Healthy versus sick myocytes: metabolism, structure and function

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The myocytes are extraordinary cells. They are immortal and contract for a lifetime, supporting the peripheral circulation. In order to do so, they have a unique ultrastructure and unique biochemical machinery that allows them to produce enough adenosine triphosphate to support the contraction. This article deals with the ultrastructure of cardiac muscle and myocytes, and with our current understanding of cardiac metabolism. In addition, the process of contraction is taken into consideration, as well as the mechanisms that allow the adult myocyte to be a terminal cell. However, even the myocytes can die; this can happen by necrosis as a result of an external insult (e.g. a lack of oxygen due to an abrupt occlusion of a coronary artery) or by apoptosis – a genetically programmed type of death that is operative in foetal life. Interestingly, and quite amazingly, under pathological conditions such as acute myocardial infarction or congestive heart failure, this genetic programme is reinstated and apoptosis can then occur, thus resembling features that occur in the embryonic phenotype. The metabolic alterations that occur under pathological conditions such as myocardial ischaemia are also addressed in depth, with several references to the so-called 'new ischaemic syndromes', such as stunning, hibernation and reperfusion paradox. The review is presented in light of an understanding of the biochemical and biomolecular changes that occur in pathological conditions, with the hope that this will provide room for innovative therapeutic intervention.

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Introduction

Ultrastructural organization in cardiac muscle is relevant to our understanding of individual cell survival *per se* and function of cell aggregates. Comparative anatomy has shown remarkable similarities among striated muscles. In cardiac muscle the transverse tubules are large, and at the Z line the junctional sarcoplasmic reticulum (SR) is reduced and continuous with multiple connections with the free SR (corbular SR in mammals). Furthermore, different from skeletal muscle, mammalian cardiac muscle has mitochondria that are more developed and positioned longitudinally with respect to the muscle structure (Fig. 1).

In the heart, the ventricles function under greatly varying pressures and, as a consequence, vary in thickness. However, the general architecture of each component (i.e. endocardium, conduction cells, capillaries, arteries, veins, nerves, etc.) is generally similar in both left and right ventricles; only the total mass differs. Also, atrial architecture[1,2] is identical in both atria except that sinoatrial and atrioventricular nodes are located in the right atrium close to the superior vena cava, and near the atrial septum close to the atrioventricular junction, respectively.

Electron and light microscopy have provided important information concerning the geometry of cardiac myocytes; each cardiac myocyte is connected longitudinally to at least two neighbouring cells and laterally to at least one. Most mammalian cardiac myocytes are loosely assembled into bundles and surrounded by extracellular space (0·2–1 µm in width). Cardiac myocytes are always connected at the intercalated disks, which are mosaics of three kinds of junctional complexes: desmosomes, nexuses and intermediate junctions. The intercalated disks are mainly positioned at cell ends, where the Z line would be expected in a sarcomere.

Myocyte structure

The plasma membrane (plasmalemma) shows specialized regions, including the intercalated disks and the transverse tubular system. The plasmalemma is covered by a laminar coat, also termed glycocalyx or basal lamina. It is approximately 50 nm in width, is separable into two layers (lamina

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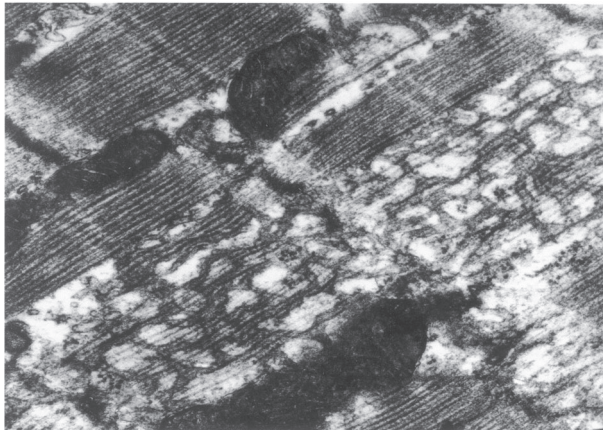
Figure 1 Contractile material of mammalian cardiac muscle in longitudinal section. Mitochondria are positioned along the muscle structure.

densa and lamina lucida) and may trap several ions, including calcium. The sarcolemma – a term that is specifically used in muscle cells – is composed of plasmalemma, glycocalyx and collagen. The plasmalemma is anchored to the intracellular cytoskeleton by anchor fibres that are approximately 10 nm in diameter, and these are mainly located at Z lines where they form grooves and the transverse tubules emanate, so as to give cardiac myocytes a scalloped surface. The transverse tubules are tubular extensions of the plasmalemma into the interior of mammalian cardiac myocytes. In most mammals they develop during the first 6–8 weeks after birth, whereas in others they are present at birth. The ratio of the total plasmalemma surface area to the total cell volume appears to be constant in cardiac muscle and correlates well with heart rate. One of the functions of transverse tubules is the propagation of the action potential. The area of existing transverse tubules increases with hypertrophy.

The cardiac SR is a tubular network that surrounds the myofibrils and has the function of sequestering calcium from the cytosol for muscle relaxation via a calcium pump (sarco(endo)plasmic reticulum Ca^{2+}-ATPase [SERCA2a]) in order to take up calcium, and calsequestrin allows high accumulation of the ion in the SR. The SR network has two main components: junctional SR (proper junctional SR, extended SR and corbular SR) and free SR (Z retes, M retes, Z tubules and longitudinal SR). The close association between two subsarcolemmal cisternae of junctional SR and one transverse tubule in cardiac muscle is called a 'triad' (Fig. 2). Junctional SR stores and releases calcium[3,4].

The contractile material in cardiac myocytes is arranged in a complex structure called 'Felderstruktur', containing cytoplasm, mitochondria and other intracellular organelles, including the cytoskeleton. The myofibrils are assembled into small repeating units called sarcomeres, which stretch between two Z lines and represent the structural base for contraction. The length of a sarcomere, although depending on the state of contraction, is approximately 2 µm. The striations are produced by the thin actin filaments, forming
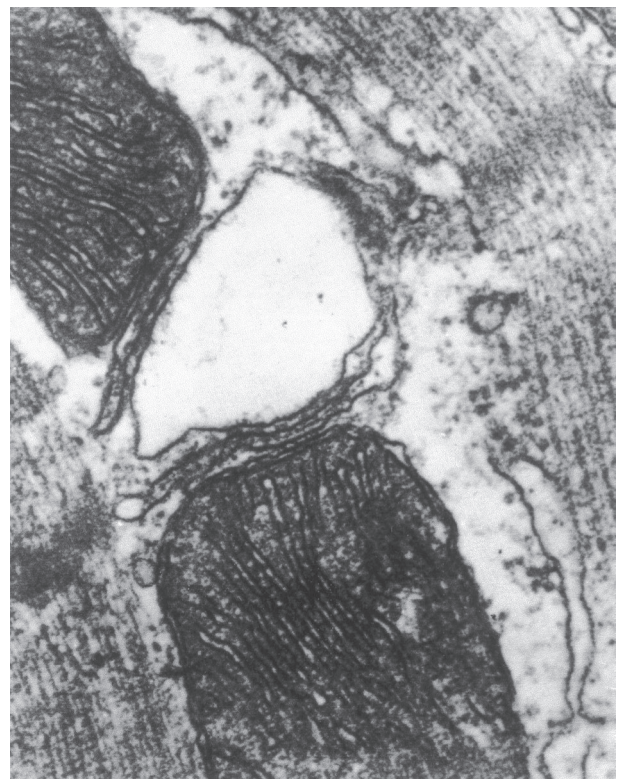

Figure 2 The sarcoplasmic reticulum forms an intricate network around the contractile material. Longitudinally orientated tubules make contact with the junctional sarcoplasmic reticulum, which, together with the transverse tubules, forms triads.

the light I bands (isotropic in polarized light), and by the thick filaments of myosin, forming the dark A bands (anisotropic in polarized light). The A band has a constant width of 1·65 µm. Analogous to skeletal muscle, and in agreement with the sliding filament theory, actin and myosin overlap in tandem during contraction, activated by calcium binding to another myofilament, namely troponin C.

Among the intracellular organelles, mitochondria comprise a large proportion of the total amount in cardiac myocytes. They are the site of the Krebs cycle metabolism, and are therefore deputed to energy production. They are composed of an inner membrane that is folded into the so-called cristae, which are separated by the matrix space containing a variety of soluble enzymes and cofactors. Mitochondria are surrounded by an outer membrane, where enzymes are involved in substrate and high energy phosphate transport (Fig. 3).

Finally, the cytoskeleton can be viewed as an intracellular scaffold, having two purposes: first, to stabilize the topography of intracellular components; and second, to control cell size and shape. The former is important for biochemical processes and the latter is crucial in defining surface to volume ratios, which influence electrical properties of excitable cells. Interestingly, alteration in ventricular size and shape (remodelling), due to pathological conditions, has negative prognostic impact.
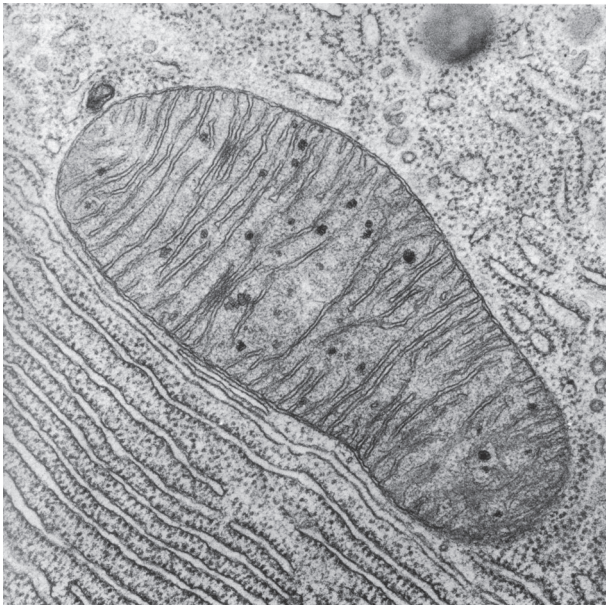
Figure 3 **Mitochondrion showing the outer and inner membranes, as well as the large number of tightly packed cristae.**

Cardiac cell metabolism

The heart is a rhythmically contracting organ, and continuously needs oxygen and metabolites such as glucose and/or free fatty acids to produce energy (i.e. adenosine trisphosphate [ATP] and creatine phosphate) and so maintain the cardiac cycle (i.e. systole and diastole). However, the need for oxygen is the conditioning factor for heart function. Intracellular oxygen reserve is enough to keep the heart beating only for a few seconds. The carbohydrates or the lipid store can support contraction for at least an hour.

Therefore, heart metabolism is aerobic and closely dependent on oxygen availability, as confirmed by the abundance of mitochondria (30% of total volume; Fig. 1) and myoglobin. The contractile process or, more precisely, myosin ATPase activity represents more than 75% of myocardial energy requirements, the remainder being covered mostly by ion homeostasis, which in turn is essential for the cardiac contraction cycle; the high energy requirement is almost exclusively covered by mitochondrial oxidative phosphorylation. This leads to high sensitivity of myocardial cells to oxygen deficiency, and mitochondria function probably plays a central role in the molecular events that lead to the tissue damage that occurs under conditions of oxygen deprivation.

In the heart, mitochondria play two main roles that are essential to cell survival: synthesis of ATP and maintenance of calcium homeostasis. These two processes are driven by the same energy source, namely the hydrogen electrochemical gradient (ΔH^+), which is generated by electron transport along the inner mitochondrial membrane[5]. Under aerobic physiological conditions, mitochondria do not contribute to the beat-to-beat regulation of cytosolic calcium, although a calcium transient in the mitochondrial matrix has been described. A molar increase in mitochondrial calcium concentration stimulates the Krebs cycle and reduces nicotinamide adenine dinucleotide (NADH) redox potential, and therefore stimulates ATP synthesis, allowing a perfect match between increase in contraction and increase in energy availability to support it.

As stated in Mitchell's chemiosmotic hypothesis[5], mitochondria energy conservation is mediated by the formation of a hydrogen electrochemical gradient (ΔH^+), which may be utilized as an energy source for ATP synthesis, via the F_1, F_0 ATPase, and for ion and metabolite transport via a specific system.

Mitochondria may directly participate in calcium homeostasis by means of separate influx and efflux pathways that are located within the inner membrane[6,7]. The total mitochondrial capacity for calcium accumulation is several times greater than that of the SR, suggesting a key mitochondrial role in calcium homeostasis only under pathological conditions when cytosolic calcium concentration increases abnormally.

Several conditions may cause a calcium-dependent increase in mitochondrial permeability to ions and solutes[6]. As a result, there is uncoupling of oxidative phosphorylation and matrix swelling.

Excitation–contraction coupling

Calcium is essential in cardiac electrical conduction and is a direct activator of the myofilaments[8]. During the action potential, calcium enters the cell through depolarization-activated channels (L-type calcium channels or dihydropyridine receptors [DHPRs]) in the sarcolemma. The inward calcium current generated contributes to the action potential plateau (Fig. 4). Intracellular calcium entry triggers calcium release from the SR, allowing (through the rise in free cytosolic calcium concentration) binding to the myofilament protein troponin C, and so starting contraction. During relaxation, free intracellular calcium must decline and dissociate from troponin C. The physiological contraction generates both isometric force (ventricular pressure) and rapid shortening (to expel blood). There are two main ways to change the strength of cardiac muscle contraction: by altering the amplitude or duration of the calcium transients; and by altering the sensitivity of the myofilaments to calcium. Stretching enhances myofilament calcium sensitivity; this is because filling of the heart with blood results in stronger contraction due to stimulation of the actin–myosin interaction[9]. This represents an important autoregulatory mechanism by which the heart adjusts to altered diastolic filling (the classic Frank–Starling response). On the other hand, myofilament calcium sensitivity is reduced by acidosis and by high levels of phosphate and magnesium, which occur during ischaemia.

Although contractile strength corresponds with calcium transients, there is a dynamic interplay between calcium and myofilaments during excitation–contraction coupling.

In order to relax, calcium must be removed from the cytosol to lower intracellular calcium concentration. In
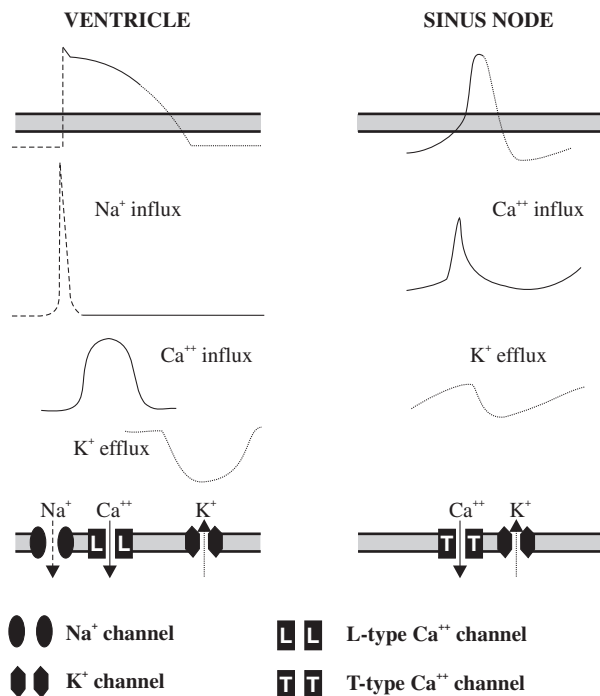
Figure 4 Schematic representation of action potentials in ventricular and sinus node cells, shown together with ion currents and channels involved.

ventricular myocytes, most calcium is removed by the SR calcium pump (SERCA2a) and partly, to a variable extent depending on the species studied[8,10–13], by the sodium–calcium exchanger in the sarcolemma. Calcium is removed only minimally by the sarcolemmal calcium pump and the mitochondrial calcium uniporter, which are collectively termed the 'slow systems'.

In human and rabbit heart failure functional expression of SERCA2a is reduced, whereas that of the sodium–calcium exchanger is increased[14], so that they may contribute more equally to the removal of cytosolic calcium[8,15]. These changes leave twitch relaxation and intracellular calcium decline unaltered, but may reduce SR calcium content, limiting calcium release and so causing systolic contractile deficit in heart failure.

Foetal ventricular myocytes exhibit two classes of sarcolemmal voltage-dependent (L-type or DHPRs, and T-type) calcium channels. In most species, at birth and later in developed adult ventricular myocytes, only L-type calcium channels are observed, and are primarily localized at the transverse tubules opposite the SR junctions, where the SR calcium release channels (ryanodine receptors [RyRs]) are present[16], macroscopically forming 'triads' (Fig. 2).

The SERCA2a is anchored to the junctional SR together with various key regulatory proteins, such as calmodulin[17], FK-506-binding protein[18], protein kinase A (PKA), phosphatase 1 and 2A [19], and sorcin[20], which binds both to the RyR and the DHPR. On the luminal surface, the RyRs are coupled to other proteins (triadin, junctin and calsequestrin)[21], which modulate the release process and/or buffer calcium in the SR.

Organized arrays of RyRs (more than 100 RyRs within an area 200 nm in diameter) at the junctions between SR and sarcolemma beneath DHPRs (10–20 DHPRs/100 RyRs), on the surface and in transverse tubules, constitute large functional calcium release complexes[22]. This local functional unit is inferred from observations of calcium sparks, or spontaneous local calcium transients, and reflects a nearly synchronous activation of a cluster of approximately 6–20 RyRs at a single junction. During excitation–contraction coupling, several thousand calcium sparks are synchronized in time by the action potential, whereas calcium sparks are rarely present at rest.

The most widely accepted mechanism underlying activation and termination of calcium release from SR in cardiac muscle is calcium-induced calcium release, particularly that mediated by the L-type calcium channel current. The T-type calcium channels, although not functional in most adult ventricular cells, appear not to be preferentially located in junctional regions, and may only represent a very minor contributor to excitation–contraction coupling because they generate much weaker inward currents than do L-type calcium channels[23,24].

Physiological sympathetic stimulation of the heart through β-adrenergic receptors increases developed contractions (inotropy) and accelerates relaxation (lusitropy) and intracellular calcium declines. Stimulation of β-adrenergic receptors activates stimulatory guanosine triphosphate binding proteins, which in turn stimulate adenylate cyclase to produce cyclic adenosine monophosphate. Cyclic adenosine monophosphate activates PKA, which phosphorylates several proteins that are involved in excitation–contraction coupling, such as phospholamban in the SR, L-type calcium channels in the sarcolemma, SR calcium release channels, troponin I and myosin binding protein C. The lusitropic effect of β-adrenergic agonists is mediated by PKA, which phosphorylates phospholamban and troponin I; the latter cause faster reuptake of calcium in the SR and dissociation of calcium from the myofilaments, respectively[25]. The faster reuptake of calcium into the SR produces an increase in SR calcium content, so that the inotropic effect of PKA activation is mediated by the combination of increased inward calcium currents and greater availability of calcium in the SR.

The adult myocyte is a terminal cell

Adult cardiac myocytes have no or insufficient replicative capacity to replace cell losses[26], which leads to cardiac functional deterioration once loss of contractile tissue is no longer compensated for by functional reserve. Cell proliferation is provided by replication of genomic DNA, mitotic nuclear division and cytokinesis, regulated by effective checkpoint controls.

Irreversible loss of vital cellular structure and function (i.e. cell death) represents both a physiological process during organogenesis in embryos and cell turnover in adults, and a pathological process in response to various injuries. There are two fundamental types of cell death: apoptosis and

necrosis. Both necrosis and apoptosis may contribute independently to myocyte cell death after infarction.

Apoptosis

During the normal development of vertebrates and invertebrates, multifocal single cell death is constantly observed and designated apoptosis, which means 'falling off'[27,28]. Apoptosis has been suggested to have a complementary but opposite role to that of mitosis in normal homeostasis. Also, it is considered to be the major process responsible for cell death not only in various physiological events, such as embryonic tissue remodelling, adult cell turnover and differentiation[27–30], but also during pathological conditions such as viral hepatitis, death of tumour cells and graft-versus-host disease[31]. Cell proliferation encompasses replication of genomic DNA, mitotic nuclear division and cytokinesis, which are regulated by checkpoint controls. Apoptosis was originally defined as an energy-dependent form of cell death, characterized by distinct phases of ultrastructural morphological features.

Morphologically, apoptosis is characterized by nuclear and cytoplasmic condensation of single parenchymal cells (shrinkage), followed by loss of the nuclear membrane, fragmentation of the nuclear chromatin and subsequent formation of multiple fragments of condensed nuclear material and cytoplasm[27,28], called apoptotic bodies. These apoptotic bodies are rapidly phagocytosed by neighbouring cells[32,33]. Therefore, flogosis does not occur. The initiation phase of apoptosis is characterized by activation and de novo synthesis of endonucleases. Several interventions (e.g. catecholamines, atrial natriuretic peptide, angiotensin II and stretch) have been shown to induce apoptosis in cultured myocytes, but their role in the clinical setting has not yet been proven. Alternatively, intracellular alterations following ischaemic and/or reperfusion injury, such as an excess of nitric oxide, may be involved. The pro-apoptotic stimuli, via two major pathways (the mitochondrial and the death receptor pathway), lead to activation of upstream followed by downstream caspases[32,34,35], which cleave an incompletely characterized number of proteins, such as nuclear proteins, proteins that are involved in signal transduction and cytoskeleton proteins. The mitochondrial pathway involves release of cytochrome c, apoptosis-inducing factor and probably other factors into the cytosol. Cytochrome c release appears to depend on the opening of the mitochondrial permeability pore, which is associated with a breakdown of the electrochemical gradient ($\Delta\Psi$) on the inner membrane of mitochondria. Cytochrome c, when complexed with apoptotic protease factor 1, activates pro-caspase 9. The death receptor pathway, instead, involves binding of Fas ligand and tumour necrosis factor-alpha to their receptors[36], thus activating pro-caspase 8. Activation of both pro-caspases 8 and 9 in turn leads to activation of caspase 3, whose target is DNA fragmentation, which can be detected using the TUNEL (terminal transferase mediated DNA nick-end labelling) technique on light microscopy[37,38].

Our understanding of the mechanisms of apoptosis in cardiac myocytes may provide new strategies to prevent myocyte loss. Although the baseline rate of apoptosis is reported to be in the range 5–10% in cultured myocytes, the impact of some stimuli may result in rates as high as 75%[39,40]. Acute and progressive myocyte loss leads to cardiac functional deterioration, because myocytes have no or at least insufficient replicative capacity to replace cell losses[26].

Necrosis

Cell death in response to an overwhelming insult is referred to as necrosis, and is characterized by the sum of morphological and metabolic changes that accompany or follow irreversible cell injury in living organisms. The basic pattern of pathological cell death that develops in response to a variety of severe injury, such as ischaemia, hypoxia, chemical toxins, infections and trauma[41], is coagulation necrosis, which is represented by denaturation and coagulation of cellular proteins. Colliquative necrosis is characterized by tissue that rapidly liquefies due to its protein-poor nature. Necrosis often involves cell swelling rather than cell shrinkage, as occurs in apoptosis. Cellular fragmentation represents a late phase that results from the degradative changes of autolysis, from activation and release of lysosomal enzymes, and of heterolysis, which is caused by the actions of inflammatory cells invading the necrotic tissue following cell death.

The evolution from reversible to irreversible injury in necrosis involves progressive derangements in energy and substrate metabolism, most importantly involving altered high energy phosphate metabolism and progressive reduction in the cellular content of ATP[42]. Potentially reversible changes include condensation and clumping of nuclear chromatin and intracellular oedema manifested by swelling of various organelles (endoplasmic reticulum, mitochondria, lysosomes and other vesicles), subsurface cell blebbing and general cell swelling. Irreversible injury is associated with additional morphological features (advanced changes in nuclear chromatin, mitochondrial lesions with electron dense calcium phosphate deposits, breaks in the plasma membrane and organellar membranes)[28,43].

Cardiac pathological conditions

In recent years, several studies have established that apoptosis, also called 'programmed cell death', occurs in various cardiac pathologies, including ischaemia and reperfusion injury, myocardial infarction and heart failure. It is known that the pathophysiology of ischaemic heart disease and congestive heart failure is multifactorial. At the level of the myocyte, contractile dysfunction, due to altered calcium handling, impaired excitation–contraction coupling, electrical instability and myocyte loss, is

believed to contribute to disease initiation and progression. At the clinical level several mechanisms have been considered, including impaired excitation–contraction coupling, altered neurohumoral balance, calcium homeostasis and extracellular matrix (i.e. connective tissue) composition, to which cardiomyocyte apoptosis must be added[44].

In myocardial infarction, apoptosis is believed to responsible for nearly all cell death. Apoptosis has been observed in the core and border region of the ischaemic area, and in viable myocardium[32]. Apoptosis is also thought to accompany ventricular dysfunction in heart failure. TUNEL-positive cardiac myocytes have been found in the hearts of patients with ischaemic and idiopathic dilated cardiomyopathy[44–46], with higher incidence of TUNEL-positive cells in the infarct border zones of ischaemic cardiomyopathy, indicating the possibility that ischaemia and increased stretch are involved[34]. Stretch is always present in ventricular overload and dilatation, which are associated with left ventricular hypertrophy.

The metabolic approach to ischaemic heart disease

Myocardial ischaemia is defined as an imbalance between fractional uptake of oxygen and the rate of cellular oxidation in the heart. This condition may have several potential outcomes. First, when ischaemia is of short duration, there is no major molecular damage and a transient post-ischaemic ventricular dysfunction occurs on reperfusion, a condition termed 'stunned myocardium'. Second, when ischaemia is prolonged and severe, irreversible damage occurs, with no recovery in contractile function upon reperfusion, and necrosis inevitably develops. Finally, when ischaemia is less severe but still prolonged, the myocytes may remain viable but exhibit depressed contractile function. Under the latter condition, termed 'hibernating myocardium', reperfusion is able to restore contractility. The key transition might occur within minutes from onset of ischaemia, or take up to several hours depending on a multitude of factors (the underlying metabolic rate probably being the most important). For the clinical cardiologist, this is determined by the extent of residual flow, the underlying heart rate, the degree of haemodynamic change (such as an increase in pre- and after-load wall stress), and the effects of any accompanying neuroendocrine activation. This physiological ischaemia, characterized by down-regulation of contraction in the absence of molecular changes, can also be considered a conservative adaptive response by the myocyte that down-regulates its contraction independently of extra cardiac signals and, in so doing, reduces its energy needs in an attempt to maintain viability.

In contrast, in biochemical ischaemia[47], possibly in response to a series of complex and dominantly extra-cardiac neurohormone signals (activated to ensure the maintenance of pump function and cardiac output), the myocyte will, at high cost, succumb to a series of cellular mechanisms to maintain contractile function, despite impairment in oxygen supply. As a consequence, the supply of energy fails to match consumption, and intracellular equilibrium (steady-state metabolism) is sacrificed, initiating a cascade of increasingly severe metabolic perturbations. The cell will then become 'metabolically distressed' and, unless interrupted by early reperfusion, biochemical ischaemia will inevitably progress toward cell death.

From ischaemia to cell death

As shown in Fig. 5, mitochondria are the organelles that are most likely to be involved in the transition from reversible ischaemia to cell death. This is perhaps not surprising because these organelles play a fundamental role in cellular energy production (the ATP turnover of the human myocardium exceeds $30 \, kg . day^{-1}$) and in maintaining intracellular ionic homeostasis, which is the other key process that is threatened by ischaemia.

Our understanding of the complexities of ischaemia and tissue injury is further complicated by the need to reperfuse the tissue to determine whether ischaemic damage is reversible or irreversible. Some, but not all, investigators believe that reperfusion itself might be detrimental and can inflict injury over and above that attributable to ischaemia[48]. Other investigators, however, question the existence of reperfusion-induced injury[49]. Ischaemia is not a static condition, and reperfusion is a part of a continuum of coronary artery disease. Such reperfusion might occur at different times during transition from angina to myocardial infarction, and may have several different outcomes, including early or delayed recovery (stunning), some recovery (hibernation) or no recovery at all.

Physiological and biochemical ischaemia

During short periods of ischaemia (e.g. in angina) there is a perfect match between biochemical and mechanical activity; this maintains viability. As shown in Fig. 6, restriction of coronary flow results in a rapid down-regulation of contraction and eventually quiescence. This is due to the effects of intracellular acidosis, which develops within seconds of induction of ischaemia and reduces calcium movements within the sarcolemma, SR and myofilaments[50]. Shortly after this, the energy charge of the myocyte is reduced, and creatine phosphate declines faster and to a greater extent than does ATP. Anaerobic metabolism, as shown by lactate release in the coronary effluent, develops and contributes to the formation of limited amounts of ATP by oxygen-independent, substrate-level phosphorylation. Taken together, these findings suggest the occurrence of biochemical as well as physiological ischaemia. Both down-regulation in contraction (and therefore in ATP consumption) and increased anaerobic ATP production explain why the fall in tissue ATP after the onset of ischaemia is not immediate.
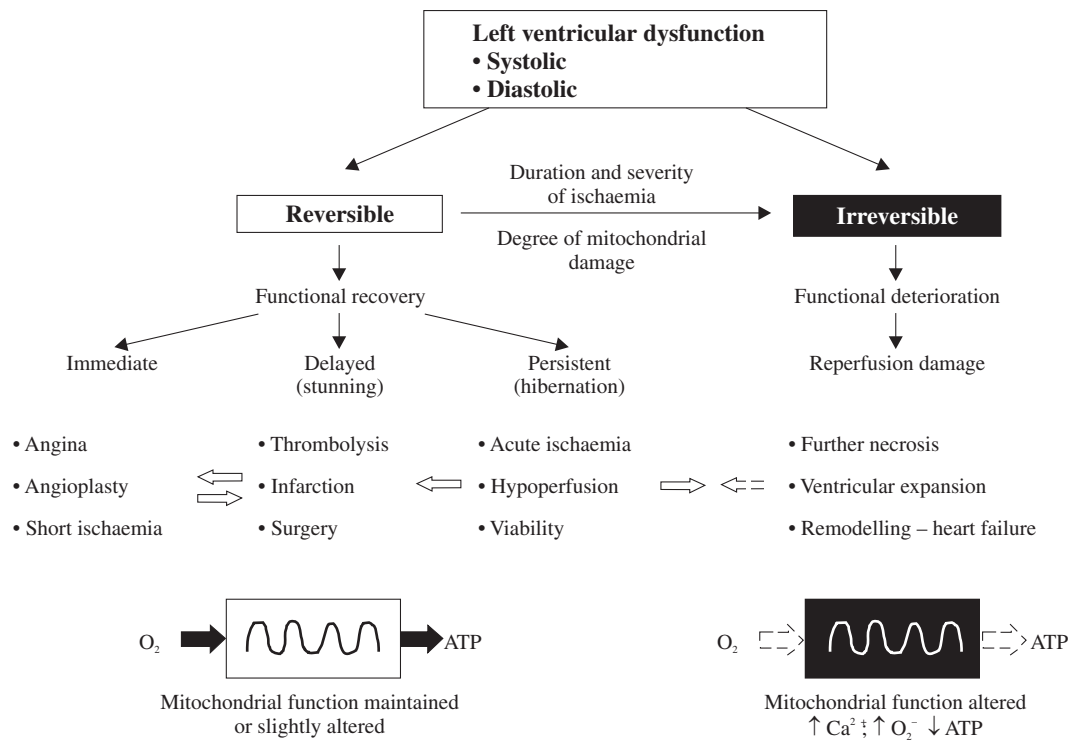
Figure 5 **Possible outcomes of myocardial ischaemia.**

The availability of this residual energy supply is essential to maintain cellular viability. Reperfusion at this stage results in a recovery of high energy phosphate production, which in turn indicates that the mitochondria are still functionally intact and capable of normal aerobic metabolism; this is linked to a recovery of mechanical function that might be either immediate or somewhat delayed (Fig. 6).

This sequence of metabolic and functional events is not restricted to experimental models but also occurs at the clinical level, for example during angina induced by atrial pacing. Figure 7 shows that in patients with coronary artery disease and angina, an increasing heart rate (and therefore increasing energy requirements of the heart to the extent that they are no longer met by supply) results in a reduction in coronary sinus pH, indicating the occurrence of myocardial acidosis. This is followed by an increase in coronary sinus lactate (indicative of the development of anaerobic metabolism) and a down-regulation of regional contraction (revealed by a reduction in the ejection fraction, suggesting systolic dysfunction). All these biochemical and mechanical events precede the occurrence of angina. Once the heart is returned to its basal level and ischaemia therefore no longer persists, coronary sinus pH and lactate release return to normal values and left ventricular systolic function improves. However, the functional recovery is not immediate because of the presence of stunning. Clearly, under these circumstances viability is maintained, although evidence of the ischaemic insult persists as long as the recovery of function fails to match the recovery of metabolism[51].

Stunning

There is now convincing evidence that the myocardium reperfused after a short period of ischaemia is characterized by a variety of unfavourable, but non-lethal, cellular changes that, given sufficient time, will return to normal. The most prominent of these changes is myocardial stunning, which is the prolonged contractile dysfunction that occurs during reperfusion despite the absence of irreversible damage[51,52]. The duration of dysfunction greatly exceeds that of the antecedent ischaemia. However, by definition, this form of injury is fully reversible, provided sufficient time is allowed. Interventions such as the use of inotropic agents can override stunning, and other interventions such as antioxidants can prevent its occurrence[52].

A number of candidate mechanisms for stunning have been investigated; these include an impaired ability to resynthesize high energy phosphates, functional sympathetic denervation, heterogeneous impairment of regional perfusion, abnormal electrical activation, loss of creatine kinase activity, damage to the collagen matrix, leucocyte activation and decreased sensitivity of myofilaments to calcium. However, the two most plausible mechanisms relate to free radical induced injury during the early moments of reperfusion and impaired calcium homeostasis.

Numerous studies suggest that oxygen-derived free radicals contribute to post-ischaemic dysfunction. Electron magnetic resonance spectroscopy has demonstrated the formation of free radicals in the stunned myocardium directly, as well as the attenuation of contractile
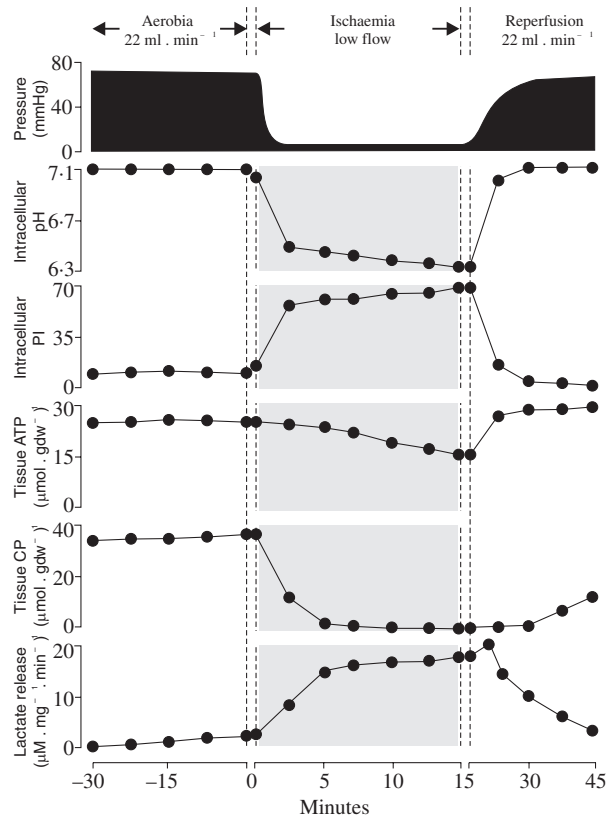
Figure 6 Effects of 15-min ischaemia followed by 30-min reperfusion on tissue inorganic phosphate (Pi), adenosine trisphosphate (ATP), creatine phosphate (CP) and lactate. Paced, isolated perfused rabbit hearts, under control and reperfusion conditions, were perfused at a mean coronary flow of 22 ml . min⁻¹. Ischaemia was induced by reducing the coronary flow to 1 ml . min⁻¹. dw=dry weight.

Figure 7 Metabolic changes during the early phases of ischaemia in patient with coronary artery disease.

dysfunction[53]. Although there is strong evidence that reactive oxygen intermediates play a major role in the pathogenesis of myocardial stunning, there is also evidence that this phenomenon is related to abnormalities in calcium homeostasis[52]. Moreover, calcium and free radical mechanisms are not mutually exclusive, but might represent two facets of the same phenomenon. Thus, Bolli[52] has suggested that oxygen free radicals might cause sarcolemmal and SR dysfunction and impairment in calcium distribution. The latter in turn could exacerbate the damage initiated by the radicals, and indeed could promote the production of further radicals[52].

Irreversible ischaemia

If coronary flow remains severely reduced, then the myocardium will remain quiescent, but nonetheless biochemical ischaemia intensifies and proceeds toward irreversible damage. In experimental studies, this is indicated by the continuing release of lactate, by increases
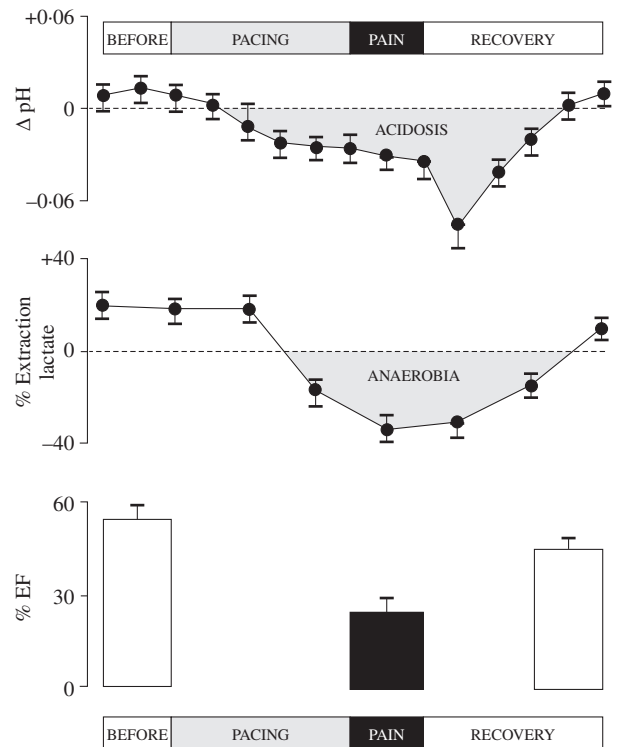
in diastolic pressure (indicating a severe perturbation of ionic homeostasis) and by signs of severe sarcolemmal damage (e.g. enzyme leakage). Under these circumstances, the mitochondria themselves become targets for ischaemic damage, which in turn decreases the possibility that reperfusion will result in recovery in both metabolism and function. Several mitochondrial alterations have been described as a consequence of either prolonged ischaemia or post-ischaemic reperfusion. Mitochondria extracted from ischaemic hearts show reduced function, decreased membrane potential and a decreased function of NADH dehydrogenase. Figure 8 shows data on the changes induced by ischaemia in mitochondrial function[54]. Thus, mitochondria appear to be quite resistant to ischaemic damage. However, the presence of residual phosphorylation capacity in mitochondria during ischaemia appears to be associated with irreversible damage during reperfusion so that, paradoxically, mitochondrial uncouplers can provide cardiac protection.

Thus, residual mitochondrial function during ischaemia might be interpreted as good or bad. This apparently contradictory concept arises from the finding that, on the one hand, intact and normally functioning mitochondria are essential for the recovery of mechanical function during reperfusion, but on the other hand the inhibition of the respiratory chain or the addition of uncouplers of oxidative phosphorylation can limit the extent of enzyme release in various models of myocardial damage[54]. These findings suggest the complex scenario that the restoration of ATP production by mitochondrial oxidative phosphorylation is
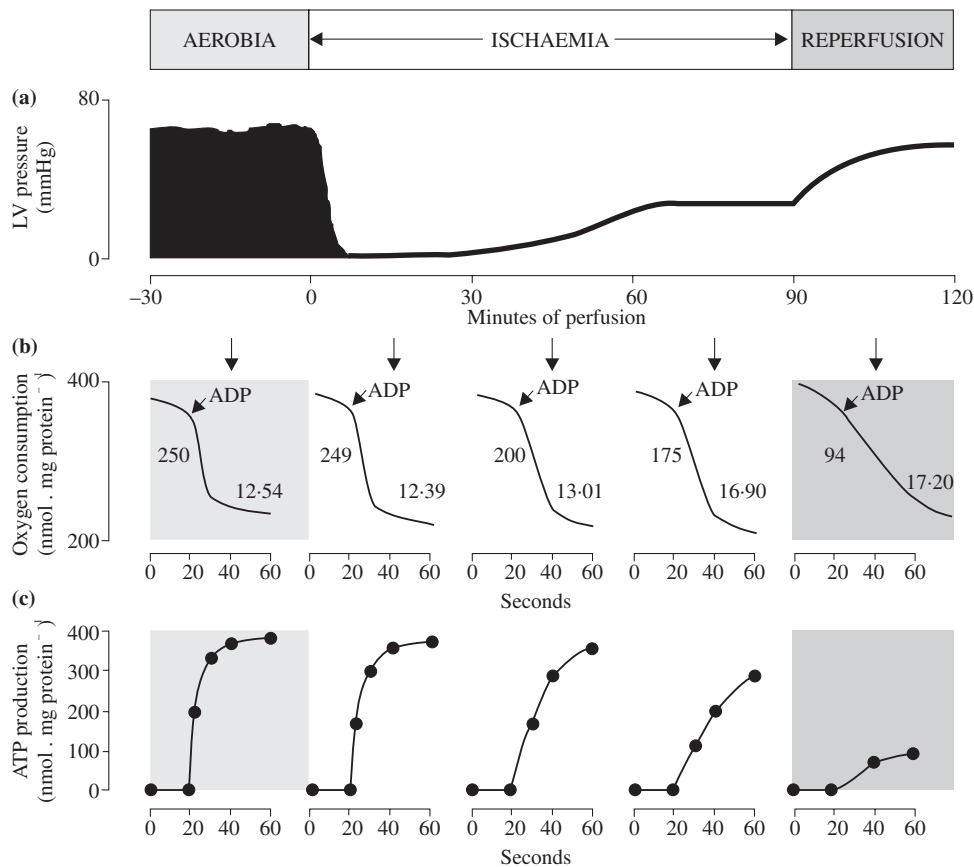
Figure 8 **Mitochondrial function in paced, isolated and perfused rabbit hearts. Under control and reperfusion conditions, the hearts were perfused at a mean coronary flow of 22 ml . min^{-1}. Ischaemia was induced by reducing coronary flow to 1 ml . min^{-1}. (a) Typical example of a left ventricular (LV) pressure tracing from a heart subjected to an ischaemia and reperfusion protocol. (b, c) Typical examples of isolated mitochondrial tracings for oxygen consumption and adenosine trisphosphate (ATP) production. The mitochondria were isolated from hearts that had been aerobic for 30 min; ischaemic for 30, 60 and 90 min; and reperfused for 30 min. The numerical values reported in the oxygen consumption tracing represent rates (nmol oxygen/mg protein per min) consumed by the isolated mitochondria during states III and IV of respiration. Glutamate was used as the respiratory substrate.**

essential for cell recovery but, at the same time, mitochondrial activity can contribute to those processes that produce cell necrosis.

The reperfusion paradox

Several factors contribute to the immediate reperfusion injury that can occur when supply of oxygen is restored to severely ischaemic tissue. All this can be considered a paradox within a paradox. The two most important factors are re-energization and rapid normalization in pH. These events are not independent, but are synergistic.

More than two decades ago, Hearse *et al*.[55] demonstrated that reoxygenation after prolonged oxygen depletion triggered sudden and major cellular injury, as indicated by massive enzyme leakage, sarcolemmal disruption and hyper-contracture. It then became apparent that re-energization sets in motion a series of paradoxes.

First, recovery of energy production reactivates the SR calcium pump, leading to excess sequestration of calcium often exceeding the capacity of the SR, which initiates a cycle of continuous release and uptake of calcium[56]. Second, the consequent excess of cytosolic calcium is taken up by the re-energized mitochondria at the expense of ATP production[54]. Finally, the resupply of energy to the myofibrillar elements in the presence of excess cytosolic calcium leads to uncontrolled, excessive force generation and hyper-contraction.

In addition, upon reperfusion, interstitial pH is rapidly normalized by the washout of protons, and a gradient is generated between the extracellular space and the cytosol that still contains a high concentration of protons. This, in turn, activates proton-extruding mechanisms, namely sodium–hydrogen exchange and sodium–bicarbonate cotransport[57]. Activation of sodium–hydrogen exchange follows, causing a net influx of sodium into the cytosol. Depending on the ability of the sodium pump to remove this excess sodium, there might be a secondary activation of the

sodium–calcium exchange mechanism which, under conditions of intracellular sodium overload, will transport sodium in the outward direction and calcium in the inward direction; this uncoupled mechanism will then further exacerbate the pre-existing calcium overload[58]. Moreover, the sodium overload will act to increase osmotic gradients with consequent cellular uptake of water, stretching and damaging the sarcolemma and further disrupting ionic homeostasis[59]; intracellular acidosis, which down-regulates myofibrillar activity, is rapidly normalized, allowing the myofibrils in the presence of excessive calcium and low ATP to hyper-contract. This whole series of complex and interacting mechanisms explains the mitochondrial paradox that characterizes reperfusion.

Hibernation

The term 'hibernation' has been narrowed from zoology and implies an adaptive reduction in energy use through reduced activity in the presence of a reduced energy supply. In the context of coronary artery disease, myocardial hibernation was originally seen as a chronic, adaptive reduction of myocardial contractile function in response to a reduction in myocardial blood flow. It was also viewed as a condition in which there would be a complete recovery of contractile function upon restoration of flow. Thus, in the concept of myocardial hibernation, the observed chronic reduction in myocardial contractile function is not regarded as the result of a persistent energy deficit, but instead as a regulatory event that acts to avoid an ongoing energy deficit, thereby maintaining myocardial integrity and viability.

Interestingly, the concept of myocardial hibernation does not originate in the laboratory, but is entirely founded on clinical grounds. In the early 1980s, Rahimtoola[60], by reviewing the results of coronary bypass surgery trials, identified a subset of patients with coronary artery disease and chronic left ventricular dysfunction who improved upon revascularization. Whereas the original idea of an adaptive reduction in contractile function in response to a reduction in blood flow was straightforward and simple, the concept of chronic yet reversible contractile dysfunction in patients with coronary artery disease was not recognized, and was seen as enormously complex and controversial.

The introduction of the concept of hibernation has challenged the traditional view that the extent of chronic contractile dysfunction necessarily reflects the amount of infarcted tissue. In hibernation, the preservation of viability rather than the occurrence of necrosis accounts for the observed reduction in function. In view of the preserved viability of the tissue, hibernation is a key factor in assessing the potential benefit from reperfusion/revascularization. Hibernating myocardium must be recognized and identified by appropriate diagnostic procedures, and requires appropriate decisions by the cardiologist responsible for the selection of patients who will benefit from the interventional reperfusion or surgical revascularization. Obviously, hibernation is only one of several important aspects that must be considered in this patient selection, and many

patients with coronary artery disease and no evidence of hibernating myocardium will also benefit.

A hibernation-like metabolic adaptation to a severe, sustained, low-flow ischaemia has been reported in studies with isolated buffer-perfused rabbit hearts in which there was a preceding short episode (10-min) of zero-flow ischaemia. The rapid decline in contractile function (physiological ischaemia) during the brief episode of zero-flow ischaemia was accompanied by a greater decrease in interstitial[50] and intracellular[61] pH, and the contractile quiescence was attributed to a faster development of myocardial acidosis. During low-flow perfusion there was no lactate release, suggesting that biochemical ischaemia did not occur. During reperfusion following sustained ischaemia, only a transient creatine kinase leakage occurred in the hearts with preceding zero-flow ischaemia. Thus, the establishment of this experimental form of myocardial hibernation requires an initial period of zero-flow ischaemia, during which rapid decreases in interstitial and intracellular pH trigger the decrease in contractile function and thereby facilitate restoration of the balance between energy supply and energy demand. Other experimental studies in pigs attribute a potentially important role to the initial stimulus of severe ischaemia in critically triggering the development of a protective state, with preserved viability during a subsequent period of sustained ischaemia[62,63]. Whether or not such an initial stimulus/trigger of severe ischaemia represents a mandatory link between hibernation and ischaemic pre-conditioning is unclear at present[64], but it supports the hypothesis that hibernating myocardium, at least most of the time, might not be biochemically but physiologically ischaemic[65].

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