Fully developed acute myocardial infarction was recognized at autopsy throughout the 19th and 20th century. Furthermore, many clinicians (e.g., Osler) were excellent pathologists and often observed acute and healed myocardial infarcts (MI) in their patients after death. However, more often than not, the acute infarcts they observed did not show a thrombus in the coronary artery supplying the infarcted region of the heart. As a result, although the infarct was usually supplied by a particular coronary artery in the myocardium and the heart usually exhibited severe atherosclerotic coronary artery disease, there was enough variation from patient to patient so that the clinicians of this day were reluctant to attribute the myocardial necrosis to interruption of the blood supply. However, by 1880, several German experimentalists had occluded major coronary arteries in the canine heart and found easily identifiable acute MI 24 hours later. However, these data failed to convince most of the experimentalists and the clinicians that human infarcts were caused by an interruption in coronary blood flow.

The problem of causality in acute MI in the human heart was not resolved in absolute terms until 1980, when DeWood et al. showed that catheterized patients with early clinical signs of acute MI almost always had a thrombus obstructing the vessel supplying the ischemic region. Furthermore, lysis...
of the thrombus restored arterial flow and reversed many of the clinical and electrocardiographic signs of ongoing infarction. It seems likely that the explanation for the failure of the early workers to find a thrombus in hearts with a well-developed acute infarct was because of the action of endothelial fibrinolysins in the adjacent endothelium dissolving the thrombus that had caused the MI. Hence, although a coronary artery had to be obstructed to cause infarction, clots were observed irregularly at autopsy in patients dying ≥24 hours after the onset.

Establishing the pathogenesis of a human disease such as acute MI relying exclusively on the autopsy is difficult because the appearance of the disease changes with time and its premortem age is difficult to establish with precision. Also, postmortem autolysis can further complicate the analysis. Because autolysis and ischemia basically involve the same mechanisms and proceed quickly, metabolic studies on human cardiac autopsy tissue are impossible. The fact that patients do not die on a schedule that allows one to sequence the events of evolving acute infarction in a reasonable period of time further complicates the analysis.

However, studies of ischemia in the experimental animal provide a good reproducible means to learn the metabolic, physiological, histological, electric, and pathological changes found in myocardial ischemia. Some of these changes are reviewed in this article.

**Experimental Ischemia and Myocyte Death**

When I began working on MI in 1953, my main aim was to find when and why myocytes died when you made them ischemic. It seemed likely that they died much more quickly than the 12 plus hours suggested by human autopsy studies. Furthermore, I had no doubt that experimental infarcts were equivalent to human infarcts and that infarcts in humans were caused by interruption of coronary blood flow by thrombi.

To answer the when and why question in the experimental animal heart, one had to have a predictable area of ischemia that could be studied directly, although the tissue sampled looked perfectly normal to the naked eye. We found that occlusion of the circumflex artery in the dog heart produced ischemia in the posterolateral wall of the dog heart and that, if cyanosis was present in this region, the posterior papillary muscle always was ischemic.4

Using this posterior papillary muscle model, we showed, for example, that K+ is lost from the tissue after 60 to 90 minutes of ischemia.5 This shift, although fairly speedy, was slower than we thought it should be because a change in K+ content can be detected in an area of low or absent flow only after the K+ had left the intracellular water of the myocyte for the extracellular fluid (ECF) and then diffused from the ischemic focus to the circulating blood. Using modern electrophysiological techniques with ion electrodes implanted in the ECF of an ischemic myocyte in the pig heart, Gettes and Cascio6 have shown this change on a continuous time scale. The K+ rises from 4 to 12 mmol/L in the ECF during the first few minutes of ischemia and then plateaus at this level for ≈50 minutes before it begins to rise exponentially as myocytes begin to die. This is at about the same time that our direct measurements showed K+ to be decreasing. Shortly before we reported the K+ data, Ladue and Wroblewski7 reported that intracellular enzymes appeared in the general circulation during MI but on a slightly slower time scale than the K+ loss. We confirmed this finding and showed that you indeed could measure a decrease in enzyme activity in the ischemic focus at about the same time that the activity of the enzyme in the serum was increasing.8 Again, diffusion surely slowed this change. The smallest enzyme studied was glutamic oxaloacetic transaminase, and it disappeared from the tissue faster than a larger enzyme such as lactate dehydrogenase, whereas the activity of succinic dehydrogenase, an enzyme that was localized in the mitochondrial cristae, changed much more slowly than the soluble enzymes. Furthermore, it did not appear in an active form in the serum. As a final step in this series of experiments, we showed that the capacity of homogenates of dying tissue to metabolize glucose to CO2 and water was inhibited greatly beginning 30 minutes after the onset of ischemia.9 This finding reinforced our belief that subendocardial myocytes were badly injured and dying early in the course of severe ischemia.

**Timing of Myocyte Death: Definition of Reversible and Irreversible Injury**

To ascertain what structural or functional change or changes caused the death of ischemic myocytes, one had to know when death occurred. A single experiment answered this question.10 We occluded the circumflex artery of the dog heart for periods of five minutes to two hours of duration and then reperfused the tissue with arterial blood to eliminate the cause of injury and thereby to return any surviving myocytes to a healthy functional state. In these experiments, it was easy to ascertain which myocytes were dead because the dead myocytes developed an unexpectedly dramatic form of disrupted architecture. They swelled markedly, developed prominent contraction bands, and showed large tracts of membrane disruption, as well as calcification in the mitochondria. This was termed contraction band necrosis (CBN) by Ganote11 and was well developed within 120 seconds of the onset of reperfusion with arterial blood.12 However, to be certain that myocyte death had occurred, we allowed some animals to survive for several months to confirm that the cells undergoing CBN were dead by showing that they were replaced by scar tissue. Thus, there was no doubt that the myocytes had died, although the pattern...
of cell death after reperfusion with arterial blood was altered greatly from that seen in permanently ischemic myocytes.

The results of these experiments showed 2 kinds of injury in the severely ischemic subendocardial myocardium. All myocytes survived if the episode of ischemia was \( \leq 15 \) minutes. This was termed reversible injury. However, increasing amounts of necrosis developed as the episode of ischemia was prolonged until, at 60 minutes, most of the subendocardial portion of the ischemic bed was dead. This was termed irreversible injury. Some 15 years later,\(^{11}\) using 10-μm radioactive microspheres to measure flow, we quantified blood flow to the subendocardial myocardium used in this model and showed that it was severely ischemic with arterial collateral flows from 0.0 to 0.09 mL/min per gram wet weight compared with the 1 to 2 mL/min per gram wet weight found in control myocardium. Once we had the timing of cell death in myocytes established, the why question was then refined to what change or changes occurring during or at the end of the episode of reversible ischemia led to the death of severely ischemic myocytes?

The primary measure of irreversibility in these experiments was the loss of structural integrity of the myocyte followed by its replacement with scar tissue. Derivative measures of cell death, such as enzyme loss, absence of oxidative metabolism, and structural disintegration by electron microscopy, are utilisable measures of cell death as well but were not used to define irreversibility in our original studies. In the final analysis, the myocyte is irreversibly injured when it fails to survive when its environment is restored to normal.

In addition to timing cell death, the results of these experiments clearly showed that ischemic myocytes were alive throughout much of the early phase of ischemia and could be salvaged by restoring arterial flow, a procedure now widely and successfully used to treat evolving acute MI. As the use of this treatment increased, it became important to ascertain where the salvageable myocytes were located. A series of experiments were performed by Reimer and Jennings\(^{14}\) on the timing and location of ischemic myocyte death in dog heart. We found that there was a transmural gradient of ischemia such that the inner layer of the heart (subendocardial myocardium) received the least flow and died first and the outer layer (subepicardial myocardium) received the most flow and often survived. If the flow was \( \geq 0.3 \) mL/min per gram wet weight in any layer, the myocardium generally survived. Thus, the decision as to life or death was related primarily to the rate of arterial collateral flow, whereas the eventual size of an MI depends primarily on the size of the arterial bed together with the volume of arterial flow delivered to this bed. The general parameters involved in survival or death in the canine heart are shown in Figure 1\(^{14}\) and demonstrate that cell death occurs quite quickly in hearts with severe transmural ischemia and eventually involves much or all of the circumflex bed, whereas cell death develops more slowly and involves much less of the ischemic bed in hearts with milder degrees of ischemia. In any event, after 6 hours of ischemia have passed, essentially no salvage occurs with reperfusion in this model. Thus, successful reperfusion therapy must be performed quickly if salvage is to be achieved.

**Role of No-Reflow**

Myocyte death is complicated in zones of severe ischemia by failure of reperfusion, the so-called no-reflow phenomenon.\(^{17}\) This is caused by the loss of the integrity of the microvasculature secondary to the effect of severe ischemia on the endothelial cells of the capillaries (Figure 2). The endothelial cells of the capillaries die on a slower time scale than myocytes, reflecting their lower energy requirements to maintain structural integrity. Areas of no-reflow prevent arterial blood and pharmacological agents from entering the ischemic region, and the tissue is unsalvageable by any therapy now available. Before reperfusion, the capillary endothelium is swollen and

![Figure 1. Mean % necrosis in the circumflex bed of the dog heart in groups of dogs subjected to 15 to 360 minutes of ischemia. Using radioactive microspheres,\(^{13}\) mean transmural collateral arterial flow was measured after 10 minutes of ischemia and infarct size was estimated by the technique of Reimer et al\(^{16}\) after the required period of ischemia had elapsed. In the analysis presented, low flow is defined as flows from 0 to 0.09 mL/min per gram wet weight, and high flow is a flow of \( >0.1 \) mL/min per gram wet weight. Animals with flows of \( >0.3 \) mL/min per gram wet weight showed little or no necrosis and were eliminated from consideration. At low flow rates, necrosis develops more quickly than at higher flow rates, and the average size of the infarct usually is greater. At higher rates of collateral flow, necrosis develops more slowly, and the resultant infarct usually is smaller. Note that necrosis is fully developed at 6 hours, and little or no salvage is possible after this period of time. Note also that the difference in the % necrosis between 3 and 6 hours of ischemia is small. Not shown in this figure is the fact that the mean % necrosis observed in all hearts in the original article after 4 days of reperfusion was 79±2.5% of the area of risk with a range of 64% to 96%,\(^{16}\) that is, virtually the same % necrosis as that seen at 6 hours in the low-flow ischemia group. This graph is reproduced from Jennings,\(^{16}\) with the permission of the publisher. © 2011 Sage Publications Inc. and the details of the sources used to provide the additional data used in this figure are given in the legend to Figure 1 in this journal.\(^{17}\) The percent of necrosis number can be changed to percent salvage by subtracting it from the average percent necrosis observed in each group at 6 or 96 hours (the 96-hour figure for all hearts in the original article is available in Reimer et al\(^{14}\) and Jennings et al\(^{15}\)).
vacuolated in this region, whereas the myocytes show the changes of irreversible injury.

As much as one third of the severely ischemic subendocardial myocardium may exhibit no-reflow. It seems unlikely that no-reflow extends into severely ischemic tissue in the subepicardial myocardium, because after reperfusion such tissue exhibits CBN. However, we have never tested this conclusion. It is of interest that no-reflow develops in the isolated perfused rat heart when it is subjected to total ischemia because of the development of contracture.18

Cause or Causes of Myocyte Death
The most likely cause of myocyte death during severe ischemia is disruption of the cell membrane or sarcolemma (SL).19–24 This allows intracellular components necessary for life to leak into the ECF and disrupts energy metabolism (Figure 3). The exact cause of the loss of membrane integrity remains unknown. It is associated with the loss of vinculin from the attachment complexes.20 In addition, there are some striking defects in energy metabolism, such as very low levels of high-energy phosphates (HEP) and destruction of the adenine nucleotide pool that occur simultaneously with the sarcolemmal changes.21–24 The ultrastructural and functional evidence for SL disruption is reviewed below.

Ultrastructural Changes
Myocytes that have just entered the irreversible state exhibit small breaks in the plasmalemma of the sarcolemma (Figure 3B–3D), plus cell swelling with occasional subsarcolemmal blebs of edema fluid (Figure 3D).19–24 For such blebs to form, the attachment complexes at the Z band have to break.20 Sarcolemmal disruption is especially common at these sites. Direct evidence of SL disruption also is provided by the use of fluorescent spheres containing anti-myosin antibodies25 or by intravenous injection of anti-myosin antibodies when the tissue is reperfused after 1 hour of ischemia.26 The spheres accumulate in the damaged myocardium by binding to the SL of irreversibly injured myocytes, and labeled antibody accumulates on the same time frame as the development of cell death (ie, at 30–60 minutes), whereas control myocytes show no binding of the antibody because the SL is intact and prevents antibody from reaching the myosin of the myofilaments. The myocytes also show peripheral aggregation of the nuclear chromatin, contain much less glycogen than control myocytes, and show disruption of mitochondrial architecture. The mitochondria are swollen and exhibit disorganized cristae.21,24 In addition, the matrix space contains fuzzy osmiophilic densities composed primarily of lipid that are termed amorphous matrix densities (Figure 2–4). These are present in all mitochondrial profiles and are characteristic of cell death in the ischemic heart. Furthermore, the changes seen in a fully developed 24-hour-old infarct are identical to those seen after 40 to 60 minutes of ischemia but are more marked (Figure 4).

Reperfusion of irreversibly injured myocytes is associated with marked reactive hyperemia. The previously ischemic cyanotic tissue develops a prominent blush because of a 4× to 5× increase in arterial flow. The reoxygenation of the tissue results almost instantly in CBN (Figure 5). Contraction bands, hydroxyapatite in mitochondria,12,17,27 and edema (Figure 6)28 are well developed after only 2 minutes of reperfusion.23 The total tissue water increases by 21% after 2 minutes and by 43% after 20 minutes of reperfusion.28,29 The 3 most dramatic ultrastructural changes seen in reperfused irreversibly injured

![Figure 2. A capillary in an area of no-reflow in myocardium that was ischemic for 90 minutes before being exposed to 10 to 12 seconds of unsuccessful reperfusion.](image-url)
tissue are, first, massive cell swelling, with large subsarcolemmal blebs of edema fluid (Figure 6) covered with the remains of a disrupted plasmalemma. Second, large contraction bands in which 3 to 10 or more sarcomeres are condensed such that the Z bands are close to each other (Figure 5). The subsarcolemmal blebs are generally located between and not over the contraction bands. The third change involves the mitochondria, which characteristically have granular densities of hydroxyapatite within them, as well as the amorphous matrix densities (AMDs) that were present before reflow (Figure 5). The ECF presumably enters the myocytes through the previously described defects in the sarcolemma and carries Ca into the myocytes where it causes massive contraction and accumulates within the mitochondria as hydroxyapatite. The increased Ca\(^{2+}\) in the myocytes originates from Ca\(^{2+}\) in the plasma reperfusing the tissue.

Reperfusion of myocytes late in the phase of reversible injury results in striking but transient changes in ultrastructure rather than contraction bands or mitochondrial Ca accumulation. All of the mitochondria swell extensively at reflow, but only 20 minutes later, the architecture of the mitochondria and the myocytes is intact and virtually indistinguishable from that of control myocytes. After 24 hours of reperfusion, widely scattered condensed mitochondria with AMDs are the only monument to the earlier episode of ischemia.

**Functional Evidence of SL Disruption: Changes in the Inulin-Diffusible Space**

Functional evidence of loss of membrane integrity is provided by study of the function of thin freehand slices of control and damaged myocardium incubated in oxygenated Krebs–Ringer phosphate containing radioactive inulin. Inulin is an inert carbohydrate with a molecular weight of 5000 kDa, which is excluded from the intracellular water of living cells. Control
slices exclude inulin just as normal living myocardium excludes it, whereas slices prepared from irreversibly injured tissue show marked increases in inulin in the intracellular water of the irreversibly injured myocyte because of the loss of integrity of SL.32,33

Metabolic Changes
Experimental studies show that metabolic changes begin to develop within a second or 2 of the cessation of flow and leave few easily detectable permanent monuments in the heart during the first 10 to 15 minutes of ischemia. For example, electrocardiographic changes develop within 10 seconds of the onset of ischemia as does cyanosis, and if a colored artificial electron acceptor such as methylene blue is present in the tissue, the blue myocardium becomes red after 10 to 18 seconds of ischemia because methylene blue is reduced to colorless leukomethylene blue.34 Contractile force diminishes quickly and ceases within 60 seconds,35 but electric stimuli continue to enter the ischemic focus. Supplies of reserve HEP in the form of creatine phosphate are exhausted within 60 seconds.36 and anaerobic glycolysis (AG) using glucose from glycogen as substrate becomes the primary source of new HEP.37 The main end product of AG is lactate, and this metabolite accumulates quickly during the first 60 to 120 seconds of ischemia. Then the rate of AG slows. Glucose-6-phosphate, α-glycerophosphate, and glucose from glycogen breakdown all accumulate but in smaller amounts than lactate.38 This increase in intracellular small particles, such as lactate and inorganic phosphate, increases the osmolality of the intracellular water.39

The tissue becomes acidic because of the accumulation of lactate. The pH falls to ≈6.6 during the first 10 minutes and equilibrates at 5.8 after 50 minutes of ischemia.40 ATP is consumed at a much faster rate than it is produced. Most of the consumption occurs in the mitochondria via the action of the mitochondrial ATPase.39,40 Because of ATP utilization, the concentration of ADP rises. The HEP of ADP is captured by adenylate kinase which converts ADP to ATP and AMP. The ATP is used and the AMP is converted to adenosine (ADO) by 5′ nucleotidase.38,41 The ADO diffuses into the general circulation. As a result of this degradation, the adenine nucleotide pool ($\Sigma$Ad) becomes much smaller. The ATP of the tissue drops to <2.0 μmole/g dry weight after an hour of severe ischemia versus the 25 to 27 μmole/g dry weight in control myocardium. The low ATP plus low levels of $\Sigma$Ad is highly associated with myocyte death in ischemia.38 The fact that myocytes die at an accelerated rate if AG is totally inhibited by iodoacetate, although no lactate is produced in the poisoned tissue, suggests acidosis is not the primary mediator of cell death42 and further supports the idea that HEP is critical in maintaining the viability of the damaged myocytes.

The reaction or reactions requiring ATP that is deficient and directly or indirectly causes irreversibility to develop have not been identified. Because the mitochondria are critical to the function of aerobic myocyte and because the mitochondria clearly function aerobically in salvaged reversibly injured myocardium, it is clear that the function of mitochondria can be restored if a substantial amount of $\Sigma$Ad pool is present in the injured tissue. However, when the pool is down...
to 25% to 30% of control, salvage is difficult or impossible. Loss of phosphorylation required to keep the cytoskeletal–sarcolemmal attachment complexes intact is a possible cause of membrane disruption. However, indirect tests of this idea using the model of total ischemia in vitro with canine myocardium treated with iodoacetic acid to inhibit AG do not directly support it. After only 5 minutes of ischemia, the ATP of iodoacetic acid–treated acontractile myocardium reached virtually zero, but the inulin-diffusible space and ultrastructural changes of irreversibility developed at the same rate in quiescent iodoacetic acid–treated myocardium as it did in quiescent control myocardium.42 Similar results were obtained in an earlier study of the changes seen in tissue slices prepared from tissue damaged by total ischemia in vitro.41 However, in the living animal, initiating ischemia in the absence of AG induced low levels of ATP and accelerated cell death.44 This suggests that the trauma of contraction of adjacent normal tissue is required to disrupt the SL on the quick time scale seen in in vivo ischemia.

A detailed study by Vander Heide and Ganote45 of the effects of anoxia on the isolated rat heart made anoxic by perfusing it with anoxic media shows exactly the same response of the SL to trauma caused by distention of the cavity of the left ventricle or contraction as do the studies with iodoacetic acid or total ischemia.42–44 No disruption of the myocyte damaged by prolonged anoxia at 37°C occurs until the damaged tissue is reperfused with an oxygenated solution in order to restart contraction of the viable tissue or is distended while still anoxic by inflating a balloon in the cavity of the left ventricle.45 Furthermore, Buckberg and colleagues46–47 have shown that reperfusion for 20 to 30 minutes with hyperkalemic blood prevents contraction and reduces myocyte death during totally vented bypass in the canine heart. Thus, several lines of evidence indicate that movement is deleterious to ischemic myocytes. The main benefit of postconditioning, which is described in the next section, also may be the result of allowing perfusion without allowing contraction. The results of these studies taken together clearly show that the injury produced by ischemia or anoxia becomes obvious much more quickly in the living contracting heart than it is does in arrested motionless hearts.

Preconditioning and Postconditioning

If one exposes the mammalian heart to ≥1 brief episodes of ischemia and reperfusion, the myocardium is protected from the effects of a prolonged episode of ischemia that is adequate to kill large numbers of myocytes. This is termed ischemic preconditioning (PC) and is the strongest protective effect to date identified.48 This effect was discovered during the course of experiments designed to ascertain the relationship of ATP depletion and acidosis in causing myocyte death. To test this relationship, we exposed myocardium to 4 cycles of 10 minutes of ischemia and 10 minutes of reperfusion, with the aim of almost completely depleting ATP in only 2 cycles of ischemia49 while projecting that lactate would continue to accumulate at the rate observed during the first episode of ischemia. The first 10-minute cycle was known to deplete the ATP from ≈27 to 10 µmol/g dry weight for a loss of 17 µmole of ATP. Reperfusion with arterial blood restored ATP to 17 µmole via rephosphorylation of the ADP and AMP that had accumulated in the tissue during the episode of ischemia. We predicted that a second episode of ischemia would decrease the ATP by another 17 µmole, leaving 0 ATP in the tissue. However, at the conclusion of the second episode of ischemia, the ATP only had fallen to 10 µmole ATP/g. Again, it was restored to 17 µmole after reperfusion. It fell from 17 to 10 µmole during the third and fourth episodes as well leaving 10 µmole of ATP in the tissue at the end of 40 cumulative minutes of ischemia. In addition, the second episode produced only ≈70% as much lactate as the first episode. This depressed lactate production persisted throughout each repeated episode of ischemia. Because we could give a cumulative 40 minutes of ischemia without depleting the ATP to the low level found in irreversibly injured myocytes after 40 continuous minutes of ischemia,48 we projected that we could prevent cell death by pretreatment with ischemia. This hypothesis proved to be correct. Pretreatment with 4 brief episodes of ischemia before the test episode of ischemia delayed cell death to a remarkable extent,49 a finding confirmed by many investigators. However, it did not permanently prevent myocyte death.48 As expected, PC preserved both ATP and the ΣAd pool and reduced the rate of AG as measured by lactate production50 or by measurement of pH.51 Preservation of ATP in the face of less AG, that is, of less activity of the primary source of new HEP in ischemic myocardium, was paradoxical and suggested that the mechanism underlying this protection would be difficult to establish. This has proved to be true.

It should be noted that the measure of effect in ischemic PC is prevention of cell death. Surrogate measures of cell death are risky end points and can be used only if they are confirmed to be effective measures of death.55

ADO Hypothesis

The mechanism underlying PC has been under study for ≈25 years and has not been fully elucidated. One of the most striking features of the PC state was discovered by Downey and colleagues,52 who hypothesized that the ADO produced during the first episode of preconditioning ischemia caused PC. They showed that pretreatment of the rabbit heart with an inhibitor of ADO binding to its receptor on the SL prevented the development of PC and that ADO could precondition in the absence of ischemia. The signaling cascade that results from ADO binding to its receptors has been studied in detail, but the findings provide no satisfactory explanation of how the changes wrought by the signaling molecules produced with the ADO stimulus prevent the death of ischemic myocytes.52,53

Diazoxide

An extensive amount of work has shown that the ATP-sensitive potassium channel may be involved in the protective effect brought about by PC. This K channel is present on both the SL and the mitochondria. Diazoxide blocks the mitochondrial KATPase and can be used to pharmacologically precondition both rodent and large animal hearts without the use of ischemia.54–57 An interesting aspect of this phenomenon is that dog hearts pretreated with diazoxide and exhibiting the PC state, when subjected to a test episode of ischemia, exhibit the same metabolic changes as hearts preconditioned with
ischemia. ATP is preserved, and less lactate is produced. This finding is as estimated by depressed lactate production. This finding is strong evidence that ATP hypothesis is involved in myocyte death, in that negative metabolic results with diazoxide would have been evidence that the metabolic effects of PC can be disassociated from its protective effects.

Pain et al have provided evidence indicating that O₂-derived free radicals may be involved in the genesis of the PC state, an idea initially proposed by Murry et al. Because O₂-derived free radicals are generated in the mitochondria, free radicals could be a cause of the changed function of the mitochondria of the PC heart.

**Postconditioning**

Vinten-Johansen and colleagues provided evidence of the existence of a new form of protection against ischemic cell death termed postconditioning (post C). They showed that protection equivalent to that afforded by ischemic PC could be achieved in the dog heart by altering the conditions of reperfusion. Instead of the abrupt onset of full reperfusion, they gave several brief episodes of reflow each followed by reocclusion. After this stuttering reperfusion, the hearts were given full unrestrained reflow. The resultant protection was unexpected and not entirely reproducible, in that 2 major laboratories have been unable to successfully post C. However, several other laboratories have been successful. This suggests that post C is protocol dependent, whereas PC can be induced with wide variations in the experimental protocol.

A simple explanation for postconditioning would be that the transient episodes of reperfusion provide brief episodes of aerobic metabolism that protect the damaged myocyte by allowing energy production sufficient to repair the SL without allowing any or enough Ca²⁺ entry to cause marked enough contraction to rupture the SL. Delayed recovery from acidosis because of transient reflow also may contribute to the beneficial effect of post C.

Small animal studies of post C, as well as some studies in humans, have suggested that the beneficial effect of post C is mediated by preventing the opening of the mitochondrial permeability transition pore (MTP). This pore is closed during ischemia, and when it opens during early reperfusion, myocyte disintegration rapidly ensues. It is of interest that pretreatment with cyclosporine also slows or prevents the opening of the MTP in the hearts of rats and rabbits. The drug is reported to be as effective as post C in preventing myocyte death in ischemia/reperfusion. Thus, cyclosporine theoretically keeps the mitochondria of badly damaged reversibly injured myocytes sufficiently intact to function after reperfusion.

The implication of the cyclosporine studies is that some myocytes, presumably those dying on the periphery of the developing area of irreversible injury, are damaged by ischemia but are reversibly injured in that they are salvageable by post C reperfusion techniques or by successful treatment with cyclosporine before reperfusion, whereas those myocytes that already are irreversibly injured develop CBN when reperfused. The hypothetical explanation of the post C effect is that the reversibly damaged myocytes that are intact before reperfusion and do not allow the MTP to open continue to survive but die if the MTP opens during unrestricted reperfusion.

Pretreatment with cyclosporine allows the drug to reach all myocytes in the myocardium destined to become ischemic, where it can prevent the opening of the MTP and thereby prevent some myocyte death during a test episode of ischemia. Administration of cyclosporine after the artery has been occluded probably restricts the action of the drug to the reversibly injured myocytes on the periphery of the advancing zone of irreversibility. However, cyclosporine has been reported to be effective in a perfused rat heart with an occluded coronary artery if the drug is given at the time of reperfusion. Ovize and colleagues have interesting preliminary evidence from a trial experiment indicating that it also might be effective in humans if given 10 minutes before reperfusion. However, these data are from a small trial in which reperfusion was initiated as long as 12 hours after the onset of pain, an elapsed time that far exceeds the period of ischemia required to kill all the myocytes at risk in a canine heart. Note the variation in infarct size with time in Figure 1 and that most of the myocytes at risk of death are dead after 3 hours of ischemia. Because it seems likely that these canine data are generally transferable to humans and because it is difficult to identify patients to treat after <3 hours of ischemia, a significant number of patients in this study likely had infarcts that were 6 to 12 hours old. At this age, few or no myocytes would be available to salvage, suggesting that the positive results are spurious because of a failure of experiment design rather than drug effects. Finally, it is impossible to measure the myocardium at risk directly in the heart of a living patient, which means that it is difficult to answer the critical question in this type of experiment, which is How big would the infarct have been in the absence of treatment? The fact is that if you do not know the answer to this question, you cannot tell whether the treatment was effective.

As noted earlier, post C demonstrates that the abrupt onset of reperfusion kills myocytes in the area of injury that will survive if the more gradual post C reperfusion technique is used. Because it seems certain that Ca²⁺ entry causes CBN and because the entry presumably comes through defects in the SL or by the inaction of a Ca transporter in the SL, one hypothetical explanation of the post C effect would be that the initial brief periods of reperfusion of post C allow sufficient SL repair to prevent Ca²⁺ entry or that increased sarcoplasmic ATP secondary to brief episodes of aerobic mitochondrial function allows excess Ca²⁺ to be pumped out before the Ca²⁺ can cause irreparable injury by causing massive contraction and inhibition of oxidative phosphorylation. The work of Buckberg and colleagues on ischemia in dogs in which the circulation was supported by pump-bypass presents a novel view of the role of contraction in causing irreversible injury. Myocytes in an area of ischemia produced by occlusion of a major artery in these hearts were reported to survive much longer if contraction was prevented by reperfusing the ischemic bed for 20
to 30 minutes with hyperkalemic arterial blood. This allowed aerobic metabolism to proceed but prevented contraction and allowed the damaged myocytes to recover. Prevention of contraction while allowing repair via new energy release may be a critical feature of how post C protects. However, because of the high Ca²⁺ of plasma, it is likely that Ca²⁺ entry through SL defects is irreparable when these defects allow enough of the high-plasma Ca²⁺ to enter the myocytes and cause contraction bands and cellular disruption. In this system, if massive entry occurs, the myocytes are disrupted to an enormous extent, and it makes no difference what the MTP does. It follows that the only salvageable myocytes are those that have not allowed massive Ca entry and that, although injured, have not opened the MTP.

The relationship between MTP opening as a potential cause of cell death in ischemia/reperfusion and the large body of evidence indicating that SL disruption and ATP depletion are the critical events leading to irreversible injury is difficult to discern at this point in time except to observe that because the MTP does not open during myocardial ischemia, cell death in permanent ischemia must be unrelated to opening the MTP. Furthermore, investigation of the role mitochondria play in the death of myocytes in areas of permanent ischemia indicates that they are involved in causing irreversibility but do not directly cause cell death. From the functional point of view, the mitochondria show no major defects during the reversible phase and maintain their ultrastructure as well. However, mitochondrial injury is important during the reversible and early irreversible phases in that ≈50% of the ΣAd pool of the myocyte is destroyed during ischemia via the action of the mitochondrial ATPase. During either in vivo or total ischemia, the action of the ATPase can be slowed greatly by inhibiting it with oligomycin. However, oligomycin treatment only slows the transition to irreversibility, it does not prevent it. Thus, the use of ATP by the ATPase is a critical factor in the speed with which irreversibility develops in severe ischemia. However, ischemic myocytes still die in hearts in which the ATPase has been inhibited because ATP is used and destroyed by other ATP-requiring reactions without replacement with new ATP.

The relationship between myocyte death caused by opening of the MTP in ischemic myocytes after reperfusion and the changes of CBN is unknown. The biology of CBN is well understood. Reperfusion of myocytes early in the phase of irreversible injury with low ATP and SL defects results in massive Ca²⁺ entry from the plasma reperfusing the tissue, enormous cell swelling, contraction bands, and mitochondrial hydroxyapatite accumulation. The mitochondria of the myocytes still can function in that they accumulate Ca²⁺ from the plasma in the form of calcium phosphate, a process that requires mitochondrial integrity and function. Thus, the mitochondria of myocytes reperfused early in the phase of irreversible injury exhibit significant energy-linked function manifest by the accumulation and deposition of calcium phosphate. After reperfusion, such mitochondria could open the MTP, thereby releasing some of the accumulated Ca²⁺ and accelerating cellular disintegration. Note, however, that after 90 minutes of ischemia, this situation no longer exists. At reperfusion, CBN develops but there is no accumulation of mitochondrial calcium phosphate. Opening of the MTP of the mitochondria of these myocytes at 90 minutes presumably would have no effect because the mitochondria are no longer functioning. In any event, it seems that there is a group of myocytes, presumably on the periphery of the zones of marked damage, that die by opening the MTP at reperfusion, but the issue is clouded by changes that occur after cellular disruption that easily can obscure the initiating events.

How the cyclosporine observations relate to PC or post C has not been established. A likely explanation may be that prevention of the opening of MTP may contribute to both these forms of protection. However, the relationship of pore formation to the deposition of mitochondrial hydroxyapatite, contraction, and contraction band formation is largely unknown and is likely to be quite complicated.

Mitochondrial Isolation From Damaged Tissue

Because the problem of ascertaining the cause of cell death during reperfusion of ischemic tissue may involve isolating mitochondria from such tissue, it is important to note a technical problem involving isolation of mitochondria from myocardium damaged by ischemia. Mitochondria are very fragile in the early phase of irreversible injury. Thus, standard techniques that work well in the isolation of mitochondria from healthy myocardium cause much mitochondrial fragmentation when used to isolate mitochondria from myocardium on the verge of cell death. Instead of isolating fragile swollen mitochondria from the badly injured cells, one isolates the more intact mitochondria found in less injured or normal myocytes. However, there are ways available to isolate the fragile mitochondria. Also, the isolates used for analysis can be assessed easily by electron microscopy, which will show, first, whether the investigator has indeed isolated intact mitochondria, and second, using AMIs as a marker, the investigator can ascertain whether the mitochondria have come from reversibly injured myocytes. Intact mitochondria containing AMs are isolated from canine heart irreversibly injured by 60 minutes of ischemia cannot perform integrated Krebs cycle metabolism. In an early study, we found that fragmented mitochondria function poorly, if at all. Figure 13 by Jennings et al shows fragmented mitochondria in an isolate prepared from canine myocardium damaged by 120 minutes of ischemia. Note that few intact mitochondria are present and that these contain the AMs of irreversible injury but that most of the isolate is fragmented mitochondria although the best technique available to isolate intact mitochondria was used. Phase contrast microscopy is helpful and will reveal whether the mitochondria is isolated chiefly. However, only electron microscopy will show whether the mitochondria have come from irreversibly injured myocytes, that is, whether they contain AMs.

Reperfusion Injury

Although the results in Figure 1 clearly show that reperfusion with arterial blood salvages large numbers of damaged myocytes, it has been proposed that reperfusion itself can injure myocytes. One example of reperfusion injury has been established in unequivocal terms. This is the phenomenon of stunning. Vatner and colleagues described stunning in the dog heart in 1975. They showed that myocardium reversibly
injured by ischemia does not contract as efficiently as control myocardium after reperfusion. In a series of elegant, well-executed experiments, Bolli et al.\(^{75,76}\) have shown that stunning results from \(O_2\)-derived free radicals impacting some portion of the contractile apparatus. In fact, stunning can be prevented totally by pretreatment with a free radical scavenger before or immediately at the time of reperfusion with arterial blood. Thus, free radicals clearly damage the myocytes and cause nonlethal reperfusion injury.\(^{75,76}\)

There are preliminary data suggesting that free radicals may cause PC as well\(^{58,59}\) by altering mitochondrial function. Bolli et al.\(^{75,76}\) have shown that stunning can cause lethal reperfusion injury.\(^{75,76}\)

It has been proposed that reperfusion with arterial blood itself can cause lethal reperfusion injury in ischemic myocytes.\(^{77}\) A significant number of findings presented earlier in this brief review suggest that many of the changes that develop during ischemia are themselves irreversible. However, the phenomenon of postconditioning suggests that one can prevent irreversible injury in some myocytes in the zone of ischemia by using several brief episodes of reperfusion prior to restoring full reperfusion. If true, I would suspect that the myocytes susceptible to lethal reperfusion injury would be on the periphery of the zone of irreversible ischemic injury, but, at the present time, there is no way available to test this hypothesis. One of the great difficulties facing scientists interested in studying myocyte salvage in post-C is the difficulty of objectively identifying the exact myocytes that are at risk before treatment and reperfusion. Such identification is required if one is going to prove both that lethal reperfusion injury exists and to establish what causes it. In any event, although proof of the existence of lethal reperfusion injury has been elusive, investigation of this concept has led to significant new insights into the nature of the changes occurring during reperfusion of myocardium damaged by ischemia.

**Sources of Funding**

This work was supported by a series of grants from the National Heart, Lung, and Blood Institute. The final grants were HL23138 and HL27416.

**Disclosures**

None.

**References**


Historical Perspective on the Pathology of Myocardial Ischemia/Reperfusion Injury
Robert B. Jennings

doi: 10.1161/CIRCRESAHA.113.300987

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/113/4/428

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/