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Charles University Hospital Prague, Czech Republic
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Cardiovascular Center Aalst
OIX Hospital
Aalst, Belgium

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Editorial

Roberto Ferrari, MD, PhD
David J. Hearse, BSc, PhD, DSc

FRIENDS AND FOES OF THE CARDIAC MYOCYTE:
“ET TU, BRUTE?”

Life and death, good and evil, yes and no; black and white; male and female; optimist and pessimist; thesis and antithesis—the enumeration could go on forever. Since the dawn of time, opposites have always held much fascination for humanity—witness, among many others, the Pythagorean “Table of Opposites” (6th century BC) with its ten pairs of contrary qualities (limited/unlimited; odd/even; unity/plurality, etc) and the Chinese Taoist philosophical paradigm, which, since the 4th-3rd century BC, lists every existing reality under the two opposite categories of Yin (陰) and Yang (陽). Originally these two characters designated the sunny side and the shady side of a valley, before becoming the two cosmic principles whose interaction and alternation preside over the genesis and evolution of the universe and constitute the “Tao” (道). But arguably none better than the Book of Ecclesiastes (written ca 250 BC) in the Bible epitomizes how our perception of life hinges on the duality of opposites:

1. To every thing there is a season, and a time to every purpose under the heaven:
2. A time to be born, and a time to die;
   a time to plant, and a time to pluck up that which is planted;
3. A time to kill, and a time to heal;
   a time to break down, and a time to build up;
4. A time to weep, and a time to laugh;
   a time to mourn, and a time to dance;
5. A time to cast away stones, and a time to gather stones together
   a time to embrace, and a time to refrain from embracing;
6. A time to get, and a time to lose;
   a time to keep, and a time to cast away;
7. A time to rend, and a time to sew;
   a time to keep silence, and a time to speak;
8. A time to love, and a time to hate;
   a time of war, and a time of peace.

Ecclesiastes 3, 1-8
These two last categories of opposites (love/hate; war/peace) take us straight to the heart of the matter since they are the perfect illustration of what **friends and foes** can be associated with. The human body, as any living organism, survives in an environment in which it is vital to distinguish what may kill it or help it thrive, harm it or benefit it. A continuous cycle between opposites, including life and death, takes place in our body cells. Nature exerts its control through sophisticated regulatory systems like an automobile driver applying, in turn, the accelerator and the brake. What is true of the organism as a whole is true of its parts, and in a very special way of its “motor,” the heart, a point abundantly underlined in this issue of *Dialogues in Cardiovascular Medicine*.

As far as the cardiomyocyte is concerned, five major entities can be identified, which are either vital or lethal—friends or foes: **oxygen**, **calcium**, **glucose**, **lipids**, and **heart rate**. It should be stressed that there is no dividing line across this group, some being friends, the others foes, but that each one individually is either a friend or a foe, depending on the context. This is where Cesar comes in, with his purported dying words *(you too, Brutus?)* on seeing his close friend among the foes about to murder him.

First, take **oxygen**: the cardiomyocyte simply cannot function without it. And yet, oxygen is a very dangerous molecule for the heart, through the highly destructive free oxygen radicals that derive from it. This Jekyll-and-Hyde–like quality was what prompted the coinage of the “oxygen paradox” concept. Yet, the heart needs and depends on oxygen to such an extent that it has developed a sophisticated defense mechanism in order to handle and eliminate its unwanted toxic side effects. Oxygen availability, in turn, depends on the coronary circulation, which is often impaired by the atherosclerotic process. It is not surprising that revascularization by any means (pharmacological or mechanic) is often an essential therapeutic procedure.

The same goes for **calcium**: if no calcium is around, you get no contraction. In contrast, calcium overload is tantamount to signing a warrant for death by apoptosis or necrosis: calcium has been dubbed the “ultimate molecular killer.”

The heart also heavily depends on **glucose** and **lipids**, which, through the aerobic and anaerobic metabolic pathways, are essential and generous friends that harness energy in the form of ATP. But there is a downside: in case of chronic excess of glucose (as in diabetes), glucose becomes probably one of the heart’s biggest enemies, causing, through atherosclerosis, major cardiovascular morbidity and mortality. Obviously, lipid disorders, again through atherosclerosis, will turn lipids into a foe. Therefore, control of diabetes and antiatherosclerotic therapy are among the most important goals of preventive medicine.
Finally, the heart, through heart rate, may be its own friend or foe. By regulating the heart’s rhythm, the sinoatrial node is not only the “pacemaker” of the heart, but also of life itself. In the animal world, there is a close relationship between heart rate and life span: species with lower heart rates live longer. For example, the mouse, with an average heart rate of 500 bpm, lives 1 to 3 years, whereas the horse, with an average heartbeat of just under 50 bpm, can live 20 to 30 years. It is likely that in future, heart rate will be considered a risk factor in its own right, as much so as high cholesterol and hypertension, and not only a reflection that something is going wrong. Control of heart rate is increasingly recognized as important in combating heart disease.

Such are the friends and foes that have been addressed at length in this issue in the Lead article and Expert Answers, but it is likely that many more will soon be identified. One lesson, however, can already be drawn—we need equilibrium: too much glucose, too little oxygen, increased heart rate, are a recipe for disaster. And yet equilibrium in all things is perhaps the most difficult goal to achieve, not only in our lives and lifetime, but also—and this is of immediate concern to us as cardiologists—in that of the myocyte.
Healthy versus sick myocyte: metabolism, structure, and function
Defining the friends and foes

Roberto Ferrari, MD, PhD
Chair of Cardiology - University of Ferrara - Ferrara - ITALY

Adult cardiomyocytes are terminal cells with minimal replicative potential. This leads to the inevitable deterioration of cardiac function once functional reserve ceases to compensate for lost contractile tissue. It follows that preservation of structure and function is the ultimate target of every therapeutic intervention. In this endeavor the cardiomyocyte has a number of friends on hand: they include stalwarts such as oxygen, calcium, and mitochondrial energy substrates (free fatty acids, carbohydrates, and glucose), but also a more recently discovered pacemaker component, the If current. Having identified the multiple friends available, the therapist must harness the services of each, while at the same time keeping a watchful eye for the cardiomyocyte’s foes. Under non-physiological conditions—primarily ischemia—erstwhile firmest friends mutate into the most formidable of foes: oxygen readmitted into ischemic tissue may no longer be used by mitochondria to drive oxidative phosphorylation, but instead converted into lethal oxygen free radicals, while mitochondria, by admitting calcium through the megapore, play a pivotal role in determining programmed cell death. Treatment is a fine balance between maximizing the potential of friends and minimizing the potential of foes.

Keywords: cardiac metabolism; ischemia; stunning; hibernation; necrosis; apoptosis

Address for correspondence: Professor Roberto Ferrari, Chair of Cardiology, University of Ferrara, Corso G. Giuseppe, 203, 44100 Ferrara, Italy (e-mail: ffr@unife.it)


SELECTED ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
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<tr>
<td>APAF-1</td>
<td>apoptotic activation factor</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>CP</td>
<td>creatine-phosphate</td>
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<tr>
<td>FLIP</td>
<td>Fas ligand inhibitory protein</td>
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<tr>
<td>IAP</td>
<td>inhibitor of apoptosis (family of proteins)</td>
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<tr>
<td>IGF</td>
<td>insulin growth factor</td>
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<tr>
<td>NAD</td>
<td>nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NADH</td>
<td>reduced nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>RyR</td>
<td>ryanodine receptor</td>
</tr>
<tr>
<td>SERCA2a</td>
<td>sarcoplasmic-endoplasmic reticulum calcium ATPase</td>
</tr>
<tr>
<td>SR</td>
<td>sarcoplasmic reticulum</td>
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<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
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the myocytes. In the following review, the metabolism, structure, and function of healthy and diseased cardiac myocytes will be described in order to identify the friends and foes of these cells.

**CARDIAC MYOCYTE ULTRASTRUCTURE**

The left and right ventricles possess the same architecture (ie, endocardium, conduction cells, capillaries, arteries, veins, nerves, etc). Only their total mass differs, as they function under a great range of different pressures. The atrial architecture is also identical in both atria, except that the sinoatrial and atrioventricular nodes are both located in the right atrium close to the superior vena cava—the sinoatrial node near the atrial septum, and the atrioventricular node near the atrioventricular junction.  

Electron and light microscopy have provided important information concerning the geometry of cardiac myocytes: each is connected longitudinally to at least two neighboring 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 or more in width). Furthermore, they 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.  

The external cardiac membrane (sarcolemma) shows specialized regions, including the intercalated disks and the transverse tubular system. It is covered by a laminar coat called glycocalyx or basal lamina, which is approximately 90 nm in width and traps several ions.
including calcium. The tubular extensions in myocytes are called transverse tubules (Figure 2A). In most mammals, they develop during the first 6 to 8 weeks after birth; in others, they are already 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. An important function of transverse tubules is propagation of the action potential. The area covered by transverse tubules increases with hypertrophy.

Cardiac sarcoplasmic reticulum (SR) is a tubular network that surrounds myofibrils and sequesters calcium from the cytosol with the help of calsequestrin, a protein that allows high accumulation of calcium. The SR network has two main components: junctional SR and free SR. The close association in cardiac muscle between two subsarcolemmal cisternae of junctional SR and one transverse tubule is called a “triad” (Figure 2B).

The contractile material is arranged in a complex structure called “Felderstruktur,” which contains 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 is approximately 2 µm; this is dependent on the state of contraction. The striations are produced by the thin actin filaments, which form the light I bands (isotropic in polarized light), and the thick myosin filaments, which form 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 the binding of calcium to another myofilament, namely troponin C. Among the intracellular organelles, mitochondria are essential for life function and also for the apoptotic death of cardiac myocytes. In cardiac tissue, mitochondria are numerous, as in all striated muscles. They occupy 30% to 38% of the entire cytoplasmic volume, and show a higher extension of the internal surface membrane (cristae), the site of the respiratory chain, oxidative phosphorylation, and production of adenosine triphosphate (ATP). Mitochondria are surrounded by an outer membrane separated from the inner membrane by the matrix space, which contains a variety of soluble enzymes and cofactors (Figure 2C).

The cytoskeleton can be viewed as an intracellular scaffold that stabilizes the topography of intracellular components and controls 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 of ventricular size and shape—called remodeling, and caused by pathological conditions—has a negative prognostic impact.

EXCITATION-CONTRACTION COUPLING

Calcium is essential in cardiac electrical conduction and is the direct activator of the myofilaments. During the action potential, calcium enters the cell through sarcolemmal depolarization-activated channels (L-type calcium channels or dihydropyridine receptors). Calci-
ment calcium sensitivity; this explains why overfilling the heart with blood results in a stronger contraction due to stimulation of the actin-myosin interaction.

This is an 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, high levels of inorganic phosphate (Pi), and magnesium. For relaxation to occur, the intracellular calcium concentration must be lowered in the cytosol and calcium dissociated from troponin C. In ventricular myocytes, most calcium is removed by the SR calcium pump (sarcoplasmic-endoplasmic reticulum calcium ATPase [SERCA2a]), and also partly by the sodium/calcium exchanger in the sarcolemma. Calcium is only minimally removed by the sarcolemmal calcium pump and the mitochondrial calcium uniporter, which are collectively called the "slow systems" and become accountable only under pathological conditions (Figure 4).

In human heart failure, functional expression of SERCA2a is reduced, whereas expression of the sodium/calcium exchanger is increased. 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. It follows that activation of the RyR may be therapeutically important for maintaining contractile function.

The SR calcium release channel is anchored to the junctional SR, together with various key regulatory proteins, which bind both to the RyR and the dihydropyridine receptor. On the SR luminal surface, the RyR is coupled to other proteins (triadin, junctin, and calsequestrin), which modulate the release process and/or buffer calcium in the SR. Calcium movements to and from the cytoplasm permit the periodicity of contraction and relaxation.

**SINUS NODE**

The action potential of specific cells in the sinoatrial node, called "pacemaker" cells, shows a different shape from the action potential of working myocytes (Figure 5). One of the major differences is that the cardiac myocytes are not able to pace spontaneously except under certain pathologic conditions, whilst the sinus node cells are. In these cells, diastolic depolarization is responsible for generation of spontaneous activity and hence of cardiac rhythm. At the end of each action potential, the repolarization ends in maximum diastolic depolarization, which in the sinoatrial node...
pacemaker cells occurs at around –60 to –70 mV. This value is greater than those typical of working myocytes, ie, below –80 mV. At this point, depolarization in pacemaker cells does not set to rest the cardiac muscle, but the myocytes slowly depolarize to a threshold that activates a fast transient, which triggers another action potential, thus producing rhythmic activity. These observations indicate that an inward component is expressed in pacemaker cells and is functioning during the slow depolarizing phase of the action potential. This current presents properties sufficient for generating action potentials, and unlike many other known currents, it is activated on hyperpolarization, before depolarization. The inward component was termed the “funny” current (\(I_f\)) because of its unusual features. The \(I_f\) current is inhibited by ivabradine which, as a result, causes pure reduction of heart rate (Figure 5). The heart can sustain its constant pump function only if a sufficient amount of energy in the form of ATP is available. Under physiological conditions, ATP is produced by aerobic metabolism, ie, by mitochondrial respiration. Oxygen is the final acceptor of electrons transported along the respiratory chain. This is why the heart is dependent on continuous oxygen availability. Its extraction from arterial blood is elevated even at rest.

**CARDIAC METABOLISM**

Cardiac metabolism is represented by an integrated network of chemical reactions with two essential aims: production of energy and production of cellular macromolecules.
physiological conditions such as starvation or hypoxia, glucose is derived from glycogen degradation. This pathway, called glycolysis, is less profitable than β-oxidation, as each molecule of substrate leads to a lower production of ATP, and it is normally inhibited when the myocardium has sufficient availability of free fatty acids. Amino acids are essential for protein synthesis, but they may also enter the Krebs cycle as substrates. The final product of the oxidation of amino acids and glucose is acetyl-CoA, which within the Krebs cycle, leads to ATP production. ATP is then carried to the cytoplasm by adenine-nucleotide translocase. Most of the ATP then reacts with creatine to produce creatine phosphate (CP). CP is then dephosphorylated by myosin ATPase to develop energy for contraction, or by other ATPases for ion transport.

During energy production, certain steps can be finely controlled. In the following section, these steps will be identified, with particular reference given to oxidative phosphorylation and mitochondrial calcium. It is surprising, however, that although glucose and free fatty acids are essential for the functioning of the heart, altered metabolism of both leads to the two major worries of the Western world: diabetes and atherosclerosis.

**Oxidative phosphorylation**

When the heart increases its workload following an increase in afterload, pressure, or heart rate or a combination of these factors, there is a concomitant increase in the need for ATP. High-energy reserves in the heart are limited, and therefore the myocardium can tolerate only short energy deficits if contractile performance is to be maintained with no structural damage. Under normal conditions, the heart produces most of its energy via oxidative phosphorylation. This process is strongly linked to ATP hydrolysis in order to maintain
a constant equilibrium, beat after beat, between energy production and energy demand. The mechanisms involved are still under debate, and information has mainly been derived from experimental studies on isolated mitochondria or in vitro cells. More recently, studies of cardiac metabolism have been carried out on isolated hearts or in vivo hearts, using noninvasive techniques such as nuclear magnetic resonance (NMR) and optic spectroscopy. Interpretation of the results obtained from intact hearts has been attempted on the basis of in vitro models. Three main steps have been identified as potential regulatory sites in oxidative phosphorylation.

**Reduced equivalent supply**

Experimental studies indicate that mitochondrial dehydrogenases such as pyruvate, isocitrate, and α-ketoglutarate dehydrogenases are the most likely regulatory sites. As catalysts of nonequilibrium reactions, these enzymes are the ideal regulatory sites within the Krebs cycle, and their activity increases following an increase in cardiac work. Many factors may modulate enzyme activity: calcium, magnesium, the adenosine diphosphate (ADP)/ATP ratio, the reduced nicotinamide adenine dinucleotide (NADH)/NAD ratio, and substrate levels. In cardiac myocytes, the most likely candidate is calcium, which is on the one hand finely bound to myosin ATPase activity and to the maximal rate of actin-myosin interaction, and on the other hand, involved in the regulation of dehydrogenase activity.

**Steps leading to phosphorylation of ADP to ATP**

In isolated mitochondria, concentrations of ADP and Pi-ATP products regulate ATP production and oxygen consumption. NMR spectroscopy has provided evidence that under normal conditions, high-energy phosphate concentration remains stable even when oxygen consumption is altered. This stability strongly depends on CP, which functions as a shuttle between ADP and ATP and as an energy store. Modulation of ATP synthase activity also represents another regulatory step in oxidative phosphorylation, catalyzing both synthesis and hydrolysis of ATP. In mammals, the hydrolytic activity is blocked by an inhibitory protein that “plays its role” when conditions do not favor ATP synthesis, such as low ADP levels and low potential difference. When there are normal levels of substrates, as well as ADP, Pi, and oxygen, and mitochondrial potential is high, ATP synthesis may occur.

**Reduction of molecular oxygen to water**

Tight control is maintained over the oxygen supply for mitochondrial oxidation regulated by cytochrome aa3, in order to adapt it to the coronary flow. Adenosine is the most likely candidate for this, since it is the terminal product of ATP degradation, and at the same time is a coronary vasodilator. If there is a high ATP breakdown, excess adenosine is produced, which dilates the coronary artery to bring more oxygen to the heart and allow the production of more ATP.

**Mitochondrial calcium**

Mitochondrial function is not only devoted to ATP production, but also to the maintenance of intracellular calcium homeostasis—in particular cytosolic calcium concentration—which is also regulated by chemosmotiologic processes. Calcium entry into the mitochondria occurs via a mono-ionic transporter called uniporter, which is likely to be a channel that opens in response to very high cytosolic calcium concentrations. Entry of calcium into the mitochondria occurs by utilizing the electrochemical gradient generated by the combination of two factors: the electrical potential of the internal mitochondrial membrane and the low calcium concentration in the matrix. The latter is maintained...
under physiological conditions by ionic exchangers, and in particular by the calcium-hydrogen and sodium-calcium exchangers.

Recently, another mechanism of calcium entry into cardiac mitochondria has been observed: rapid high-affinity entry. This calcium movement could specifically contribute to mitochondrial responses to brief physiological stimuli, and is represented by the mitochondrial transient permeability pore.

![Diagram of ion balance and metabolic processes](image)

**Figure 10. The progression of ischemic damage.**

**Abbreviations:** ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; Ca²⁺, calcium ion; CO₂, carbon dioxide; NE, norepinephrine. After reference 6: Ferrari R, Opie L. Atlas of the Myocardium. Copyright © 1992, Raven Press.

Opening of a multiprotein channel, the so-called mitochondrial megapore, is one of the factors that contributes to increased calcium permeability and activation of various caspases responsible for apoptosis. This, in turn, leads to the release of some anti-apoptotic proteins, particularly Bcl-2 and Bcl-X(L), which bind to the megapore, facilitate its closure, and reduce increases in mitochondrial membrane calcium permeability. This means that mitochondria-associated microdomains can act as regulators and catalysts of cell fate. By producing ATP mitochondria play a central role in determining life, equally, by opening the megapore and allowing calcium entry, they play a pivotal role in determining programmed cell death. Thus mitochondria and calcium are both friends and foes of the myocyte according to different circumstances. Equally, oxygen is by far the best friend, but under certain conditions it becomes a very toxic poison, producing oxygen free radicals able to destroy cells.

**HYPOXIA AND ISCHEMIA**

The term ischemia describes a condition of reduced oxygen availability in the cell such that it is insufficient to maintain the mitochondrial oxidative processes. In humans, ischemia is an indication of reduced coronary perfusion subsequent to an alteration in coronary vessel diameter.

As soon as the supply of energy fails to match the needs of intracellular metabolism, a cascade of increasingly severe metabolic perturbations commences. The cell becomes "metabolically distressed," and unless interrupted by early reperfusion, ischemia will inevitably progress toward cell death and necrosis (Figure 10).

During short periods of ischemia, e.g., in angina, the match between biochemical and mechanical activity is maintained; the mechanical activity in the ischemic areas is drastically reduced (hypokinesia and akinesia), and the residual delivery of oxygen may just be sufficient for a short period to maintain cell viability. As shown in Figure 11, in the isolated heart, complete abolition of coronary flow (global ischemia) results in a rapid downregulation of contraction and, eventually, quiescence. This is due to intracellular acidosis, which develops within seconds of induction of ischemia and reduces calcium movements within the sarcolemma, SR, and myofilaments. Shortly after this, the energy charge of the myocyte is reduced, and CP levels decline faster and to a greater extent than ATP. Anaerobic metabolism develops, leading to lactate production, and contributes to the formation of limited amounts of ATP by oxygen-independent, substrate-level phosphorylation. Taken together, these effects suggest the occurrence of biochemical ischemia (anaerobic metabolism) as well as physiological ischemia (contractile defects). Both contractile downregulation (and therefore decreased ATP consumption) and increased anaerobic ATP production are cellular cardioprotective mechanisms, occurring, however, at the expense of contractions. Thus, these two processes are very friendly. The availability of the residual ATP is essential for maintaining cellular viability and, as shown in Figure 12, is strictly dependent on the heart rate before ischemia.

Reperfusion at this stage results in 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 can be either immediate or somewhat delayed (Figure 11).

This sequence of metabolic and functional events is not restricted to experimental models, but also occurs at a clinical level, for example during angina induced by atrial pacing. In Figure 13, it can be seen that in patients with coronary artery disease, increased energy requirement from the heart due to increased heart rate, to the extent that it can no longer be met by supply, results in a reduction in coronary sinus pH; that is, in the occurrence of myocardial acidosis. This is followed by an increase in the coronary sinus concentration of lactate, indicative of anaerobic metabolism, and a downregulation of regional contraction demonstrated by a reduction in the ejection fraction, suggesting regional systolic dysfunction. All these biochemical and mechanical events precede the occurrence of the symptoms of angina. Once the heart rate has returned to its basal level and ischemia therefore no longer persists, coronary sinus pH and the release of
lactate return to their normal values and left ventricu-
lar systolic function improves. However, functional
recovery may not be immediate because of the pres-
ence of the stunning phenomenon. The term "stunning"
normally refers to a typical post-ischemic condition in
which contractile recovery is delayed by hours or even
days. The molecular mechanism that characterizes
the stunning phenomenon has not been completely
clarified. Several hypotheses have been taken into con-
sideration, such as extracellular collagen alterations,
myofilament malfunction, reduced sympathetic re-
sponse, reduced energetic reserve, calcium metabolism
alterations, and cellular damage mediated by reactive
oxygen species. Thus in this context, oxygen, which
is the number one friend of the myocyte, can also
bring about impaired contraction, at least in the is-
chemic and reperfused area. Clearly, in the
stunned myocardium, viability is maintained,
although evidence of the ischemic insult per-
sists until the metabolic recovery is matched
by functional recovery. Thus, even stun-
ning can be considered a "friend."

HIBERNATION

Repetitive episodes of stunning following
more prolonged periods of ischemia may in
some patients result in chronic regional mal-
function, which will recover upon reperfu-
sion. This condition is normally referred to
as hibernation. The term "hibernation" has
been borrowed from zoology, and implies
an adaptive reduction in energy use in the
presence of a reduced energy supply,
through reduced activity. In the early 1980s,
Rahimtoola, by reviewing the results of
coronary bypass surgery trials, identified a subset of
patients with coronary artery disease and chronic left
ventricular dysfunction that improved upon revascu-
larization. The rapid amelioration of myocardial func-
tion obtained by revascularization ruled out the hy-
pothesis that the reduced dysfunction was due to
histological modification of the myocardium, and left
the scientific community with the dilemma of explain-
ing this phenomenon.

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 pa-
ients with coronary artery disease was not easy to
recognize and was seen as enormously complex and
controversial.

The introduction of the concept of hibernation has chal-
 lenged 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 functional reduction. In view of the
preserved viability of the tissue, hibernation is a key
factor in assessing the potential benefit of reperfu-
sion/ revascularization. Two hypotheses have been put
forward: the mechanical alteration is due to "chronic
ischemia" or better, to chronic coronary blood flow re-
duction with chronic intracellular acidosis, downregu-
lating contraction to allow the maintenance of viability
via reduced ATP production; or it is due to repeti-
tive ischemic insults that maintain a chronic state of
myocardial stunning.

Whatever the underlying mechanism, hibernated my-
ocardium represents a peculiar state of the myocyte, chronically hypoperfused and akinetic, although viable.

Thus, hibernation can in general be considered a "friend of the myocyte, as it preserves its struc-
ture, but this is at the expense of its function. Thus it
is not so "friendly" when considering the heart as an
organ, because a viable yet not contracting heart is not
useful to the body!

IRREVERSIBLE ISCHEMIA
AND CELL DEATH

Experimentally, late myocardial reperfusion does not
bring about any contractile recovery. Instead, it caus-
es accumulation of tissue and mitochondrial calcium,
Healthy versus sick myocyte: metabolism, structure, and function - Ferrari

Mitochondria are the cellular organelles most likely to be involved in the transition from reversible ischemia to cell death. This is perhaps not surprising, since these organelles play a fundamental role in cellular energy production and in cell death by apoptosis and even necrosis. When the driving force of mitochondrial metabolism collapses, the megapore for calcium opens, and calcium, which accumulates in the cytoplasm, starts to enter the mitochondria, allowing the release of cytochrome c and activation of caspases, thus leading to apoptosis (Figure 15). If, by any chance, oxygen is readmitted at this stage, either because of spontaneous development of collaterals or because of induced reperfusion (by thrombolysis or primary angioplasty or surgery), it is no longer used by the mitochondria to support oxidative phosphorylation and ATP production. On the contrary, it is converted into oxygen free radicals, which are potentially destructive entities, and cell death will occur from necrosis (Figure 14). Once again, calcium, mitochondria, and oxygen thought to be the real “friends” of the heart turn out to be the real enemies (Figure 16)!

If coronary blood flow remains severely reduced, the myocardium will remain quiescent; biochemical ischemia will nonetheless intensify, and proceed toward irreversible damage. In experimental studies, this is indicated by the continuing release of lactate, increases in diastolic pressure (indicating a severe perturbation of ionic homeostasis), and signs of severe sarcolemmal damage (e.g., enzyme leakage). Under these circumstances, mitochondria themselves become targets for ischemic damage, which in turn, decreases the possibility that these changes are a consequence of either

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**Figure 15. Fundamental events characterizing apoptosis and necrosis.**

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**Figure 16. Intrinsic pathway of apoptosis caused by oxidative stress.**

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**Abbreviations:** AIF, apoptosis-inducing factor; APAF-1, apoptotic protease activating factor-1; ARC, apoptotic inhibitor; Endo G, endonuclease G; HSP-70, heat shock protein–70; IAP, inhibitor of apoptosis protein; PARP, poly (ADP-ribose) polymerase; ROS, reactive oxygen species; Smac/DIABLO, second mitochondria-derived activator of caspases/direct inhibitor of apoptosis-binding protein with low pI; zVAD-fMK, broad-range caspase inhibitor.
prolonged ischemia or post-ischemic reperfusion. Mitochondria extracted from ischemic hearts show reduced function, decreased membrane potential, and decreased activity of NADH dehydrogenase. Residual mitochondrial function during ischemia 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, if calcium has abnormally accumulated in the cytosol, reactivation of the mitochondrial electron transport chain will buffer cytosolic calcium by transporting the ion into the matrix at the expense of ATP production, thus causing irreversible necrotic damage.

Our understanding of the complexities of ischemia and tissue injury is further complicated by the need for tissue reperfusion to determine whether ischemic damage is reversible or irreversible. Some, but not all, investigators believe that reperfusion itself might be detrimental and able to inflict injury over and above the level attributable to ischemia.\textsuperscript{39} Other investigators, however, question the existence of reperfusion-induced injury.\textsuperscript{40}

Numerous studies suggest that oxygen-derived free radicals contribute to post-ischemic dysfunction. Electron magnetic resonance spectroscopy has directly demonstrated the formation of free radicals in the stunned myocardium as well as during reperfusion after prolonged ischemia, leading to the so-called reperfusion paradox.\textsuperscript{41}

**THE REPERFUSION PARADOX**

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

More than two decades ago, Hearse\textsuperscript{42} demonstrated that reoxygenation of the myocardium after prolonged oxygen depletion triggers sudden and major cellular injury, as indicated by massive enzyme leakage, sarcolemmal disruption, and hypercontracture. Re-energization then sets in motion a series of paradoxes: (i) recovery of energy production reactivates the SR calcium pump, leading to excess sequestration of calcium often exceeding the capacity of the SR, thus initiating a cycle of continuous release and uptake of calcium; (ii) excess cytosolic calcium is taken up by the re-energized mitochondria at the expense of ATP production, and (iii) resupply of energy to the myofibrils in the presence of excess cytosolic calcium leads to uncontrolled force generation and hypercontraction.

In addition, upon reperfusion, interstitial pH is rapidly normalized by proton washout, 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 co-transport.\textsuperscript{43} Activation of sodium/hydrogen exchange causes a net influx of sodium into the cytosol. Depending on the ability of the sodium pump to remove this excess, there may be a secondary activation of the sodium/calcium exchanger that, under conditions of intracellular sodium overload, will transport sodium outside and calcium inside the cell, a mechanism that will further exacerbate the pre-existing calcium overload.\textsuperscript{44}

Moreover, sodium overload increases osmotic gradients, with consequent cellular uptake of water, stretching and damaging of the sarcolemma, and further disruption of ionic homeostasis.\textsuperscript{45} Intracellular acidosis, which downregulates myofibrillar activity, is rapidly normalized, leaving the myofibrils in the presence of excessive calcium and low ATP, and thus hypercontracting. It clearly appears that after prolonged ischemia, the typical friends of the myocytes such as oxygen and calcium, become deleterious and lead to apoptosis and/or necrosis. Thus the only way to save the myocyte is to keep the ischemic process as short as possible: it follows that duration of ischemia is its real friend or foe.

**NECROSIS AND APOPTOSIS**

Whereas in the 1980s, attention was more focused on necrosis as the major cause of death following cardiac myocyte ischemia, in the 1990s, evidence of an alternative cause of death accumulated, ie, apoptosis (Figure 15).

Necrosis is a rapid and irreversible process that most cells undergo after major damage. The process does not require energy, explaining why necrosis comes into play only in severe ischemic conditions. The last step of necrosis is the “explosion” of the cell pouring out its whole cytoplasmic content and causing an inflammatory response. Necrosis involves millions of myocytes at the same time and it is considered an inci-
Apoptosis, by contrast, involves a single cell at a time, represents a genetically programmed death, is a nuclear process, and is energy-dependent. The cell “implodes” and destroys its contents, with no membrane disarrangement, and therefore no inflammation response. It divides itself into small apoptotic bodies phagocytosed by adjacent cells: thus the self-programmed apoptotic cell does not leave any trace (Figure 16).

Evidence of programmed cell death contributes to the characterization of different pathologies, such as heart failure, hypertensive disorders, viral myocarditis, sudden death, and transplant rejection, etc. The connecting line of these pathologies is the increased workload in cardiomyocytes, leading to a stretching of the sarcolemma, so that the myocytes respond by activating two distinct and yet biochemically similar processes; one leading to hypertrophy, and the other to apoptosis. Hypertrophy is expression of life, apoptosis of death.

Mixed forms of cellular death have been observed in the literature, thus it is not yet clear whether the death process is managed by a unique mechanism, or whether different types follow different mechanisms. Mechanisms of differentiation appear to be the metabolic and energetic states of the cell, intracellular pH and concentration of calcium ions, as well as the cellular redox state. Current studies indicate that the pathways of apoptotic activation are similar to those of other undifferentiated cells, the most studied are the death receptor (extrinsic) and the mitochondrial (intrinsic) pathways.

The extrinsic pathway

This pathway requires activation of the death receptors belonging to the tumor necrosis factor (TNF) superfamily, located on the cell surface, via specific ligands, and permits an immediate response to the environment. To date six factors have been characterized: Fas, TNF-R1, DR3, TRAIL (ligand bound to TNF, favoring apoptosis) —TRAIL-R1 or DR4, TRAIL-R2 or DR5, and DR6. Via intracellular death dominions, these six death receptors trigger a chain of cytoplasmic events that lead to cell death by activation of specific proteases, such as caspase 8 and 10. However, this process is not always activated by Fas or TNF, since it can be modulated by other proteins such as, for example, the Fas ligand inhibitory protein (FLIP) or the GIP130.

The intrinsic pathway

In the mitochondrial pathway, caspase 9 is the effector of cell death. Cytochrome c, involved in a regulatory step in mitochondrial respiration, is also one of the fundamental factors in this apoptotic pathway. Under physiological conditions, cytochrome c is hidden in the intermembrane space of the mitochondrion, away from the cytoplasmic apoptotic activation factor (APAF-1). Following stress and alter disarrangement and permeabilization of the outer mitochondrial membrane to calcium, cytochrome c binds to APAF-1, allowing the activation of caspase 9, which in turn activates caspase 3. Permeabilization of the outer mitochondrial membrane is one of the characteristic events of physiologic and pathologic cell death, which is regulated at different levels. It is induced by several second pro-apoptotic messengers, such as calcium ions, reactive oxygen species, and lipid messengers (like ceramide).

Members of the Bcl-2 family may modulate mitochondrial permeabilization processes; this family is composed of both promoters and inhibitors of apoptosis. Recent studies have revealed that mitochondrial pro-apoptotic proteins may be released by mechanisms independent of pore formation. Pore openings dissipate membrane potential (ΔΨm), and high calcium together with reactive oxygen species appears to trigger the changes. Prolonged pore opening causes transient permeability and a fall in the protonic gradient essential for energy production, and ATP hydrolysis. In accordance with this observation, translocation of cytochrome c into the cytoplasm and/or a fall in mitochondrial ΔΨ are associated with apoptosis of cardiomyocytes induced by different stimuli, such as hydrogen peroxide, hypoxia/re-oxygenation, serum/glucose deprivation, and ischemia/reperfusion.

Although caspases are considered the more important proteases contributing to the death program, in some cases, apoptosis may be activated in their absence by other proteases such as cathepsins and calpains.

The initiation events in apoptosis are multiple and different, but the final step is normally activation of the caspase family. Many regulatory factors have caspases as their target, these include the FLIP and Inhibitor of Apoptosis (IAP) families, also present in the heart.

The Bcl-2 family members are targets of other regulatory agents, since overexpression of these proteins in rats, and in particular in ventricular myocytes, helps...
Insulin growth factor (IGF)-1 is another important growth and survival factor in cardiomyocytes. Its ability to inhibit apoptosis induced by ischemia/reperfusion or infarction, limiting ventricular remodeling and hypertrophy, has been reproduced and its therapeutic potential is encouraging.

Furthermore, heat shock proteins, such as Hsp70s and Hsp27, appear to inhibit cytochrome c release and caspase 3 activation, whereas Hsp60s and Hsp10 protect cardiomyocytes against ischemia/reperfusion damage by maintaining mitochondrial integrity.

Calcium ions, through changes in cytosolic concentration, are often associated with apoptosis, and many effectors of the apoptotic process are calcium-dependent. By contrast, antioxidants represent one of the most potent agents against oxidative stress. They remove reactive oxygen species and reduce oxidative damage derived from ischemia/reperfusion injury. In cardiac cells, many antioxidants are present and active: glutathione, glutathione-peroxidase, superoxide dismutase, catalase, and vitamins E and C.

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Friends & Foes of the Cardiac Myocyte

*Expert Answers to Three Key Questions*

1. How best to control the lack of oxygen in the ischemic cardiomyocyte?
   
   *F. Crea, G. A. Lanza*

2. How best to achieve heart rate control in coronary artery disease patients?

   *N. Danchin*

3. Diabetes: what’s the best strategy for blood glucose?

   *U. Ayyagari, P. Dyson, D. Matthews*
Continuous oxygen supply is crucial to cardiomyocyte function and integrity. Lack of oxygen leads to rapid myocardial cell damage. In the clinical setting, the most frequent cause of lack of oxygen in myocardial cells is ischemia. This may occur because: (i) coronary artery stenoses impede adequate increase in oxygen supply to match increased myocardial consumption; or (ii) a primary reduction in coronary blood flow (CBF) occurs resulting from acute coronary thrombosis or spasm. Definitive treatment of myocardial ischemia relies on prompt restoration of CBF. However, other treatments aim to increase the resistance of cardiomyocytes to ischemia and delay cell death in case of prolonged ischemia, through: (i) reduction in myocardial oxygen consumption; (ii) optimization of cardiomyocyte metabolism; (iii) preconditioning and postconditioning; or (iv) prevention of apoptosis.

Keywords: oxygen supply; cardiomyocyte; myocardial ischemia; stenosis; coronary blood flow; preconditioning; postconditioning; apoptosis; trimetazidine; perindopril; ivabradine; ranolazine; glucose-insulin-potassium; angiotensin receptor blocker; β-blocker; insulin-like growth factor I

Address for correspondence:
Prof Filippo Crea, Istituto di Cardiologia, Università Cattolica del Sacro Cuore, Rome, Italy (e-mail: filippo.crea@rm.unicatt.it)

Reduction in myocardial oxygen consumption

The most obvious way to prevent the deleterious effects of myocardial ischemia is to reduce oxygen consumption by reducing myocardial work. This ensures prolonged survival by reducing cardiomyocyte energy consumption and catabolite production. As heart rate is by far the most important factor affecting myocardial oxygen consumption (which is halved by halving heart rate), reducing the heart rate represents a crucial means of protection against myocardial ischemia. In the clinical setting, heart rate reduction has been classically achieved with β-blockers, which reduce myocardial work by reducing blood pressure and
myocardial contractility. β-Blockers have been shown to reduce the extent of myocardial necrosis following prolonged acute coronary occlusion both in experimental studies and in patients.\(^1\)

However, other drugs, such as ivabradine, are capable of achieving pure heart rate reduction, without affecting other determinants of oxygen consumption. Ivabradine, used in the treatment of patients with stable angina, is a selective and specific inhibitor of the \(I_f\) sinus node current.\(^2\) The ASSOCIATE study (evaluation of the Antianginal efficacy and Safety of the aSsociation Of the \(I_f\) Current inhibitor IvabrAdine with a beTa-blockEr) showed that ivabradine, given on top of the β-blocker atenolol in patients with stable angina, is safe and provides significant improvement in exercise capacity, making this one of the best evidence-based combination therapies for angina patients.\(^3\) The recently reported findings from the BEAUTIFUL trial (morBidity-mortality EvAlUa-Tion of the \(I_f\) inhibitor ivabradine in patients with coronary disease and left ventricULar dysfunction) have suggested that the benefits of ivabradine might extend beyond its antianginal properties. Indeed, in this study the drug significantly reduced coronary events (with a 36% reduction in the risk of fatal or non-fatal myocardial infarction) in the subgroup of patients with heart rate above 70 beats per minute (bpm), a post-hoc observation that merits investigation in appropriately designed prospective trials.\(^4\)

**IMPROVEMENT IN OXYGEN UTILIZATION BY MYOCARDIAL CELLS**

While myocardial ischemia has traditionally been treated with drugs that reduce myocardial oxygen consumption and/or improve CBF in recent years, new drugs have been developed with the aim of improving oxygen utilization by myocardial cells.

In normal heart, free fatty acids (FFA) are the predominant substrate used by the heart as energy source (60% to 90%), while glucose metabolism contributes only 10% to 40% to energy generation. During ischemia, heart metabolism shifts toward an increased use of glucose further depress myocardial function in ischemic segments. Furthermore, FFA alters cellular ion homeostasis, leading to increased arrhythmogenic substrate production.

**Trimetazidine**

Drugs such as trimetazidine, which partially inhibit FFA oxidation and favor glucose utilization during ischemia, are thus expected to have a beneficial effect on myocardial cells.\(^5\) Trimetazidine has been reported to exert anti-ischemic properties without affecting myocardial oxygen consumption and blood supply.\(^6-8\) The beneficial effect of this agent has been attributed to preservation of intracellular levels of phosphocreatine and ATP,\(^9,10\) and reduction in ischemia-related cell injury caused by cell acidosis, calcium overload,\(^11\) and free radicals.\(^12\)

Specifically, trimetazidine has been shown to reduce FFA oxidation rate and increase glucose oxidation during low-flow ischemia, via hi-
The efficacy of trimetazidine in stable angina pectoris has been extensively assessed in several clinical studies, both in monotherapy\(^{14}\) and in combination. TRIMPOL II (Second TRIMetazidine in POLand trial), a randomized, multicenter, double-blind, placebo-controlled study in 347 stable angina patients insufficiently controlled by a β-blocker, showed that addition of trimetazidine led to a significant improvement in all ergometric and clinical parameters, including a significant reduction in the mean number of angina attacks, as well as a significant increase in time to 1-mm ST-segment depression and in total exercise duration (Figure 1).\(^{15}\)

These findings have been confirmed in a recent meta-analysis of 18 randomized, controlled double-blind, parallel-group studies, which has shown, in 2713 patients with angina pectoris, that trimetazidine is effective and safe in reducing symptoms, and that it also improves ischemic threshold and exercise capacity.\(^{16}\)

Several clinical trials have also reported additional benefits of trimetazidine in patients with stable angina, showing improvement in left ventricular ejection fraction and in regional wall-motion abnormalities during dobutamine stress echocardiography\(^{17}\) together with improvement in exercise-induced angina and ischemic ECG changes. Moreover, extensive evidence in the literature indicates that trimetazidine may improve cardiac function and functional capacity in patients with ischemic cardiomyopathy.\(^{18-21}\)

Finally, although no benefit in terms of survival was observed in a large trial using 48-hour intravenous trimetazidine infusion in patients admitted for acute myocardial infarction (AMI), in the subset of nonthrombolyzed patients there was a significant decrease in mortality associated with trimetazidine treatment.\(^{22}\)

### Glucose-insulin-potassium (GIK)

GIK—a solution including glucose, insulin, and potassium—has long been used with the aim of increasing glycolysis and reducing FFA uptake and metabolism in myocardial cells during AMI. Insulin alone has also been proposed in this context. However, the efficacy of GIK or insulin alone in preserving myocardial cell integrity during AMI remains controversial, with some studies (eg, DIGAMI [Diabetes mellitus Insulin Glucose infusion in Acute Myocardial Infarction trial])\(^{23}\) and some GIK studies suggesting favorable effects on survival with this approach, both in diabetic and non-diabetic patients, while others have failed to confirm these results.\(^{24}\)

### Ranolazine

Recent findings suggest that ranolazine may act through inhibition of the ventricular late inward sodium current. This would reduce \(\text{Na}^+\)-\(\text{Ca}^{2+}\) exchange and intracellular calcium concentrations, thus improving ischemia-induced myocardial dysfunction.\(^{25}\) Conflicting data have been reported in clinical trials. In an early trial in patients with stable angina, the effects of ranolazine (30 to 120 mg tid) were similar to placebo with respect to exercise-induced myocardial ischemia and angina symptoms.\(^{3}\) In contrast, two studies using higher doses of ranolazine (up to 1500 mg twice a day) showed more favorable effects. In the Monotherapy Assessment of Ranolazine In Stable Angina (MARISA) trial, exercise duration and time to angina and to 1-mm ST-segment depression were longer on ranolazine than on placebo.\(^{26}\)

In the Combination Assessment of Ranolazine In Stable Angina (CARISA) trial, which randomized 823 patients with stable angina receiving either a β-blocker or a calcium-channel blocker, to ranolazine (750 or 1000 mg bid) or placebo, duration of exercise and time to ST-segment depression and to angina were longer, and weekly angina episodes were fewer, on ranolazine than on placebo, with no significant side effects being reported.\(^{27}\)

### MYOCARDIAL PRECONDITIONING AND POSTCONDITIONING

#### Ischemic preconditioning

The term ischemic preconditioning (IPC) indicates the ability of one or more brief periods (≤5 minutes) of myocardial ischemia to induce protection against cardiomyocyte injury following a subsequent prolonged period of ischemia. IPC decreases infarct size by 40% to 75% and the period of protection usually lasts 1 to 2 hours after the IPC stimulus (early IPC)(Figure 2, page 250).\(^{28}\)

The mechanisms of IPC are complex and have not yet been completely elucidated. Some key events, however, have been clearly identified.\(^{29-31}\) Thus, protein kinase C (PKC), particularly its isoform PKCe, is a key mediator of IPC. Various substances that accumulate in the interstitium during myocardial ischemia have been shown to be involved in IPC (eg, adenosine, bradykinin, norepinephrine, opioids), and these substances activate PKC through activation of phospholipase C. However, other kinases, such as protein kinase A, which is activated by β-adrenergic agonists, are also involved in IPC.
Reactive oxygen species (ROS) also activate PKC and other kinases. Recent data suggest that ROS, in spite of their well-known detrimental effects on cell metabolism, may have a crucial role in the mechanisms triggering IPC. Indeed, substances able to generate ROS (e.g., acetylcholine, bradykinin, opioids, volatile anesthetics) are also known IPC agonists, whereas free radical scavengers and antioxidants block IPC.

Adenosine, which accumulates in the heart during ischemia, has also repeatedly been shown to be a mediator of IPC through PKC activation. Further, in Kir6.2 knockout mice, which lack surface K<sub>ATP</sub> channels, the protective effect of IPC is abolished. Opening of mitochondrial K<sub>ATP</sub> channels, which regulate matrix volume during ischemia and reperfusion, also seems to play a key role in IPC.

Another key step in IPC is the opening of surface K<sub>ATP</sub> channels, mediated by phosphorylation of the channel protein by PKC and resulting in reduction of Ca<sup>2+</sup> influx and energy expenditure. IPC is blocked by the K<sub>ATP</sub> channel antagonist glibenclamide and induced by K<sub>ATP</sub> channel openers (aprikalim, pinacidil, diazoxide). Furthermore, in Kir6.2 knockout mice, which lack surface K<sub>ATP</sub> channels, the protective effect of IPC is abolished. Opening of mitochondrial K<sub>ATP</sub> channels, which regulate matrix volume during ischemia and reperfusion, also seems to play a key role in IPC.

Further relevant mechanisms in IPC include a slight (2.5% to 4%) mitochondrial swelling and modulation of the mitochondrial permeability transition pore (MPTP), a complex protein channel of the mitochondrial inner membrane.

IPC has been suggested to be involved in several clinical settings, including the reduced extent of myocardial necrosis and the better outcome of patients with AMI preceded by unstable angina, as compared with unheralded AMI, and the reduced ischemic damage during the second of two successive episodes of transient myocardial ischemia in the setting of coronary balloon angioplasty or exercise stress testing.

Several drugs, including K<sup>+</sup> channel openers (diazoxide, nicorandil), volatile anesthetics (halothane, isoflurane), agonists of G-protein–coupled receptors (adenosine, bradykinin, catecholamines, opioids), and blockers of the Na<sup>+</sup>/H<sup>+</sup> exchanger (NHE) (cariporide, amiloride) have been shown to reproduce the effects of IPC, a phenomenon known as pharmacological preconditioning. It remains to establish whether the beneficial effects, observed in clinical trials for some of these drugs, are, at least in part, mediated by myocardial preconditioning.

Notably, a second, late “window of protection” has been demonstrated as a consequence of ischemic preconditioning stimuli. This delayed IPC occurs about 24 hours after the preconditioning stimulus and may persist for about 72 hours, but is less effective, typically decreasing infarct size by about 25%. Delayed IPC is related to the activation of genes that transcript for cardioprotective proteins. PKC, nitric oxide synthase (NOS), and cyclooxygenase (COX)-2 seem to play a role in this form of IPC.

**Postconditioning**

As previously observed, timely reperfusion is the definitive treatment for myocardial ischemia. Reperfusion, however, particularly when delayed, may have deleterious effects on myocardial cells, which contributes significantly to the persistence of myocardial dysfunction in spite of the fact that complete reperfusion has been achieved. The mechanisms of reperfusion injury are complex, but seem to involve rapid ROS generation, NHE-1 activation, an inflammatory response to reperfusion, and mitochondrial per-
meability transition pore (MPTP) opening. Several therapeutic options have been tested to prevent reperfusion injury in experimental models (eg, NHE-1 inhibitors, regional hypothermia, and gradual reperfusion), but none has been successfully tested in patients with AMI.

Very recently, an endogenous form of myocardial protection able to limit reperfusion, rather than ischemic, injury, named "postconditioning" (post-C), has been described. Post-C consists of a series of brief coronary occlusions applied at the time of reperfusion after a prolonged myocardial ischemia. In the original study, dogs subjected to 1-hour myocardial ischemia following left anterior descending coronary artery occlusion were randomized to abrupt reperfusion (controls), 3 cycles of 30 seconds of reperfusion, and 30 seconds’ reocclusion before definitive reperfusion (post-C), or to IPC prior to the 1-hour occlusion.

Compared with controls, infarct size was reduced by approximately 40% both in IPC and in postconditioned dogs (Figure 3). The effect of post-C has been confirmed in other studies. The duration of reperfusion/reocclusion time seems to be critical to cardioprotection. Shorter ischemia-reperfusion episodes (eg, 10 to 30 s) appear to be more effective than longer periods (eg, 1 min), whereas the number of reperfusion cycles applied does not seem to have any relevant impact on the achievement of significant post-C cardioprotection. It is not clear whether post-C is related to the short ischemic periods or to the short reperfusion periods of the post-C sequence.

The mechanisms of post-C remain to be clarified. Interestingly, however, several systems shown to be involved in IPC have also been suggested to be involved in post-C. Thus, adenosine seems to play an important role in post-C, which is prevented by A2a and A3 receptor blockade. Surface and mitochondrial KATP channels also have been suggested to play a role, as glibenclamide and 5-hydroxydecanoate prevent post-C. Prevention of MPTP opening during reperfusion may also be involved. Differently from early IPC, NOS seems to play a role in post-C, as NOS blockade with L-NAME (nitro-L-arginine methyl ester) inhibits its cardioprotective effects. Finally, the involvement of reperfusion injury survival kinases in post-C has recently received much attention. Some studies suggest that post-C is mediated through the PI3-kinase-Akt pro-survival pathway, although this mechanism must be verified in further studies.

Compared with IPC, post-C could theoretically be applied more easily in clinical practice, in particular in patients with AMI undergoing primary coronary intervention. While there are both practical and ethical issues about triggering post-C by repeated mechanical occlusion following reperfusion of the infarct-related artery, the demonstration of the role played by some agents in IPC (eg, adenosine, NO, insulin, statins, opioids) suggests it would be interesting to test them in this clinical setting.

**INHIBITION OF APOPTOSIS**

In recent years, cell death by apoptosis (programmed cell death) has been claimed to play a relevant role in the progressive loss of cardiac myocytes and deterioration of left ventricular (LV) function in ischemic regions. This suggests that inhibition of apoptosis could limit ischemia-induced cardiomyocyte loss, in particular in chronically ischemic myocardium, thus reducing LV remodeling and improving clinical outcome.

The key enzyme pathway leading to apoptotic cell death is the caspase cascade, a group of cysteinyl-aspartate-directed proteases, which, in healthy cells, reside in the cytosol as inactive proforms. When activated, caspases cleave several proteins critical to cell life. Two major path-
ways are known to activate the apoptotic sequence in mammalian cells, although other less known pathways of activation exist (Figure 4). The “intrinsic” pathway is triggered by ischemia-reperfusion, hypoxia, and oxidative stress and is mediated by injured mitochondria, which release substances able to activate the caspase cascade and translocate them to the nucleus, where they induce, either directly or indirectly, DNA fragmentation. The “extrinsic” pathway of apoptosis involves the activation of the so-called death receptors, such as Fas and TNFα (tumor necrosis factor-alpha) receptors, which trigger formation of a death-inducing signaling complex through caspase-8 activation. This pathway is more likely activated when the membrane Fas and TNFα receptors increase, as in heart failure.

Besides caspases, several other families of proteins, such as the Bcl-2 family, have been shown to be involved and modulate apoptosis (either as activators or inhibitors). As previously observed, the decrease in apoptosis rate could limit ischemia-induced myocardial cell loss. Some drugs known to have favorable effects on ischemic cardiomyopathy, including angiotensin-converting enzyme (ACE) inhibitors, might act through this mechanism.

The ant apoptotic effect of ACE inhibitors has been investigated in endothelial cells in an in vivo animal model. In this model, however, only perindopril, among five ACE inhibitors tested (enalapril, perindopril, quinapril, ramipril, and trandolapril), was associated with a significant decrease in the rate of endothelial cell apoptosis (P<0.001) (Figure 5).

These findings are in line with the recent PERindopril-Thrombosis, InflammatioN, Endothelial dysfunction and Neurohormonal activation Trial (PERTINENT), in which patients with coronary artery disease received perindopril 8 mg/day for 1 year. The PERTINENT substudy, which assessed both clinical and biological markers of endothelial function, showed a 31% (P<0.05)
reduction in the rate of apoptosis with perindopril. Treatment with perindopril concomitantly restored the balance between angiotensin II and bradykinin levels toward normal, reduced indices of inflammation (TNFα levels returned to normal, -13%, P<0.05 vs baseline), and, in doing so, prevented endothelial apoptosis from occurring. Angiotensin II blockers and β-blockers have also been shown to have anti-apoptotic effects in animal models, due to the inhibitory effect on the renin-angiotensin system and the sympathetic system, which, under certain conditions, may activate apoptosis.41

Antioxidant agents could also act as antiapoptotic substances, since oxidative stress and ROS are able to trigger the “intrinsic” apoptotic pathway. Accordingly, in a rat infarct model, the antioxidant probucol was shown to prevent upregulation of several proapoptotic molecules.44

Specific potential targets to limit apoptosis include caspases and endonucleases. Inhibitors of these enzymes have been shown to reduce infarct size and LV remodeling in experimental models of ischemic injury.49 Finally, insulin-like growth factor I (IGF-I) has also been shown to improve cardiac function in animal models of cardiomyopathy through an antiapoptotic effect mediated by caspase-3 inhibition.44

Some potential limitations in applying therapeutic strategies designed to specifically inhibit apoptosis in clinical practice should, however, be acknowledged, in particular with regard to the potential procarcinogenic activity of apoptosis inhibition. Furthermore, while dosing and scheduling of antiapoptotic drugs have been precisely specified in animal models, what should be for use in patients is at present still unknown.

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Attenuation of ischemia/reperfusion injury in rats by a caspase inhibitor. 
Numerous epidemiological studies have found strong correlations between resting heart rate and long-term outcomes, both in normal populations and in coronary disease patients. The recently published fourth set of European guidelines on cardiovascular disease prevention recommend that assessment of heart rate form an integral part of the assessment for total cardiovascular disease risk.

With regard to patients with coronary artery disease (CAD), there are many reasons to believe that heart rate reduction should form an important part of optimal CAD management. Obviously, a high heart rate induces or exacerbates myocardial ischemia and angina, because it both increases oxygen demand and decreases myocardial perfusion. The latter occurs as a result of shortening of the duration of diastole, which is when the larger part of coronary perfusion takes place due to considerably less wall stress during diastole than systole. For the clinician, heart rate control is associated with functional improvement in symptoms in patients with angina, concomitant with a decrease in myocardial ischemia.

Experimental data and clinical observations demonstrate the important role played by heart rate in the pathophysiology of atherosclerosis, which through the stress exerted on the vascular endothelium leads to atherosclerotic lesion formation and plaque rupture. The recent results of BEAUTIFUL (morBidity-mortality EvAlUaTion of the I inhibitior ivabradine in patients with coronary disease and left ventricULar dysfunction) have shown that elevated heart rate is a strong marker of adverse cardiovascular outcomes. Slowing heart rate may thus be a valid therapeutic target in coronary artery disease patients. β-Blockers are the most widely used medication able to decrease heart rate. They improve cardiovascular outcomes in patients after acute myocardial infarction, but their use remains fraught with difficulties, mainly related to their potential side effects. There are also the heart rate-lowering calcium channel blockers diltiazem and verapamil, but their effect is difficult to predict and data on their clinical benefit are rather scant. The If current inhibitor ivabradine is a potent antianginal agent that acts through pure heart rate reduction and is devoid of other hemodynamic properties. Beside medication, exercise training is an efficacious means of lowering heart rate if maintained over time.

**Keywords:** angina; coronary artery disease; exercise training; heart rate; If current inhibitor; β-blocker; calcium channel blocker

**Address for correspondence:** Professor Nicolas Danchin, Department of Cardiology, Hôpital Européen Georges Pompidou, 20 rue LeBlanc, 75015 Paris, France (e-mail: nicolas.danchin@egp.ap-hop-paris.fr)

**SELECTED ABBREVIATIONS AND ACRONYMS**

BEAUTIFUL — morBidity-mortality EvAlUaTion of the If inhibitior ivabradine in patients with coronary disease and left ventricULar dysfunction

bpm — beats per minute

CAD — coronary artery disease

CIBIS — Cardiac Insufficiency Bisoprolol Study

COMMIT — Clopidogrel and Metoprolol in Myocardial Infarction Trial

DAVIT — Danish Study Group on Verapamil in Myocardial Infarction

INVEST — INternational VErapamil-trandolapril STudy

MDPIT — Multicenter Diltiazem Post Infarction Trial

PCI — percutaneous coronary intervention

SHIFT — Systolic Heart failure treatment with the If-inhibitor ivabradine Trial

SPECT — single photon emission computed tomography

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hibitor ivabradine in patients with coronary disease and left ventricular dysfunction (and left ventricular systolic dysfunction) provided prospective confirmation that high heart rate (70 beats per minute [bpm] or greater) is an independent risk factor for adverse outcomes in patients with CAD and left ventricular systolic dysfunction (Figure 1), and extended the evidence to a wide range of coronary events such as admission to hospital for fatal and nonfatal myocardial infarction, and coronary revascularization. Additionally, because the use of background evidence-based therapy—including importantly, β-blockers—was very high in BEAUTIFUL, at levels far above those of previous surveys in stable CAD, the study constitutes the first clear demonstration that elevated resting heart rate places patients at risk for cardiovascular events, even if they are apparently well treated. The study data indicate that heart rate should be considered in every patient with coronary heart disease as a simple measurement with prognostic implications. In the present article, we will review the methods available in clinical practice to control and reduce heart rate, and their potential impact on the control of myocardial ischemia and the prevention of cardiovascular events. Essentially, three classes of medications are available with the ability to decrease resting heart rate: β-blockers, heart rate–lowering calcium channel blockers, and the newer agents, the If current inhibitors. In addition to pharmacological methods, specific interventions—in particular exercise training—also decrease heart rate and are likely to bring substantial benefits to CAD patients.

NONPHARMACOLOGICAL INTERVENTIONS

Nonpharmacological interventions such as physical training and cardiac rehabilitation can lower heart rate, both at baseline and during exercise, resulting in an increase in exercise capacity. Malfatto et al studied 53 patients with stable CAD and a recent myocardial infarction. Three groups were examined: 14 patients who received β-blocking agents and did not participate in the rehabilitation program (group 1), 19 patients who participated in the rehabilitation program but received no β-blockers (group 2), and 20 patients who both underwent cardiac rehabilitation and received β-blockers. At baseline, exercise duration, maximal workload, and rate-pressure product were similar in all 3 groups. After 3 months, however, exercise duration and maximal workload increased and the rate-pressure product decreased in a similar way in groups 2 and 3, while all 3 variables remained unchanged in the group that received β-blocking agents but did not participate in the rehabilitation program. Such benefits from physical training may result in definite clinical improvement, both in terms of symptoms and coronary events. Recently, physical training was compared with percutaneous coronary intervention (PCI) in a controlled trial including 101 patients aged ≤70 years with stable CAD and an index coronary artery stenosis amenable to percutaneous intervention. Patients in the exercise training group first had a 2-week hospital program of physical training, and were then asked to exercise daily (20 minutes of bicycle ergometry per day). At 2 years of follow-up, resting heart rate decreased from 71 to 65 bpm in the exercise-training group, but was unchanged in the PCI group. There was an equivalent improvement in the Canadian classification of angina for both groups. Maximal exercise tolerance, however, increased in the
exercise-training group but remained unchanged in the PCI group. Event-free survival was higher in the rehabilitation group (88% versus 70%, \( P=0.02 \)), mainly because of interventions in the group having undergone coronary angioplasty.

**β-BLOCKING AGENTS**

β-Blockers represent the class of anti-ischemic medication with the most widely recognized impact on heart rate and the broadest clinical experience. Typically, a β-blocking agent such as atenolol decreases resting heart rate by 12-15 bpm. During exercise, heart rate is similarly decreased with β-blockers compared with subjects not receiving β-blockers.

In the recent past, data have suggested that β-blockers might have additional protective effects compared with other antianginal medications that have similar anti-ischemic properties. In a series of 352 patients with stable CAD having undergone thallium single photon emission computed tomography (SPECT), Marie et al.\(^\text{14}\) analyzed the influence of different medications given immediately after the exercise test. The presence of myocardial ischemia documented by thallium SPECT was a strong predictor of major coronary events in the whole population; in patients who received β-blocking agents after the test, however, the prognostic impact of exercise ischemia was no longer evident, whereas it remained present in patients who received additional antianginal medications other than β-blockers. Whether the additional protection conferred by β-blockers is partially or totally related to their heart rate–lowering capacity remains speculative. Concordant data do suggest, however, that heart rate reduction might be one of the main reasons for their efficacy. Experimental data in the past have shown that in animals subject to pacing, the anti-ischemic effect of β-blockers is no longer present.\(^\text{15}\) After myocardial infarction, there is ample evidence that β-blocking agents improve long-term prognosis. In a meta-analysis by Freemantle et al.\(^\text{16}\) administration of β-blockers was associated with a 23% reduction in the risk of death over the following years. However, when β-blockers were classified according to their pharmacological properties, those with intrinsic sympathomimetic activity appeared to have a lesser degree of efficacy than those without intrinsic sympathomimetic activity (odds ratio [OR], 1.19; 95% confidence interval [CI], 0.96-1.47). Likewise, in randomized trials with β-blocking agents in the post–myocardial infarction setting, a very strong correlation has been observed between reduction in mortality with β-blockers and decrease in heart rate.\(^\text{17,18}\) Lastly, Aupetit et al.\(^\text{19}\) analyzed the impact of propranolol on the fibrillation threshold in pigs subjected to increasing periods of ischemia. Under spontaneous heart rate conditions, propranolol significantly increased the fibrillation threshold for all durations of coronary occlusion. When the animals were paced, however, the antifibrillatory effect of propranolol completely disappeared, suggesting that this major protective property of β-blocking agents might be solely related to their impact on heart rate.

The additional properties of β-blockers, however, might also contribute to their beneficial effects. In the Cardiac Insufficiency Bisoprolol Study II (CIBIS-II), which assessed the role of β-blockade with bisoprolol in patients with congestive heart failure,\(^\text{20}\) changes in heart rate were recorded in patients alive 2 months after inclusion in the trial, and the impact of the changes on long-term survival was assessed. Using multivariate analyses, both a lower baseline heart rate and a higher degree of heart rate reduction during the first 2 months were independently correlated with late mortality. In patients with sinus rhythm at baseline, treatment with bisoprolol was an additional predictor of improved survival, independent of baseline heart rate and heart rate reduction, suggesting independent roles for heart rate reduction and use of the β-blocking agent bisoprolol in this setting.

Experiments in nonatherosclerotic dogs also suggest that the anti-ischemic efficacy of β-blockers is not solely due to their heart rate reduction properties, but also to their negative inotropic effects. Colin et al.\(^\text{21}\) compared myocardial oxygen consumption in a series of 8 instrumented dogs given saline, atenolol, or ivabradine infusion. Both atenolol and ivabradine decreased oxygen consumption at maximal workload, though the β-blocker had a more profound effect. When the dogs were paced, however, ivabradine failed to improve oxygen consumption while atenolol still had an impact, suggesting that in this model, the anti-ischemic effect of atenolol was equally due to its action on heart rate and inotropism. However, the prolongation of diastolic time, which is an important determinant of myocardial oxygen supply, was significantly shorter with atenolol than with ivabradine, despite similar reduction in heart rate. This smaller prolongation of diastolic time with atenolol compared with ivabradine could be explained by a significant increase in left ventricular ejection time resulting from the negative inotropic effect of atenolol. In addition, atenolol extends the relaxation process due to a negative lusitropic effect.
**β-Blockade** could increase or unmask α-vasoconstriction, particularly in situations of increased sympathetic activity such as stress and exercise, which could, as a consequence, reduce coronary blood flow and contribute to the precipitation of acute myocardial ischemia. Unmasked α-adrenergic coronary vasoconstriction is particularly evident in atherosclerotic coronary vessels; it is seen at the level of epicardial conduit coronary arteries where it is mediated by α₁-adrenoceptors, and it is even more prominent in the coronary microcirculation, mediated by α₂-adrenoceptors. Whatever their mechanism of action, β-blockers remain rather difficult to use in everyday life. Recent data from registries in patients with acute myocardial infarction show that the proportion of patients receiving β-blocking agents at hospital discharge ranges from 60% to 80%. In the Euro Heart Survey on stable angina, which was carried out in 2002 in patients with stable CAD throughout Europe, 67% of patients were treated with β-blockers, with only small differences between geographical regions; among patients not receiving β-blockers, only 24% were reported as having contraindications. In patients with signs of heart failure or with moderate to severe left ventricular dysfunction, the initial dose of β-blocker must be low and the dose must then be increased very progressively; this explains why target doses are often not achieved in such patients. In addition, β-blockers slow intracardiac conduction and they may cause atrioventricular block. By contrast, they are particularly useful in patients with rapid atrial fibrillation. In patients with sick sinus node syndrome, however, implantation of a pacemaker is usually necessary before prescribing β-blocking agents. All of these reasons may explain why in patients in whom β-blocker therapy was initiated following acute myocardial infarction, only 60% still used them 1 year later.

Very recently, the results of the CLOTidogrel and Metoprolol in Myocardial Infarction Trial (COMMIT) emphasized the limits of using β-blockers in acute situations such as acute myocardial infarction. In this large controlled trial, the potential benefits of the early administration of the β-blocker metoprolol were counteracted by a 30% increase in the risk of cardiogenic shock and a 12% increase in the risk of heart failure, obviously related to the negative inotropic effects of metoprolol. In addition, there was a threefold increase in respiratory problems and a twofold increase in conduction disturbances or atrial arrhythmias. Overall, the positive effects of metoprolol on reinfarction and ventricular fibrillation were masked by the negative impact of the drug, mainly due to its effects on cardiac function.

**CALCIUM CHANNEL BLOCKERS**

The long-acting formulations of the nondihydropyridine calcium channel blockers diltiazem and verapamil both decrease heart rate. In the Danish Study Group on Verapamil in Myocardial Infarction II (DAVIT II) trial, verapamil improved outcomes in patients with a history of myocardial infarction without heart failure. A slight prolongation of the PR interval is common with either verapamil or diltiazem. For these reasons, the combination of heart rate-lowering calcium antagonists with β-blockers is not recommended. In real-world registries, only a minority of patients with CAD is treated with calcium channel blockers, particularly after myocardial infarction, and the proportion has considerably decreased in the past decade.

**I₄ CURRENT INHIBITORS: IVABRADINE**

Recent years have seen the development of the I₄ current inhibitors. The I₄ current regulates the slope of diastolic depolarization in the sinus node, and its inhibition results in a marked decrease in heart rate. Previous agents such as zatebradine had an action both on the I₄ current and on the I₃K current, which regulates the duration of the action potential, this resulted in an increased QT duration, and the clinical devel-
Development of zatebradine was therefore stopped. Ivabradine has virtually no effect on the duration of the action potential at the recommended doses, and electrophysiologic studies have shown that its effect on corrected QT duration is neutral. The advantage of its mode of action lies in its ability to reduce heart rate without impairment of key cardiovascular or hemodynamic parameters such as myocardial contractility, ventricular repolarization, cardiac conduction, blood pressure, and coronary vascular resistance.

In man, ivabradine decreases resting and exercise heart rate in a dose-dependent manner, as clearly demonstrated in the study by Borer et al (Figure 2). Several large clinical trials in patients with stable angina have documented its anti-anginal and anti-ischemic efficacy, which appears comparable to that of atenolol (Figure 3) and amlodipine. Results from a recent study demonstrated the anti-anginal and anti-ischemic efficacy of ivabradine in combination with a β-blocker. The addition of ivabradine to therapy for patients with angina pectoris already receiving atenolol 50 mg/day reduced heart rate by 9 bpm and significantly improved all parameters relating to exercise capacity compared with placebo.

The medication appears easy to use and is well tolerated: in clinical trials to date, less than 1% of patients have developed profound bradycardia <40 bpm. This is due to the fact that the propensity of ivabradine to decrease heart rate is dependent on the basal activity of the If channels: in patients with a spontaneously slow heart rate, ivabradine will further reduce heart rate by only a small extent, while in patients with a high heart rate, it will provide a much greater decrease (Figure 4).

Some patients may experience visual symptoms (phosphenes) over the first weeks of treatment. In a recent study, visual symptoms were reported as an adverse event in 1.8% of patients in the ivabradine group and 0.9% of patients in the placebo group. These symptoms usually disappear after a few weeks of treatment, do not have an impact on the daily activity of patients, and have raised no specific safety concerns.

The large morbidity mortality trial BEAUTIFUL was conducted to assess whether heart rate reduction with ivabradine could improve the prognosis in CAD patients. The BEAUTIFUL investigators added...
substantially to current knowledge concerning the prognostic value of elevated heart rate by also carrying out analysis of the prospective data from the placebo arm of the study to assess the association between heart rate and different outcomes. Elevated resting heart rate (≥70 bpm) was associated with a 34% increase in relative risk for cardiovascular mortality (P=0.0041), a 53% increase in relative risk for heart failure (P<0.0001), a 46% increase in relative risk for myocardial infarction (P=0.0066), and a 38% increase in relative risk for coronary revascularization (P=0.037). Ivabradine did not affect the primary composite end point, which was driven mainly by heart failure outcomes. However, in patients with a heart rate of 70 bpm or greater, ivabradine did reduce end points related to CAD—a 36% reduction in admission to hospital for fatal and nonfatal myocardial infarction (P=0.001) and a 30% reduction in coronary revascularization (P=0.016) (Figure 5). These benefits were recorded in patients receiving optimal preventive therapy, including β-blockers in 84% of patients. Our knowledge of ivabradine will be enhanced by the results of the Systolic Heart Failure treatment with the If inhibitor ivabradine Trial (SHIFT), which is studying a typical heart failure population.

It must be remembered, however, that the mode of action of ivabradine requires the presence of sinus rhythm: in patients with atrial fibrillation, the medication therefore has no detectable effect.

CONCLUSION

Beyond exercise training, several classes of medication have the ability to decrease heart rate in CAD patients. The most extensively studied is the β-blocker, which improves outcomes after acute myocardial infarction. Unfortunately, contraindications and side effects such as fatigue, asthma, bronchospasm, or erectile dysfunction are not uncom-
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Beta-blockers in acute myocardial infarction intervention trials.


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Minimizing macrovascular and microvascular complications is a major goal in the management of diabetes. For this to be achieved, it is necessary to adopt a multiple risk-factor interventional approach. This involves lifestyle choices, careful education, and strategies addressing the reduction of blood glucose, control of blood pressure, and optimization of lipid profiles. Here we discuss the general strategy, with particular emphasis placed on blood glucose control. A number of recent studies have investigated the effects of tight blood glucose control on macrovascular outcomes in patients with diabetes, and these are also reviewed. Although current guidelines provide a suggested approach to management of diabetes, therapeutic targets as well as treatment regimens should be individually tailored and agreed upon between physician and patient.

Keywords: diabetes; complication; macrovascular; intervention; blood glucose; cardiovascular; risk factor; lifestyle; education; therapy

Address for correspondence:
Professor David Matthews, Professor of Diabetes Medicine, National Institute for Health Research, Oxford Biomedical Research Centre, Oxford, UK
(e-mail: david.matthews@ocdem.ox.ac.uk)

SELECTED ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACCORD</td>
<td>Action to Control CardiOvascular Risk in Diabetes (study)</td>
</tr>
<tr>
<td>DAFNE</td>
<td>Dose Adjustment For Normal Eating (education program)</td>
</tr>
<tr>
<td>DCCT</td>
<td>Diabetes Control and Complications Trial</td>
</tr>
<tr>
<td>DESMOND</td>
<td>Diabetes Education and Self Management for Ongoing and Newly Diagnosed (education program)</td>
</tr>
<tr>
<td>DIGAMI</td>
<td>Diabetes Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction (study)</td>
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<tr>
<td>DPP-4</td>
<td>Dipeptidyl peptidase-4</td>
</tr>
<tr>
<td>EDIC</td>
<td>Epidemiology of Diabetes Interventions and Complications</td>
</tr>
<tr>
<td>GIP</td>
<td>gastric inhibitory peptide</td>
</tr>
<tr>
<td>GLP-1</td>
<td>glucagon-like peptide-1</td>
</tr>
<tr>
<td>NICE</td>
<td>National Institute for Clinical Excellence (UK)</td>
</tr>
<tr>
<td>PROactive</td>
<td>PROspective pioglitAzone Clinical Trial In macroVascular Events</td>
</tr>
<tr>
<td>RECORD</td>
<td>Rosiglitazone Evaluated for Cardiovascular Outcomes in oral agent combination therapy for type 2 Diabetes</td>
</tr>
<tr>
<td>UKPDS</td>
<td>United Kingdom Prospective Diabetes Study</td>
</tr>
<tr>
<td>VADT</td>
<td>Veterans Affairs Diabetes Trial</td>
</tr>
</tbody>
</table>

The clinical picture of diabetes mellitus is defined by hyperglycemia leading to macrovascular and microvascular complications.1,2 Macrovascular disease (coronary artery disease, cerebrovascular disease, and peripheral vascular disease) is the leading cause of morbidity and early mortality in diabetes mellitus. Up to 80% of patients with type 2 diabetes die from cardiovascular complications, and average life expectancy is reduced by approximately 10 years.3 The increased risk of macrovascular disease associated with diabetes cannot solely be explained by the presence of associated cardiovascular risk factors, and persists even after adjustment for other risk factors such as smoking, hypertension, and hyperlipidemia.4,5
ments in microvascular end points in patients with diabetes (type 1 diabetes in DCCT and EDIC and type 2 diabetes in UKPDS) with improvement in blood glucose control. In type 1 diabetes, intensive blood glucose control has also been shown to reduce the risk of any cardiovascular event by 42%, and the risk of nonfatal heart attack, stroke, or death from cardiovascular causes by 57% (EDIC). In UKPDS, which compared dietary management alone with intensified treatment using oral antihyperglycemic agents or insulin in patients with type 2 diabetes, each 1% reduction in the updated mean HbA1C level (for any level of blood glucose) was associated with reductions in risk of 21% for any diabetes-related end point, 21% for death related to diabetes, 14% for myocardial infarction, and 37% for microvascular complications. No threshold of risk was observed for any of the end points. The study also showed a decrease in the development of macrovascular complications in the intensive treatment group, although in the main study, the end points did not reach statistical significance.

Compared with those receiving conventional treatment in UKPDS, the group treated with metformin showed a 36% reduction in all-cause mortality, a 39% reduction in myocardial infarction, and a 30% reduction in all macrovascular disease (myocardial infarction, sudden death, angina, stroke, and peripheral disease).

Although the cardiovascular outcomes of the PROspective pioglitAzone Clinical Trial In macroVascular Events (PROActive) were positive, doubts remained about the totality of benefits, as the main reported result was inconclusive. The RECORD trial (Rosiglitazone Evaluated for Cardiovascular Outcomes in oRAl agent combination therapy for type 2 Diabetes) showed no effect on outcome. With conflicting evidence in type 2 diabetes in favor of tight glycemic targets to optimize macrovascular outcomes, three trials were designed to address this—Action to Control CardiOvascular Risk in Diabetes (ACCORD), Action in Diabetes and VAscular disease: Preterax and DiamicroN MR Controlled Evaluation (ADVANCE), and Veterans Affairs Diabetes Trial (VADT). Their aim was to determine the effect on primary cardiovascular end points of lowering blood glucose levels to the near-normal range in type 2 diabetics with and without pre-existing cardiovascular disease. Unfortunately, these trials have not laid the question to rest. Although the results of VADT indicated a favorable trend toward reduction of all cardiovascular end points except death, none of the trials provided unequivocal evidence that blood glucose control to near-normal levels improves macrovascular outcomes.

Not only did ACCORD fail to show improvement in cardiovascular outcomes, there was a troubling finding of significantly increased incidence of death from any cause and death from cardiovascular causes in the intensive control arm. This finding was not borne out by the ADVANCE data, but there were significant differences between the two trials.

Although the intensive glucose control arms of both the trials achieved an almost equivalent HbA1C level (ACCORD 6.4% compared with ADVANCE 6.5%), ACCORD used an aggressive treatment strategy. In addition: (i) subjects in ACCORD reached their nadir HbA1C in 8 months, compared with 3 years in ADVANCE, (ii) mean weight gain in ACCORD was 3.5 kg in those receiving intensive glucose control, compared with no weight gain in the ADVANCE intensive glucose control arm; (iii) a higher proportion of subjects in ACCORD eventually received insulin (77% ACCORD compared with 40% ADVANCE) and thiazolidinediones (92% compared with 19%); and (iv) subjects in ACCORD had a higher incidence of severe hypoglycemia (six times the rate in ADVANCE), perhaps reflecting the more aggressive glucose-lowering strategy and higher proportion of patients receiving insulin.

**Long-term tight blood glucose control**

Together, the data from the three trials did not show any significant improvement in the risk of major cardiovascular events over the trial duration of 4 to 6 years. This perhaps suggests the need for longer-term trials in diabetes to evaluate the cardiovascular benefits of intervention.

Data from the 10-year follow-up of UKPDS showed that as expected, the between-group differences in HbA1C levels were lost within 1 year of the end of the study, as all patients were converted to tighter control. Despite this convergence, relative reductions in risk persisted at 10 years for the sulfonylurea-insulin group regarding any diabetes-related end point and microvascular disease, and risk reductions for myocardial infarction and death from any cause also emerged over time. In the metformin group, significant risk reductions persisted for any diabetes-related end point, myocardial infarction, and death from any cause also emerged over time. This “legacy effect” of intensive blood glucose control was also demonstrated by the DCCT/EDIC data. During the mean 17 years of follow-up, although differences in HbA1C levels between the treatment groups were lost, initial
intensive blood glucose treatment reduced the ultimate risk of any cardiovascular event by 42% and the risk of nonfatal myocardial infarction, stroke, or death from cardiovascular disease by 57%. This perhaps points toward a need to treat patients to tight blood glucose targets from early in the course of both type 1 and type 2 diabetes.

**Control of associated risk factors**

Evidence is also accumulating to suggest that optimizing the control of associated risk factors (hypertension, hypercholesterolemia) may play a key role in cardiovascular risk reduction in diabetes. The Steno 2 diabetes study showed the benefit of multifactorial risk reduction in type 2 diabetes. The intensive control arm aimed for stepwise introduction of lifestyle measures (reduced intake of total and saturated dietary fat, dietary supplementation with vitamin E, C, folic acid, and chromium, regular exercise, and smoking cessation) and pharmacological intervention. Antihyperglycemic therapy was intensified as necessary, with the aim of achieving an HbA1C level of <6.5%; all patients were prescribed angiotensin-converting enzyme inhibitor or angiotensin receptor blocker therapy aimed at achieving blood pressure of 130/80 mm Hg, lipid-lowering therapy was prescribed with the aim of achieving fasting serum cholesterol <175 mg/dL, and triglycerides <150 mg/dL. Aspirin was initially prescribed for secondary prevention, and eventually in all subjects in the intensive control arm. After 7.8 years of follow-up, subjects in the intensive control arm showed risk reductions of 55% for developing cardiovascular disease, 61% for developing nephropathy, 58% for developing retinopathy, and 63% for developing autonomic neuropathy.

**Acute intervention for the reduction of blood glucose**

The evidence for acute intervention to lower blood glucose levels is less well established, although it is recognized that stress hyperglycemia increases the risk of death in myocardial infarction. The Diabetes Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction-I (DIGAMI 1) study was a randomized prospective study in which patients with suspected acute myocardial infarction and a blood glucose level ≥11 mmol/L were randomized to receive insulin therapy or standard treatment. The study found a 30%-50% decrease in long-term mortality in the insulin-treated patients, although it remained uncertain whether the benefit was due to the insulin-glucose infusion in the first 24 hours of admission, or the follow-up longer-term subcutaneous insulin therapy and subsequent improvement of HbA1C by 1.5%. These findings were not substantiated in the DIGAMI 2 study, although this study suffered from a loss of power and clinical events more commonly observed in the intensive control arm than expected.

**Therapeutic targets for blood glucose control**

The risk of cardiovascular disease and microvascular complications in those with diabetes is known to be related to the extent of hyperglycemia over time, although target levels for control of hyperglycemia are the subject of continuing debate. At the ends of the spectrum, we know that proliferative and sight-threatening retinopathy is not a feature of those with normal glucose homeostasis, and at the other extreme, those with the poorest control are liable to multiple tissue complications including serious retinopathy and early death. The consensus is that in type 2 diabetes, aiming for near-normal blood glucose levels, lowering systolic blood pressure to about 130 mm Hg, and reducing cholesterol concentrations can improve microvascular and macrovascular outcomes. However, there is evidence from ACCORD that in patients with established type 2 diabetes and a background of high cardiovascular risk, overaggressive reduction of blood glucose (to 6.5% rather than 7.5%) increases total mortality.

Over the years, guideline-recommended HbA1C targets for diabetes control have been lowered. Current views suggest that 7.0% is a reasonable target, and 6.5% may be obtainable in some patients—but there are certain caveats to this recommendation, especially regarding the elderly and those with established cardiac disease. The UK National Institute for Health and Clinical Excellence (NICE) has issued guidelines on the achievement of optimal blood glucose control, and they suggest that one should: (i) involve the person in decisions about their individual HbA1C target level, which may be above the level of 6.5% set for people with type 2 diabetes in general; (ii) encourage the person to maintain their individual target unless the resulting side effects (including hypoglycemia) or their efforts to achieve the target impair their quality of life; (iii) offer therapy (lifestyle and medication) to help achieve and maintain the HbA1C target level; (iv) inform a person with a higher HbA1C level that any reduction in HbA1C toward the agreed target is advantageous for future health; and (v) avoid pursuing highly intensive management to levels of less than 6.5%.
With regard to the targets outlined above, one should mention that the units of HbA1C are likely to be reported differently (Table 1), as proposed by the International Federation of Clinical Chemistry and Laboratory Medicine.23

With regard to diabetes pharmacotherapy, there are no strong data indicating the order in which one should use particular therapies, although each individual agent has been tested in monotherapy against other regimens. The lean type 2 diabetic patient may benefit from treatment with an insulin secretagogue or insulin therapy, whereas those who are overweight may benefit more from an insulin sensitizer. Owing to the progressive nature of β-cell failure in type 2 diabetes, blood glucose control will deteriorate on monotherapy alone, and most patients need multiple agents to achieve glycemic control in the long term.24 NICE has produced a treatment algorithm (Figure 1, page 268)22 that is applicable to most patients with type 2 diabetes, although individuals may tolerate agents differently and can experience a range of side effects.

### THERAPIES FOR REDUCING BLOOD GLUCOSE LEVELS

#### Education, diet, and lifestyle

Education and the acquisition of skills for self-management of diabetes form the cornerstones of diabetes management. Structured education programs for type 1 and type 2 diabetes (InSight, Dose Adjustment For Normal Eating [DAFNE], Diabetes Education and Adjustment For Normal Eating [DESmOND]) aim to impart knowledge and skills to the learner, their family, and carers to enable them to make appropriate lifestyle, dietary, and management choices.22 Lifestyle factors, especially diet and physical activity, are effective therapies in treating diabetes, with evidence showing that medical nutrition therapy decreases HbA1C levels, low density lipoprotein (LDL) cholesterol, and blood pressure in individuals with diabetes.25 Lifestyle interventions in people with type 2 diabetes result in improvements in blood glucose control26 and significant weight loss in overweight and obese individuals.27

#### Diet

Dietary advice should be tailored to the individual, and should address personal, cultural, and religious preferences, taking into account the individual's willingness to change their customary lifestyle. There is now a broad consensus on the type of diet that is most beneficial for people with diabetes, and this conforms to the idea of what constitutes a healthy diet for the nondiabetic population.

#### Weight management

Weight management is especially important for the 80%-90% of people with type 2 diabetes who are overweight or obese. Modest weight loss (5%-10%) has been shown to improve both insulin sensitivity and blood glucose control.28 Weight loss of 11% reduces mortality in type 2 diabetes by 25%.29

Realistic targets for weight loss should be agreed upon, as many people with diabetes are unable to reduce their body weight to within the normal range.

It is recommended that weight reduction be undertaken through a combination of diet and increased physical activity. There is no convincing evidence as to the most effective method for weight loss in people with diabetes, and the best strategy is that which matches the individual's food preferences and lifestyle.

#### Physical activity

Regular daily physical activity is of benefit in people with both type 1 and type 2 diabetes, regardless of body weight. Physical activity improves blood glucose control in people with type 2 diabetes,30 reduces cardiovascular risk,31 and reduces the risk of diabetes-related complications in people with type 1 diabetes.32
Insulin regimens

Results from DCCT and UKPDS as well as follow-on data from these studies demonstrate the benefits of tight blood glucose control in the long term. Insulin is the mainstay of treatment in type 1 diabetes, and needs to be given within a few years of diagnosis of type 2 diabetes. UKPDS data show that β-cell function is reduced to 50%-60% of its normal level at diagnosis of type 2 diabetes. This declines further at a rate of approximately 4% per year, and 5%-10% of type 2 diabetics will convert to insulin therapy each year. However, all too frequently in

**Figure 1.** Current treatment algorithm from the National Institute of Health and Clinical Excellence (NICE) for type 2 diabetes. After reference 22: National Collaborating Centre for Chronic Conditions. Type 2 Diabetes. National Clinical Guideline for Management in Primary and Secondary Care (Update). Copyright © 2008, Royal College of Physicians.
type 2 diabetes, patients perceive initiation of insulin therapy as a “failure” and an “end-of-the-road” option. This association with negative connotations, along with the fear of insulin injections, leads to a reluctance to commence insulin, resulting in reduced uptake and delay in initiation of therapy. Poor concordance impacts on physical, social, and psychological aspects of quality of life.

The choice of the ideal insulin regimen is hotly debated, and should be adjusted to patient preference, lifestyle, and desired control. Many patients combine insulin with other antihyperglycemic agents—either oral agents (although not in combination with thiazolidinediones, as this increases the risk of congestive cardiac failure) or glucagon-like peptide-1 (GLP-1) agonists. The most popular regimens include twice-daily premixed insulin, or basal (long-acting insulin) and prandial (rapid and short-acting insulin) doses.

Sulfonylureas
Sulfonylureas were the first pharmacological agents discovered to have insulinotropic effects in man. They were discovered as a side effect of sulfonamide production in 1942, and have been used for the treatment of type 2 diabetes for over 50 years. They have a successful track record in major outcome trials—most notably in UKPDS, where there was strong evidence to support their use.

Sulfonylureas stimulate insulin secretion by binding to the sulfonylurea receptor-1 in the plasma membrane of the pancreatic β cells. This causes closure of the adenosine triphosphate–sensitive potassium channel (K_ATP), preventing potassium efflux, leading to membrane depolarization and the opening of voltage-dependent calcium channels. The influx of calcium raises intracellular calcium concentrations and activates exocytosis of insulin. Sulfonylureas have a rapid onset of action, a duration of action lasting between 12 and 24 hours, and can reduce fasting plasma glucose by 2-4 mmol/L and HbA1C by 1%-2%.

The two marketed glinides are repaglinide and nateglinide. Repaglinide has a short half life of less than 1 hour. It is metabolized in the liver by CYP3A4. This is inhibited by gemfibrozil, which can exacerbate the hypoglycemic effects of repaglinide. The glinides are rapid-acting insulin secretagogues with a short half-life, and are dosed at meal times. They act by binding to the sulfonylurea receptor-1. In theory, the mealtime dosage is meant to confer flexibility and convenience of dosing—in practice this is less so, and leads to problems with concordance.

The principal side effect with both sulfonylureas and glinides is hypoglycemia.

Biguanides
The mode of action of metformin remains obscure and the main molecular mechanism of action has not been fully elucidated, although it is know that it acts as an insulin sensitizer in the liver and promotes glucose uptake in the muscle. It lowers fasting plasma glucose by 2-4 mmol/L and HbA1C by 1%-2%. UKPDS demonstrated excellent and significant effects on outcome in overweight patients. The main side effects with both sulfonylureas and glinides is hypoglycemia.

The glinides
The glinides are rapid-acting insulin secretagogues with a short half-life, and are dosed at meal times. They act by binding to the sulfonylurea receptor-1.

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Table II. Pharmacokinetic properties of sulfonylureas. Based on data from reference 37.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Total daily dose (mg)</th>
<th>Half-life (hours)</th>
<th>Duration of action*</th>
<th>Main route of elimination</th>
<th>Active metabolites?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpropamide</td>
<td>100-500</td>
<td>36</td>
<td>Long</td>
<td>Urine &gt;90%</td>
<td>Active</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>500-2000</td>
<td>4.5 - 6.5</td>
<td>Short</td>
<td>Urine ~ 100%</td>
<td>Inactive</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>2.5 - 15</td>
<td>10</td>
<td>Intermediate to long</td>
<td>Bile &gt;50%</td>
<td>Active</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>1 - 6</td>
<td>5</td>
<td>Intermediate to long</td>
<td>Urine ~ 60%</td>
<td>Active</td>
</tr>
<tr>
<td>Glipizide</td>
<td>2.5 - 20</td>
<td>2 - 5</td>
<td>Short to intermediate</td>
<td>Urine ~ 70%</td>
<td>Inactive</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>40 - 320</td>
<td>12</td>
<td>Intermediate</td>
<td>Urine ~ 65%</td>
<td>Inactive</td>
</tr>
<tr>
<td>Gliclazide MR</td>
<td>30 - 120</td>
<td>16</td>
<td>Intermediate to long</td>
<td>Urine ~ 65%</td>
<td>Inactive</td>
</tr>
<tr>
<td>Gliquidone</td>
<td>15 - 180</td>
<td>0.4 - 3</td>
<td>Short to intermediate</td>
<td>Bile ~ 95%</td>
<td>Inactive</td>
</tr>
</tbody>
</table>

*short <12 hours; intermediate 12-24 hours; long >24 hours
dominal discomfort, diarrhea, nausea, flatulence), but remit with time. Approximately 10% of patients cannot tolerate metformin. A newer modified-release preparation has a more gastrointestinal-friendly profile. Lactic acidosis is rare (0.03 cases per 1000 patient-years), but when it occurs, it has a high mortality rate. Metformin is contraindicated in renal failure (creatinine ≥130 μmol/L or estimated glomerular filtration rate <45 ml/minute/1.73 m²).

**Thiazolidinediones**

Thiazolidinediones (gliptazones) are highly selective agonists of the peroxisome proliferator-activated receptor-γ found in adipose tissue, skeletal muscle, and liver. Thiazolidinediones improve sensitivity to insulin in muscle, liver, and adipose tissue, increase peripheral glucose uptake, inhibit hepatic gluconeogenesis, and, some months into their use, improve β-cell function. They lower HbA1C by 0.5%-1.5%. Thiazolidinediones cause fluid retention and edema and are therefore contraindicated in heart failure. Anemia, an increased incidence of fractures, and liver failure are other recognized side effects.

**Acarbose**

Acarbose is an α-glucosidase inhibitor that reduces digestion and the consequent rate of absorption of carbohydrates. It reduces postprandial hyperglycemia and lowers HbA1C by 0.2%. In Chinese subjects with impaired glucose tolerance, acarbose caused reductions in postprandial glucose, body weight, and conversion to type 2 diabetes, indicating a potential benefit in the delay of type 2 diabetes. It causes dose-related gastrointestinal side effects such as flatulence, abdominal discomfort, and diarrhea, and therefore it is generally advised to start with a low dose and gradually increase the dose to the desired amount/maximum tolerated dose.

**Incretin-based therapies**

In healthy individuals, it is known that for a given rise in plasma glucose, the subsequent rise in plasma insulin will be threefold greater when the glucose is administered orally compared with intravenously. This effect is mediated by the incretin hormones GLP-1 and glucagon-dependent insulinotropic peptide—also known as gastric inhibitory peptide. In type 2 diabetes, the β-cell response to GIP is lost, but preserved to GLP-1. The levels of endogenous GLP-1, however, are reduced. Replacement of GLP-1 either with synthetic analogues (exenatide, liraglutide) or through inhibition of the half-life of endogenous GLP-1 (sitagliptin, vildagliptin) forms the cornerstone of incretin-based therapies.

**GLP-1 mimetics: exenatide and liraglutide**

Exenatide, originally found in the venom of the Gila monster (Heloderma suspectum), a South American lizard, has a half-life of about 3-4 hours and needs to be administered twice daily to gain its full therapeutic effect. Liraglutide shares 97% homology with the native human GLP-1 peptide chain, with the addition of a 16-C fatty acid chain to Lys26. It has a longer half-life than exenatide, and is suitable for once-daily administration. GLP-1 mimetics show marked insulinogenic effects in the presence of glucose, with enhanced β-cell function and concomitant lowering of HbA1C levels by 0.6%-0.8%. As GLP-1 mimetics stimulate insulin release only in the presence of blood glucose, the risk of hypoglycemia is low. The side effect is nausea in a significant number of subjects. The nausea can be transient, but in some patients it continues, and it can be a cause of discontinuation of the medication. There have been reports of an increase in pancreatitis in those taking exenatide, and there have also been reports of some deaths. Longer acting (once-weekly) preparations are under assessment. GLP-1 mimetics also cause weight loss as a desirable side effect.

**DPP-4 inhibitors: sitagliptin and vildagliptin**

Dipeptidyl peptidase-4 (DPP-4) inhibition is a rational approach to enhancing GLP-1 function, because endogenous GLP-1 is present in significant quantities but is normally degraded by DPP-4, so that its half-life is normally only about 2 minutes. The advantage of using inhibitors of DPP-4 is that they are not peptides and can thus be given in tablet form. They lower HbA1C levels by about 0.5%-1.0%. The side effects are few—there is little or no nausea and no increase in the rates of hypoglycemia. DPP-4 inhibitors are weight neutral, but recent comparator trials with liraglutide over 26 weeks have shown weight loss of up to one kilogram.

**DIABETES IN THE ELDERLY**

Type 2 diabetes in the elderly may be a metabolically discrete entity, although as in the young and middle aged, it is characterized by a combination of β-cell dysfunction and insulin resistance. Lean elderly patients predominantly exhibit deficits in insulin secretion, whereas in the obese elderly, the problem concerns decreased insulin sensitivity. Hepatic glucose output is generally not increased. The risk of severe hypoglycemia increases with age due to multiple factors—
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Several years ago, I received an announcement of a meeting in Germany of a "World Conference on Magic Bullets—Celebrating Paul Ehrlich's 150th Birthday." Who was Paul Ehrlich, what were his magic bullets, and what is the significance of Ehrlich's work for the 21st century?

Paul Ehrlich was born in 1854 in Upper Silesia, then Germany. He studied medicine at the Universities of Breslau, Strasbourg, Freiburg, and Leipzig. In 1878, he obtained his doctorate of medicine. He then worked at a medical clinic in Berlin. In 1882, he became titular professor in Berlin and joined Robert Koch, the discoverer of the tubercle bacillus. The rest of his life Ehrlich spent in Frankfurt as director of a scientific institute where he received the Nobel Prize. He died in 1915 from a cerebral vascular accident. Ehrlich was a man obsessed by his work, burning the candle on both ends, but he was also gentle and caring, beloved by his staff and his family. Ehrlich was addicted to Havana cigars. Cigar smokers of today can only envy him; in Ehrlich's time there was no embargo on imported cigars from Cuba.

As a medical student Ehrlich became fascinated by the use of aniline dyes in the staining of blood and tissue. In his first publication still as a medical student, he described the staining qualities of tissues and cells. In his first publication still as a medical student, he described the staining qualities of tissues and cells. Soon after in his doctoral thesis, he formulated ideas on the chemical relationship between cells and dyes. Much of his subsequent work originated from these early studies, as he expressed it, "corpora non agunt nisi fixata," substances do not interact unless fixed (bound).

Ehrlich developed techniques for the staining of blood cells and first identified mast cells and eosinophils. Later, when working with Robert Koch, he perfected the staining of the tubercle bacillus. Koch, who had not been able to demonstrate these bacilli in blood, received a slide in which a blood smear contained numerous tubercle bacilli. Ehrlich discovered that the slide had been superimposed on another which had not been sufficiently cleaned and which contained numerous tubercle bacilli in infected tissue. Ehrlich's scientific career was not with-
Paul Ehrlich and his magic bullets

Ehrlich’s work for which he received the Nobel Prize began with studies on the plant toxin ricin, a poison which is much in the news today. As he wrote, “The most noteworthy finding that surprised me is the sudden—I might say critical—appearance of immunity on the sixth day.” His demonstrations that protective immunity is not limited to bacterial toxins, that high titers of antiserum can be obtained by starting with low initial amounts of antigen, that actively induced immunity is long-lasting and has a sudden onset, had a remarkable impact on immunology. Ehrlich worked on the protective action of maternal milk and established that the presence of antibodies explains the immunity against some infectious diseases during the first year of life. Soon afterwards, Ehrlich began to standardize toxins and antitoxins, leading him to the quantification of diphtheria antitoxin production. Ehrlich viewed the antigen-antibody interaction as specific, depending on the chemical joining of two complementary structures.

When I went to medical school, I learned about Ehrlich’s side-chain theory of antibody formation. He pictured the interaction as a chemical process involving stereochemical structures that fit together as lock and key. The cell has receptors, in the form of side-chains, that are to become antibodies. As he expressed it: “one may therefore rightly assume that these toxophile protoplasmic groups (receptors) in reality serve normal functions in the animal organisms and that they only incidentally and by pure chance possess the capacity to anchor themselves to this or to that toxin.”

At that time the cause of syphilis, Treponema pallidum, was discovered and Ehrlich set about to treat syphilis by chemotherapeutic agents. He considered these substances as magic bullets, aiming only at the pernicious invaders of the organism. Ehrlich already had prepared a large number of derivatives of arsanilic acid and its reduction products. In 1907, he tested the 606th preparation, which had been put aside because it was thought to be ineffective. Hata from Japan, working with Ehrlich, found that this preparation was highly effective in the treatment of syphilis-infected animals. In
1910, the clinical treatment of syphilis was announced and 606 was given its name, salvarsan.

What happened to Ehrlich’s concept of the magic bullet in the 21st century? Within the last years, magic bullets have been devised primarily for the treatment of specific cancers. They are now referred to as smart bullets, targeted drugs, therapies that zero in on specific targets such as specific receptors on the cell surface. They assault a patient’s cancer cells while steering clear of normal cells. So far, targeted compounds have been tried in colon, lung, breast, and kidney cancers, and in chronic lymphocytic leukemia. Many of them are monoclonal antibodies, their action based on modulation of angiogenesis through inhibition of vascular endothelial, fibroblast, epidermal, and platelet-derived growth factors.

Fundamentally, many of these compounds act by blockading the receptor tyrosine kinase activity of vascular endothelial growth factor receptors. In cardiology, promotion of angiogenesis is needed, rather than its inhibition. For example, vascular endothelial growth factor protein is used in stable angina and cell-based therapy is attempted, based on the fact that postnatal vascular genesis contributes to blood vessel formation in adults. Yet, molecular changes in cancer are the results of faulty gene expression. In heart muscle on the other hand, hypoxia and ischemia result in nonspecific injuries.

Paul Ehrlich’s faith in specific treatments of disease, his magic bullets, has been vindicated, another step in the endless fight against disease.

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Friends & Foes of the Cardiac Myocyte

Summaries of Ten Seminal Papers

Michael J. Shattock
Cardiovascular Division - King's College London - St Thomas' Hospital - London SE1 7EH - UK
(e-mail: michael.shattock@kcl.ac.uk)

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Dept of Cardiology - Azienda Ospedaliero Universitaria - Università di Ferrara
Ferrara - ITALY

Highlights of the years by Ian Mudway, MD
Lung Biology - Division of Life Sciences - Franklin Williams Building
150 Stamford Street - London SE1 9NN - UK
some evolutionary biologists argue that we evolved despite oxygen rather than because of it. In fact, the demise of the dinosaurs has been attributed, by some, to their inability to deal with rising atmospheric PO$_2$. It has also been argued that the high metabolic rates and increasing body size in small mammals were only possible because of the increasing availability of oxygen.

Whatever the truth, it is clear that mammals have evolved potent antioxidant defenses to protect their cells against the incredible reactivity of molecular oxygen. So, in the context of this issue, it is difficult to decide—is oxygen a friend or a foe? One of the first studies to show that reoxygenation might exacerbate damage to the hypoxic or ischemic heart was provided by this study from Hearse and colleagues.

To place this study in its historical context, it is useful to appreciate that, in the early 1970s, the attention of cardiologists was firmly focused on the deleterious effects of ischemia and oxygen deprivation. At this time many centers around the world were investigating the mechanisms underlying ischemic injury. The concept that, in the absence of reperfusion, infarct size and ischemic damage could be limited by pharmacological intervention was slowly starting to evolve. This understandable fixation on the ischemic process and its manipulation, however, distracted attention from reperfusion or reoxygenation. But in 1972, Hearse and colleagues reported a series of studies, which, in retrospect, were to focus attention on the possible damaging effects of reoxygenation and reperfusion, and these studies were undoubtedly the catalyst for many others investigating “reperfusion injury.”
While Hearse and colleagues specifically set out to investigate the effects of reoxygenation on hypoxic tissue injury and enzyme leakage, their thinking was that “... enzyme release could be reduced or even halted by reoxygenation.” They were, however, clearly surprised by their results. At the end of their Introduction, they whet the reader’s appetite to their intriguing observations by stating “In the course of this study the unexpected observation was made that reoxygenation during glucose free [hypoxic] perfusion exacerbated enzyme release.” Although they did not coin the term “oxygen paradox” until a later paper, this sentence marks the birth of this concept—that is, reoxygenation, while undoubtedly essential for the long-term survival of the tissue, can paradoxically in itself cause massive tissue injury.

To investigate reoxygenation, Hearse and colleagues used the isolated perfused rat heart preparation. During hypoxia, they showed that enzymes typically leak from the heart in two phases. Firstly, there is a small “blip” of enzyme release after about 40 to 50 min, but this small release (just 2% to 5% of the total release) is a pretaste of a much larger and more sustained release. In this second phase, enzymes are progressively lost from the hypoxic heart as the cell membranes become increasingly damaged. This second phase of enzyme release peaks at around 3 to 4 hours and represents the loss of the major portion of soluble intracellular proteins as cells literally fall apart and injury becomes severe and irreversible.

The two distinct phases of hypoxic tissue injury are important as between phase 1 (=50 min) and the peak of phase 2 (=200 min), lies the transition from reversible to irreversible myocardial injury. It is during this period that reoxygenation was shown to have its most dramatic effects. Reoxygenation induced a massive enzyme loss from the heart, which peaked after about 2 min of reoxygenation at levels 100 to 200 times the immediately preceding hypoxic level. Hearse and colleagues concluded that this massive release of myocardial enzymes reflected “...sudden and major ultrastructural damage.” They showed that irrespective of which myocardial enzyme they measured (or even if they measured total protein) the profile of release was largely similar, allowing them to conclude that the release process is not some selective change in membrane permeability, but rather a massive nonspecific disruption of cellular architecture, loss of membrane integrity, and cell lysis. Hearse and colleagues made a further important observation. They estimated the integrated total enzyme loss from hypoxic hearts and compared it with the amount lost from reoxygenated hearts. They found that although reoxygenation accelerates enzyme release, the total amount of enzyme release was similar to that seen with sustained hypoxia.

Thus started the controversy that has yet to be definitively resolved—does reoxygenation induce new cell death per se or does it simply accelerate the demise of cells already destined to die? Irrespective of whether reoxygenation induces new injury or simply accelerates cell death, perhaps one of the most prescient conclusions of their study was that it was the readmission of molecular oxygen and the associated oxidant stress that may induce these effects.

They concluded that their paper had led them “to question some of the basic assumptions concerning the advantages of reoxygenation or reperfusion” and the much debated concept of reoxygenation/reperfusion injury was thus born, with all of its subsequent implications for stunning, arrhythmias, contractile dysfunction, and necrosis.

This leaves us with the conundrum—reoxygenation/reperfusion is essential for the survival of the ischemic myocyte, but may in itself cause damage—thus molecular oxygen remains both a friend and a foe to the ischemic cell.

1973

The last US soldier leaves Vietnam;
The World Trade Center is officially inaugurated in New York City on April 4, with a ribbon cutting ceremony; and Chilean poet and Nobel Prize laureate Pablo Neruda dies, aged 69
Effect of alpha-tocopherol on hypoxic-perfused and reoxygenated rabbit heart muscle

C. Guarnieri, R. Ferrari, O. Visioli, C. M. Caldarera, W. G. Nayler

*J Mol Cell Cardiol.* 1978;10:893-906

As reviewed in the preceding review, in 1973 Heanse and colleagues had shown that reoxygenation of the heart after a period of hypoxia results in oxidative injury and enzyme leakage. In 1978, they coined the term “the oxygen paradox.” Despite awareness of the possible role of molecular oxygen in myocardial reperfusion injury and of the deleterious effects of lipid peroxidation, few studies had tried to ameliorate these effects. However, at the same time as Heanse et al were investigating the “oxygen paradox,” Guarnieri and colleagues were busy studying the effects of α-tocopherol on hypoxia/reoxygenation injury. Their aim was to investigate “...whether α-tocopherol protects the myocardium when it is partially deprived of oxygen, and then reoxygenated.” They stated that they chose α-tocopherol for its properties “... as a lipid-soluble antioxidant” and “... as a stabilizer of biological membranes” (whatever that means!)

In this study, Guarnieri et al perfused isolated rabbit hearts with α-tocopherol prior to, and throughout, a 30-minute period of hypoxia (with substrate deprivation) and reoxygenation (40 min). They showed that: (i) the hypoxia-induced depression of contraction (measured with a piece of string and an apical strain gauge) was reduced with α-tocopherol; (ii) enzyme and protein leakage were minimized; and (iii) ATP reserves and mitochondrial function were preserved.

With the benefit of hindsight, one of the striking observations in this study was that the effects of α-tocopherol were evident right from the start of the hypoxic period and were not simply confined to the reperfusion phase (where one might expect the oxidative stress to be most severe). This raises an interesting question, which even today has not been properly answered. That is, do free radicals primarily damage the heart during the ischemic/hypoxic phase or during the reperfusion/reoxygenation phase? Dogma would have it that all the oxidative action is at reperfusion/reoxygenation. We now happily accept that there is a burst of free radical production at the time of reperfusion (see review of the Zweier et al paper later in this series) and this may be damaging. However, much of the evidence for the timing of this radical burst is based on observations made using techniques such as electron paramagnetic resonance (EPR), where spin-trapping agents that have sat in the nonperfused ischemic tissue for many minutes are then suddenly washed from the heart on reperfusion! Maybe it is not surprising that these techniques measure a burst of “free radical production” on reperfusion. This interpretation is perhaps a little disingenuous to the many studies that show manipulating oxidative stress in the early minutes of reperfusion is cardioprotective. However, observations such as the ones in this Guarnieri study point to the idea that oxidative stress during hypoxia or ischemia may also be important in the overall pattern of injury. In support of this, for example, Eaton and colleagues (2001) have shown that the oxidative modification of proteins during ischemia/reperfusion is essentially complete during the ischemic phase and is not exacerbated by subsequent reperfusion.

Whatever the temporal profile of oxidative stress, or the molecular targets for injury, this study remains seminal in that it was the first of many aimed at demonstrating that reactive oxidant species play a role in myocardial injury and that their reduction is cardioprotective. Sadly, despite this initial study, many other studies of α-tocopherol (both in vitro and in vivo), and in particular many large randomized clinical trials, have not lived up to this early promise—but that's another story!

**1978**

US Army sergeant Walter Robinson “walks” across the English Channel in 11 h and 30 min using homemade “water shoes”; the Solomon Islands become independent from the United Kingdom; and Daniel arap Moi becomes president of Kenya
The “wavefront phenomenon” of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow

K. A. Reimer, R. B. Jennings

Lab Invest. 1979;40:633-644

By 1979, the idea that it may be possible to salvage the acutely ischemic myocardium by pharmacological intervention had received much attention. The general, but unsubstantiated, perception was that cell death started somewhere toward the center of the subendocardium of an ischemic territory and then gradually expanded concentrically moving both laterally and transmurally. The concept that there were lateral “border zones” of jeopardized tissue (in which blood flow may be intermediate between the severely ischemic center of an evolving infarct and the adjacent normal tissue) was the basis for the idea that this tissue could provide the target for “infarct-limiting” drugs. In a series of experiments investigating the temporal, spatial, and flow determinants of evolving myocardial infarction in the dog heart, Reimer and Jennings carefully and unequivocally debunked this idea.

In these studies, using mongrel dogs subjected to coronary artery ligation, Reimer and Jennings observed that after short periods of ischemia (40 min), the necrosis was largely confined to a thin subendocardial zone, but crucially extended laterally to the limits of the occluded zone. That is, even after this short period of ischemia, there was no evidence of a lateral border zone of salvable tissue. As ischemia progressed, the area of necrosis gradually expanded toward the endocardium such that by 4 days 79% of the occluded territory had infarcted, with the small surviving rim of tissue being confined to the epicardial surface. This transmural (rather than lateral) wavefront of cell death was shown to be a primary determinant of the amount of tissue that can be salvaged by reperfusion. At 40 min, the wavefront had not progressed very far, and thus reperfusion limited the size of the infarct to about 64% of the total area at risk of infarction. By 3 hours, however, little or no tissue could be salvaged by reperfusion, with infarcts being largely similar in size to those seen after a permanent occlusion. This experimental observation has important implications for the clinical application of angioplasty and thrombolysis and is borne out by clinical observation.

Boersma (1996), for example, reported in the Lancet that the clinical benefit of thrombolytic reperfusion 2 to 3 hours after symptom onset is disappearingly small. This study went on to investigate the relationship between the transmural evolution of infarction and what is now accepted to be its principal determinant—collateral blood flow. They showed that in the ischemic territory, there was always a gradient of collateral blood flow from subendocardium to subepicardium. Subendocardial flow in the ischemic territory was invariably very low (about 3% of normal), while that of the subepicardium could be as high as 40% (average 17%). This transmural gradient of flow was shown to closely correlate with both the rate of development and the eventual extent of cell death.

Reimer and Jennings concluded that, since there is no lateral border zone, the size of an infarct at any moment in time is determined by the size of the occluded zone and the extent to which the wavefront of cell death has spread transmurally toward the epicardial surface. The rate of spread of this wavefront is essentially determined by the gradient of transmural collateral blood flow.

Thus, this landmark paper demonstrates the importance of two of the best friends of the ischemic cardiac myocyte: (i) significant collateral flow; and (ii) early reperfusion.

1979

Dutch electronics company Philips makes the first demonstration of the compact disk (CD);
Mother Teresa of Calcutta is awarded the Nobel Peace Prize; and snow falls in the Sahara desert—for only 30 minutes;
Having previously observed an outward current induced by metabolic inhibition in cardiac cells, in this paper Akinori Noma describes for the first time the identity of the underlying channel—the ATP-sensitive potassium channel ($K_{ATP}$). Similar channels had previously been observed in liver mitochondrial inner membrane—the significance of this former observation to cardiac pathology only became clear many years later (see below).

In this paper, Noma used the patch-clamp technique to probe the characteristics of this channel. Initially, he showed that in the cell-attached mode of patch clamping, ATP depletion with cyanide opens a large conductance potassium channel. In this technique, a small patch of membrane is isolated under an electrode while remaining attached to the underlying cell. In order to study this in more detail, Noma went on to use the excised-patch mode—in which this small patch of membrane is “ripped” from the cell, leaving its cytosolic face freely accessible to the bathing solution. Using this approach, Noma demonstrated the opening of this channel was inhibited by cytosolic ATP with an IC$_{50}$ of around 100 $\mu$M. This paper concludes that, during hypoxia or metabolic inhibition, the activation of this channel would shorten the action potential, decrease contraction and ATP consumption, and "... may prevent further depletion of ATP and protect the cell from irreversible impairment of its energy metabolism." The idea that the $K_{ATP}$ channel was a "friend" to the cardiac myocyte was thus born.

While, in this article, we focus on the cardiac role of $K_{ATP}$ channels, it is worth stressing in passing that the channel identified by Noma has since been identified in a range of cells; most notably in the pancreatic $\beta$ cell, smooth muscle, skeletal muscle, and in neurons. In the pancreas, the $K_{ATP}$ channel plays a role in linking glucose metabolism to insulin secretion (and hence provides a therapeutic target) and is thus not only seminal in cardiac electrophysiology, but in many other areas, including diabetology. In the 23 years since the publication of this article, we now know that this channel does not open under normal conditions in healthy myocytes, but its opening can be modulated by many intracellular factors including ADP, AMP, pH, lactate, anions, and, of course, a growing list of $K_{ATP}$ channel-opening or channel-blocking drugs. In line with the idea that this channel may protect against ischemic injury, $K_{ATP}$ channel-opening agents (such as pinacidil, cromakalim, nicorandil, etc) were shown to be extremely cardioprotective. However, in this context, two observations were to take this into whole new areas.

Firstly, Downey showed that the potent protection afforded by ischemic preconditioning could be blocked by $K_{ATP}$ channel blockers and mimicked by openers. Secondly, the protection mediated by $K_{ATP}$ channel opening was evident in the cardioplegically arrested heart and was independent of sarcolemmal channels and action potential shortening. Thus, while Noma’s perfectly sensible and, in fact, verifiable proposal that opening the channel may protect by shortening the action potential, it seems that $K_{ATP}$ channels elsewhere may be the primary targets of both preconditioning and K channel openers. This is where those mitochondrial $K_{ATP}$ channels come in. Despite a significant amount of research effort (hampered significantly by the specificity of the agents purported to selectively target mitochondrial $K_{ATP}$ channels) all we can confidently say at present is that agents claiming to selectively open $K_{ATP}$ channels are incredibly cardioprotective. While the mechanism of this cardioprotection will keep us occupied for years to come, we can confidently say that, in the context of cardioprotection, the $K_{ATP}$ channel first described by Noma in 1983 is an important friend to the ischemic myocyte.

Red rain falls in the UK; the color is caused by the presence of Saharan sand in the raindrops; a new Disneyland opens in Tokyo; and Michael Jackson performs his first “Moonwalk”

1983
**Direct measurement of free radical generation following reperfusion of ischemic myocardium**

**J. L. Zweier, J. T. Flaherty, M. L. Weisfeldt**

*Proc Natl Acad Sci U S A. 1987;84:1404-1407*

By 1987, many pharmacological studies had implicated oxygen-derived free radicals in the genesis of reperfusion injury. However, since radicals exist for only the tiniest fraction of a second, their detection and quantification in intact beating hearts was, to say the least, challenging! In 1987, Jay Zweier and colleagues in Baltimore and Pamela Garlick and colleagues in London used electron paramagnetic resonance (EPR) spectroscopy techniques to detect radical production in isolated hearts during early reperfusion. This paper by Zweier and colleagues was the first direct measurement of a burst of radicals during early reperfusion. Later that year, Garlick and colleagues used spin-trapping agents to report similar findings in the isolated rat heart.

Adult rabbit hearts were perfused in the Langendorff mode and were freeze-clamped during aerobic perfusion, after 10 min of normothermic ischemia, or at various times during the first 60 seconds of reperfusion. Signals corresponding to oxygen- and nitrogen-centered radicals increased during ischemia and showed a further increase on reperfusion peaking after 10 seconds.

The authors went on to build on this initial study and, like Garlick and colleagues, used spin traps to show that the primary oxygen-centered species generated during early reperfusion were superoxide and hydroxyl radicals. The authors perfused hearts with recombinant superoxide dismutase (SOD), which was crucially administered at the time of reperfusion and not prior to ischemia. In the SOD-treated group, radical adduct formation on reperfusion was reduced by over 80% and, importantly, this radical scavenging was associated with an increase in functional recovery. The authors then reasoned that the detection of signals reflecting hydroxyl radical formation may occur as a consequence of iron-catalyzed Fenton chemistry. In later experiments, where SOD-treatment was combined with an iron chelating agent (chelex), the radical signal on reperfusion was totally eliminated and functional recovery was again inversely correlated with the fall in oxidant stress.

This paper was the first to demonstrate radical production during the early seconds of reperfusion. Subsequent studies using antioxidants showed that the magnitude of this radical burst was inversely correlated with the recovery of contractile function. Many studies before and since have failed to distinguish between the therapeutic benefits of an agent added during ischemia and reperfusion, and one added during reperfusion alone. In studies in 1988, both Zweier and Bolli showed that radical scavengers administered at the time of reperfusion could both scavenge these radicals and improve functional recovery. The initial observations in 1987 and the application of EPR spectroscopy have led to many subsequent studies both from Jay Zweier and others. These include the classic experiments of Roberto Bolli showing the role of oxygen-derived free radicals, generated during the early seconds of reperfusion, as mediators of myocardial stunning.

**1987**

The NASDAQ system at the New York Stock Exchange breaks down for several hours after a squirrel chews through electrical wiring in Connecticut; the World’s population reaches the 5 billion mark; and a supernova is observed with the naked eye, the first since 1604.
Michel Lazdunski came up in 1985 with what became known as the Lazdunski Hypothesis. In the course of describing the properties of the Na/H exchanger, Lazdunski proposed a mechanism by which Na and Ca load during reperfusion may exacerbate injury. He suggested that during ischemia a combination of Na/K pump inhibition and transient Na/H exchange activation loads the cell with Na. During ischemia, however, the effect of this is muted by the intracellular and extracellular acidification, which inhibits both Na/Ca exchange and, eventually, Na/H exchange. The washout of the extracellular space on reperfusion, however, reveals a large outward proton gradient; this activates Na/H exchange, which in turn adds to the intracellular Na load. Thus, early on during reperfusion an increased Na load may promote cellular Ca load by diminishing the driving force for forward mode Na/Ca exchange or even promoting reverse mode exchange and Ca influx. This, in a nutshell, is the Lazdunski Hypothesis.

By 1989, a number of studies had provided support for this hypothesis but the lack of measurement of intracellular ion concentrations with sufficient resolution and temporal precision, in genuine ischemia/reperfusion, limited direct confirmation. The study of Tani and Neely provided the breakthrough that confirmed the Lazdunski Hypothesis.

Isolated rat hearts were subjected to 15 or 30 min of global ischemia followed by 30 min of reperfusion. At various times throughout the protocol, tissue Na and Ca content was estimated in frozen homogenates using Na-selective electrodes and 45Ca uptake. Using careful corrections to account for the volume of the extracellular space, Tani and Neely provided intracellular ionic concentrations. The seminal observation of this paper boils down to a single point in a single graph. After 2 min of reperfusion, intracellular Na (that had risen 4-fold during ischemia) did not rapidly recover, but in fact was further elevated. The authors went on to reason that anything that limits acidosis during ischemia should limit Na load on reperfusion. Thus, they used glycogen depletion to reduce ischemia-induced acidosis by restricting lactate production. Interestingly, this had only a small effect on ischemia-induced Na load (suggesting that the activation of Na/H exchange does not contribute substantially to the Na load during ischemia in this model)—an observation supported by a similar small effect on ischemic Na load of amiloride. However, the big effect was seen on the rise in Na during early reperfusion—this was not only abolished, but reversed by both glycogen depletion and by amiloride pretreatment. A clear inverse correlation was demonstrated between reperfusion Na and Ca load and the recovery of function.

This paper has been followed by many others, using a variety of techniques, some of which support these observations and some of which do not. However, there is no doubt that the Lazdunski Hypothesis, and this seminal study, influenced the thinking of a generation of researchers in this area—a fine legacy for a fine man (Bob Neely 1936-1988).

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Japan’s emperor Hirohito dies of duodenal cancer aged 87 after a 62-year reign; the first female bishop, Barbara Clementine Harris, is consecrated by the US Episcopal Church; and NBC airs the first episodes of the Seinfeld sitcom, a “show about nothing,” hailed as the “greatest television program of all time”
It's a lesson to us all—if you want your discovery to make an impact, it needs a snappy name! In 1980, Rahimtoola tried to raise awareness of what he referred to as "chronic painless persistent severe reversible myocardial ischemia at rest"—no wonder no one took any notice! Even though this syndrome was both quite common and widely recognized, it was generally believed that persistent left ventricular dysfunction was due to necrosis, and prolonged ischemia (with or without pain) was not possible. However, in 1984, Rahimtoola coined the term "hibernating myocardium"—and the rest, as they say, is history.

Fortunately, for us, Rahimtoola was no zoologist. Had he been, he would have known that the correct term for normothermic 37°C metabolic downregulation is aestivation (estivation in the USA), while hibernation is reserved for the hypothermic condition. However, in the interests of snappyness (and minimizing spelling-induced transatlantic confusion) hibernation is by far the better choice.

While many original articles, reviews, and editorials appeared in the 1980s, this review article encapsulates both the concept and the state of understanding and has rightly become a citation classic. In it, Rahimtoola defines the key features of hibernation. That is, to be truly defined as hibernating, the myocardium has to be (i) metabolically viable; (ii) underperfused; and (iii) reversibly akinetic (either in response to inotropes or revascularization). Rahimtoola uses a series of questions to explore the prevailing understanding of this syndrome: these are worth reviewing here as many still have not been definitively answered to date. They were:

(i) What is the evidence that abnormal left ventricular function at rest can recover?
(ii) What is the mechanism by which hibernation occurs?
(iii) Why do not all severely ischemic hearts hibernate?
(iv) How can one detect the hibernating myocardium?
(v) What clinical syndromes does hibernating myocardium occur in and how common is it?
(vi) Is the hibernating myocardium different from the stunned myocardium?
(vii) How does one treat the hibernating myocardium?
(viii) How long can the myocardium hibernate?
(ix) How soon after treatment does the hibernating myocardium normalize function?

Like all seminal concepts, this has generated even more questions to add to this list and some rather heated debate. For example, is the hibernating myocardium really ischemic? What do we actually mean by ischemia? Is a reduction in coronary flow invariably associated with hibernation? This latter question has lead to much angst in the field with recent advances in imaging and blood-flow measurements showing hibernation without a flow deficit at rest. Given this observation, the key feature distinguishing hibernation from stunning (persistent coronary flow reduction) seems less definitive. This then re-raises the possibility that hibernation may be simply repetitive stunning. Finally, as a basic scientist, the fascinating question of how the myocardium matches its contractility and energy requirements to the prevailing coronary blood flow remains largely unanswered.

Nonetheless, despite all of these questions, this much-cited seminal paper encapsulates the concepts of hibernation and has and continues to stimulate much research.

1989
The first national park in The Netherlands is established in Schiermonnikoog:
Spanish painter Salvador Dali dies, aged 85; and Antifeminist Marc Lépine guns down 14 young women at the École Polytechnique in Montreal
Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction

M. S. Marber, D. S. Latchman, J. M. Walker, D. M. Yellon

Circulation. 1993;88:1264-1272

Synthesis of heat-shock or stress proteins (HSPs) in response to environmental stress is evolutionarily ancient and was well known and described in the late 1970s. In 1988, Bill Currie and colleagues showed increased ischemic tolerance in hearts excised from rats that 24 hours previously had been subjected to 15 minutes of heat stress at 42°C. While these results were striking, their relevance to the clinical condition was uncertain (tempting though it might be to ascribe the Mediterranean resistance to heart disease to temperature rather than diet?).

In 1993, Marber and colleagues showed that HSPs could be elevated, and ischemic tolerance conferred, by the more clinically relevant stimulus of repeated short sublethal ischemic episodes (a situation likened to unstable angina). Anesthetized rabbits were subjected to 4 cycles of 5-min coronary artery occlusion/10-min reperfusion or heat stress (42°C for 15 minutes) and then allowed to recover. Rabbits were then reanesthetized 24 hours later and a coronary artery reoccluded for 30 min and reperfused for 120 min. Hearts were then taken for infarct sizing and, in separate experiments, were excised after 24 hours and processed for HSP expression (measured using quantitative immunoblotting). Both the "preconditioning" ischemic cycles and heat stress increased expression of HSP65 and reduced infarct size. Interestingly, there was a dissociation between infarct size reduction and recovery of function (assessed as rate-pressure product)—this latter observation suggesting that, in this model, infarct size does not appear to be a major determinant of contractile function.

This paper appeared at a time when interest in acute preconditioning was in full swing. Many studies had described cardioprotection afforded by multiple cycles of sublethal ischemia, but this protection was shown to wane after 2 hours of reperfusion. This study from Marber and colleagues therefore provided two important observations: (i) that the synthesis of cardioprotective stress proteins could be activated, not just by heat stress, but also by sublethal ischemic episodes, and (ii) that such preconditioning-like protocols could reactivate a "second window of protection (SWOP)" 24 hours after acute protection had waned.

This paper, therefore, heralded a large number of studies investigating this "second window of protection." Studies from Boll and colleagues, for example, have shown that sublethal ischemia releases all manner of endogenous substances including nitric oxide, cytokines, and adenine, and these trigger the delayed expression of a whole plethora of cardioprotective proteins including iNOS (inducible nitric oxide synthase), HSPs, catalase, HIF (hypoxia-inducible transcription factors), heme oxygenase-1, cyclooxygenase-2, aldose reductase, and superoxide dismutase.

While such protein expression has been described in patients with stable angina, the importance of this endogenous stress response, first described by Marber and colleagues, back in 1988, has yet to be definitively demonstrated in coronary syndromes.

1993

European television news channel
EuroNews goes on air;
Intel Corporation produces the first Pentium chips;
and the WHO declares tuberculosis
a Global Emergency
Reperfusion injury induces apoptosis in rabbit cardiomyocytes

R. A. Gottlieb, K. O. Burleson, R. A. Kloner, B. M. Babior, R. L. Engler

J Clin Invest. 1994;94:1621-1628

The seminal papers selected for this volume tell a story. Reimer and Jennings clearly demonstrated that early reperfusion is the only way for severely ischemic myocytes to survive, and the exact timing of the transition from reversibly injured to irreversibly injured cells is largely determined by the extent of residual collateral flow. Hearse and colleagues (and later Zweier, Guarnieri, Tani and Neely, etc) all showed us that reperfusion or reoxygenation may not be entirely benign and may either induce de novo injury or accelerate the necrotic process in cells already doomed to die. This debate is a surprisingly difficult one to resolve. This paper by Gottlieb et al makes two important contributions: (i) it demonstrates that apoptosis can kill cells during reperfusion, and (ii) it argues that since this apoptotic cell death is unique to reperfusion (and is not seen in ischemia alone), this is indicative of a reperfusion-specific event and thus may be potentially preventable.

In this study, rabbit hearts were subjected to either in vivo left anterior descending coronary artery (LAD) occlusion (30 min) and reperfusion (4 h) or continuous ischemia (30 min or 4.5 h) with no reperfusion. Hearts were then perfused to remove blood and the ischemic and normal territories defined by monastral blue perfusion. DNA was extracted from normal, ischemic, and reperfused tissue and apoptosis assessed by terminal deoxynucleotidyl transferase nick-end labeling (TUNEL) and DNA fragmentation. To eliminate a contribution from granulocytes trapped in the ischemic territory, controls were performed with neutropenic animals pretreated with nitrogen mustard.

DNA fragmentation was found only in reperfused tissue and not in tissue that had only been ischemic (albeit for 4.5 hours!). This nucleosomal DNA cleavage was shown to be predominantly in cardiac myocytes by in situ TUNEL staining and was unaffected by neutropenia.

Necrosis is characterized by ATP depletion, cell swelling, and loss of membrane integrity, while apoptosis is typically only seen under conditions where ATP remains high. Cells undergoing programmed cell death typically have intact membranes and are subject to cell shrinkage and phagocytosis. The conclusion of this paper is, therefore, that apoptosis is only seen during reperfusion, when cells can be reenergized. The authors conclude that apoptosis is a “reperfusion-specific process” and may hence be amenable to adjunct therapy administered at the time of reperfusion.

Despite these conclusions, it remains difficult to unequivocally argue that the cells undergoing apoptosis on reperfusion in this study may not have already been condemned to die by delayed necrosis before they were reenergized and voluntarily “fell on their swords.” However, this was the first study to demonstrate apoptosis in the intact adult myocardium in response to injury. Tanaka and colleagues, in the same year, showed apoptosis in hypoxic cultured rat myocytes, but the relevance of such observations to ischemia/reperfusion remains debatable. The study by Gottlieb and colleagues did not attempt to assess the fraction of cells dying by apoptosis compared with those dying through necrosis. While this latter issue is still a matter for debate, it seems that necrotic cell death is not only by far the largest contributor to infarction, but may, like apoptosis, be amenable to intervention at the time of reperfusion. This study, showing apoptosis in the adult heart, has lead to many others not only in ischemia/reperfusion, but also in heart failure where apoptosis may be a significant contributor to the slow and inexorable progression of the disease.

Kansai International Airport opens in Osaka, Japan; US President Bill Clinton delivers his first State of the Union address, calling for health care reform; and ice hockey becomes Canada’s official winter sport.
Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion

E. J. Griffiths, A. P. Halestrap

In the mid 1990s, the search for the Holy Grail of a single “end-effector,” either responsible for injury or capable of protecting against injury, following myocardial ischemia/reperfusion was still booming. The studies of Downey, O’Rourke, and many others had implicated the mitochondria in the signaling pathway underlying preconditioning. The mitochondrial K$_{ATP}$ channel was initially proposed as that elusive “end-effector” of protection. However, when it became clear that mitochondrial ROS production downstream from mitoK$_{ATP}$ channel opening was an essential part of the signaling cascade, the K$_{ATP}$ channel was relegated to the role of a bit player in the signal transduction pathway. However, the mitochondria would not lie down and Halestrap and colleagues had long appreciated that all of the conditions necessary for the opening of the mitochondria permeability transition pore (MPTP), which had been described back in the 1980s by Crompton and colleagues, occur during ischemia and reperfusion. Opening of this channel could either de-energize the mitochondria (by uncoupling the proton gradient) and hence accelerate necrosis, or could result in mitochondrial cytochrome C loss and the activation of apoptotic programmed cell death.

The key prerequisites for MPTP opening are: (i) Ca overload; (ii) ATP depletion; (iii) oxidative stress; and (iv) elevated inorganic phosphate concentration. As has been described in the other summaries earlier in this issue, without exception, these conditions prevail during ischemia. However, the MPTP is also kept closed by acidosis and hence it is only during reperfusion that the Ca overload, normalization of pH, oxidant stress, Pi (inorganic phosphate) accumulation, and ATP depletion are likely to combine to open the MPTP.

This is all good in theory, but how can this be demonstrated? Using a neat trick, Griffiths and Halestrap showed us how. They used radiolabelled 2-deoxyglucose ([${}^{3}$H]DOG—“hot DOG”—which loads into cells via the cell membrane glucose transporters, but is not metabolized beyond DOG-6P and remains trapped in the cytosol. Mitochondria isolated from such cells will not contain [${}^{3}$H]DOG. However, if the MPTP opens, [${}^{3}$H]DOG can enter the mitochondria. By rapidly isolating the mitochondria and measuring their [${}^{3}$H] content relative to citrate synthase, it is possible to measure the extent of MPTP opening. Using this approach, Griffiths and Halestrap demonstrated for the first time that the MPTP opening does not occur during ischemia, but does occur within 2 minutes of reperfusion when pH has returned to normal (and, incidentally, ROS generation and Ca load may also be at their peak).

In subsequent studies, Halestrap and colleagues have demonstrated that a proportion of the MPTP channels that are open at the time of reperfusion subsequently close and that functional recovery correlates well with the extent of this closure. They have shown that agents that block MPTP (such as cyclosporin A or sanglifehrin A) can profoundly protect against ischemia/reperfusion and they, and others, have suggested that the enhanced closure of the MPTP may indeed be the elusive end-effector of preconditioning.

The development of effective ways of blocking the MPTP on reperfusion may provide important therapeutic options during reperfusion following thrombolysis, cardiac surgery, or revascularization.

The World Trade Organization (WTO) replaces the General Agreement on Tariffs and Trade (GATT); a rocket launched from a space exploration center in Norway is briefly interpreted by the Russians as an incoming attack; and Hungarian actress Eva Gabor dies, aged 76.
**Friends & Foes of the Cardiac Myocyte**

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*selected by Roberto Ferrari, MD, PhD*

Chair of Cardiology - University of Ferrara - Ferrara - ITALY

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