"An affair of the heart"

Inaugural Professorial Lecture

by

Professor Roger Willis

Rotary Centre for
Cardiovascular Research

GRIFFITH
UNIVERSITY
GOLD COAST CAMPUS

Introduction

Popular culture attributes special qualities to the heart, including romantic love. These notions are enshrined in our language and reinforced each St Valentine's Day. In fact, the heart is a pump, and its special functional role in the body has been appreciated since antiquity.

My "affair of the heart" began shortly after arriving at Griffith University in 1980. The special environment in the School of Science at the time, my earlier exposure to the principles of cardiovascular physiology at the University of Queensland and Case Western Reserve University, and a fortuitous grant from the National Heart Foundation of Australia launched me on a project to better understand the relationship between cardiac metabolism and function.

I wish to tell the story of my research as it has unfolded since then. In so doing, I will endeavour to develop the following themes; normal and abnormal heart function, pathogenesis of ischaemic injury, natural cardioprotective mechanisms, paradoxes and controversies of heart function, technology and experimentation in cardiovascular research, new paradigms and research directions.

The Author

Born in Monto, Queensland in 1947, Roger Willis grew up and was educated in Brisbane. He studied science at the University of Queensland and graduated with a Doctor of Philosophy in Physiology in 1976. From 1976-1980 he worked as a Research Associate and later an Assistant Professor of Physiology at the Medical School of Case Western Research University in Cleveland, Ohio. Imbued with the American "nothing is impossible" philosophy, he returned to Queensland in 1980 to take up a position of Lecturer in the School of Science at the Nathan Campus of Griffith University. Eleven years later he was an Associate Professor, serving as Head of the School of Science and Founding Director of the Rotary Centre for Cardiovascular Research. In 1994 he moved to the Gold Coast Campus as Head of the new School of Health Science. From 1995 to 1997 he served as Dean of the Faculty of Nursing and Health Sciences. He was promoted to Professor in 1996.

die of a broken heart - win a person's heart - to have no heart - to lose heart - the very heart of the matter - to clasp a person to one's heart - dear heart - after one's own heart - at heart - be all heart - break the heart of - by heart - eat one's heart out - from the bottom of one's heart - have a heart - have one's heart in one's mouth - heart and soul - hearts of hearts - heart of oak - lose one's heart - set one's heart on - take heart - to one's hearts content - wear one's heart upon one's sleeve – affair of the heart - with all one's heart - heartache - heart break - heartburn - hearten - heartfelt - hearth - heartless - heart rending - heart searching - heartsick - heart sore - heart stricken - heart strings - heart throb - heart to heart - heart warming - hearty

Normal heart function

The heart and circulation

The circulation is comprised of two circuits (Figure 2.1). The right heart pumps venous blood

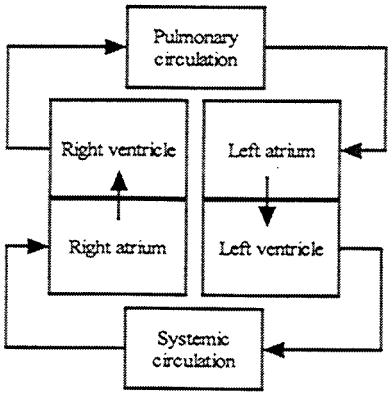

Figure 2.1 Pulmonary and systemic circulations. Arrows show the direction of blood flow

to the lungs where it is oxygenated and returned to the left heart via the pulmonary circulation. The left heart pumps this arterial blood to the rest of the body where it supports tissue metabolism and is deoxygenated in the process. Systemic venous blood is collected by the superior and inferior vena cava and returned to the right heart. The amount of blood pumped by each side of the heart is termed the cardiac output and is about 5 litres per minute in a resting adult.

Cardiac structure

The heart consists of two muscle masses of unequal size, separated by a fibrous plate. This plate acts as an electrical insulator and also supports the four cardiac valves (Figure 2.2). The

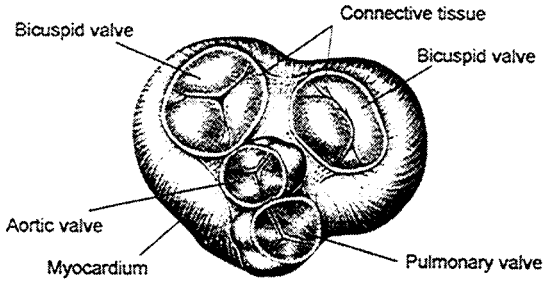

Figure 2.2 Fibrous plate that contains the cardiac valves and separates the atria from the ventricles

smaller muscle mass above the fibrous plate is comprised of the right and left atrial chambers which are connected to the pulmonary and systemic circulations by the vena cava and pulmonary veins respectively (Figure 2.3). The AV

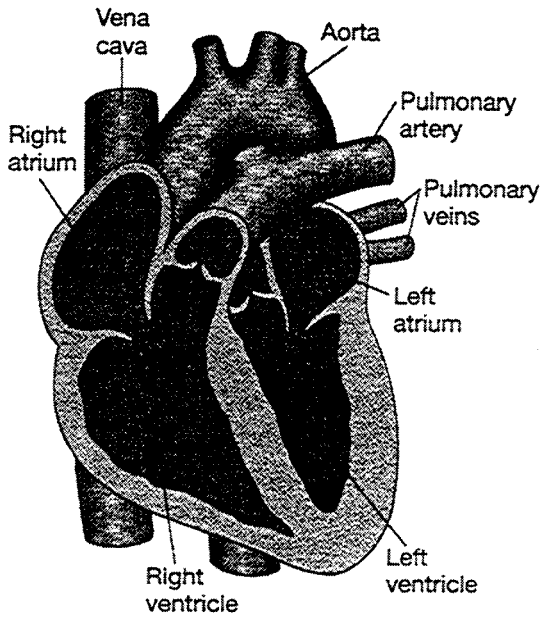

Figure 2.3 The four cardiac chambers and associated large blood vessels

valves connect the atrial chambers to the corresponding ventricular chambers, which together constitute the larger muscle mass below the fibrous plate. The semi-lunar valves separate the right and left ventricular chambers from the pulmonary artery and aorta respectively. Because pressures in the systemic circulation are five-fold greater than that in the pulmonary circulation, the wall of the left ventricle is much thicker than the right (Figure 2.4).

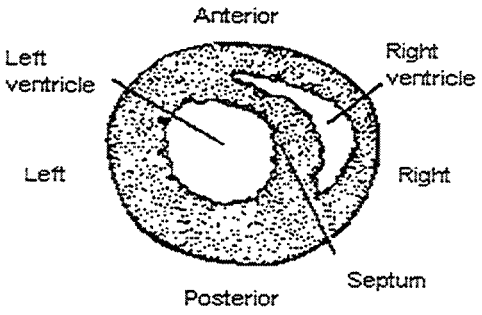

Figure 2.4 Cross-sectional view of the ventricles

The SA node is located in the wall of the right atrium and is the origin of the cardiac electrical impulse that triggers cardiac contraction. The cardiac conduction system (Figure 2.5) links the SA node to the AV node and bundle of His which is the normal pathway for passage of electrical impulses from the atria to the ventricles. The electrical signal is delayed at the AV node and then distributed to all parts of the ventricle by the

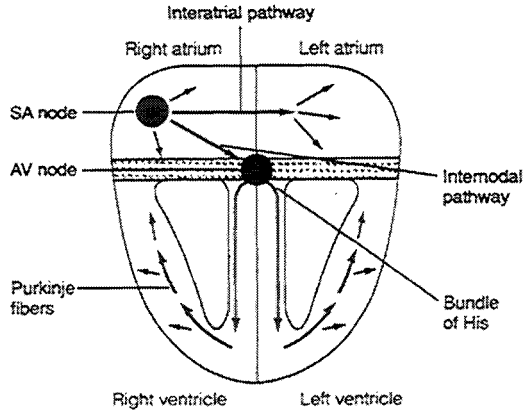

Figure 2.5 The cardiac conduction system

various bundle branches and Purkinje fibres. The passage of the electrical signal in the heart is further assisted by the fact that individual cells are connected by low resistance electrical connections called intercalated discs. These discs are not found in other types of muscle. Cardiac muscle cells have to be electrically stimulated in order to contract.

Heart muscle is composed of individual cells called myocytes

The muscle mass of the heart is comprised of individual cells called myocytes. The structure of these cells reflects their specialized function as mechanochemical transducers (Figure 2.6). The

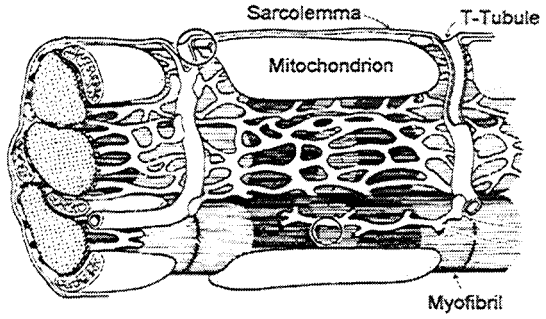

Figure 2.6 Structure of the myocyte

cell is enveloped in an electrically excitable membrane (sarcolemma) which can propagate a cardiac action potential. This event is associated with the entry of extracellular calcium into the myocyte through calcium slow channels. This "trigger" calcium mobilizes additional calcium from the sarcoplasmic reticulum causing free intracellular calcium to rise from 10^{-7} to 10^{-6}M. The ten-fold increase in intracellular calcium activates the contractile apparatus thus causing the cell to shorten (Figure 2.7). The contractile apparatus, with its regular array of actin and myosin microfilaments, is a major cellular component and gives the cell its characteristic

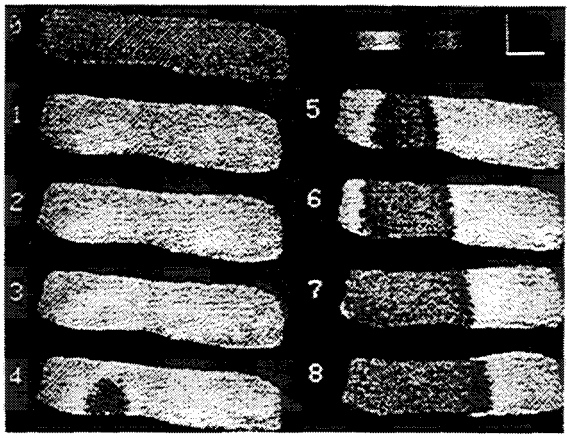

Figure 2.7 The increase in intracellular calcium as the action potential spreads over the myocyte

striped appearance. While calcium remains elevated and adenosine triphosphate (ATP) is present, the myocyte continues to contract. ATP is the energy currency of the cell and is expended in order to produce the sliding of actin and myosin filaments. ATP is produced by mitochondria that are aligned in the myocyte next to the contractile apparatus.

The heart turns chemical energy into mechanical work

In his classic text "Physiology in health and disease", Dr Carl Wiggers described what became known as the "Wiggers diagram" which shows in graphical form the series of electrical and mechanical events which describe the pumping action of the heart. My first exposure to the "Wiggers Diagram" was in an inspirational lecture by Dr Ron Cross who presented the events of the cardiac cycle to second year medical students. I had no idea at the time that I would eventually become an Assistant Professor in Carl Wiggers' Department of Physiology at Case Western Reserve University. The heart fills with blood during diastole, and ejects blood during systole. Rhythmic contraction of the heart is controlled by the pacemaker that initiates an electrical impulse which causes a sequential contraction of the atria and ventricles. At the body surface, these electrical events are recorded as the ECG (Figure 2.8).

The story of the cardiac cycle is usually begun in mid-diastole when both the atria and ventricles are relaxed. At this time in the right heart, venous blood from the systemic circulation is flowing from the vena cava into the right atrium, through the open tricuspid valve and into the right ventricle. In the left heart, oxygenated blood from the pulmonary veins is moving through the left atrium and the open bicuspid (mitral) valve and into the left ventricle. This blood flow is due to a

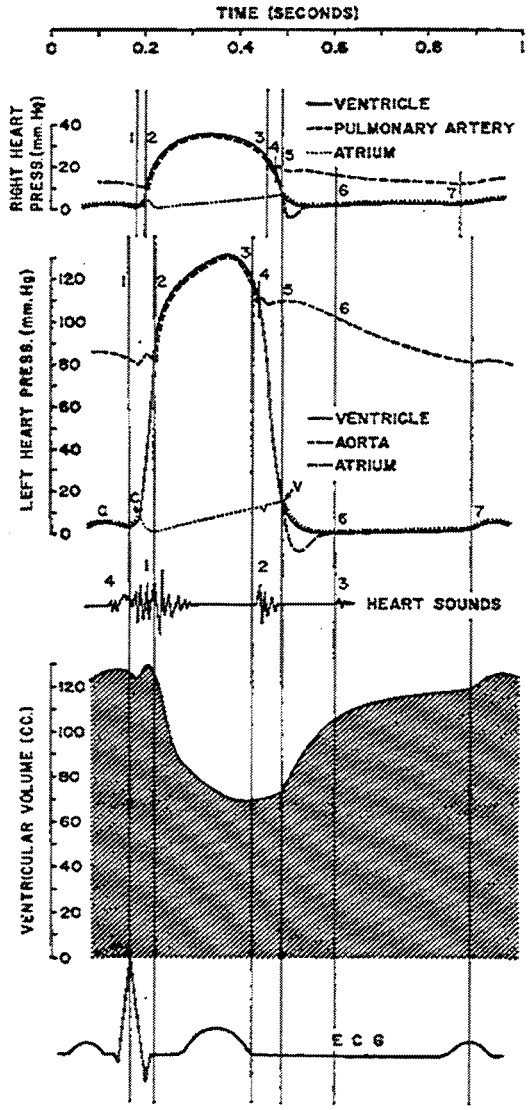

Figure 2.8 The "Wigger's diagram" which shows the events of the cardiac cycle

slightly higher pressure in the veins compared to the ventricles, due to the previous heart beat. Towards the end of diastole, the SA node (pacemaker) discharges triggering the contraction of both atria. The atria are anti-chambers to the ventricles, and their contraction increases ventricular volume by a further 30% thus ensuring that the ventricular pump is primed with the full end-diastolic volume. The P wave in the ECG is associated with atrial systole. The cardiac conduction system delays electrical activity at the AV node for about 0.1s to permit ventricular filling before the ventricles are electrically activated. The hallmark of ventricular activation is the appearance of the QRS complex in the ECG. This waveform signals the beginning of ventricular systole. As soon as ventricular pressure exceeds atrial pressure, the AV valves shut producing the first heart sound (lub), and a period of isovolumic contraction ensues. When right ventricular pressure exceeds the pressure in the pulmonary artery, and when left ventricular pressure exceeds the pressure in the aorta, the pulmonary and aortic valves open and blood is ejected from the heart into the pulmonary and systemic circulations. Toward the end of ventricular systole, the T wave is seen in the ECG, the ejection of blood slows, and ventricular pressure drops. When ventricular pressure drops below arterial pressure, the semilunar valves shut producing the second heart sound (dup), and a period of isovolumic relaxation follows. The diastolic filling commences when ventricular pressures fall below atrial pressures thus opening the AV valves.

Cardiac work and loading conditions

Each time the heart beats, a volume of blood called the stroke volume is ejected under pressure from the left and right ventricles. Figure 2.9 shows the work diagram of the left ventricle depicted as a pressure-volume loop.

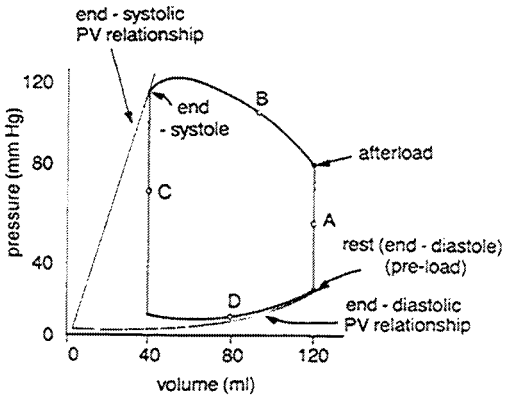

Figure 2.9 Pressure-volume loop of the left ventricle

The loading conditions of the ventricle determine its work output. Pre-load is the extent to which the ventricle fills with blood during diastole, and after-load the arterial pressure against which the ventricle must pump. The area of the curve A, B, C, D represents the external mechanical work preformed by the left ventricle.

Pressure-volume loops can be obtained by introducing a catheter into the ventricle to measure pressure and using an imaging technique like echocardiography (Figure 2.9) to measure ventricular volume. Figure 2.10 shows sequential images of the left ventricular cavity of a sheep during one cardiac cycle. Note that a significant residual volume of blood remains in the heart after the ejection of the stroke volume. The ejection fraction is defined as the ratio of the stroke volume to end-diastolic volume. Normal values range between 0.55 and 0.75.

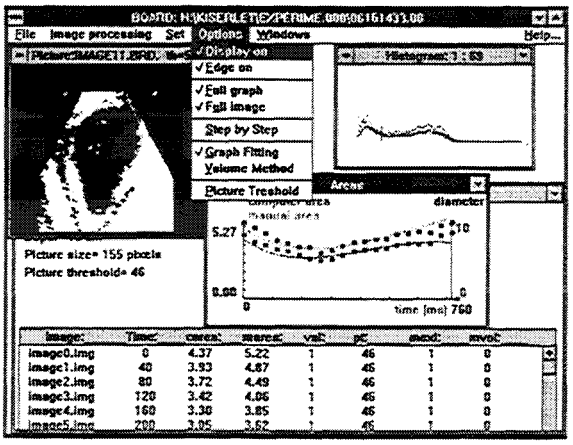

Figure 2.10 Windows interface for an echocardiographic image analysis system

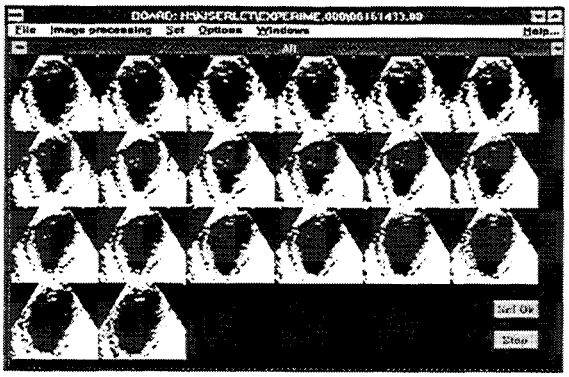

Figure 2.11 Sequential long-axis views of the left ventricle during the cardiac cycle in the sheep

The Frank-Starling relationship

A remarkable property of the heart first recognized by Frank and Starling around the early 1900s is its capacity to autoregulate its own pumping action. Within limits, the heart pumps all the blood delivered to it from the veins. Graphically, this is depicted as a ventricular function curve (Figure 2.12).

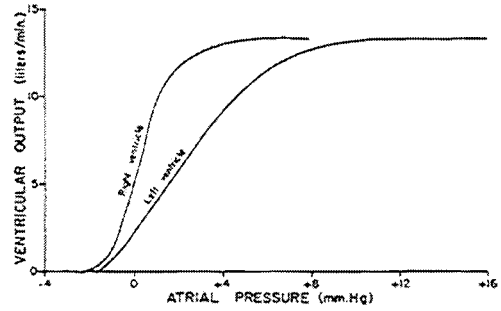

Figure 2.12 Left and right heart ventricular function curves in the human

The coronary blood vessels

Supporting the mechanical activity of the heart and its requirement for ATP, is the supply of oxygen and energy substrates to the myocytes by blood in the coronary vessels (Figure 2.13). The

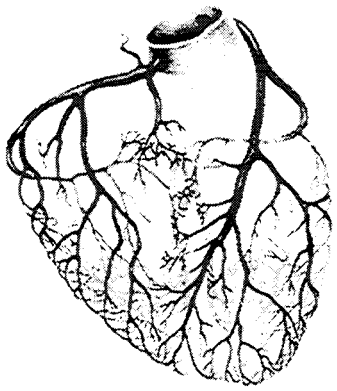

Figure 2.13 Coronary blood vessels

left and right coronary arteries exit the aorta close to the aortic valve. These arteries and their branches are located at specific locations on the surface of the heart. At intervals they plunge into the myocardial mass to form nutrient capillaries. Deoxygenated blood is collected in veins, which run parallel to coronary arteries. Most venous blood collects in the coronary sinus that empties into the right atrium. Four percent of systemic blood is directed to the heart. There is significant variation in the anatomy of the coronary vessels

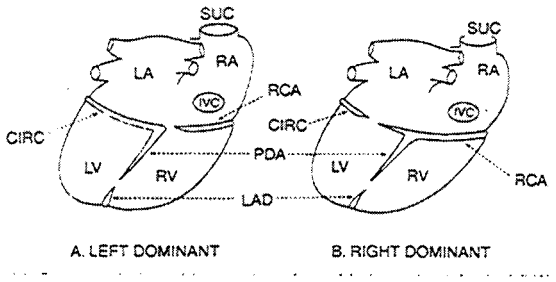

Figure 2. 14 Posterior view of the human heart showing variation in coronary artery supply

in the human (Figure 2.14).

The heart is an aerobic organ

The heart pumps blood through the circulation under pressure and consumes chemical energy (ATP) in the process. The ATP required for contraction is produced aerobically in the myocyte by the oxidization of fat and glucose. In the absence of oxygen, cardiac contraction quickly fails because anaerobic metabolism can supply only 5% of the total cardiac ATP

requirement. The energy substrates oxidized by heart are fatty acids and glucose (Figure 2.15).

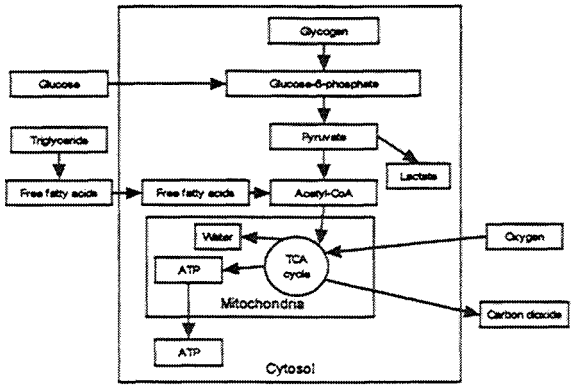

Figure 2.15 Utilization of energy substrates by the heart

Fatty acids are presented to the myocyte from the blood both as free fatty acids (attached to albumin) and triglyceride (enveloped in lipoprotein). Once inside the myocyte, metabolic processes in the cytosol reduce fatty acids by β-oxidation and glucose by glycolysis to acetyl-CoA that then enters the Kreb's cycle in the mitochondria. Acetyl-CoA is further degraded to carbon dioxide and water. In both the cytosol and the mitochondria, reducing equivalents are produced predominantly as NADH. Reducing equivalents are then converted in the mitochondria to ATP by the process of oxidative phosphorylation. Some ATP is produced by substrate level phosphorylation.

The mechanism of cardiac contraction

Heart muscle exhibits a prominent banding pattern when viewed under the microscope and for this reason is termed striated muscle (Figure 2.16). The banding is due to an orderly array of overlapping actin (thin) and myosin (thick)

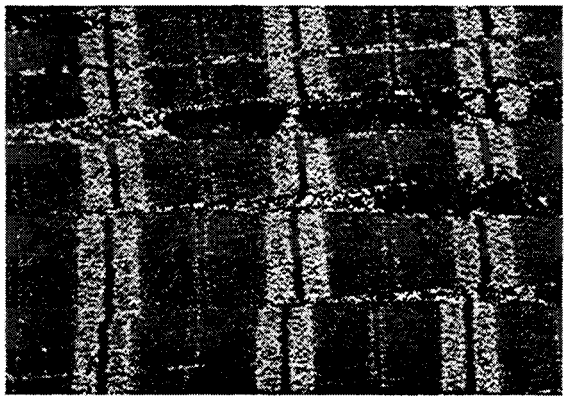

Figure 2.16 Striated appearance of cardiac muscle

filaments. The sliding filament theory proposed by Huxley and Handson in the mid-1950s describes the relative movement of thick (myosin) and thin (actin) filaments to produce muscle cell shortening (Figure 2.17). In the presence of increased cytosolic calcium ion, a cross-bridge is

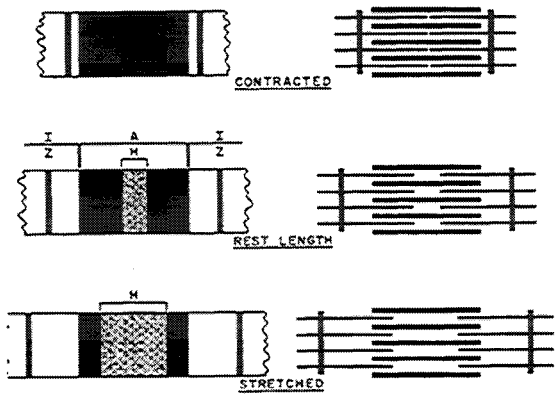

Figure 2.17 The sliding filament theory of muscle contraction

able to form linking myosin to actin (Figure 2.18). At this time, a molecule of ATP that was

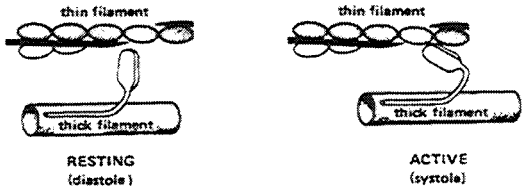

Figure 2.18 Operation of the cross-bridge between thick and thin filaments during contraction

previously attached to the bridge is split to ADP and inorganic phosphate (P_i). This causes a twisting of the cross-bridge and a sliding together of the thick and thin filaments. A second ATP is required to displace the ADP and break the bridge. Cross-bridge cycling and muscle shortening continues while ATP is present and calcium remains elevated.

Coupling of cardiac work, myocardial energy metabolism, and coronary blood flow

Cardiac function is controlled by, and subservient to, the needs of body metabolism and related processes. For example, in exercise the activation of skeletal muscle increases muscle metabolism and blood flow and cardiac output is increased to meet this requirement. The maximum cardiac output that can be achieved in strenuous exercise is about 30 L/min, 6-fold more than the resting value of 5 L/min. Both haemodynamic changes and autonomic nerve activity associated with exercise directly stimulate cardiac output by increasing heart rate, stroke volume, and the vigour of cardiac contraction.

When cardiac output is elevated, the contractile apparatus is working to a greater extent and more frequently. Consequently, more ATP is

consumed and synthesized. Mitochondrial function is supported in turn by an increased coronary blood supply, which delivers additional oxygen, fatty acids, and glucose for aerobic metabolism. Since these processes are normally closely coupled, specific signals or control factors must link each process in turn. Controversy still surrounds the nature of the signals that link cardiac work to myocardial energy metabolism (intracellular signals), and energy metabolism to coronary blood flow (extracellular signals).

Intracellular signals

A normal heart is able to respond to an external stimulus to increase cardiac work and energy metabolism. Calcium links excitation to contraction, but what links contraction to mitochondrial function? One possibility is that the hydrolysis products of ATP, ADP or P_i may control ATP synthesis. Another possibility is that calcium stimulates ATP synthesis (Figure 2.19).

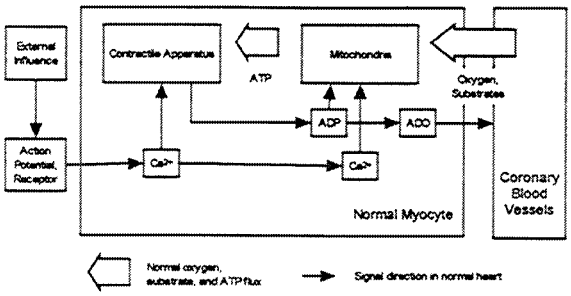

Figure 2.19 The supply and control of chemical energy in a heart at rest

In a heart which has an increased work load due to positive inotropic stimulation metabolic signals would be expected to be increased (Figure 2.20).

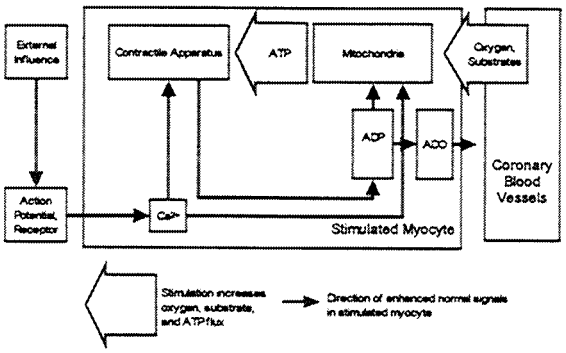

Figure 2.20 The supply and control of chemical energy in a heart at elevated work load

Extracelluar signaling and adenosine

Adenosine is formed by the hydrolysis of ATP (Figure 2.21). Since the 1920s, it has been known to have profound biological actions, including the ability to slow the heartbeat, reduce the strength of cardiac contraction, and dilate the

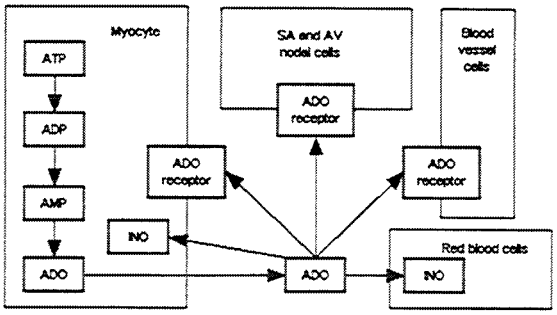

Figure 2.21 The origin, fate, and some actions of adenosine (ADO) in the heart. INO=inosine, an inactive, breakdown product of ADO

coronary blood vessels (Figure 2.22). In the 1970s, Berne proposed that adenosine might couple coronary blood flow to myocardial

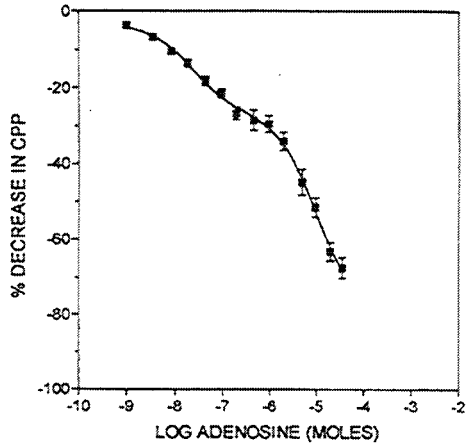

Figure 2.22 Dilation of the coronary vessels with adenosine. The biphasic response indicates the presence of at least 2 receptors

metabolism. Evidence now suggests that adenosine acts as a local hormone, modulating heart function in response to changes in myocardial metabolism. It exerts its biological effects by interacting with specific cell surface receptors and several types of adenosine receptor have now been described Figure 2.22.

Recent work suggests that adenosine may adjust the function of the heart at elevated work loads in order to minimize oxygen consumption. Figure 2.23 shows the relation between myocardial oxygen consumption (MVO_2) and total cardiac work (PVA) in anaesthetized sheep either treated (+8PT) or untreated (-8PT) with the adenosine antagonist drug 8-phenyltheophylline. More oxygen is consumed at a given work load when endogenous adenosine is blocked.

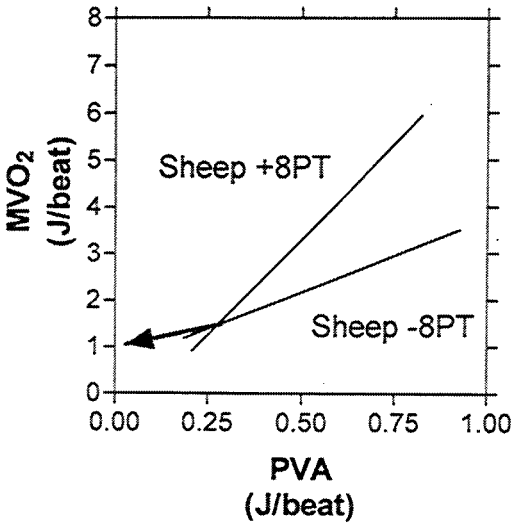

Figure 2.23 Relation between myocardial oxygen consumption (MVO_2) and cardiac work (PVA)

Abnormal heart function

Normal heart function may be disturbed by a variety of different conditions. This section will be restricted to a consideration of functional disturbances associated with myocardial ischaemia due to coronary artery disease.

Coronary artery disease

The response-to-injury theory for the pathogenesis of atherosclerosis is depicted in Figure 3.1. The disease is characterized by the

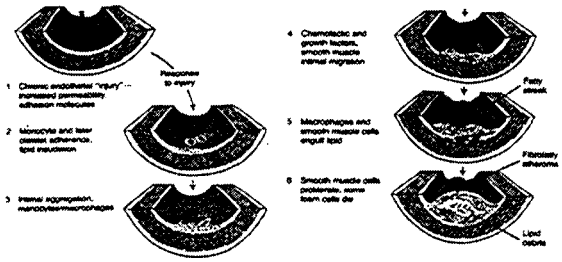

Figure 3.1 Following injury to the endothelium, atheroma forms progressively occluding the artery.

deposition of fibro-fatty plaques (atheroma) in the intima of the artery. In this scheme, the initiating event is some form of injury to the endothelial lining of the artery. Injury may be due to physical factors such as high blood pressure, or to chemical factors like oxidized low density lipoprotein (LDL). Figure 3.2 shows the deleterious effects of oxidised LDL on the vasodilator response of the coronary vessels.

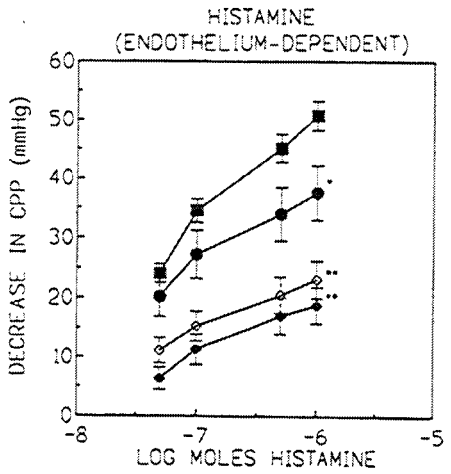

Figure 3.2 The effect of oxidized LDL on the dilator response of the rat coronary vessels

Chronic ischaemia

Chronic ischaemic heart disease is almost always the result of the obstruction of the coronary arteries by atheroma due to the presence of coronary artery disease (Figure 3.3). With

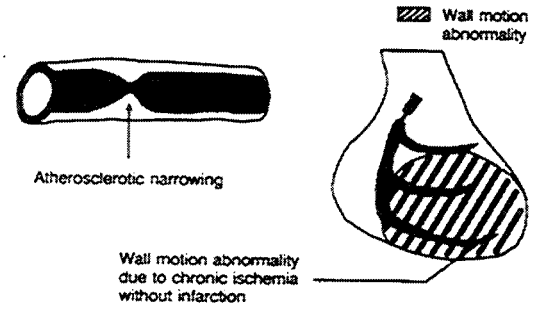

Figure 3.3

insufficient oxygen delivery to the myocardial muscle, both systolic and diastolic function is compromised and imaging techniques reveal wall motion abnormalities (Figure 3.4).

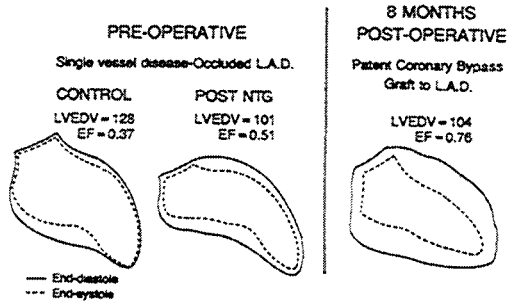

Figure 3.4 Abnormal wall motion associated with regional myocardial ischaemia

Stunning

Stunning is defined as the temporary and reversible reduction of contractile function seen on reperfusion after a period of ischaemia. Figure 3.5 illustrates the phenomenon in the perfused rat heart after 5, 10, and 15 min of total, global ischaemia.

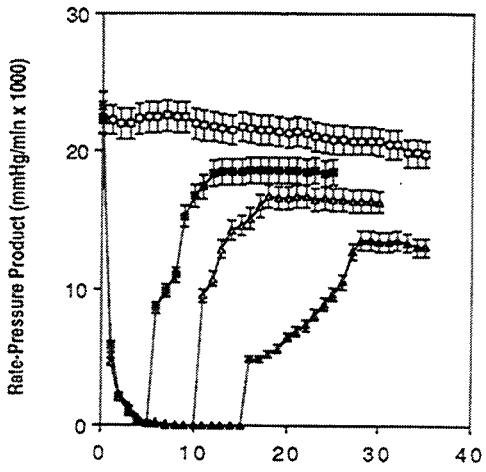

Figure 3.5 Myocardial stunning in the perfused rat heart

Signaling during ischaemia

During ischaemia, obstruction of the coronary blood supply restricts the delivery of oxygen and substrates for cytosolic and mitochondrial metabolism, thus limiting ATP synthesis. While normal signals are present, they are ineffective. Also, pathophysiological signaling may assume importance (Figure 3.6). For example, is P_i a signal to limit contraction and induce hibernation?

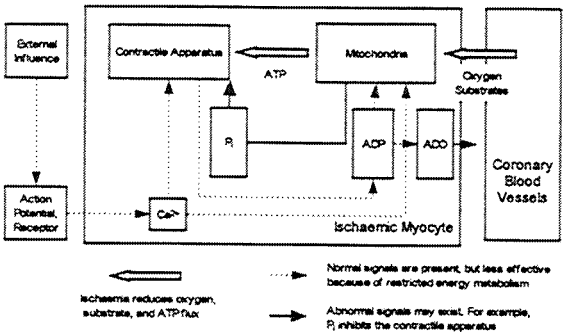

Figure 3.6 The supply and control of chemical energy in an ischaemic heart

Interestingly, the ^{31}P NMR spectrum of a dysfunctional, ischaemic heart (Figure 3.7) is similar to a heart performing extra work (Figure 6.5).

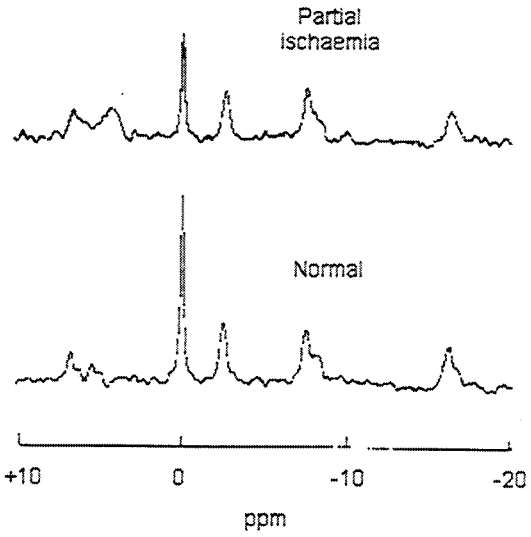

Figure 3.7 Changes in the ^{31}P NMR spectrum of a perfused rat heart during partial ischaemia

Pathogenesis of ischaemic injury

Ischaemia is defined as a diminution of tissue blood supply. An immediate consequence of myocardial ischaemia is the loss of aerobic energy metabolism and contractile function. If the condition continues, the myocytes will die in a process called necrosis. The pathogenesis of ischaemic injury describes the sequence of steps linking the failure of energy metabolism to irreversible cell death (Figure 4.1). Ischaemic pathogenesis is considered below at two levels of biological organization; the whole heart and the myocyte.

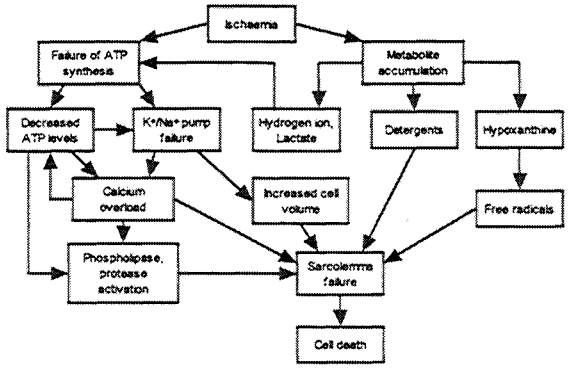

Figure 4.1 Possible steps in the development of ischaemic cell injury

Myocardial infarction

When a coronary artery is narrowed by atheroma, it is vulnerable to complete occlusion due to spasm or thrombosis. Depending on where the blockage occurs, a region of myocardium will become totally dysfunctional. If the artery remains blocked, but the heart continues to function, the myocytes in the ischaemic region will die and be replaced by non-functional scar tissue.

Timely intervention can reduce the extent of necrosis. If flow can be quickly restored, significant salvage of myocardium may be possible. Even if the blockage remains, infarct size can be reduced by establishing conditions that favour the recovery of tissue in the border zone of the infarct

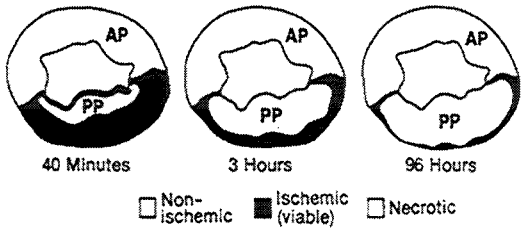

Figure 4.2 Cross-sectional view of the ventricle during myocardial infarction. AP=normal tissue; PP=necrotic tissue: dark=salvaged tissue

The pathogenesis of myocardial infarction has been studied in the dog (Figure 4.2). Following coronary artery occlusion, the infarct progresses from the endocardium to the epicardium in a wavefront. Twenty minutes of coronary occlusion followed by reflow produces no myocyte necrosis, all the injury being reversible at this time. After 40 minutes of occlusion followed by reflow, subendocardial necrosis is seen involving 35% of the coronary bed at risk. Permanent occlusion results in 75% necrosis. The margin of the area at risk (25%) is spared because of colateral blood flow. The window for significant myocardial salvage is about 3 hours in the dog.

The transition between cell injury and death

Cells can be injured by a variety of noxious insults including the failure of energy metabolism. While it might be expected that the early steps in the injury process will be specific to the particular noxious insult, it has been suggested that a "final common pathway" of injury may exist in all cells.

The "point of no return" is another concept in cellular pathogenesis. It is a common observation that depending on the severity of the noxious insult and the effectiveness of cellular protective mechanisms, an afflicted cell may recover or die. Is there a step in the pathogenic sequence that marks the transition from reversible to irreversible injury?

In the heart, we are concerned specifically with ischaemic cell injury. Considerable evidence now suggests that failure of intracellular calcium concentration regulation is not only an important step leading to necrosis but may also be the "point of no return".

Ischaemic cell injury

Living cells use energy as ATP to perform various specialized functions and to maintain themselves in an organized state. In the myocyte, 80% of the ATP produced by aerobic metabolism is used for contraction, and the balance is used to maintain cell integrity. If myocytes are deprived of the capacity for ATP synthesis, they cease to contract and become progressively disorganized. At some point, even if energy supply is restored, they will have passed the "point of no return" and salvage is impossible. This point in myocytes may be the failure to control intracellular free calcium concentration. In globally ischaemic hearts the point of no return correlates well with the rise in

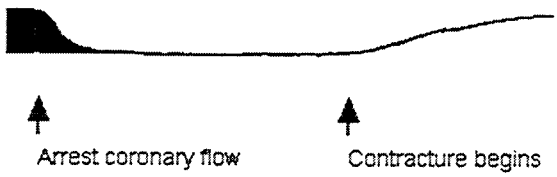

Figure 4.3 Ischaemic contracture begins 15 to 20 min after arresting coronary flow in perfused heart

diastolic pressure associated with an increase in cytosolic calcium (Figure 4.3).

The calcium antagonist drugs have found use in treating coronary artery disease. These drugs block calcium slow channels and thereby reduce cardiac contraction and dilate blood vessels. Additional benefits may be due to their ability to delay calcium overload.

Natural cardioprotective mechanisms

Early contractile failure and myocardial stunning are functional changes that are observed during the early stages of myocardial ischaemia. Undeniably they represent specific functional deficits leading inexorably to heart failure. They may also be thought of as adaptive mechanisms that may enhance cardiac survival under certain circumstances.

For example, early contractile failure may represent a mechanism to conserve ATP for the maintenance of cell viability at the expense of contraction in small regions of ischaemic tissue. Also, myocardial stunning may have the benefits of hibernation during partial ischaemia conserving ATP by depressing contraction.

Ischaemic preconditioning was first reported in 1990. Murray showed that myocyte death due to ischaemia could be delayed by preconditioning the myocardium with brief episodes of ischaemia prior to the main ischaemic insult. This natural protective mechanism is more effective than any other intervention in affording protection against ischaemic injury.

The challenge is to harness natural cardioprotective mechanisms for the treatment of ischaemic heart disease.

Early contractile failure

When the coronary blood supply is occluded, myocardial contraction quickly fails. This phenomenon is called early contractile failure. It may be observed on a regional or global scale.

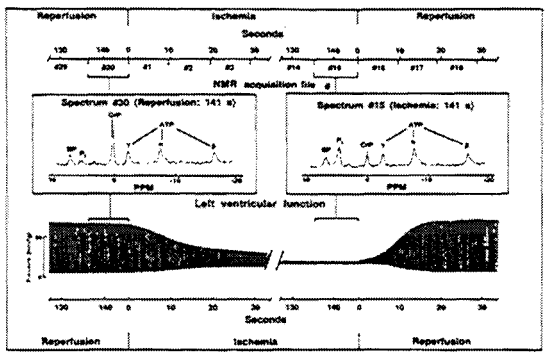

Figure 5.1 Early contractile failure and functional recovery in the globally ischaemic guinea-pig heart

Paradoxically, this is not due to lack of ATP (Figure 5.1), but rather to an inhibition of the contractile apparatus by inorganic phosphate (P_i) (Figure 5.2). By arresting contraction, the ATP consumption of the myocyte is reduced by 80%

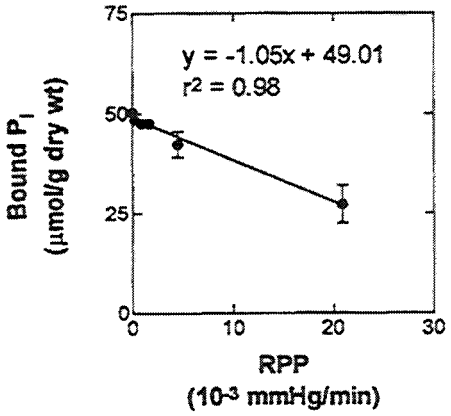

Figure 5.2 The rate-pressure product (RPP) correlates closely with bound Pi in the heart

thus preserving ATP for the maintenance of cell integrity. Early contractile failure can be thought of as a cardioprotective mechanism that operates when coronary blood flow is impaired, to conserve ATP and enhance cell viability.

Hibernation

Hibernation is a term used in zoology to describe a reversible state of inactivity in animals with associated benefit of energy conservation. It is an adaptive state to enhance survival during winter, when food sources and energy supply are limited. Myocardial hibernation may represent an adaptive response of the heart to the reduced energy supply associated with coronary artery disease. Stunning and hibernation are two different views of the same phenomenon.

Preconditioning

Murray reported that myocyte death was delayed by preconditioning the myocardium with brief episodes of ischaemia. Also, delayed were myocardial acidosis and cardiac arrhythmias. The mechanism of preconditioning is unknown but proposed triggering mechanisms are oxygen free radicals, heat stress proteins generated by preconditioning, attenuation of energy demand during preconditioning, and adenosine receptor activation. Most evidence supports the adenosine theory. Myocardial stunning and preconditioning appear to be unrelated.

Paradoxes and controversies of heart function

The response of hearts to some experimental interventions is so counter-intuitive that the observed phenomena are referred to as paradoxes of heart function. The calcium paradox and the oxygen paradox are such examples. The early contractile failure produced by ischaemia can also be considered a paradox, because it is not due to exhaustion of ATP.

In other situations, heart experiments yield results that are controversial in that they conflict with established dogma. A good example is in the area of the control of mitochondrial function discussed below.

Calcium paradox

Calcium is present in the extracellular fluid at a concentration of 2.5 mM. Half of this calcium is bound to protein, and the rest is free in solution. Bound and free calcium perform different functions. For example, some bound calcium has a structural role attached to the glycocalyx on the surface of the myocyte. It forms part of the glue that anchors cells together at the intercalated disc (Figure 6.1). On the other hand, free

Figure 6.1 Transmission electron micrograph of an intercalated disc.

extracellular calcium is required to trigger excitation-contraction coupling and its removal is a practical way to eliminate myocardial contraction. While this would never happen naturally, it can be easily achieved experimentally by perfusing heart with fluid which contains no calcium. In fact this is a strategy used to arrest hearts during surgery. However, when calcium is restored an unexpected and dramatic consequence sometimes occurred. Rather than the desired outcome of a resumption of contraction, the heart exhibited a sustained contraction (Figure 6.2), accompanied by a loss of intracellular contents which is characteristic of

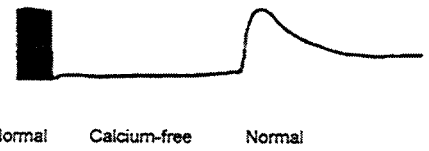

Figure 6.2 Left ventricular pressure of a perfused rat heart. Calcium is removed from the perfusate for 5 min and restored. A contracture develops

massive cell injury. This is the stone heart or calcium paradox phenomenon. The explanation elucidated some years later was that when extracellular calcium falls below 50 µM for a few minutes, calcium is lost from intercalated disc. When contraction is initiated by restoring extracellular calcium, the weakened intercalcated discs tear apart producing large holes in the sarcolemma. Massive amounts of calcium enter the cell and cytosolic components are lost. The conditions required for the calcium paradox can now be avoided.

Oxygen paradox

Early reperfusion remains the most effect way to reduce infarct size and mortality after myocardial infarction. However, reperfusion may have harmful consequences. Hearse in 1977 introduced the concept of reperfusion injury and the oxygen paradox. It is associated with several general pathological features, including reperfusion arrhythmias, myocardial stunning, and myocyte necrosis. The oxygen paradox may be defined as the conversion of reversibly injured myocytes to irreversibly injured cells on reperfusion. It should be distinguished from the accelerated disruption of severely injured cells on reperfusion by contraction band necrosis. Factors which may be important to its pathogenesis are calcium overload, neutrophil accumulation, oedema, hemorrhage, and oxygen free radical generation. Microvascular injury (endothelial cell injury) may jeopardize blood flow to potentially viable myocytes, thus expanding the infarct. This was termed no-reflow injury by Kloner in 1974. Endothelial cells are more resistant to ischaemic injury than myocytes.

The oxygen paradox is a significant practical problem when hearts are either reperfused after surgical arrest, or when the coronary flow to portions of heart muscle is restored after blood vessel grafting, clot dissolution, plaque removal, or relaxation of coronary spasm. A better understanding of the mechanism of the oxygen paradox will be needed to optimize conditions for injury minimization. The experiment depicted in Figure 6.3, suggests the involvement of free radicals and a role for vitamin E.

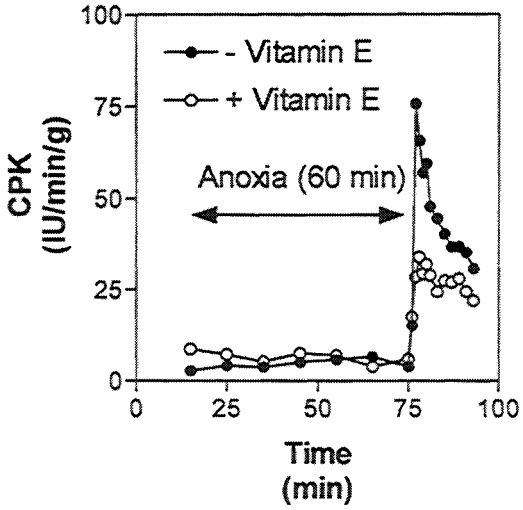

Figure 6.3 Leakage of enzymes from a perfused heart when oxygen is introduced into the perfusate after a period of anoxia.

Contol of mitochondrial function

Experiments with isolated mitochondria suggest that the rate of ATP synthesis is determined by ADP and P_i in a simple kinetic control mechanism. The rate of ATP synthesis can be expressed mathematically as follows:

$$V/Vmax = \frac{([ADP] \times [P_i])/(K_{ADP} \times K_{Pi})}{1 + [ADP]/K_{ADP} + [P_i]/K_{Pi} + (([ADP] \times [P_i])/(K_{ADP} \times K_{Pi}))}$$

But are mitochondria regulated in this way *in vivo*? To test the kinetic control hypothesis in intact animals, magnetic resonance techniques have been used to measure myocardial ADP levels at different cardiac work loads. Surface coil techniques are used to obtain spectra (Figure 6.2).

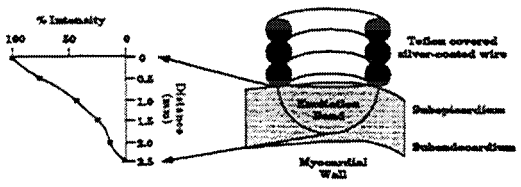

Figure 6.4

However, controversy surrounds the results of these experiments. In large animals, changes in ADP and P_i levels appear to be too small to account for the observed rates of ATP synthesis. Current dogma now favours the redox state (NAD/NADH ratio) and intracellular Ca^{2+} as more important regulators of mitochondrial function than ADP and P_i. In contrast, small animals show changes in the ^{31}P NMR spectrum of heart that are indicative of increases in ADP when cardiac work is increased (Figure 6.5). This supports the kinetic control hypothesis. A novel solution to this contradiction is the possibility of metabolic scaling.

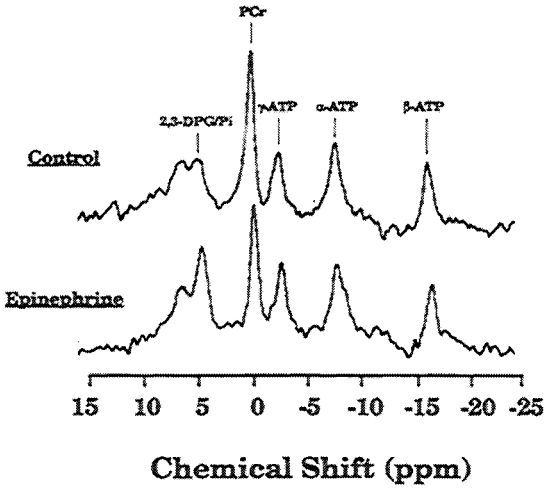

Figure 6.5 Changes in the ^{31}P NMR spectrum of the in situ rat heart due to increased cardiac work

Functional and biochemical scaling

ADP levels scale across species of different sizes (Figure 6.6) in the same way that basal metabolic rate and heart rate is scaled.

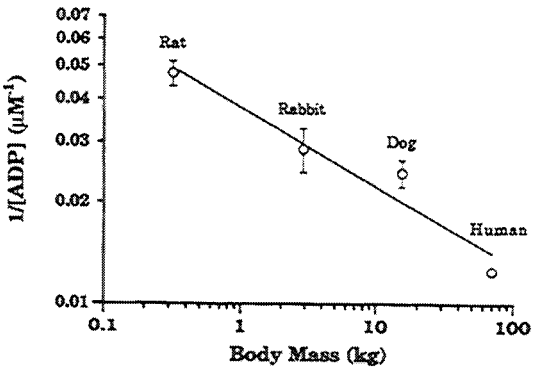

Figure 6.6 Relation between ADP levels and body size in four species

If it is assumed that the K_m for ADP remains constant at about 35 μM across species, then ADP must assume less importance as a mitochondrial control in larger animals (Figure 6.7).

Alternatively, if K_m increased with body mass, then ADP would continue to be an effective control (Figure 6.8).

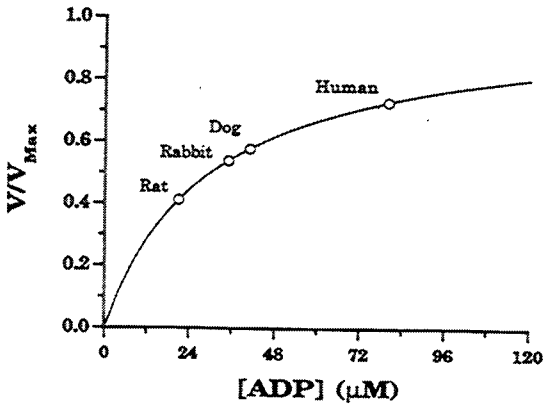

Figure 6.7 ADP would be a poor kinetic control in large species if the K_m for ADP was fixed at 35 μM

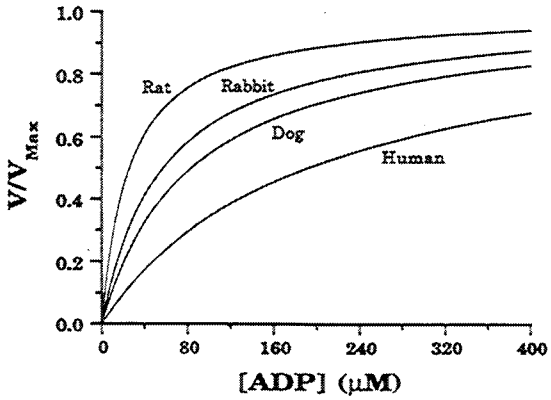

Figure 6.8 ADP may be an effective kinetic control if the K_m for ADP scaled with body mass

Technology and experimentation in cardiovascular research

Arthur Guyton's computer model of the circulation

The maintenance of arterial blood pressure is essential for adequate organ perfusion and the brain is particularly susceptible. For example, loss of consciousness can result from the hypotension caused by postural change or blood loss. Not surprisingly, several powerful control systems have evolved to regulate arterial blood pressure. Each control system operates with a different response time. For example, powerful nervous control mechanisms act within seconds to restore low blood pressure. Within minutes to hours several additional pressure controls begin to operate, but these short and medium term controls fatigue after several days, and long term hormonal mechanisms become important.

Because hypertension is a major risk factor for heart disease and stroke, considerable effort has been expended in understanding how blood control systems are disturbed in this condition. With a plethora of data on individual nervous and hormonal blood pressure control systems it became difficult to make progress.

In 1972 Guyton published a systems engineering model of the circulation which was amenable to computer analysis (Figure 7.1). The model was

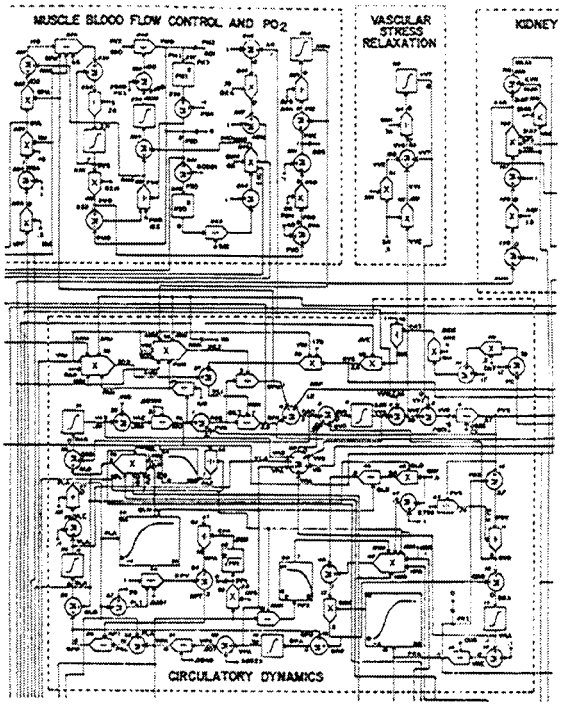

Figure 7.1 A portion of the system engineering analysis of the circulation

comprised of 18 different systems containing 354 blocks. It required a PDP-9 computer with 16K of memory programmed in FORTRAN to run the simulations!

The model quickly confirmed abnormal kidney function as the most likely cause of chronic hypertension (Figure 7.2).

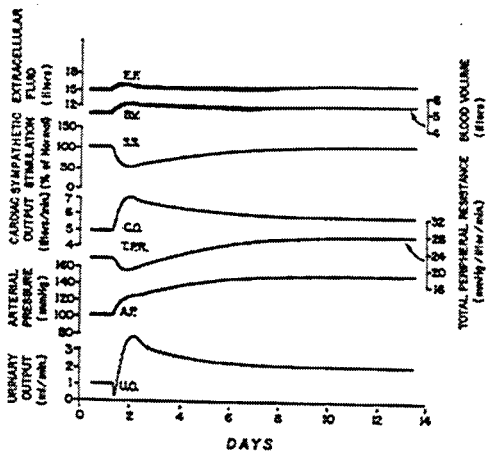

Figure 7.2. In this simulation, hypertension develops when kidney mass is reduced to a third of normal and salt intake is increased to five times normal

Magnetic resonance and the biological uncertainty principle

Nuclear magnetic resonance spectroscopy had been used since the 1950s to determine the structure of chemical compounds. However, it was not until the advent of powerful computers in the 1980s that magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) found application in biology and medicine.

MRS went a long way to removing the uncertainty principle as it operates in biology in a number of important areas of investigation, and as such was immediately embraced by many physiologists like myself. In physics, Heisenberg's uncertainty principle states that it is impossible to determine simultaneously the exact position and momentum of a particle. For example, observation of the position of an electron required an uncertain change to its momentum. A similar problem exists in experimental biology, but on a molecular and cellular scale. In order to study the biological system, it must be disturbed in an unpredictable way. For example, the classical approach to understanding of the chemical reactions of energy metabolism required destruction of the cell and analysis of its energy metabolites. Because the compounds of interest are labile, elaborate methods to quickly freeze tissue to the temperature of liquid nitrogen were developed. There are obvious uncertainties

associated with using these static views of frozen tissue to construct a dynamic movie of energy metabolism. This doubt is removed by MRS, which permits the non-destructive observation of high-energy phosphate compounds, including ATP in intact, functioning hearts.

The technique is made possible by the fact that certain atomic nuclei resonate at specific radio frequencies when placed in a magnetic field. Of biological relevance are the naturally occurring isotopes of hydrogen, carbon, and phosphorus that exhibit this behaviour. Moreover, the chemical environment of a nucleus influences its precise frequency of resonance. This environment is determined by the position of the nucleus in the molecule relative to the nuclei of other atoms. For example, Figure 7.3 shows the

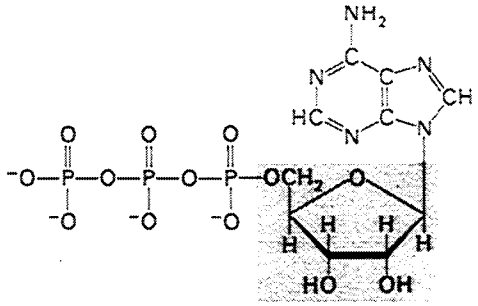

Figure 7.3. Structure of adenosine triphosphate (ATP)

structure of ATP. Because each phosphorus atom is in a different chemical environment, three phosphorus resonances would be expected.

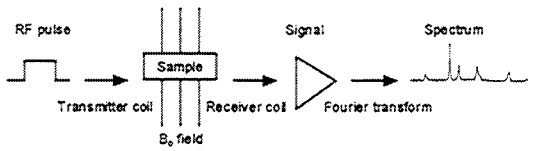

Figure 7.4 The NMR experiment

Figure 7.4 depicts the magnetic resonance experiment. In this example, the heart sample containing ATP is placed in a strong, uniform magnetic field and exposed to a single pulse of a packet of radiofrequencies clustered around the phosphorus resonant frequency. The pulse is generated by a radio transmitter and delivered to the sample by a tuned transmitter coil. The transmitter is then switched off and a radio receiver switched on. The coil becomes a receiver coil to pick up radiofrequency signals coming from the resonant nuclei. The signal decays over time, as energy is lost from the system. After this relaxation process, another pulse can be delivered. Fourier transformation is used to convert the signal from the time to frequency domain and provides the means to add data from

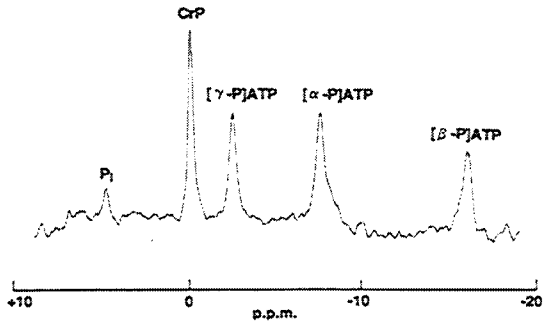

Figure 7.5 ^{31}P NMR spectrum of a perfused rat heart

successive pulses to enhance coherent signal and eliminate random noise. The phosphorus spectrum of a perfused, beating rat heart is shown in Figure 7.5. It is remarkable that it is so simple with only 6 broad peaks corresponding to the expected 3 resonances of ATP, the resonance of creatine phosphate, another high energy phosphate in the myocyte, and those of sugar phosphate, and inorganic phosphate. To be seen in the spectrum, compounds have to be free in solution and in mMolar concentrations. For example, most of the inorganic phosphate in the myocyte is bound and MRS invisible.

Cardiac microdialysis

The technique of cardiac microdialysis was introduced by Van Wylen in 1990. A microdyalisis probe is constructed from a fibre drawn from a kidney dialysis canister (Figure 7.6), and is

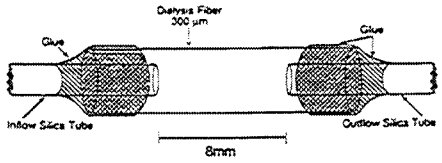

Figure 7.6 Microdialysis probe construction

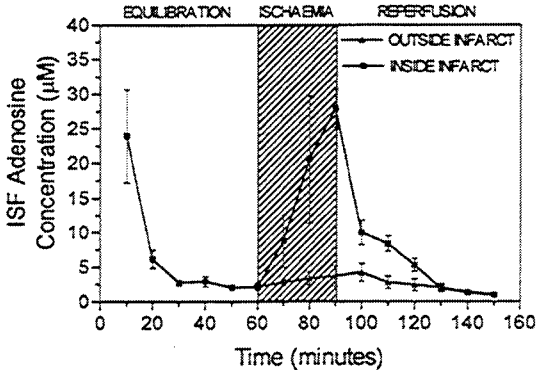

Figure 7.7 Microdialysis is used to monitor interstitial adenosine during myocardial infarction

inserted into the wall of the ventricle. Fluid is pumped slowly through the probe providing time for small molecules in the interstitial fluid to equilibrate across the semipermeable wall of the dialysis fibre. Effluent from the probe is assayed for components like adenosine. The use of the technique to monitor adenosine formation in an infarcted region of a rabbit heart is shown in Figure 7.7.

Isolated heart cells

Many types of experimental design would be more convenient if it were possible to work with isolated myocytes. However, there are several difficulties with this approach. To prepare myocytes from an adult heart, it is necessary to treat the heart with enzymes to digest the intercellular matrix. The extracellular calcium concentration is lowered in order to separate cells at the intercalated discs. This treatment alters the composition of the glycocalyx and creates the conditions of the calcium paradox. Poor cell viability may result. Also, because mature cells can not divide in culture, new preparations must be made daily. Even when successfully prepared, the cells are no longer electrically connected to each other and possess no resting tension. Foetal heart cells can be cultured and they exhibit contractile activity, but because they are immature, they are not necessarily good models for the mature myocyte. Even with these difficulties, isolated myocytes (Figure 7.8) have found a place in cardiac experimentation (see Figure 2.7).

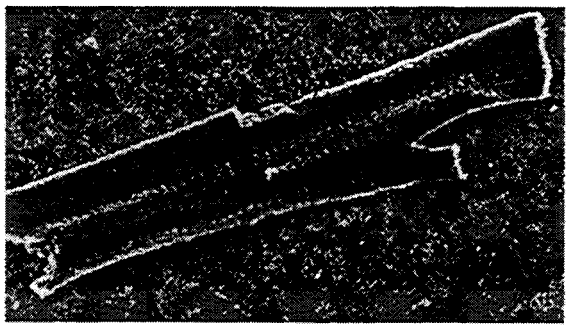

Figure 7.8 The angular appearance of isolated myocytes suggests a well developed cytoskeleton

Drug discovery

Many xanthines, including caffeine, are adenosine receptor antagonists. Theophylline has found use in treating asthma and several other xanthines are used experimentally to block adenosine receptor function in biological systems. A model compound for this purpose is 8-phenyltheophylline. In 1991 a series of non-xanthine compounds were synthetised and one of the compounds, GU285 (Figure 7.9) was

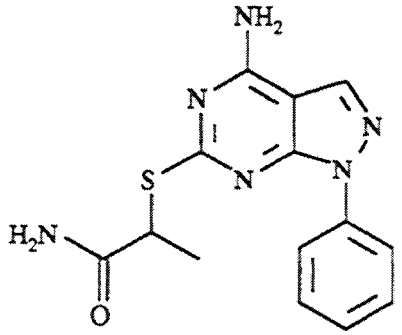

Figure 7.9 The structure of GU285

identified as the most potent inhibitor of the binding of adenosine receptor ligands to their receptor sites. It was found to be a non-selective, competitive, adenosine antagonist drug. More potent and more soluble than 8-phenyltheophylline.

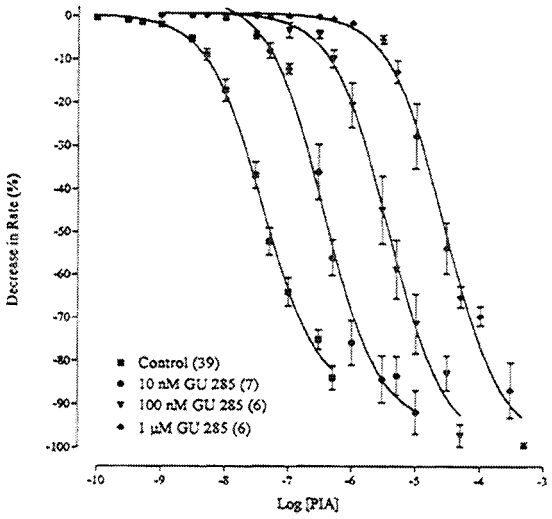

Figure 7.10 GU285 shifts the PIA dose-response curve to the right in rat right atrial preparation

New paradigms and research directions

Arnold Katz cites (1) the Frank-Starling Law, (2) the concept of contractility, and (3) altered myocardial phenotype as the three paradigms that have dominated thinking about heart function in the 20th century.

The Frank-Starling Law or volume-pressure relationship describes the intrinsic property of the heart to pump the blood delivered to it via the veins. It can be explained in terms of a greater cross-bridge recruitment and force development as a result of increased initial stretching of the contractile apparatus due to enhanced cardiac filling.

The idea of contractility developed later, and was necessary to explain the effects of compounds like adrenaline that increased the force of cardiac contraction without altering the initial stretch of the heart. In fact, increased contractility is defined as an increase the strength of cardiac contraction at constant fibre length. It can be explained in terms of both cross-bridge recruitment and cycling rate.

Another way for a heart to alter its function is to alter the expression of its genetic material to make myocytes larger with more intracellular machinery including contractile proteins and mitochondria. Cardiac hypertrophy (Figure 8.1) is seen in athletes and patients with compromised heart function. Understanding altered genetic expression and controlling genetic expression is the paradigm to take us into the next century.

Approaches to the amelioration of heart disease.

Strategies for the prevention and treatment of heart disease have undergone considerable refinement in the last half of this century. Since the 1960s there has been a downward trend in coronary heart disease mortality in many developed nations including Australia. The reasons for this are many-fold. The recognition of the importance of diet and life-style factors in delaying the onset of heart disease and the application of these principles in the form of effective community health promotion is one reason. Another is the development of improved surgical techniques and post-operative care. The development of heart-lung by-pass, vascular grafting, and heart transplantation techniques are examples. More sophisticated and targeted medical interventions, including use pharmaceuticals such as ACE inhibitors and

Figure 8.1 Morphological changes observed in cardiac hypertrophy. The sequence progresses from the early changes in the top panel to advanced changes in the bottom panel

calcium antagonists or percutaneous angioplasty are major developments. Improved detection and diagnosis of heart disease with a full range of imaging methods and other clinical assessments are additional factors.

There is no doubt that further major developments will occur in all these areas as we move into the next century. Diet and life-style factors will be better understood, drug discovery will continue, surgical and diagnostic techniques will be refined, and natural cardioprotective mechanisms will be harnessed for benefit.

However, it is important to recognize the limitations of what can be done with existing approaches. For example, cutting the aberrant conduction pathways that cause certain types of arrhythmia is a surgical approach that provides a complete and permanent cure for this type of heart disturbance. In other situations, while surgery can provide dramatic benefits, the underlying disease process continues. An example is coronary artery bypass surgery, which is very effective for relieving angina and improving quality of life for people with ischaemic heart disease. However, because atherosclerosis continues, the grafts may become blocked and the angina return at a later date. Another example, is heart transplantation. In certain types of cardiomyopathy, the diseased heart can be replaced with a transplant. The outlook of the recipient is changed from impending death due to heart failure, to enjoyment of a full and active life. Nevertheless, success of the procedure depends on drug therapy to prevent tissue rejection.

Heart tissue regeneration

After myocardial infarction, necrotic heart tissue is replaced by non-functional scar tissue. This happens because the adult heart can not grow new myocytes.

However, what if non-cardiac cells could be induced to become myocytes, and then be introduced into the injured region to form a functional patch? If the person's own cells were used, tissue rejection could be avoided. Since all body cells contain the necessary genetic information to produce myocytes this approach is theoretically possible. An experiment to transform heart cells into skin cells is depicted in Figure 8.2. Already in experiments, skin fibroblasts have been be turned into skeletal muscle cells and in mice, immature foetal heart cells have been shown to integrate into damaged adult heart to form a stable, and functional graft.

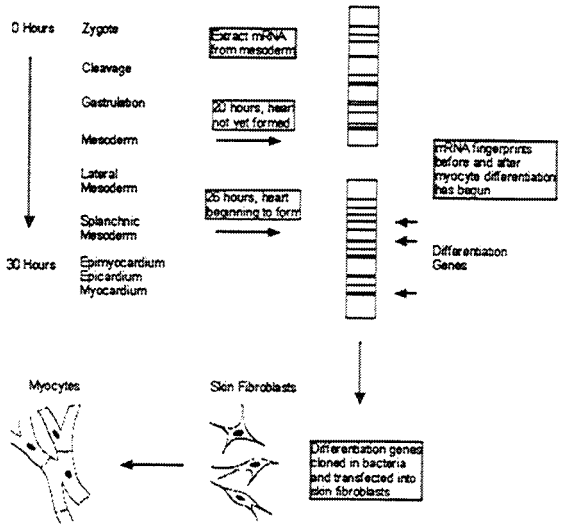

Figure 8.2 An experiment designed to transform skin fibroblasts into myocytes

Genetic modification and repair

The fact the some individuals have poor diet and lifestyle habits, and yet remain free of cardiovascular disease during a long life, is evidence of the importance of having been born with the correct genetic information. Conversely, some individuals are very susceptible to heart disease.

All evidence suggests that cardiovascular disorders are complex genetic diseases that exhibit different degrees of severity depending on environmental influences. The hope is, that once the genetic defects causing a particular disease are identified, it may be possible for them to be repaired.

Another approach might be to enhance natural cardioprotective mechanisms. For example, it would appear that important protective mechanisms are mediated by adenosine working through specific receptors. Although effective, these mechanisms may be limited by the amount of adenosine receptor present. Experiments have been performed with transgenic mice, in which

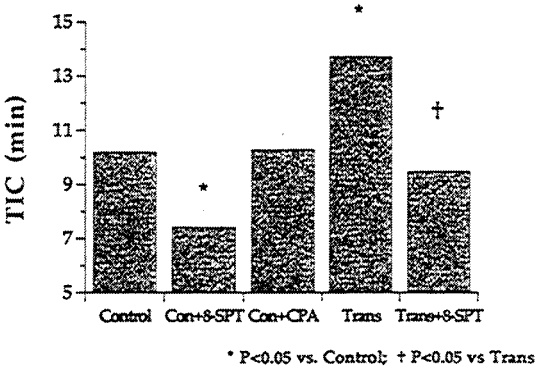

Figure 8.3 Increased adenosine receptors in transgenic mice increases time to ischaemic contracture (TIC)

expression of adenosine receptor has been specifically enhanced. The hearts of these mice show delayed times to ischaemic contracture (Figure 8.3) which may be explained by the preservation of ATP (Figure 8.4).

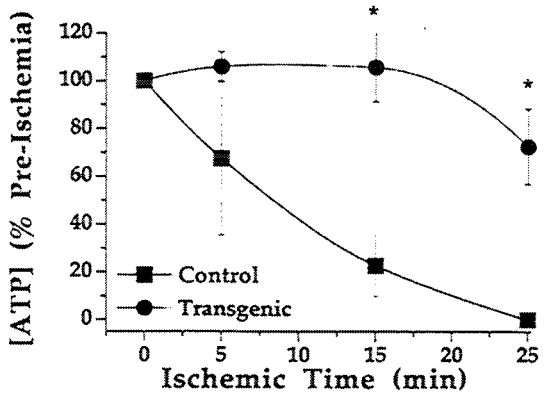

Figure 8.4 Transgenic mice conserve myocardial ATP during ischaemia.

Rotary Centre for Cardiovascular Research

History

The Council of Griffith University established the Rotary Centre for Cardiovascular Research in March 1991. It was officially opened by the Administrator of the State of Queensland and Chancellor of the University, Justice John Macrossan, in June 1991. The Centre is located in the Health Building on the Gold Coast Campus of the University (Figure 9.1).

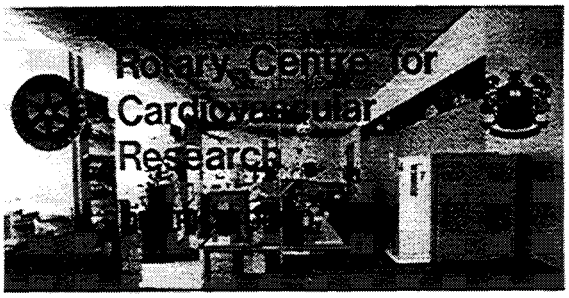

Figure 9.1 Entrance to the laboratory of the Integrative Cardiovascular Research Group of the Rotary Centre for Cardiovascular Research

The Centre has enjoyed substantial community support and acted as a focus for the development of basic and strategic cardiovascular research in Queensland. Significant numbers of well-performed cardiovascular researchers are now active in laboratories on both the Nathan and Gold Coast campuses of Griffith University. The Centre receives support from the National Health and Medical Research Council and the National Heart Foundation of Australia.

The challenge

Ischaemic heart disease due to obstruction of the coronary arteries causes more disability, deaths, and economic loss in western countries than any other disease. It is but one of a number of cardiovascular diseases which account for about half of all deaths in Australia. In addition to causing premature death, ischaemic heart disease reduces the quality of life of those afflicted. The incidence of this disease peaked in industrialized countries in the 1960s and has since been falling. Unfortunately, death rates from heart disease are increasing in developing countries. More must be done to fight cardiovascular disease.

Goals

The formal goals of the Centre are to:

(1) Target within a single Institute in Queensland, cardiovascular disease at all levels from the molecule, through the cell, organ, and organ system, to the individual, clinic and lifestyle.

(2) Direct and support a range of research projects that are designed to increase our understanding of cardiovascular disease with a view to improving methods of detection, treatment, and prevention of heart and blood vessel disease.

(3) Disseminate new knowledge about cardiovascular disease to the medical and scientific community in Queensland by the presentation and publication of research findings of the Institute, as well as timely presentations of developments overseas in the form of seminars or courses

(4) Train and develop the careers of young Queenslanders interested in cardiovascular research

(5) Co-operate with other Centres or Institutes in Australia and overseas having similar aims and objectives.

Project groups

Table 9.1 shows the functional groups within the Centre. Research is conducted on both the Gold Coast and Nathan campuses of Griffith University.

Table 9.1 Research groups and laboratories within the Rotary Centre for Cardiovascular Research

Group	Campus
Integrative Cardiovascular Research Group	Gold Coast
Exercise Science Research Laboratory	Gold Coast
Free Radical Injury Group	Gold Coast
Heart Tissue Differentiation and Regeneration Group	Nathan
Vascular Growth and Control Laboratory	Gold Coast

Staff and student profile

Table 9.2 lists staff and students who have an association with the Centre in 1997.

Figure 9.2 Staff and students associated with the Rotary Centre for Cardiovascular Research

Name	Name
Dr Jay Browning	Dr Wayne Murrell
Dr Denis Crane	Postdoctoral Fellow (NHMRC)
Dr Philip Gaffney	Ms Rachel Jones
Ms Elizabeth Gass	Mr Benjamin Hack
Professor Greg Gass	Ms Bronwyn Garnham
Dr Darren Grice	Postdoctoral Fellow, (NHMRC)
Dr Fiona Harden	Mr Clint Mainwaring
Dr John Headrick	Mr James McKirdy
Dr Helen Massa	Mr Dan Dwyer
Dr Tony Perkins	Ms Helen Naug
Dr Roslyn Rose'Meyer	Ms Chris Wylie
Dr Don Schneider	Ms Andrea Hinschen
Professor Roger Willis	Mr Shane Weston