

# Induced Ischemic Arrest of the Heart: Metabolic Considerations

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## INTRODUCTION

Normothermic ischemic arrest of the heart as an adjunct to open-heart surgery has gained widespread clinical acceptance. This form of arrest, achieved by simply cross-clamping the ascending aorta to eliminate coronary perfusion, is convenient in that it provides a bloodless operative field and a relaxed myocardium. The upper limit of the safe period of such ischemia is generally considered to be in the range of 30 to 45 minutes.<sup>1</sup> Clinical experience has revealed that, in some cases, it is possible to exceed these limits with relative impunity. However, about half of the deaths during cardiac surgery and in the early post-operative period are associated with some degree of ischemic necrosis of the inner third of the myocardium, the characteristic lesion of prolonged ischemic arrest of the total heart.<sup>2,3</sup> Safety in elective arrest of the heart thus becomes a problem of great importance in cardiac surgery.

Previous attempts to establish a safe time limit for the interruption of the coronary circulation during open-heart surgery have been based on hemodynamic measurements of the recovery of myocardial function and histologic examination of altered cardiac ultrastructure following fixed periods of ischemic arrest in groups of experimental animals. This approach does not take into account the marked variation in the metabolic response to ischemia from one heart to another. In contrast, we have sought a direct measurement of the individual's heart metabolic response to ischemic arrest.

The preservation of myocardial cellular viability during ischemia depends on the capacity of the heart to produce energy through anaerobic metabolism. To characterize the individual heart's response to this metabolic stress, one can monitor the transition from aerobic to anaerobic metabolism and the rate at which the anaerobic metabolic response proceeds. We have accomplished this by monitoring intramyocardial carbon dioxide tension during the ischemic period.

## CARDIAC METABOLISM IN ISCHEMIC ARREST

A schematic overview of the two major energy-producing metabolic pathways of the heart is shown in Figure 1. In aerobic metabolism, glucose is first phosphorylated to glucose-6-phosphate and then undergoes further reactions which result in the formation of pyruvic acid. These reactions make up part, but not all, of the Embden-Meyerhof pathway of glycolysis. The pyruvic acid is then converted to acetyl-co-

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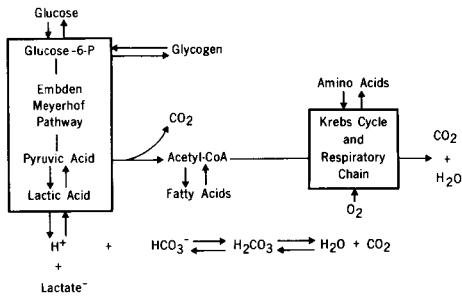


Fig. 1.

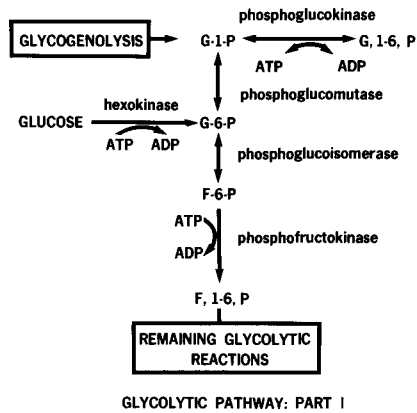


Fig. 2.

Figure 1. Overview of myocardial metabolic pathways.

Figure 2. Glycolytic pathway: Part I, Reactions consuming ATP. The failure of the glycolytic pathway to continue to generate ATP in ischemia is caused by a shortage of ATP as a substrate in the early steps of glycolysis. Part II, Reactions producing ATP. These later steps of glycolysis produce more ATP than the early steps consume such that under normal circumstances there is a net production of ATP by the entire glycolytic sequence.

enzyme-A with the liberation of carbon dioxide. Acetyl-coenzyme-A marks the entrance to the Kreb's Cycle and Respiratory Chain, a complex set of enzyme-catalyzed reactions which take place in the mitochondria of the heart. The result of these mitochondrial reactions is that oxygen is combined with carbon and hydrogen atoms of the entering acetyl groups to produce carbon dioxide and water with the liberation of adenosine triphosphate (ATP), the high-energy phosphate which is the common currency of metabolic energy exchange. Fatty acids can also function as fuel for aerobic metabolism through their conversion to acetyl-coenzyme-A. In fact, they tend to be used in preference to glucose when myocardial oxygenation is good.<sup>4</sup>

In anaerobic metabolism, glucose continues through the Embden-Meyerhof pathway one step beyond pyruvic acid and is converted to lactic acid. Fatty acids, by contrast, cannot be used in anaerobic metabolism because the acetyl-coenzyme-A formed from the fatty acids cannot be converted to pyruvic acid. Metabolic transformations are also available which allow amino acids to be used in both aerobic and anaerobic energy production. However, as glucose or fatty acids are available under aerobic conditions and glucose is available under anaerobic conditions, amino acid consumption is negligible.

As long as oxygen is available and acetyl units continue to move through the Kreb's Cycle and Respiratory Chain, pyruvic acid is not converted to lactic acid and aerobic metabolism predominates. In the absence of oxygen, the Embden-Meyerhof glycolytic pathway remains the only set of reactions capable of significant ATP production. Glucose becomes the sole fuel and may be available exogenously through perfusion of the coronary artery perfusion, and therefore no exogenous glucose supply, glycogen becomes the only energy source for glycolysis.

With this background, it is possible to predict what will happen to the myocardial carbon dioxide level when coronary perfusion ceases. The heart demands a steady supply of ATP, even when it is vented and the load of supporting the circulation is removed from it, because the maintenance of transmembrane electrical

potentials and cellular integrity continue to require energy. With no coronary blood flow, the available oxygen is rapidly used up and its concentration falls to zero within one or two minutes. The Embden-Meyerhof pathway becomes the only source of ATP production and, when this occurs, carbon dioxide begins to accumulate in significant quantities.

Although carbon dioxide is a product of the aerobic pathway, it is normally washed out by the coronary circulation. However, when aerobic metabolism ceases for lack of oxygen and there is no myocardial perfusion to wash out metabolic products, the carbon dioxide level increases because carbon dioxide is also a product of anaerobic metabolism. Specifically, myocardial intracellular lactic acid, the end product of the anaerobic pathway, dissociates into hydrogen and lactate ions and the resulting increased hydrogen concentration drives the intracellular bicarbonate buffer system towards a higher concentration of carbonic acid which itself is in balance with the concentration of carbon dioxide free as a gas in solution (Figure 1). Carbon dioxide is highly lipid-soluble and easily crosses cell membranes. Thus, for practical purposes, intracellular and extracellular carbon dioxide concentrations can be considered to be identical. Therefore, the rate of extracellular carbon dioxide accumulation is directly linked to intracellular ATP production during ischemic arrest and indicates the metabolic capacity of the heart to cope with this stress.

The production of ATP from anaerobic glycolysis is inadequate to meet the consumption of ATP required by the myocardium under all known circumstances. Since the consumption of ATP is in excess of its production, the concentration of ATP continually falls. Carbon dioxide continues to accumulate until further production of ATP ceases completely. What causes the ultimate cessation of glycolytic flux in ischemic arrest of the heart? One might guess that the heart simply runs out of fuel, that is, the limited stores of myocardial glycogen become totally depleted. This, however, is not the case. The full explanation requires a detailed examination of the early reactions of the Embden-Meyerhof pathway.

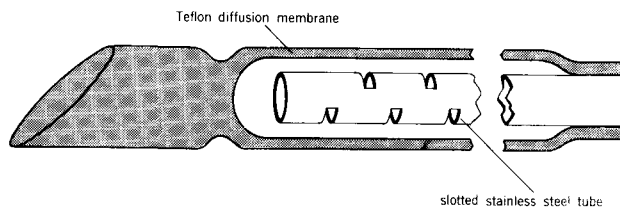
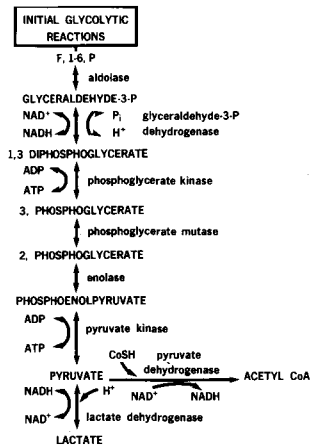


Fig. 3.

Fig. 2.

GLYCOLYTIC PATHWAY: PART II

Figure 3. The stainless steel sampling catheter and Teflon diffusion membrane (overall diameter 1.4 mm). Tissue gases can diffuse through the membrane into the stainless steel tube through its end hole and wall slots.

In Figure 2, the reactions of glycolysis are illustrated.<sup>5</sup> The phosphorylation of fructose-6-phosphate to fructose-1, 6-diphosphate, catalyzed by the enzyme phosphofruktokinase, is the first irreversible step in glycolysis and is shown in Part I. It has been proven by Kubler and Spieckermann to be the rate limiting reaction for

the final failure of ATP production in ischemia.<sup>6</sup> Phosphofructokinase is inhibited by hydrogen ions. The accumulation of hydrogen ions from the dissociation of the lactic acid which builds up as the end product of anaerobic glycolysis must, therefore, produce some degree of inhibition of phosphofructokinase. The idea of a metabolic system, closed due to lack of perfusion, poisoning itself with its own reaction products is attractively simple. However, phosphofructokinase inhibition by a lowered pH is not the entire answer to the failure of ATP production.

Looking at the reactants, one notes that ATP is used as a reactant in these early reactions in the glycolytic pathway. The phosphofructokinase reaction requires that ATP be present to donate a phosphate group to fructose-6-phosphate and be reduced to ADP. The conversion of glucose-1-phosphate, obtained from glycogen breakdown, to glucose-6-phosphate is catalyzed by the enzyme phosphoglucosmutase. This enzyme requires glucose-1,6-diphosphate as a cofactor and cannot function without it. An adequate concentration of this cofactor can only be produced from glucose-1-phosphate, the reaction being catalyzed by phosphoglucokinase only if sufficient ATP is present.

Examination of the later steps in glycolysis, as shown in Figure 2, Part II. reveals that, in contrast to the earlier reactions shown in Part I, none of these require ATP as a substrate. For each half molecule of fructose-1,6-diphosphate, two molecules of ATP are produced, one by the phosphoglyceratekinase reaction and the second by the pyruvate-kinase reaction. The result is that for every molecule of glucose-6-phosphate used, four molecules of ATP are produced from these later reactions. The subtraction of one molecule of ATP, used up in the earlier phosphofructokinase reaction, still leaves a three molecule net ATP production by the entire glycolytic sequence for each molecule of glucose obtained from glycogen breakdown. Therefore, cessation of glycolysis must be related to a shortage of ATP. Once the ATP concentration falls below a critical level, the fructose-1,6-diphosphate required for ATP production in the later steps in the glycolytic sequence is generated progressively more slowly and the gap between net production and consumption of ATP in the cell rapidly widens. The resulting lower ATP levels cause additional slowing of fructose-1,6-diphosphate production, thus perpetuating the vicious cycle. The role of cellular acidosis is that it precipitates the failure of glycolysis at a higher ATP concentration and therefore at an earlier time during cardiac arrest than would otherwise occur.

In summary, the failure of the glycolytic pathway to continue to generate ATP is caused by a shortage of ATP as a substrate in the reactions leading to its further production. Although the early steps of glycolysis consume ATP (Part I) the later steps (Part II) produce it in excess so that there is a net production of ATP by the entire glycolytic pathway. At critically low ATP concentrations, the generating capacity of the early steps of glycolysis breaks down completely and the remaining steps can no longer provide ATP for the cell.

The biochemistry of ischemic arrest of the heart with no coronary artery perfusion differs from that of anoxic arrest of the heart with coronary perfusion. We feel that it is worth stressing the distinction between ischemia and anoxia. Ischemia refers to a condition of absent or, more loosely, deficient blood flow, whereas anoxia simply means a total lack of oxygen. Total ischemia produces anoxia in a very short time since no oxygen is delivered to the tissues and tissue oxygen stores are minimal. However, anoxia can be produced by perfusion with blood or any solution which is carrying no oxygen. The fundamental difference between ischemia and anoxia with perfusion is that, in the former, the end products of metabolism build up, whereas in the latter, they are washed away.

Clinically, the term "anoxia arrest of the heart" is frequently applied to total ischemic arrest of the heart. Therefore, it is useful to use the term "perfused anoxic arrest" to avoid ambiguity. In perfused anoxic arrest, glycolysis also fails due to inadequate ATP as a substrate in reactions leading to its own production. However, because the metabolic end products are washed out, intracellular acidosis is not marked and failure of ATP production is somewhat delayed.

This review of the biochemistry of cardiac ischemia points out the desirability of being able to measure intramyocardial extracellular carbon dioxide concentration because the carbon dioxide accumulation is linked directly to ATP production. Because the partial pressure of any gas in solution is directly proportional to its concentration, gas tension measurements can be viewed as essentially determinations of concentration.

### INTRAMYOCARDIAL CARBON DIOXIDE TENSION MEASUREMENTS

In order to measure the partial pressure of carbon dioxide ( $p\text{CO}_2$ ) within the myocardium during ischemic arrest we have used a mass spectrometer adapted for tissue measurements by means of a special sampling catheter. The basic elements of the measurement system are the sampling catheter, the mass spectrometer and a chart recorder.

Most important to an understanding of how the system works are the physical principles underlying the operation of the sampling catheter. Figure 3 shows the sampling catheter which consists of a stainless steel tube, approximately six feet in length, tipped by a Teflon diffusion membrane. The other end of the catheter, now shown here, is connected to the interior of the mass spectrometer which is maintained at a near vacuum ( $10^{-6}$  mm Hg). Gases can diffuse through the Teflon membrane into the diffusion chamber which is formed between the stainless steel tube and the Teflon membrane, and enter the stainless steel tube through its end hole and wall slots. However, liquids cannot pass across the Teflon membrane and therefore the catheter tip can be placed in tissue without interfering with tissue liquids. Steel is used as the catheter material because it is essential that no gases or liquids enter the catheter lumen between the tissue sampling site and the mass spectrometer. The diameter of the sampling catheter is about 1.4 millimeters and is small enough to insert into the wall of the heart without causing excessive damage to the myocardium.

For each gas diffusing through the Teflon membrane, the number of molecules which cross the membrane per second is determined by the pressure difference that drives that gas across the membrane, that is, the difference in partial pressure for that gas from one side of the membrane to the other. Because there is a vacuum on the inside of the membrane the partial pressure of all gases are zero within the diffusion chamber. Thus, the rate of flow for each gas that crosses the membrane is determined by its partial pressure in the tissue surrounding the catheter tip. It is by means of the mass spectrometer that these flow rates are measured separately for each gas.

The gas molecules arriving at the mass spectrometer are ionized and accelerated in an electromagnetic field which focuses ions of the same mass (44 daltons for  $\text{CO}_2$ ) on a target electrode to which they transfer their charge. The number of charges transferred per second equals the electrode current which in turn is proportional to the number of molecules of gas entering the mass spectrometer per second since it is the entering gas molecules that provide the charges. It has already been pointed out that the number of molecules of gas per second passing down the sampling catheter is proportional to the partial pressure of that gas in the tissue surrounding the catheter tip. Thus, the target electrode current, amplified for output to the recorder, is proportional to the partial pressure of an individual gas in tissue.

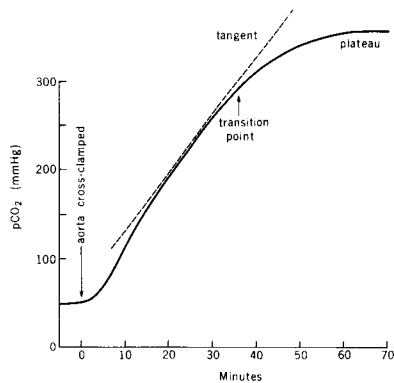


Fig. 4.

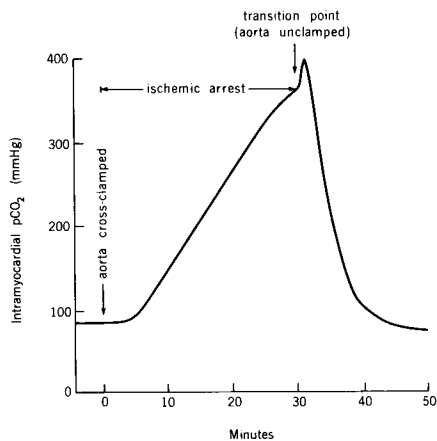


Fig. 5.

Figure 4. A typical carbon dioxide accumulation curve during ischemic cardiac arrest. Following cessation of coronary blood flow there is an early acceleration in anaerobic carbon dioxide generation followed by a period of sustained production at a constant rate. This is followed by a period of decline in the rate of rise of carbon dioxide tension until it is zero at which time the "plateau" of the carbon dioxide accumulation curve is reached. The "transition point", marking the onset of deterioration in carbon dioxide generation, is the point on the carbon dioxide accumulation profile where the curve falls below a tangent drawn through its linear portion.

Figure 5. If the aorta is unclamped at the transition point of the carbon dioxide accumulation curve, the intramyocardial carbon dioxide tension falls to normal and the heart can be easily resuscitated.

#### USING $pCO_2$ MEASUREMENTS TO DETERMINE THE SAFE LIMITS OF ISCHEMIC CARDIAC ARREST

A typical myocardial carbon dioxide accumulation curve during ischemic arrest of the heart is shown in Figure 4. Following aortic cross-clamping, which interrupts the coronary circulation, there is an early acceleration of anaerobic carbon dioxide generation and then a period of carbon dioxide accumulation at an essentially constant rate. This is followed by a period of decline in the rate of rise of carbon dioxide tension until it is zero and the "plateau" of the carbon dioxide accumulation curve is reached.

Since the rate of carbon dioxide accumulation is linked to the rate of ATP generation, the profile of carbon dioxide accumulation provides a continuous record of the heart's ability to continue to provide high-energy phosphate to maintain cellular viability. Our approach has been to use the information concerning ATP production contained in the carbon dioxide accumulation curve to develop a criterion for estimating a safe upper limit for the period of ischemic arrest which takes into account the metabolic capabilities of the individual heart.

A geometrical construction is included in the carbon dioxide accumulation curve shown in Figure 4. A tangent is drawn through the linear portion of the curve which corresponds to the sustained period of essentially constant carbon dioxide accumulation. The point at which the rate of carbon dioxide generation begins to deteriorate we term the "transition point" and is determined by noting where the carbon dioxide accumulation curve falls below a tangent drawn through its linear portion. We believe this transition point corresponds to the initiation of the rapid deterioration of ATP production, which, in turn, is determined by inadequate levels of ATP as a substrate in the reactions leading to its further generation. In the face of the widening energy supply-demand imbalance which occurs, the structural integrity of the myocardium cannot be long maintained.

We have recently carried out a series of experiments in which the transition point criterion for the safe termination of ischemic arrest of the heart was put to the test.<sup>7</sup> Dogs were placed on total cardiopulmonary bypass with venting of the left ventricle so that the heart was no longer performing significant mechanical work. The intramyocardial temperature was continuously monitored by an indwelling electronic thermometer and the circulating blood temperature was adjusted to keep intramyocardial temperature absolutely constant at either 38°C for normothermia (in the dog, normal body temperature is 1°C higher than in man) or 28°C for moderate hypothermia as required. Pump flow was adjusted to maintain a mean arterial pressure of 80 mm Hg to assure adequate coronary perfusion.

Under both normothermic and hypothermic conditions, when the ischemic arrest was terminated at the transition point of the carbon dioxide accumulation curve, the intramyocardial pCO<sub>2</sub> rapidly fell to normal (Figure 5) and all hearts could be easily resuscitated. Termination at the plateau of the carbon dioxide accumulation curve yielded very little decrease of intramyocardial pCO<sub>2</sub> and none of the hearts could be resuscitated. In normothermic hearts the transition point was reached after a mean of 30 minutes of ischemic arrest whereas moderate hypothermia approximately doubled the time to transition point, extending it to a mean of 57 minutes. In the normothermic hearts the plateau was reached after a mean of 66 minutes whereas moderate hypothermia extended the plateau to a mean of 126 minutes. There was a marked variation from heart to heart in both the range of times taken to reach the transition point (25 to 35 minutes at 38°C; 44 to 66 minutes at 28°C) and the range of carbon dioxide tensions at the transition point (260 to 355 mm Hg at 38°C; 154 to 374 mm Hg at 28°C). These observations support the contention that there is considerable variation in the metabolic response of the individual heart to ischemia.

Histological examination of hearts in which the ischemic arrest was terminated at the transition point of the carbon dioxide accumulation curve was essentially normal. In contrast, if the ischemic arrest was terminated at the plateau of the carbon dioxide accumulation curve, definite histological changes of early necrosis were observed, these being most marked in the inner one-third of the left ventricular wall.

The results of these experiments indicate that by monitoring intramyocardial carbon dioxide tension it is possible to determine when myocardial anaerobic metabolism begins to become inadequate to support continued cellular viability during ischemic cardiac arrest. Reperfusion of the coronary arteries at this time should allow recovery of myocardial function.

In the clinical setting, mass spectrometric measurements of the myocardial carbon dioxide tension during ischemic arrest potentially offer the surgeon sufficient information to reperfuse every patient's heart before irreversible damage occurs, even in the difficult case where the myocardial response to ischemic stress is poor. These measurements would document for the first time the considerable variation in metabolic response to ischemia we believe exists from one human heart to another. One would expect that pathological states such as left ventricular hypertrophy or dilatation and co-existing coronary artery disease would have a further impact on the variation in response to ischemia we have already demonstrated in our studies in normal dogs.

#### ISCHEMIC CONTRACTURE OF THE LEFT VENTRICLE

Ischemic contracture of the left ventricle may occur following prolonged periods of ischemic arrest of the heart. In this state fiber shortening is so extreme that there is

virtual obliteration of the left ventricular cavity and the left ventricle is rock-hard to palpation. Cooley and his co-workers<sup>8</sup> have descriptively termed this phenomenon the "stone heart". When a surgeon faces the problem of a stone heart, he is, with present knowledge, unable to obtain a beating left ventricle despite prolonged bypass support or any pharmacologic manipulation. Recently, hypothermia and beta-adrenergic receptor blockade have both been advocated as possible means of preventing or reversing this phenomenon.<sup>9</sup>

While ischemic contracture is an uncommon occurrence clinically (only 13 of 4,732 cases in the clinical review by Wukasch and colleagues<sup>10</sup>) we found that we could consistently produce it experimentally if we adhered to strict normothermic conditions and did not terminate the ischemic arrest until the plateau of the carbon dioxide accumulation curve was reached.

In every experiment in which the intramyocardial temperature has been kept constant at 38°C, severe left ventricular rigor has been observed after the carbon dioxide accumulation curve has reached its plateau. During these experiments it was observed that the left ventricle was very firm to palpation by the time the plateau of the carbon dioxide accumulation curve was reached and did not beat or fibrillate after reperfusion of the coronary arteries. Gross examination of the heart sliced transversely between base and apex revealed a markedly thickened left ventricular wall with blanching of its deeper layers and near obliteration of the left ventricular cavity. In contrast to the left ventricle, the free wall of the right ventricle was usually not contracted and right ventricular contractions could often be achieved by defibrillation.

Considerable controversy exists regarding the mechanism of ischemic contracture, but two suggestions have been put forward. The first is that contracture is due to an interaction between actin and myosin filaments resulting from low concentrations of ATP.<sup>11</sup> The second is that, under conditions of intracellular metabolic alkalosis, calcium may become bound to the proteins which regulate the contraction of cardiac muscle to produce a calcium-activated contracture.

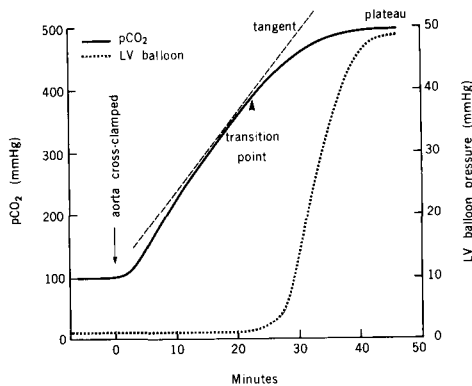


Fig. 6.

Figure 6. The onset of ischemic contracture, detected by a rise in hydrostatic pressure in a constant volume liquid-filled balloon filling the resting volume of the left ventricular cavity, is compared to the simultaneously monitored intramyocardial carbon dioxide accumulation curve.

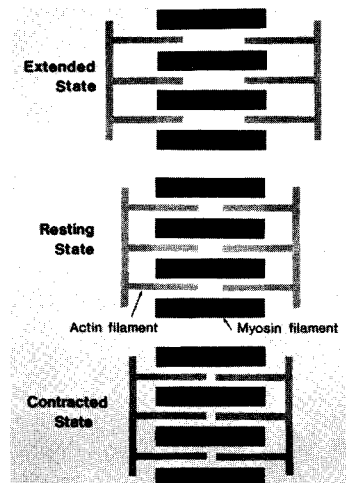


Fig. 7.

Figure 7. Muscle contraction occurs due to the sliding of thick (myosin) protein filaments along thin (actin) protein filaments.



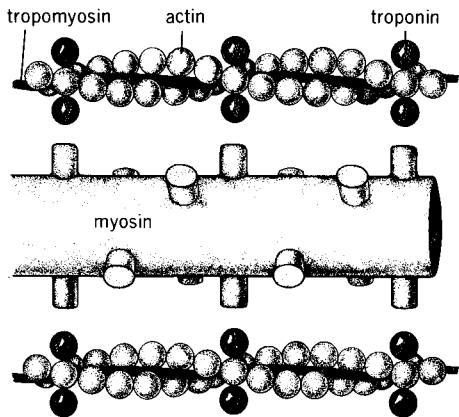


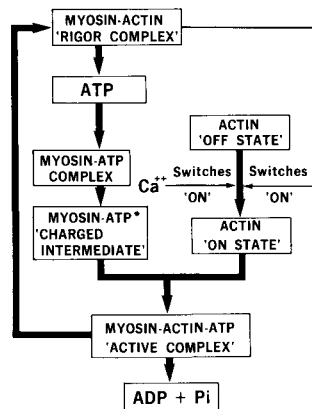
Fig. 8.

Figure 8. A schematic representation of an individual myosin filament and the two closest actin filaments in the same plane. The regulator proteins troponin and tropomyosin lie in the grooves of the twisted double stranded actin filament. The projecting heads of the myosin molecules which form the “crossbridges” are depicted, simplistically, as cylindrical projections.

Figure 9. In the presence of the regulator proteins tropomyosin and troponin, the actin and myosin filaments of vertebrate muscle interact as shown in this flowchart. Both calcium ions, under normal conditions, and “rigor complexes”, in the state of ATP depletion, “turn on” actin for contraction.

We believe that the balance of evidence favours the first hypothesis. Our measurements of intramyocardial carbon dioxide accumulation are consistent with the occurrence of contracture in the ATP depleted state. We have been able to determine precisely the initiation of contracture by measuring the hydrostatic pressure developed in a constant volume, liquid-filled balloon filling the resting volume of the left ventricular cavity. In Figure 6, the onset of contracture as determined by an initial increase in the balloon hydrostatic pressure is compared with the simultaneously monitored intramyocardial carbon dioxide accumulation curve. The increase in hydrostatic pressure, the earliest indication of rigor development, has been found to consistently occur at the transition point of the carbon dioxide accumulation curve.

The contraction of all muscle, whether skeletal or cardiac, is dependent on the interaction between the molecules actin and myosin arranged as thin and thick filaments respectively in an interdigitating fashion (Figure 7). A more detailed schematic view (Figure 8) of an individual myosin filament and the two closest actin filaments in the same plane illustrates the structural arrangements that govern the contraction process. The actin thin filament consists of globular monomers of action polymerized and twisted into a double stranded filament. Troponin, a regulator protein, is bound to a second regulator protein, tropomyosin with a periodicity of about seven actin monomer units. Tropomyosin is, in turn, bound to the stranded actin. The myosin thick filament consists of individual myosin molecules woven together. Each myosin molecule consists of a long tail attached to a globular head bent at about 45° to the axis of the tail. The core of the thick filament corresponds to the interwoven tails of the myosin molecules and the projections, shown schematically as cylinders in Figure 8, correspond to the globular heads which project out around the circumference of the thick filament spaced at 60° intervals. These are the “crossbridges” which, under certain conditions, can interact with the surrounding actin filaments, also spaced at 60° intervals around the thick filament, to cause contraction.



CONTRACTION: Interaction of muscle proteins, ATP and Calcium  
Fig. 9.

Under normal circumstances muscle contraction requires ATP, coupled with the myosin filament crossbridge, to form a myosin-ATP complex. This, in turn, spontaneously converts to a "charged" intermediate form (myosin-ATP\*) capable of binding with an actin molecule on the thin filament. The further addition of calcium ions, which "turn on" the actin filament, allow the formation of a myosin-actin-ATP complex termed the "active complex". The turning on of actin by calcium ions require the presence of the regulator proteins, troponin and tropomyosin. Troponin has an avid calcium binding site and it is through a change in conformation of troponin that occurs when calcium is bound that actin is allowed to interact with myosin. The role of tropomyosin is to carry the message of calcium binding along the actin monomer chain, thus turning on the actin. As soon as the active complex (myosin-actin-ATP) is formed, the ATP is hydrolyzed to ADP and phosphate which separate leaving a myosin-actin complex, the "rigor complex". Provided ATP is available, the myosin can separate from actin to reform a myosin-ATP complex and start again the cycle of crossbridge attachment and subsequent detachment that causes contraction.

In the special case of the ATP depleted state there is evidence that contraction takes place by a special mechanism. Under normal conditions virtually all of the myosin crossbridges are lined with ATP forming myosin-ATP complexes, but at low ATP concentrations myosin crossbridges are exposed with no linked ATP. The result is that rigor complexes (myosin-actin) form, not as a result of the removal of ATP from active complexes by hydrolysis as happens in the course of normal contraction, but because myosin crossbridges, in the absence of ATP, bind directly to actin filaments. This cannot be prevented by the regulator proteins troponin and tropomyosin and is independent of calcium ion concentration. The rigor complexes, in turn, are capable of turning on the actin filament, even in the absence of calcium ions. With actin turned on, the remaining myosin-ATP is hydrolyzed and then add to the number of rigor complexes (myosin-actin). The only way to reduce the mounting number of rigor complexes is by a supply of ATP to combine with them and replenish the available myosin-ATP complexes, in the process of which actin is released from the myosin crossbridges allowing relaxation.

The experimental evidence for the molecular control of muscle contraction as outlined above, for both normal and ATP depleted conditions, has been reviewed in detail by Murray and Weber.<sup>12,13</sup> In Figure 9 the cycle of muscle complex formation in contraction, valid for both normal and ATP depleted circumstances, is shown in flow chart format.

What is the role of calcium in the process of contracture? The formation of rigor complexes is certainly independent of the calcium ion concentration, but once formed, they would potentiate the effect on contraction of any calcium which is present. In the relaxed myocardium, calcium ions are removed from the vicinity of the actin and myosin filaments by the continual hydrolysis of ATP to drive a calcium "pump" in the membranes of the sarcoplasmic reticulum of the cell. Without ATP this pump must break down and calcium ion concentration must increase. Therefore, it is possible that calcium-activated contraction may play a role in rigor development. The balance between this process and the rigor-complex induced contracture is not yet clear. However, to the extent that calcium might play a role in contracture, it would be due to the effect of low ATP concentrations on the calcium pump and is included within the ATP depletion hypothesis.

The most obvious criticism of the alkalotic calcium-activated contracture hypothesis is that during prolonged ischemia intracellular acidosis, not alkalosis,

develops. It is this acidosis that is responsible for the accumulation of carbon dioxide, the basis of our measurements of the anaerobic state of the heart.

Regarding interventions to prevent the development of ischemic contracture we have observed that, under conditions of moderate generalized hypothermia (28°C), left ventricular contracture does not take place over the entire period during which anaerobic metabolism continues (until the plateau of the carbon dioxide accumulation curve is reached) nor after the release of the aortic clamp at the end of this period. We do not know the mechanism whereby hypothermia provides protection against contracture in this ATP depleted state. It is also possible that the protection is not absolute. Gott and his co-workers observed left ventricular contracture at 27°C after ischemic arrest prolonged to three hours.<sup>13</sup> However, since hemodynamic recovery is not possible when the plateau of the carbon dioxide accumulation curve is reached,<sup>7</sup> protection from contracture beyond this point has no practical significance. On the basis of our observations we recommend the use of moderate hypothermia clinically whenever prolonged periods of ischemic arrest are anticipated.

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