wilkie). 19 Under these conditions, it is thought that the low concentrations of Pi and ADP are capable of stimulating ATP production by the mitochondria at a rate equal to consumption (for discussion, see Hansford). 22 It is worth noting from Figure 1 that a 20% fall in [PCr] from the normal cellular level is associated with a 200% increase in [ATP]. 23,24 These substantial changes in [ADP] and [Pi], coupled with the sensitivity of mitochondrial ATP production to [ADP] and [Pi], explain how large increases in ATP consumption, which accompany an increased work load, can occur with only a small fall in [PCr] and with no detectable change in [ATP]. 25,26 During ischemia or anoxia, ATP production by oxidative phosphorylation falls to zero and P-metabolite concentrations move towards the right of the diagram and continue to move to the right until either ATP production can be increased, e.g., by increased anaerobic glycolysis, and/or ATP consumption can be decreased, e.g., by a fall in developed tension. Many studies have now shown that the general features of this model are correct and that the maintenance of [ATP] while [PCR] falls and [Pi] rises results from the resynthesis of ATP from PCR catalyzed by creatine kinase. 17,22 It has been suggested in the past that the relative constancy of [ATP] while [PCR] falls is evidence of "compartmentation" of ATP production, 5,26,27 but this model shows that it is due simply to buffering by PCR in the presence of creatine kinase.

Although this model is helpful in understanding the P-metabolite changes when ATP consumption exceeds production, other processes occur in ischemia and anoxia that complicate the situation. The effect of these will be to change both the end point at which the ATP consumption and production are again equal and the P-metabolite concentrations present at this new steady state. These processes include the following: 1) Anaerobic glycolysis is accelerated twofold within the first minute of ischemia or hypoxia 15,16 and at this rate 0.1–0.2 mM/sec of ATP will be produced. During ischemia this high rate of glycolysis is not sustained and decreases despite the presence of glycogen stores. 28 The mechanism of this reduction is not established although both intracellular acidosis and lactate accumulation are thought to play important roles. 29 During hypoxia, glycolysis continues at a high rate. If glucose is removed from the perfusate, glycolysis will continue until the available glycogen is consumed. 30 2) Tension falls rapidly during ischemia and hypoxia so that the major component of ATP consumption may fall. 3) AMP is further degraded to inosine monophosphate (IMP) and adenosine, which are eventually lost from the cellular pool. These processes take place over 30–60 minutes (for review see Jennings and Steenbergen). 4) Changes in pH (see later section) will modify the equilibrium constant for creatine kinase and myokinase and lead to small changes in the equilibrium P-metabolite concentrations.

However, there are studies that do not seem to support this model, even allowing for the complications described above. For instance, Doorey and Barry, 31 in studies of cultured embryonic chick ventricular cells, have shown that changes in [ATP] occur very rapidly (10–20 seconds) after application of CN and precede changes in [PCr]. However, in this preparation the ratio of PCr/ATP under control conditions was only 0.7 compared with 2–3 in most studies on intact adult hearts. The reason for this unusually poor initial metabolic status is not clear but examination of Figure 1 shows that for such starting conditions a rapid early decline in ATP, which is more or less proportional to the decline in PCr, is the expected result on the basis of this model.

Compartmentation

Mammalian hearts contain approximately 10⁹ muscle cells. Inevitably, the metabolic status of these cells will vary due to local variations in work load, blood supply, etc. For instance, differences in metabolic levels between endocardial and epicardial layers of the left ventricle are well recognized. 32 Similarly, in many isolated heart preparations the left side of the heart will experience a greater work load than the right either because the left ventricle is stretched by a balloon or because, in the working heart, it is contracting against a load and performing external work. These regional variations represent one kind of compartmentation of metabolite levels in the heart.

Within the cardiac cell there are also well-defined compartments surrounded by internal membranes, notably the sarcoplasmic reticulum and the inner mitochondrial matrix. These compartments are known to have different ionic and metabolic concentrations from the myoplasm. 33,34 This is one reason why both biochemical and NMR measurements of metabolites that represent spatially averaged results from whole hearts need careful interpretation.

In recent years, there has been much interest in a third kind of compartmentation, which arises because ATP is synthesized mainly in the mitochondria but utilized mainly in the myofibrils. In cardiac muscles, the myofibrils are 1–2 μm in diameter and are surrounded by columns of mitochondria. Concentration gradients must therefore exist across the myofibrils. The magnitude of this kind of "myoplasmic compartmentation" can be calculated in the steady state using the diffusion equation 35 that describes diffusion into a cylinder with consumption uniformly distributed across it. Assuming an external [ATP] of 7 mM, ATP consumption of 1 mM/sec in a beating heart, 2 ATP
diffusion coefficient of $1 \times 10^{-4}$ mm$^2$/sec$^{36}$ and a myofibrillar diameter of 2 μm, then in the steady state the [ATP] in the center of the myofibril is 6.9975 mM. A larger gradient will exist transiently during systole when ATP consumption is higher, but the time-averaged gradient will be close to the above value. On this model, ADP must diffuse in the opposite direction and because its concentration outside of the myofibril is lower, perhaps 10 μM, the required concentration at the center is 12.5 μM. However, even these small gradients are an overestimate because the enzyme creatine kinase will hold [ATP] and [ADP] more nearly constant and produce gradients of [PCr], [Pi], and [Cr]. This leads to the situation of facilitated diffusion that has been recently analyzed in detail by Meyer et al.37 Their calculations show that a very large proportion of the high-energy phosphate flux is in fact carried by PCr and that the gradients of [PCr] are even smaller than those calculated above for [ATP].

It should be stressed that there is no direct evidence for any substantial myoplasmic compartmentation and the theoretical considerations above suggest that it should be small. Nevertheless, myoplasmic compartmentation has been invoked to explain a number of puzzling phenomena. For instance, as discussed on pages 159–162, acute hypoxic failure has been attributed to myofibrillar compartmentation though subsequently other explanations have emerged. (For further discussion of phenomena attributed to myoplasmic compartmentation, see discussion in Meyer et al.37) Further progress in this area will occur only if direct evidence can be obtained demonstrating the presence and magnitude of this type of compartmentation.

$[\text{ATP}]$ vs. $A_{\text{ATP}}$

There is general agreement in the literature that some consequence of ATP depletion plays a crucial role in the development of the effects of ischemia on cell function. ATP has at least two roles in any energetic process which it fuels. In the first place, ATP must bind to a site on the enzyme for which it is the substrate. Even when there is more than one site with different affinities, this role can be quantified by the apparent $K_m$, i.e., the concentration of ATP that leads to half maximum saturation of the process under consideration. In the second place, the hydrolysis of ATP can drive a reaction in the forward direction only if the free energy change of hydrolysis of ATP is greater than the free energy required for the process under consideration. The free energy change of ATP hydrolysis is a differential quantity whose magnitude depends on the advancement of the hydrolysis reaction. Its nomenclature in the literature is confused. It is commonly designated $\Delta G_{\text{ATP}}$, although recently $dG/dc$ has been used to indicate its differential nature; however, both of these are negative quantities for ATP hydrolysis and can be misleading. We have therefore chosen an alternative form known as (thermodynamic) affinity or $A_{\text{ATP}}$, which has the advantage of being a positive quantity for spontaneous reactions such as ATP hydrolysis.38 The relation between these quantities is

$$A_{\text{ATP}} = -\Delta G_{\text{ATP}} = -dG/dc = -\Delta G^o_{\text{ATP}} + RT \ln ([\text{ATP}]/([\text{ADP}] \times [\Pi]))$$

where $\Delta G^o_{\text{ATP}}$ is the standard free energy change of ATP hydrolysis under specified conditions of pH, $[\text{Mg}^{2+}]$, etc., and $R$ and $T$ have their usual meanings. Note that the ability of ATP to bind to a site depends principally on [ATP] whereas the (thermodynamic) affinity depends on [ADP] and [Pi] in addition to [ATP]. Figure 1 shows that while [ATP] is held essentially constant during the early part of metabolic decline, [ADP] and [Pi] rise considerably so that $A_{\text{ATP}}$ falls substantially over this period. Table 1 lists values of $K_m$ taken from the literature for various ATP-dependent processes in the myocardial cell. Table 2 shows calculations of the work per ATP consumed in the operation of various ion pumps in the myocardial cell. It is worth noting that the $K_m$ for all the pumps and for rigor tension development are less than 0.2 mM and much lower than the normal level of 6–8 mM. An important point that emerges from Figure 1 and Tables 1 and 2 is that operation of the various ion pumps is likely to be affected by the reduction of $A_{\text{ATP}}$ before [ATP] falls below the $K_m$ of any of the pumps. The real situation may be more complex than this analysis suggests if ADP competes with ATP for the binding site, since under these circumstances the apparent $K_m$ of ATP for its binding site will decline during metabolic depletion.

**Ionic Consequences of Ischemia and Hypoxia**

In this section we consider the ions Ca$^{2+}$, Na$^+$, H$^+$, and K$^+$: each has a major role in cellular function and the regulation of contraction. Furthermore, these ions are known to change their intracellular concentration during anoxia or ischemia and these changes are thought to be involved in the functional consequences of anoxia or ischemia. Ca$^{2+}$, Na$^+$, and H$^+$ exist in the myoplasm at concentrations below electrochemical equilibrium so that active (energy consuming) processes are required to extrude them from the cell. Thus, on first principles, one would expect the intracellular concentrations of these ions to increase towards electrochemical equilibrium as the pathways for ATP production are blocked. Intracellular [K$^+$] is higher than electrochemical equilibrium so that it tends

### Table 1. ATP Concentrations at Which Various Processes Occur at Half-maximal Rates

<table>
<thead>
<tr>
<th>Process</th>
<th>ATP concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR Ca pump</td>
<td>0.18 mM</td>
<td>Shigekawa et al.</td>
</tr>
<tr>
<td>Na-K pump*</td>
<td>0.1 mM</td>
<td>Glynn and Karlish</td>
</tr>
<tr>
<td>SM Ca pump*</td>
<td>0.03 mM</td>
<td>Caroni and Carafoli</td>
</tr>
<tr>
<td>Rigor attachment of crossbridge†</td>
<td>0.05 mM</td>
<td>Fabiato and Fabiato</td>
</tr>
</tbody>
</table>

*Study on red blood cells containing choline chloride.
†The number of rigor crossbridges attached is decreased at higher concentrations and increased at lower concentrations.
Intracellular Calcium

Intracellular calcium is highly compartmentalized within the cardiac cell, and it remains difficult to obtain reliable estimates of its concentration in the various cellular compartments. Measurements of total cell calcium are technically easier and were the earliest measurements to be made in ischemia. Total cell calcium does not change dramatically during the first hour of ischemia, so that metabolic consequences of anoxia do not appear to be a direct consequence of mitochondrial calcium loading (for further discussion, see Williams). However, if the free energy of hydrolysis of ATP decreases below a critical level (see Table 2), the ability of the sarcoplasmic reticulum to take up calcium may be compromised. In this case, the calcium transient would be expected to decrease and diastolic [Ca2+], would be expected to increase, unless another mechanism, such as the Na-Ca exchanger or the mitochondria, can continue to remove calcium from the myoplasm.

The Sarcoplasmic Reticulum. The sudden increase in [Ca2+], that initiates contraction (the calcium transient) is thought to be caused principally by the release of calcium stored in the sarcoplasmic reticulum, although the fraction of the activator calcium coming from this source probably varies between species. The amount of calcium in the sarcoplasmic reticulum will depend on [Ca2+]j and on the Ca-pump in the sarcoplasmic reticulum, whose properties are relatively well defined. However, if the free energy of hydrolysis of ATP decreases below a critical level (see Table 2), the ability of the sarcoplasmic reticulum to take up calcium may be compromised. In this case, the calcium transient would be expected to decrease and diastolic [Ca2+], would be expected to increase, unless another mechanism, such as the Na-Ca exchanger or the mitochondria, can continue to remove calcium from the myoplasm.

The Mitochondria. Mitochondria constitute about one-third of the myocardial cell volume and have a very large capacity for calcium (possibly as high as 4-10 mmol/l cell volume). The inner mitochondrial membrane has a Na-Ca exchange mechanism, a Na-H exchange mechanism, and a calcium uniporter. The mitochondria have the potential, therefore, to play a substantial role in the calcium metabolism of the cell. It has been suggested that an increase of myoplasmic [H+], such as occurs during hypoxia and ischemia, may release calcium from the mitochondria. However, it now appears that the mitochondria contain little calcium under normal conditions, so that even if it was all released it would have a relatively small effect on [Ca2+]. Nevertheless, under conditions of prolonged ischemia, the mitochondria may become loaded with calcium. This has led to speculation that calcium loading of the mitochondria and the resulting uncoupling of oxidative phosphorylation might be a factor in ischemic cell damage. However, loss of respiratory control over oxidative phosphorylation can occur in the absence of calcium loading of mitochondria, so that metabolic consequences of anoxia do not appear to be a direct consequence of mitochondrial calcium loading (for further discussion, see Williams).

Binding to Proteins and Membrane Phospholipids. Troponin is probably the most quantitatively important myoplasmic Ca2+ buffer. Other ions in the myoplasm, particularly H+, may compete with Ca2+.
for binding sites. Such competition will move the concentration-effect curve for calcium on troponin to the right, so that a given concentration of Ca\(^{2+}\) will have less effect, e.g., less tension will be developed for a given calcium release, but more calcium will remain free in the myoplasm.

The role of membrane-bound calcium in excitation-contraction coupling remains controversial. Calcium from this source may be released by the action potential and contribute to contraction; alternatively, these sites may simply act as passive buffers.

**Intracellular Sodium**

The free myoplasmic concentration of Na\(^{+}\) ([Na\(^{+}\)]\(_{i}\)) is close to 10 mM while the total cellular sodium is 20–30 mmol/kg cells (see discussion in Ellis). Efflux of sodium depends principally on the Na pump, which ejects 3 Na\(^{+}\) in exchange for 2 K\(^{+}\) for the hydrolysis of one ATP molecule. Current knowledge about the Na pump in cardiac muscle is summarized by Eisner. Influx of sodium into the cell can occur by various routes: 1) voltage-dependent channels, such as the fast Na channel, and 2) exchangers, such as the Na–H exchange and Na–Ca exchange. These exchangers are potentially reversible, the direction of operation depending on the gradients for each ion, the stoichiometry, and the membrane potential. Under normal resting conditions, Na\(^{+}\) will enter the cell under the influence of the electrochemical gradient for Na\(^{+}\) and either H\(^{+}\) or Ca\(^{2+}\) will be ejected from the myoplasm, but under other conditions, e.g., Na pump inhibition (see Dietmer & Ellis), the Na–Ca exchange may reverse and allow Ca\(^{2+}\) entry in exchange for Na\(^{+}\) efflux.

Small changes in [Na\(^{+}\)]\(_{i}\) have no direct effect on the contractile proteins, but changes in [Na\(^{+}\)] affect [Ca\(^{2+}\)] by means of Na–Ca exchange. Thus, when [Na\(^{+}\)]\(_{i}\) rises, the inward gradient for Na\(^{+}\) is reduced and Na\(^{+}\) influx and Ca\(^{2+}\) efflux on the Na–Ca exchange are both reduced. Consequently, calcium rises in the myoplasm and is taken up into the sarcoplasmic reticulum store. This leads to larger calcium release and stronger contractions. The net effect is that developed tension shows a very steep dependence on [Ca\(^{2+}\)] and Na\(^{+}\) and depends on the gradients for each ion, the stoichiometry, and the membrane potential. Activity of the Na pump may also be inhibited if ATP falls below the K\(_m\) (0.1 mM, Table 1) or if A\(_{NtP}\) falls below the minimal level of energy required for maintenance of the electrochemical gradients (44 kJ/mol under aerobic conditions, Table 2). Inspection of Figure 1 suggests that the latter may occur first, though changes in intracellular K and membrane potential during ischemia complicate the issue. Total sodium has been shown to rise both during prolonged (60 minutes) ischemia and hypoxia. Although there is some compartmentation of sodium, this probably means that [Na\(^{+}\)] should also rise. There are now several studies of myoplasmic [Na\(^{+}\)] that examine this issue. A study by Kleber on isolated, ischemic hearts showed that [Na\(^{+}\)] was unchanged or fell slightly after 15 minutes of ischemia. The investigator suggested that a rise of [Na\(^{+}\)] due to reduced activity of the Na pump might have been offset by the reduced influx associated with depolarization. This interpretation has been supported by more recent work in which papillary muscles were exposed to conditions designed to mimic ischemia (hypoxia, raised K\(_{o}\), extracellular acidosis, and glucose removal). When all these conditions were combined, [Na\(^{+}\)] changed little over 12 minutes. However, when [K\(^{+}\)] was maintained constant but the other conditions imposed, [Na\(^{+}\)] showed a small rise over 12 minutes. A study by Guarnieri on hypoxic but perfused papillary muscles (in which [K\(^{+}\)] will hardly change and depolarization should be relatively small) also showed a rise of [Na\(^{+}\)], provided glucose was absent from the perfusate. In addition, Guarnieri showed that over the same time, [Ca\(^{2+}\)], did not rise, suggesting that Na–Ca exchange may be inhibited during this period (see pages 163–165).

Further studies of the changes in [Na\(^{+}\)], in ischemia and hypoxia are needed and are of particular importance for assessing the contribution that Na–Ca exchange makes to calcium regulation.

**Intracellular pH**

For an extracellular pH of 7.4, the intracellular pH (pHi) at electrochemical equilibrium would be about 6.1. Many studies have shown that pHi is about 7.0, so some active pump must be ejecting protons. Several mechanisms seem to contribute to pH regulation, including a Na–H exchange that is mainly active in the presence of an acid load and a HCO\(_3^-\)/Cl\(^-\) exchange that is mainly active under alkaline conditions.

When interventions lead to changes in pHi, the various pump mechanisms lead to recoveries of pHi with time courses of 10–20 minutes. It follows that over periods of a few minutes, such as the time course of acute ischemic failure, the effects of the pump are small and the changes in pHi reflect net production/consumption of H\(^+\) in the presence of intracellular buffers. A number of metabolic reactions involve H\(^+\); these include:

\[
\text{ATP} \rightarrow \text{ADP} + \text{Pi} + n\text{H}^+ \quad n = 0.8
\]

\[
\text{PCr} + n\text{H}^+ \rightarrow \text{Cr} + \text{Pi} \quad n = 0.4
\]

\[
\text{glucose} \rightarrow 2 \text{lactate} + n\text{H}^+ \quad n = 2.0
\]

Estimates of the stoichiometry of H\(^+\) production under physiological conditions of pHi, [Mg\(^{2+}\)], etc. are shown for each reaction (for discussion see Gevers and Wilkie). NAD + H\(^+\) → NADH is ignored because the total amounts of these substances are so small (less than 1 mM). The intracellular buffering power of ventricular tissue is around 70 mM/pH unit, so that provided the net change in the above metabolites is known it is possible to calculate the expected changes in pHi and compare them with the measured changes. During ischemia or hypoxia, PCR breakdown is rapid; complete breakdown from the normal level of 20 mM would be expected to give a maximum alkalosis of 0.11 pH units (20 × 0.4/70). Allen et al have observed a transient alkalosis of 0.1 pH unit in the first
1–2 minutes after hypoxia. When anaerobic glycolysis can occur, the initial alkalosis is rapidly overwhelmed by a much larger acidosis associated with lactate production. For instance, if all glycogen stores (20–50 mM glucose units, depending on species) were converted to lactate, it would lead to an acidosis of 0.6–1.4 pH units. In ischemia, when lactate cannot leave the heart, intracellular acidosis of 1.0 unit has been observed. In hypoxia when perfusion continues, the acidosis is much smaller because lactate leaves the cardiac cells at a moderate rate. In addition, as expected, if glycolysis is prevented, acidosis is abolished.

Over longer periods, such as occur in ischemic contractions, the situation is much more complex since the mitochondria and surface membrane H pumps will be involved and changes in [Na⁺], and membrane potential may affect both passive fluxes of H⁺ and the H pumps. In ischemia, the acidosis associated with lactate accumulation will dominate the measured pH, but this situation has not yet been investigated or analyzed.

**Intracellular and Extracellular Potassium**

The onset of ischemia or hypoxia leads to an increase in potassium efflux from myocardial cells so that intracellular [K⁺] tends to fall and extracellular [K⁺] tends to rise (for review, see Kleber). Recent work suggests that the mechanism of this increased efflux is linked to the additional metabolic anions associated with anaerobic metabolism. In ischemia, potassium cannot leave the extracellular space, and its concentration rises over about 10 minutes to 10–15 mM; in hypoxia, potassium is carried away by the perfusate so that extracellular concentration changes will be small and transient. Increased [K⁺] leads to depolarization that has a crucial role in slowing conduction and the production of arrhythmias.

**Possible Mechanisms Underlying Acute Ischemic Failure**

A striking feature of acute ischemic failure is its rapidity. A substantial decline in developed tension is apparent after one minute of ischemia, and the decline is generally complete within 5–10 minutes. In this section, the mechanisms that may underlie this rapid decline of tension are considered. The consensus of a wide range of studies (for reviews, see Kubler and Spieckermann and Gibbs) is that [ATP] falls by only a small amount during this period. This fact has led some groups to speculate that there may be compartmentation of ATP within the myoplasm, with [ATP] falling to much lower levels in some critical region of the cell, but an alternative conclusion is that the decline in tension is not directly related to [ATP]. In this context, it is worth noting from Figure 1 that at the time when [ATP] is little changed, there are substantial falls in [PCr] and A₁₅ and rises in [ADP] and [Pi]; any of these might conceivably inhibit tension production.

Part of the very rapid phase of the decline of developed pressure in ischemia probably has a mechanical origin. This phase of decline is synchronous with the fall in perfusion pressure and is usually attributed to reduced stiffness and stretch associated with the fall of perfusion pressure in the vascular system. This component will of course be absent from preparations that are made hypoxic while perfusion continues.

Current understanding of the control of tension production in cardiac muscle suggests that it can be conveniently considered under three headings: the action potential, the amount of calcium delivered into the myoplasm, and the properties of the myofibrils.

**The Action Potential**

Not only is the action potential the trigger for contraction, but its amplitude and duration exert some control over the magnitude of contraction. In particular, many workers (for review, see Morad and Goldman) have shown that developed tension is a function of the duration and amplitude of the action potential (or of a voltage clamp pulse of comparable magnitude). Changes in action potential duration and amplitude have long been known to occur in ischemia and hypoxia (for review, see Carmeliet) and in this section, we consider the contribution such changes make to the sudden fall of developed tension in ischemia or hypoxia.

Action potentials have been measured in ischemic tissue. The changes observed are variable between different studies (see discussion in Downar et al) and include a slowing of the rate of rise of the action potential and of conduction velocity, and a reduction in the duration and amplitude of the action potential. When these changes are pronounced, as in the study of Downar et al, they lead to a failure of conduction within 10–15 minutes. Detailed studies of the mechanisms that underlie the above changes in the action potential have generally used perfused, isolated preparations so that the various components of ischemia can be separated.

The extracellular potassium accumulation of ischemia has been noted above and leads to depolarization of the membrane potential and a small shortening of the action potential (see Carmeliet for discussion of the mechanism involved). However, such changes in [K⁺], lead to only a small reduction in developed tension in isolated cardiac preparations. So that the membrane potential changes associated with increased [K⁺], probably make only a small contribution to the rapid decline of tension in ischemia.

McDonald and MacLeod studied action potentials under a variety of hypoxic conditions. In general, they found substantial decreases in action potential duration but only small depolarizations of the resting membrane potential. With 5 mM glucose present in the perfusate, the action potential duration during anoxia fell to about 40% of control over 60 minutes. However, over the 5 minutes in which the rapid fall of tension occurred, the reduction in action potential duration was less than 5% and seems unlikely to have had a major effect on ten-
sion production. Thus, the rapid fall in tension that occurs during anoxia, when glycolysis is accelerated, does not seem to be attributable to changes in action potential duration.

When anoxia is produced in muscles perfused with substrate-free solutions, a much greater shortening of the action potential occurs. Only recently, however, has the question been studied of whether this shortening occurs sufficiently rapidly to account for the decline of developed tension. Recent work suggests that when glycolysis has been prevented, either by glycogen depletion or by 2-deoxyglucose treatment, anoxia leads to a shortening of the action potential duration to < 20% control within 2-3 minutes and this was coincident with a rapid fall of tension to 0-5% of control. Such a dramatic shortening of the action potential would be expected to lead to reduced calcium release from the sarcoplasmic reticulum and could explain the reduced calcium transients reported under these conditions.

A number of possible mechanisms for the decline in action potential duration were discussed by Carmeliet. The plateau of the action potential represents a balance between small inward and small outward currents so that a decrease in the inward current or an increase in the outward current would lead to shortening of the action potential. Early work showed that CN, dinitrophenol, or intracellular acidosis led to a reduction of the inward Ca++ current. Both the shortening of the action potential and the reduced inward current have now been observed in single cells and can be triggered by a fall in [ATP], and reversed by injection of ATP into the cell. However, the contribution of reduced inward Ca++ current to the shortening of the action potential is still uncertain. Reasons include: 1) Recent investigations in single cells show that the Ca++ current is briefer in single cells than measurements in multicellular preparations suggested, so that the contribution of the Ca++ current to the plateau of the action potential may be small. 2) Experiments by Vleugels et al. suggest that increases in outward K+ currents are quantitatively more important than reductions in inward current. 3) The timecourse of the decrease in [ATP], or pH, in hypoxia may not be sufficiently fast to account for the shortening of the action potential by means of their known effects on the Ca++ current.

A mechanism that may explain the increased outward K+ currents seen in hypoxia is the recently described K+ channel that is activated when [ATP] falls. The K+ for the suppression of this current was found to be 0.1 mM in excised patches in perfused whole cells. It is not yet clear what the effective K+ in undisturbed cells might be, but it appears that significant increases in K+ conductance are unlikely until [ATP], has fallen below 1-2 mM. In most situations where direct measurements have been made, [ATP], does not fall to this level at the time when the rapid decline of tension occurs and when rapid shortening of the action potential may be important. Simultaneous measurements of action potential duration, developed tension, and [ATP], are needed to resolve this point.

In summary, substantial changes in action potential duration and magnitude can occur rapidly in ischemia and hypoxia with glycolysis inhibited. While the mechanism of these changes is not yet well understood, it is likely that they contribute to early contractile failure.

Ca++ Delivery to Contractile Proteins

The tension developed in each contraction of the heart depends mainly on the amount of Ca++ delivered to the myoplasm (see pages 157-158). Changes in this Ca++ delivery are a major source of the variations in tension production that are so characteristic of the heart. For this reason, and because of the known reduction in the Ca++ current under hypoxic conditions, it has often been suggested that there might be a reduction in Ca++ delivery during ischemia and hypoxia and that this might contribute to the rapid decline of tension.

Unfortunately, at present there is no direct way of measuring Ca++ delivery to contractile proteins. Developed tension is not a satisfactory indicator because the properties of the myofibrils can also change (see next section). The myoplasmic [Ca+++] during contraction (the Ca++ transient) is the nearest approximation available at present. If both the calcium transients and the tension are measured simultaneously it is possible to distinguish, at least qualitatively, between changes in Ca++ delivery, which change both the Ca++ transient and the developed tension in a parallel fashion, and changes in apparent sensitivity to [Ca+++], which lead to [Ca++] and developed tension changing in opposite directions.

There are at present no studies of the Ca++ transients in ischemic cardiac tissue, but Allen and Orchard have observed the effects of anoxia, both in the presence and the absence of glycolysis, on the Ca++ transients of ferret ventricular muscle. With glycolysis intact, the amplitude of the Ca++ transient was unaffected by preventing oxidative phosphorylation, by either anoxia or cyanide, despite the fact that developed tension fell to 30% of control. This result makes it unlikely that there are major changes in the Ca++ delivery to the contractile proteins; it suggests instead that either the ability of the contractile proteins to produce tension or their sensitivity to calcium has been reduced.

However, when glycolysis was prevented, anoxia led to a quite different result. Under these conditions, the amplitude of the Ca++ transients declined rapidly with a time course that was comparable to the decline of developed tension. Thus, failure of Ca++ delivery to the contractile proteins is apparently one of the causes of the decline of tension. The mechanism of this failure of Ca++ delivery has not been conclusively determined; it is not known, for instance, whether there is Ca++ stored in the sarcoplasmic reticulum but the release mechanism has failed or whether sarcoplasmic reticulum Ca pump has failed and there is less calcium stored and available for release. At present, the authors think
that the most likely explanation is that changes in the action potential described in the previous section lead to a failure of the calcium release mechanism. An alternative possibility is suggested by the work of Smith et al.\textsuperscript{109} They have identified an ATP-activated Ca channel in isolated sarcoplasmic reticulum. Obviously, if this channel is involved in Ca\textsuperscript{2+} release, it must normally be inhibited by some other mechanism except during activation. However, when [ATP] falls to low levels, the channel may be inhibited and Ca\textsuperscript{2+} release prevented.

Two other possibilities for the failure of Ca\textsuperscript{2+} release in anoxia with glycolysis prevented have been experimentally tested and rejected. Allen et al.\textsuperscript{13} measured the [ATP] by NMR under similar conditions and showed that [ATP], at the time of the rapid fall of tension was close to normal and certainly much greater than the apparent K\textsubscript{m} of the sarcoplasmic reticulum Ca-ATPase. They also calculated A\textsubscript{ATP} to test the suggestions of Kammermeir et al.\textsuperscript{104} and Allen and Orchard\textsuperscript{93} that free energy had fallen below the level that would allow the sarcoplasmic reticulum Ca pump to operate. These experiments did not support this hypothesis because the fall in A\textsubscript{ATP} was similar in hypoxia with glycolysis present and in hypoxia with glycolysis absent despite the fact that in the former case the Ca\textsuperscript{2+} transients were little affected, while in the latter case the Ca\textsuperscript{2+} transients were abolished.

The Properties of Myofibrils

Studies with skinned cardiac muscle preparations have identified a range of interventions that affect either the sensitivity of the myofibrils to calcium or the maximum Ca-activated tension (for review, see Rupp\textsuperscript{109}). Such changes can occur by a variety of mechanisms. 1) Covalent modification of the contractile or regulatory proteins, the best known example of which is phosphorylation of the inhibitory subunit of troponin,\textsuperscript{110} which leads to a reduction in the affinity of troponin for calcium. The actomyosin ATPase activity of myofibrils extracted from ischemic hearts has similar Ca sensitivity to that of myofibrils from normal hearts;\textsuperscript{111} making this mechanism unlikely. 2) Competition between another cation and calcium for the troponin binding site, which should lead to a parallel shift in the pCa-tension response curves, i.e., a change in apparent sensitivity to Ca\textsuperscript{2+} with no change in the maximum Ca-activated tension. It is now established that at least part of the inhibitory effects of acidosis on tension production is caused by this mechanism.\textsuperscript{59} 3) Ions or metabolites may affect one of the other contractile proteins, leading to changes in apparent sensitivity or maximum Ca-activated tension. The effects of ATP and Pi on the contractile proteins are probably of this variety.

In this account, we will mention only those interventions that seem to be of importance in ischemia.

pH. In 1969, Katz and Hecht\textsuperscript{108} suggested that an intracellular acidosis was responsible for the decrease of tension observed during ischemia. This was an attractive hypothesis since myocardial anoxia was known to lead to accelerated glycolysis and increased lactic acid production\textsuperscript{13} and hence, presumably, to a decrease of pH\textsubscript{i}. It had been known since 1880 that acid solutions led to a decrease in the amount of tension developed by the heart,\textsuperscript{109} and recent studies\textsuperscript{110} show that much of this effect can be explained by the effects of intracellular acidosis on the myofibrils. Qualitatively, therefore, it appeared possible that a decrease in pH\textsubscript{i} was responsible for the decreased tension observed during ischemia. This hypothesis received further support when nuclear magnetic resonance (NMR) studies showed that pH\textsubscript{i} did indeed decrease when intact, working heart muscle was made ischemic or hypoxic.\textsuperscript{80}

However, it was also necessary to show that the decrease of pH\textsubscript{i} was both large enough and fast enough to account for the decrease of tension. In an attempt to answer this point, several groups have used \textsuperscript{31}P NMR to monitor pH\textsubscript{i} in isolated hearts during hypoxia and ischemia. Jacobus et al.\textsuperscript{111} showed that pH\textsubscript{i} and tension both decreased monophasically during ischemia. However, the decrease in mechanical performance during ischemia was greater than could be accounted for by the acidosis. Studies of hypoxia have also shown that the contribution of acidosis to the decline of tension is small. Allen et al.\textsuperscript{13} showed that when glycolysis was present, hypoxia led initially to an alkalosis (see pages 158-159 for discussion) followed after several minutes by an acidosis. Since developed pressure fell more or less monotonically, the contribution of pH changes to the tension change was clearly small. Furthermore, when glycolysis was prevented, hypoxia led to a rapid fall in developed tension but without any acidosis.\textsuperscript{13}

In conclusion, there is a decrease of pH\textsubscript{i} during ischemia and hypoxia that undoubtedly makes some contribution to the observed decrease in tension. However, it seems unlikely that it is the major cause of tension decline, the development of acidosis being too small and too slow to explain the decline of tension.

Phosphate Compounds. Reducing [ATP] (in reality, MgATP\textsuperscript{2-}) has a variety of effects on the tension development of skinned cardiac muscle.\textsuperscript{42} Maximum Ca-activated tension increases slightly (at least down to 0.3 mM) and Ca sensitivity also increases. Note that these effects will result in increased developed tension when [ATP] falls from its normal myoplasmic level to submillimolar levels. The rigor-promoting effects of low [ATP] will be considered in the next section.

PCr and ADP have very small direct effects on tension development in skinned fibers.\textsuperscript{112} Inorganic phosphate (Pi) has, however, recently been shown to have major effects on skinned cardiac fibers, and it seems likely that the substantial increases in [Pi] that occur in ischemia and hypoxia exert a major depressant effect on tension development. Herzig and Ruegg\textsuperscript{113} showed that Pi exerted a pronounced inhibitory effect on maximum Ca-activated tension. Kentish\textsuperscript{112} has shown that, in addition, Pi leads to a reduction in Ca sensitivity. Both effects are large. For instance, if the normal [Pi] is 1-3 mM and it increases to 20 mM during ischemia or hypoxia, maximum Ca-
activated tension will be reduced to about 50% control. The effect on Ca sensitivity leads to a further reduction, so that developed tension would be about 20% of the original control. Not only is this effect large, it is also rapid in onset. Figure 1 and many experimental results have shown that [Pi] starts to rise as soon as ATP consumption exceeds ATP production and well before there is a significant change in [ATP].

Although Pi depresses tension production, it does not seem to reduce ATPase activity of the myofilaments. Thus, it may be misleading to assume that ATP consumption by the heart during ischemia or anoxia is reduced in proportion to the decline in tension production.

Summary

The factors responsible for the rapid fall of developed tension in ischemia and hypoxia fall into three categories.

Metabolic factors. These are probably the most important contributors to early ischemic and hypoxic failure. The earliest effect arises from the inhibitory effects of Pi on the contractile proteins. Subsequently, the acidosis, particularly in ischemia in which reten-
tion of lactate causes a very large fall in pH, leads to a further reduction in tension.

Mechanical factors. The fall in perfusion pressure during ischemia leads to reduced muscle stretch and tension development with a time course similar to the fall in perfusion pressure.

Activation factors. Rapid shortening of the action potential duration and reduced calcium release have both been observed in hypoxia with glycolysis inhibited and probably contribute to the rapid decline of tension under these conditions. A similar mechanism may operate in ischemia.

Possible Mechanisms Underlying Ischemic Contracture

The contracture that develops in ischemia or in hypoxia with glycolysis prevented has been attributed to two mechanisms.

Rise in \([Ca^{2+}]\). Evidence for this possibility was the small rise in total calcium measured over this period. Skinned fiber experiments at normal [ATP] suggest that [Ca\(^{2+}\)] would need to rise to 5-10 \(\mu\)M to explain the observed developed tension.

Fall in [ATP], leading to rigor crossbridge formation. Skinned fiber experiments suggest that at the normal resting [Ca\(^{2+}\)], [ATP], would have to fall to 0.1 mM or below before rigor development became significant. Although discussed as if they were independent, there is evidence that the two mechanisms may interact with each other. Thus, Bremel and Weber\(^{13}\) showed that under conditions that led to rigor attachment, the binding constant of troponin for calcium was increased, so that only a small rise in [Ca\(^{2+}\)], from the normal resting level might lead to considerable Ca-dependent tension production. Fabiato and Fabiato\(^{42}\) showed, for instance, that 0.1 \(\mu\)M [Ca\(^{2+}\)], which at normal [ATP] does not cause any tension develop-

ment, led to 10% of Ca-activated tension when [ATP] was lowered to 0.3 mM.

\([Ca^{2+}]\), has now been measured by a variety of techniques in cardiac cells exposed to hypoxic conditions. There is general agreement that metabolic depletion leads to increases in [Ca\(^{2+}\)], but the conditions that lead to this rise and the magnitude and time course of the rise show considerable variability. A report by Dahl and Isenberg,\(^{16}\) who used Ca\(^{2+}\)-sensitive micro-electrodes to measure [Ca\(^{2+}\)], in Purkinje fibers, has been quoted as demonstrating a large and rapid rise in [Ca\(^{2+}\)], on metabolic inhibition. In fact, the investigators used dinitrophenol (DNP), a proton ionophore, that eliminates the proton gradient in mitochondria. This agent prevents ATP production by the mitochondria, which may then actually consume ATP, so that the decline in [ATP], may be larger than when oxidative phosphorylation is prevented by CN or anoxia. Dinitrophenol will also allow any calcium stored in the mitochondria to be released. In “fresh” preparations, stored for less than 3 hours, application of DNP led to a slow rise in [Ca\(^{2+}\)], that reached 10 \(\mu\)M in 20-30 minutes. Older preparations showed much faster rises, reaching 10 \(\mu\)M in 2-4 minutes. It is not clear from these experiments whether the increases in [Ca\(^{2+}\)], are due to the decline in [ATP], and consequent inhibition of Ca pumps or to the release of calcium stored in mitochondria. In either case, the result is probably not what would be expected in ischemia or anoxia.

Snowdowne et al\(^{17}\) also showed a very rapid rise in [Ca\(^{2+}\)], on metabolic inhibition. These workers first isolated cells by a collagenase method and then loaded them with aequorin, using an osmotic shock technique. Large numbers of such cells were then exposed to either hypoxia in the absence of external glucose or to FCCP, another proton ionophore. Either treatment produced rapid and reversible increases in [Ca\(^{2+}\)]. There are two reasons to doubt whether this rapid rise in [Ca\(^{2+}\)], is representative of healthy cells under these conditions. First, the metabolic status of these cells is unknown but is likely to be poor since collagenase isolation is known to lead to loss of P-metabolites.\(^{44}\) The subsequent osmotic lysis of cells seems likely to lead to further deterioration. Second, about 30% of the loaded cells were already rounded up and these cells, which are already damaged, may have contributed disproportionately to the recorded increases in [Ca\(^{2+}\)].

In contrast, there are studies from several laboratories indicating that metabolic inhibition leads to rises in [Ca\(^{2+}\)], after a delay of only 20-40 minutes and that a contracture of intact preparations or rounding up of isolated cells precedes the rise in [Ca\(^{2+}\)]. Allen and Orchard\(^{93}\) showed that during short exposures to hypoxia after inhibition of glycolysis, a substantial contracture could develop with no significant increase in resting [Ca\(^{2+}\)]. Subsequent studies with more complete inhibition of glycolysis confirmed this finding\(^{118}\) and showed that, at about the time that the contracture was close to maximal, the resting [Ca\(^{2+}\)], started to
rise. Similar findings have been reported by Cobbold and Bourne, who used single isolated ventricular cells microinjected with aequorin. They showed that during hypoxia with glycolytic inhibition, single cells took 10–20 minutes to show signs of contracture, and only 5–10 minutes later were increases in [Ca\(^{2+}\)] recorded. As mentioned earlier, studies by Guarnieri using ion-sensitive electrodes showed that [Ca\(^{2+}\)] did not change in 20 minutes of hypoxia in the absence of glucose, despite the fact that [Na\(^+\)], rose (for discussion, see pages 163–165).

We suggest that the disparities in the time required for [Ca\(^{2+}\)], to rise on hypoxia reflect differences in the initial metabolic status of the preparations, although further tests of this point will require simultaneous measurements of [Ca\(^{2+}\)] and metabolic status during ischemia and hypoxia. All the results cited are consistent with the following conclusions. When [ATP], or perhaps A\(_{ATP}\), falls below a certain level, [Ca\(^{2+}\)] rises quite rapidly, presumably because of failure of the various Ca pumps that remove calcium from the myoplasm. [ATP], does not fall below this level in cardiac cells with oxidative phosphorylation blocked, provided anaerobic glycolysis can continue. When glycolysis is blocked in addition to oxidative phosphorylation, [ATP], will, of course, eventually fall below this level, but the threshold for rigor production seems to occur at a significantly higher level than that necessary to block Ca pumps and lead to elevated [Ca\(^{2+}\)]i.

There are a number of experimental observations that suggest that ischemic and hypoxic contractures are due to the attachment of rigor crossbridges, precipitated by the low [ATP]. Thus, interventions that lead to greater tension production and therefore increased ATP consumption lead to enhanced rigor production. The stiffness of muscle in ischemic or hypoxic contracture increases considerably above that associated with a normal contraction, consistent with rigor bridge attachment. Heat measurements have shown that the heat/tension ratio is similar in a twitch and a K+ contracture but smaller in hypoxic contractures. Since ATP consumption associated with crossbridge cycling is the major cause of heat production, these findings suggest that the crossbridges in hypoxic contractures are turning over at a slower rate, as expected for rigor crossbridges.

[ATP], has been measured in contractures by both biochemical and NMR methods, and the consensus is that it is at very low levels during contractures. It is difficult, however, to prove quantitatively that [ATP], has fallen to the level shown in skinned fiber studies to cause rigor for two main reasons: 1) Present NMR and analytical methods cannot measure myoplasmic levels of ATP with confidence in the range of 0.1 mM. 2) Problems of tissue homogeneity exist in both intact preparations and in the large numbers of isolated cells that are required for such measurements. For instance, in NMR studies on whole hearts, contractures can sometimes be recorded from the left ventricle at a time when the NMR signal from the whole heart suggests a mean [ATP], of several mM. This may mean that in the nonworking right ventricle, [ATP], is close to normal while in the working left ventricle it has declined to levels capable of producing rigor.

A different explanation for the possible appearance of rigor at [ATP], higher than 0.1 mM is suggested by the work of Miller and Smith on skinned cardiac muscle. They showed that in the presence of an elevated [ADP], the rigor tension at any given [ATP] was increased.

**Summary**

The present weight of evidence supports the idea that ischemic and hypoxic contractures are produced by low ATP, leading to rigor. Further reduction in ATP leads to a rise in [Ca\(^{2+}\)], that may precipitate other cell damage (see next section) but is not itself the initiating cause of the contracture.

**Reperfusion Damage**

 Interruption of coronary flow to a region of the myocardium for more than about 1 hour leads to permanent damage. Return of coronary flow is essential to survival of the myocardium, but it was realized in the early 1960s that reperfusion can also accelerate the appearance of myocardial damage. Early studies of this subject were reviewed by Hearse, who drew attention to the similarities between the calcium paradox and reperfusion damage. (The term calcium paradox describes the cell damage observed when extracellular Ca\(^{2+}\) is returned to the perfusate after a period of Ca-free perfusion.) In both situations, there is a large, rapid increase in total cell calcium, a contracture develops, and there is eventual cellular damage with release of intracellular enzymes. It is now accepted that calcium accumulation precipitates many kinds of muscle damage. The evidence for this statement and the mechanism by which increased Ca\(^{2+}\) leads to cell damage have been extensively reviewed (e.g., Jennings and Steenber, Nayler et al, and Poole-Wilson) and will not be repeated here. Instead, discussion will be focused on two issues — the mechanism of the Ca\(^{2+}\) increase and the ionic interactions that may occur during reperfusion.

**Mechanism of Calcium Influx**

The mechanism of Ca\(^{2+}\) accumulation in the calcium paradox is now well established. During a period of low Ca\(^{2+}\) perfusion, [Na], rises; thus when Ca\(^{2+}\) is returned to the extracellular space, operation of the Na-Ca exchanger will lead to increased Na+ efflux, accompanied by an increased Ca\(^{2+}\) influx leading to Ca\(^{2+}\) accumulation. If this mechanism represents the main route for Ca\(^{2+}\) influx, then both Ca\(^{2+}\) accumulation and tissue damage should depend on the level of [Na\(^+\)], that exists at the time when Ca\(^{2+}\) is returned to the perfusate. Such a correlation was demonstrated by Ruano-Arroyo et al. What has recently become clear is that the mechanism by which Na+ enters the cell during the
low Ca\(^{2+}\) perfusion is not primarily by the Na channel or by Na–Ca exchange, but via the Ca channels, which become highly permeable to Na \(^{+}\) when the external Ca\(^{2+}\) is very low. In accordance with this theory, Ca-channel blocking drugs exert a protective effect if applied during the period of Ca-free perfusion but not when external Ca\(^{2+}\) is replaced. Thus, it seems that the Ca\(^{2+}\) influx that triggers damage after a period of Ca-free perfusion is mediated by the Na–Ca exchanger and is caused by the elevation of [Na\(^{+}\)].

Clearly, the same sequence of events cannot operate in reperfusion or reoxygenation damage because external Ca\(^{2+}\) is present, though in ischemia the amount of extracellular Ca\(^{2+}\) is limited and could conceivably limit Ca\(^{2+}\) influx during prolonged ischemia. However, it has been established that Ca\(^{2+}\) influx does increase dramatically on reperfusion or reoxygenation and that this influx is not simply due to a nonspecific increase in cell permeability nor does it occur via Ca channels. The idea that the influx occurs via Na–Ca exchange was proposed by Grinnwald. Isolated hearts were subjected to ischemia and reperfusion after maneuvers designed to raise or lower [Na\(^{+}\)]. The increase in total Ca\(^{2+}\) on reperfusion was determined to be dependent on [Na\(^{+}\)]. This idea was confirmed and extended by Renlund et al., who showed that the degree of mechanical and metabolic recovery was inversely related to the level of [Na\(^{+}\)], before reperfusion but could not be accounted for by changes in the level of PCR or ATP before reperfusion.

As discussed earlier, there is evidence that [Na\(^{+}\)], rises during ischemia, probably because the Na pump is inhibited by low levels of ATP or reduced A\(_{ATP}\). However, the reason why Ca\(^{2+}\) influx occurs at a rapid rate only after reperfusion or reoxygenation when elevated [Na\(^{+}\)], is present both before and after reperfusion remains a mystery. The influx of Ca\(^{2+}\) could still occur by Na–Ca exchange if the exchange is inhibited during ischemia but reactivated on reperfusion. There are two possible mechanisms that could allow this to happen: 1) Intracellular pH is known to inhibit Na–Ca exchange. Since ischemic or hypoxic muscle is acidotic and this acidosis recovers on reperfusion and reoxygenation, acidosis could inhibit Na–Ca exchange during ischemia and allow it to reactivate on reperfusion. However, the fact that damage also occurs on reoxygenation after a period of anoxia without glucose or a period of anoxia with glycolysis inhibited implies that intracellular acidosis is not essential since there will be little acidosis when lactate production is prevented. 2) In squid axon, both Na\(_{app}\)-dependent Ca\(^{2+}\) efflux and Na\(_{app}\)-dependent Ca\(^{2+}\) influx have been shown to be dependent on intracellular ATP, with a K\(_{m}\) of around 0.2 mM. If Na–Ca exchange in the heart has the same properties, Na–Ca exchange will be inhibited during ischemia by the low level of ATP and reactivated when ATP rises in reperfusion. This mechanism could also explain why total Ca\(^{2+}\) and [Ca\(^{2+}\)], do not rise rapidly during ischemia despite substantial rises in [Na\(^{+}\)].

The authors believe that Na–Ca exchange is the most likely route for Ca\(^{2+}\) entry on reperfusion, on the basis of present evidence. For alternative views, see Poole-Wilson and Nayler et al.

### Interactions Between Na\(^{+}\), Ca\(^{2+}\), and H\(^{+}\)

The intracellular concentrations of Na\(^{+}\), Ca\(^{2+}\), and H\(^{+}\) are each elevated during reperfusion, and there is some evidence that a regenerative cycle of the sort shown below may occur.

\[
\begin{align*}
\text{[Na}^{+}\text{]} & \rightarrow \text{[Ca}^{2+}\text{]} & \rightarrow \text{[H}^{+}\text{]} \\
\end{align*}
\]

The raised [Na\(^{+}\)], leads to a raised [Ca\(^{2+}\)], by Na–Ca exchange. The mechanism by which an increased [Ca\(^{2+}\)], leads to an increased [H\(^{+}\)], is the least clear though there is good experimental evidence for its existence. Possible mechanisms include direct competition between Ca\(^{2+}\) and H\(^{+}\) for binding sites on proteins or for entry to mitochondria. Alternatively, the raised [Ca\(^{2+}\)], may trigger anaerobic glycolysis and lactic acid production, either directly or indirectly, by activating tension production and ATP consumption. The raised [H\(^{+}\)], leads to increased [Na\(^{+}\)], by Na–H exchange. The important feature of this cycle is that it shows positive feedback; once threshold levels of some or all of the ions are exceeded, the cycle will tend to continue until prevented by some extraneous mechanism. Thus, once a substantial rise of [Ca\(^{2+}\)], occurs, it may continue to rise until it causes cellular damage.

Why should this potentially damaging cycle occur during reperfusion but not under normal conditions or during ischemia? Under normal conditions, an elevated [Ca\(^{2+}\)], is rapidly pumped down to the diastolic level by the sarcoplasmic reticulum Ca pump, and [Na\(^{+}\)], is regulated by the Na pump. In ischemia, if ATP or A\(_{ATP}\) falls sufficiently low, both the Na pump and the SR Ca pump will fail, but as noted above, inhibition of Na–Ca exchange may prevent operation of the cycle. Lazdunski et al. have pointed out that high extracellular [H\(^{+}\)] occurs during ischemia and the competition between extracellular H\(^{+}\) and Na\(^{+}\) inhibits the Na–H exchange; this will also tend to prevent operation of the cycle. After a period of ischemia, then, both [Na\(^{+}\)], and [H\(^{+}\)], will tend to be elevated, and [Ca\(^{2+}\)], may also be elevated if the period of ischemia is long. However, inhibition of Na–Ca exchange in the above cycle prevents these levels from rising rapidly to damaging levels.

When reperfusion occurs, oxidative phosphorylation will restart and P-metabolites will show some recovery. However, the recovery of ATP is limited. There is loss of total adenine nucleotides, which is related to the duration of ischemia, and ATP consumption is potentially at a very high rate because the Na pump, the sarcoplasmic reticulum Ca pump, and the myofibrillar ATPase are all near maximal activation. Reactivation of the Na–Ca exchange will lead to substantial Ca\(^{2+}\) influx so that the sarcoplasmic reticulum will soon be saturated with calcium and no longer help to keep [Ca\(^{2+}\)], low. Activation of the Na–H exchanger
will tend to keep \([\text{Na}^+]\), high and maintain the \([\text{Ca}^{2+}]\) influx. Thus, whether or not recovery can occur will depend on whether the Na pump, in the face of reduced [ATP], and increased \([\text{Na}^+]\), can lower \([\text{Na}^+]\), to a level that prevents the above regenerative cycle from becoming established.

Cell damage also occurs on reoxygenation after a period of anoxia in which glycolysis is reduced or prevented. Under these circumstances, the accumulation of intracellular and extracellular H\(^+\) will be reduced. This implies that reactivation of the Na-H exchanger\(^7\) and Na\(^+\) entry in exchange for H\(^+\) are not essential to the activation of the pathological cycle indicated above. In the absence of anaerobic glycolysis, the fall in [ATP] in anoxia is likely to be more rapid than in ischemia, and the consequences of a longer period with a very low [ATP] may lead to larger increases in \([\text{Na}^+]\), and \([\text{Ca}^{2+}]\), which compensate for the reduction of Na\(^+\) entry in exchange for H\(^+\).

**Summary**

There is good evidence that elevated \([\text{Ca}^{2+}]\), produced by an influx of \([\text{Ca}^{2+}]\) in exchange for Na\(^+\), is the underlying pathology in reperfusion or reoxygenation damage. Further measurements of \([\text{Na}^+]\), and \([\text{Ca}^{2+}]\), during ischemia and reperfusion, coupled with information about metabolic levels, are needed to confirm or refute this hypothesis. Contributions to cell damage by other mechanisms, e.g., oxygen free radicals, certainly cannot yet be excluded.

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**References**

30. Hearse DJ, Chain EB: The role of glucose in the survival and recovery of the anoxic isolated perfused rat heart. *Biochem J* 1972;128:1125–1133
34. Geisbuhler T, Alischuld RA, Trewyn RW, Ansel AZ, Lamba
39. Shigekawa M, Finegan JM, Katz AM: Calcium transport ATPase of canine cardiac sarcoplasmic reticulum; a comparison with that of rabbit fast skeletal muscle sarcoplasmic reticulum. J Biol Chem 1976;251:6894-6900
42. Fabiato A, Fabiato F: Effects of magnesium on contractile activation of skinned cardiac cells. J Physiol (Lond) 1975;249:497-517
50. Guarnieri TF: Relationship between intracellular calcium activity (Ca2+) and intracellular sodium activity (Na+) during hypoxia (abstract). Circulation 1985;72(suppl III, part II):III-327
52. Fabiato A: Calcium-induced release of calcium ions from the cardiac sarcoplasmic reticulum. Am J Physiol 1983;245:C1-C14
55. Fry CH, Poole-Wilson PA: Effects of acid-base changes on excitation-contraction coupling in guinea-pig and rabbit cardiac ventricular muscle. J Physiol (Lond) 1981;313:141-160
64. Deitmer JW, Ellis D: Changes in the intracellular sodium activity of sheep heart Purkinje fibres produced by calcium and other divalent cations. J Physiol (Lond) 1978;277:437-453
68. McDonald TF, MacLeod DP: Metabolism and the electrical activity of anoxic ventricular muscle. J Physiol 1973;229:559-582
73. Deitmer JW, Ellis D: Interactions between the regulation of the intracellular pH and sodium activity of sheep cardiac Purkinje fibres. J Physiol (Lond) 1978;277:1029-1042
76. Gevers W: Generation of protons by metabolic processes in heart cells. J Mol Cell Cardiol 1979;8:867-874
78. Ellis D, Thomas RC: Direct measurement of the intracellular pH of mammalian cardiac muscle. J Physiol (Lond) 1976;262:755-771
81. Kleber AG: Extracellular potassium accumulation during...
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... contains adenine nucleotide-activated calcium channels.


123. Smith JS, Coronado R, Meissner G: Sarcoplasmic reticulum...
sion in the isolated rat heart: Influence of extracellular sodium. *J Mol Cell Cardiol* 1982;14:359-365
131. DiPolo R: Calcium influx in internally dialyzed squid giant axons. *J Gen Physiol* 1979;73:91-113

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