Reperfusion injury is an issue of concern to every physician treating patients with acute myocardial infarction in the 1990s. For patients without specific contraindications to therapy presenting for medical care early in the course of myocardial infarction, an attempt at reperfusion of ischemic, but not yet necrotic, myocardium is the current standard of clinical care. Whether a patient is treated with a thrombolytic agent to lyse an intracoronary thrombus or undergoes emergency coronary angioplasty to reperfuse the myocardium, it is the hope, and usually the case, that reversibly ischemic tissue will be reperfused. However, it remains uncertain whether simply restoring blood flow to an area of myocardium lacking adequate blood supply is in fact the optimal strategy for salvage of myocardial tissue. Paradoxically, this seemingly straightforward approach may lead to reperfusion injury and necrosis of tissue that was potentially viable. Almost invariably, a patient with an evolving myocardial infarction who undergoes reperfusion therapy with either a thrombolytic agent or by angioplasty develops evidence for myocardial necrosis as evidenced by release of creatine kinase–MB. While it is convenient to attribute this myocardial necrosis to ischemic damage that had occurred prior to reperfusion, the very act of reperfusion may make the infarct larger than it was before reperfusion was begun. Thus, with our very best efforts to salvage myocardium, we may at times be increasing infarct size. Each year in the United States, 150,000–200,000 patients receive thrombolytic therapy for acute myocardial infarction and additional thousands undergo emergency angioplasty for actual or threatened acute myocardial infarction. Therefore, the issue of reperfusion injury and infarct extension is of broad clinical importance.

In this issue of Circulation, Quaife et al assess how reperfusion injury by examining mechanisms of reoxygenation-induced injury in an in vitro cultured myocyte model of hypoxia and glycolytic blockade. Their experiments help to clarify mechanisms of reoxygenation-induced injury and point toward reperfusion strategies that may prove eventually to be useful clinically.

In 1972, Shen and Jennings put forward the concept that intracellular calcium accumulation was the pivotal event in the transition from reversible to irreversible ischemic myocardial injury. For many years the widely accepted working hypothesis has been that the coup de grace for an ischemically injured cell could be administered by reperfusion of ischemic or severely hypoxic tissue, leading to influx of calcium, an abrupt increase in cytosolic calcium concentration, and a subsequent chain of events leading to rigor and eventually to contraction band necrosis. The current study by Quaife et al complements work from other laboratories and casts serious doubt on the concept of massive calcium overload early in reperfusion injury.

In gaining a perspective on the role of calcium in cellular injury, differences in experimental approaches must be borne in mind. Hypoxia is by no means pathophysiologically identical to ischemia, nor is reoxygenation identical to reperfusion. Quaife et al have examined two important elements of ischemia: hypoxia and blockade of glycolytic metabolism. The latter they produced with inhibition of glycolysis rather than by substrate exhaustion, to reflect the situation in acute ischemia in vivo. Elements of ischemia that may modulate cytosolic [Ca\(^{2+}\)] that were not systematically altered in the experiments of Quaife et al include extracellular and intracellular pH, [K\(^+\)], and [adenosine]. Furthermore, reoxygenation and resupply of glycolytic substrate are critical events in reperfusion, but are not the only changes that occur with in vivo reperfusion.

The introduction of second-generation calcium-sensitive fluorescent dyes by Tsien and colleagues has permitted noteworthy advances in studies of calcium homeostasis at the cellular level. Cytosolic free calcium concentration ([Ca\(^{2+}\)]) can be measured with a fluorescent dye such as indo-1 or fura-2 and compared to cellular calcium content determined by isotopic labeling with \(^{45}\)Ca or chemical mass determinations using atomic absorption spectrometry.
try. It has been demonstrated convincingly⁸ that under nonischemic conditions cellular calcium content can be increased at least fivefold above normal physiological levels without inducing irreversible injury. Indeed, nonischemic cells have a remarkable ability to defend against injury from elevated levels of \( [\text{Ca}^{2+}] \),⁸,¹³,¹⁴ Quaife et al² now report the simultaneous recording of cell contraction and calcium transients in myocardial cells during hypoxia and reoxygenation to examine contractile behavior and \( [\text{Ca}^{2+}] \), in combination with the use of biochemical and isotopic techniques to examine calcium homeostasis during reoxygenation. They have taken a reductionist approach to examination of ischemia and reperfusion, studying under carefully controlled circumstances certain critical elements of ischemia, hypoxia and blockade of glycolysis, rather than trying to study calcium homeostasis in the midst of all the complexities of in vivo ischemia. While this approach has limitations, as noted above, it provides useful insights.

The primary experimental approach of Quaife et al² was to make cultured chick embryo ventricular myocytes hypoxic for 2–3 hours in the presence of glycolytic blockade while simultaneously measuring contractility and \( [\text{Ca}^{2+}] \), then reoxygenating the cells under a variety of conditions. They demonstrate the phenomenon of oxygen paradox in this preparation, similar to that observed in intact mammalian myocardial preparations.¹⁵,¹⁶ That is, most of the injury as judged by lactate dehydrogenase release occurs after reoxygenation and not during hypoxia. This observation is consistent with findings from our laboratory⁹,¹⁰ and also confirms observations by Murphy et al.⁸ A central finding of the study by Quaife et al is that upon reoxygenation, \( [\text{Ca}^{2+}] \) actually increases (albeit not to normal levels) at a time when hypercontracture is developing. This observation helps lay to rest the concept that reoxygenation invariably produces further \( [\text{Ca}^{2+}] \) overload. Moreover, Quaife et al were able to confirm reports from other laboratories that upon reoxygenation (or reperfusion following ischemia), total cellular calcium content increases.⁹,¹⁰,¹⁶ When the rapidly exchangeable \( ^{45}\text{Ca}^{2+} \) pool size was measured, Quaife et al found that reoxygenation produced a marked increase in cellular \( ^{45}\text{Ca}^{2+} \) content at the same time that \( [\text{Ca}^{2+}] \) was declining. This implies that even with the relatively low [ATP] prevailing at the end of hypoxia, cells were defending \( [\text{Ca}^{2+}] \), in the face of increasing calcium influx. It is presumed that the increased amount of calcium entering the cell at the time of reoxygenation was being effectively sequestered by the sarcoplasmic reticulum or by mitochondria. While there is no direct proof of this in the experiments of Quaife et al, it is a reasonable postulate.

At the time when the reoxygenated cells developed hypercontracture, \( [\text{Ca}^{2+}] \) was elevated to levels somewhat higher than those normally occurring in systole; this degree of elevation of \( [\text{Ca}^{2+}] \) at least permits development of contracture. When crossbridge cycling of the contractile proteins was inhibited by 2,3-butanedione monoxime, hypercontracture was prevented and hypoxic injury was limited. Thus, in the presence of reduced [ATP], but under conditions in which ATP could be resynthesized, elevation of \( [\text{Ca}^{2+}] \), appears to activate contractile proteins and contribute to cellular injury, possibly by mechanical stress on the cytoskeleton and sarcolemma.

An additional important finding of Quaife et al² is that in this pure myocyte preparation, devoid of neural, vascular, and leukocyte elements, free radical scavengers ameliorated cellular injury upon reoxygenation. It has been proposed that oxygen-derived free radicals generated by neutrophils, vascular endothelial cells, or neural elements contribute importantly to reperfusion or reoxygenation injury, at least in some model systems.¹⁵ The report by Quaife et al confirms work from our laboratory⁹ indicating that in systems that contain only myocardial cells, free radical scavengers still have a protective role. This implies that myocytes themselves, at least from chick embryo, can be an important source of oxygen-derived free radicals under the experimental conditions studied.

The study by Quaife et al² has some limitations. It is known that the acetoxymethyl ester forms of the fluorescent dyes indo-1 and fura-2 distribute not only to the cytosol of cells but also to sarcoplasmic reticulum and to mitochondria in varying degrees, depending upon the cell type and loading conditions.¹² Intracellular compartmentation of indo-1 was not dealt with by Quaife et al. Almost certainly part of the calcium signal they recorded was not from the cytosol but rather from indo-1–calcium complexes in mitochondria and/or sarcoplasmic reticulum. Dye compartmentation may actually have led to an overestimation of true \( [\text{Ca}^{2+}] \) during hypoxia and reoxygenation. It is likely that this quantitative problem does not change the qualitative result.

The preparation studied was an immature avian ventricular cell culture system. Quantitative differences in calcium homeostasis and sensitivity to hypoxia and glycolytic blockade may occur between this primary cell culture system⁵,¹⁷ and adult mammalian myocytes,¹⁴ but there is little evidence for fundamental differences in mechanisms of calcium homeostasis and response to hypoxia or ischemia.⁶,¹⁸ The oxygen paradox has been described in intact mammalian tissue, and a decline rather than an increase in \( [\text{Ca}^{2+}] \) has been reported upon reperfusion of ischemic adult mammalian myocardial tissue.¹⁹ Quaife and colleagues² measured cell motion rather than developed force of contraction of intact tissue, but these measurements of contractile state are closely related.⁷ Finally, Quaife et al, were studying hypoxia plus glycolytic blockade and not in vivo ischemia. Their observations highlight the process of rapid resynthesis of high energy phosphates following relief of hypoxia or ischemia. In the presence of only moderately elevated \( [\text{Ca}^{2+}] \), a rapid increase in ATP synthesis was associated with reoxygenation-induced injury. Kusuoka et al⁶ have suggested that upon
reperfusion of ischemic adult mammalian myocardium there is increased ATP utilization for Ca\(^{2+}\) sequestration, contributing to the slow recovery of initial [ATP]. Recently Marban et al\(^{19}\) using an intact mammalian heart model of ischemia and NMR approaches entirely distinct from the methods of Quaife et al have shown a small rise in time-averaged [Ca\(^{2+}\)], followed by a decline in [Ca\(^{2+}\)], at the time of reperfusion, in good qualitative agreement with the hypoxic avian myocyte model. Thus, it is likely that the mechanisms described by Quaife et al are generalizable to some degree and are not merely peculiarities of the system studied. Further advances in understanding calcium homeostasis and its role in reperfusion injury await application of techniques for digital imaging of calcium-sensitive fluorescent dyes in cell ischemia models and better definition of compartmentation of these dyes. Work is proceeding in this direction in several laboratories, and further progress is anticipated.

Even if the study by Quaife et al\(^{2}\) of hypoxic myocytes is likened to the proverbial blind men each examining one part of the elephant, they have provided useful new information about how one element works. Taken together with studies examining other aspects of hypoxia, ischemia, and reperfusion injury, a more complete picture of this clinically important process should emerge. One hopes that clues to effective therapeutic strategies will emerge as well.

**References**

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