The Nature and Effects of Myocardial Magnesium Depletion Following Orthotopic Heart Transplantation in Man: Implications for the Role of Magnesium in Cardiac Conduction

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ABSTRACT

Background: Magnesium is essential to life as a vital part of the respiratory chain in both the animal and the plant kingdoms. As a result the effects of magnesium deficiency in metabolically active tissue, such as the heart and brain, are profound. Little is known of the results of a disturbance of magnesium physiology within the human heart, but, in animal studies, serious disorders of mitochondrial calcium handling are described, resulting in significant myocyte calcium overload. Electrophysiologically, the effects of magnesium deficiency on cardiac conduction are poorly defined, and the mechanism for the beneficial action of magnesium in ventricular tachycardia is unknown.

Cyclosporin, an immunosuppressive agent used therapeutically to reduce the risk of graft rejection after cardiac transplantation, is known to induce hypomagnesaemia, and consequent myocardial magnesium depletion would be anticipated. Circumstantially, cardiac transplant recipients are therefore an ideal group in which to study the nature and effect of magnesium depletion in the myocardium in man. Furthermore, the denervated state of the transplanted myocardium is particularly appropriate for the study of the electrophysiological effects of magnesium deficiency or excess.

The Measurement of Magnesium in the Myocardium: No suitable method for the analysis of the minute samples (less than 0.4mg dry weight) of myocardium for magnesium was available and a new method utilising pepsin digestion was developed, optimised and validated. The assay was subsequently adapted for calcium analysis.

Magnesium and the Transplanted Heart: Serum and myocardial magnesium were measured serially in nineteen consecutive cardiac transplant recipients over the first nine months following transplantation. Hypomagnesaemia occurred in all recipients with the development of myocardial magnesium depletion in 94%. There was a significant delay (up to six weeks) in repletion of myocardial magnesium after normalisation of serum levels.

Magnesium Depletion and Myocardial Calcium Content: In a biochemical and histological study (using electron microscope techniques), myocardial calcium in biopsies from the transplanted heart was found to be generally higher than that in control hearts. Mitochondrial calcium deposition was noted, which was more marked in association with prolonged myocardial

magnesium depletion, suggesting a possible risk factor for accelerated graft vasculopathy. In discussion, a role for magnesium as a myocardial protectant in ischaemic calcium-mediated myocyte injury was postulated.

Magnesium and Cardiac Arrhythmia: In electrophysiological studies, prolongation of the ventricular action potential was produced by pharmacological doses of magnesium in the transplanted heart suggesting one mechanism for the beneficial anti-arrhythmic effect of magnesium; isolated hypomagnesaemia was not found to be arrhythmogenic. These data were incorporated with recent advances in studies of cellular electrophysiology to suggest that magnesium exerts its anti-arrhythmic action via potassium channel modification, in addition to its role as a calcium channel blocker.

The Future: Appreciation of magnesium as an anti-arrhythmic agent must be substantiated by proper controlled, comparative trials. An increasing realisation of the effectiveness of magnesium as an anti-ischaemic agent is at present the subject of a major clinical trial. The mechanisms by which this extraordinary ion exerts its omnipotent effects have yet to be fully elucidated, promising exciting future research.

Declaration

No portion of the work referred to in this thesis
has been submitted in support of another degree or qualification
of this or any other university or institute of learning.

Education and Research Experience

After qualifying MB ChB from the University of Manchester in 1983, Dr Millane gained full registration in 1984 with the General Medical Council after pre-registration House Officer posts at Manchester Royal Infirmary.

General Professional Training was undertaken at the North West Regional Cardiothoracic Centre, Wythenshawe Hospital, Manchester, East Birmingham Hospital, West Midlands and Windsor Hospital, Berkshire culminating in Membership of the Royal College of Physicians in 1987.

Specific training toward specialist accreditation in Cardiology and General Medicine continued as part of the Regional Medical Registrar Rotation at St George's Hospital, London (1987-1989).

Research experience was gained during a full-time clinical research post in the Departments of Medicine and Cardiological Sciences of St George's Hospital Medical School, University of London under the supervision of Professor A John Camm (1989-1991). Apart from the work presented in this Thesis, close involvement with research into risk stratification after acute myocardial infarction was maintained, and included collaboration with the International Studies for Infarct Survival based in Oxford, as part of the pilot study for the ISIS 4 trial (1991-93).

Dr Millane is currently a Clinical Registrar in Cardiology at the South-West Thames Regional Cardiothoracic Unit, St George's Hospital, London.

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I am grateful to Dr David Bennett who encouraged my fledgling interest in magnesium, a subject which was at that time was barely explored. However, the subsequent development of the thesis led me rapidly to the Department of Cardiological Sciences and Professor John Camm, who undertook to supervise the clinical component of the thesis. I recognise that I have been extremely fortunate to work under his supervision, a supervision applied gently but firmly! I shall always be grateful for the opportunities offered by my sojourn under his directorship.

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To my Parents

CHAPTER ONE

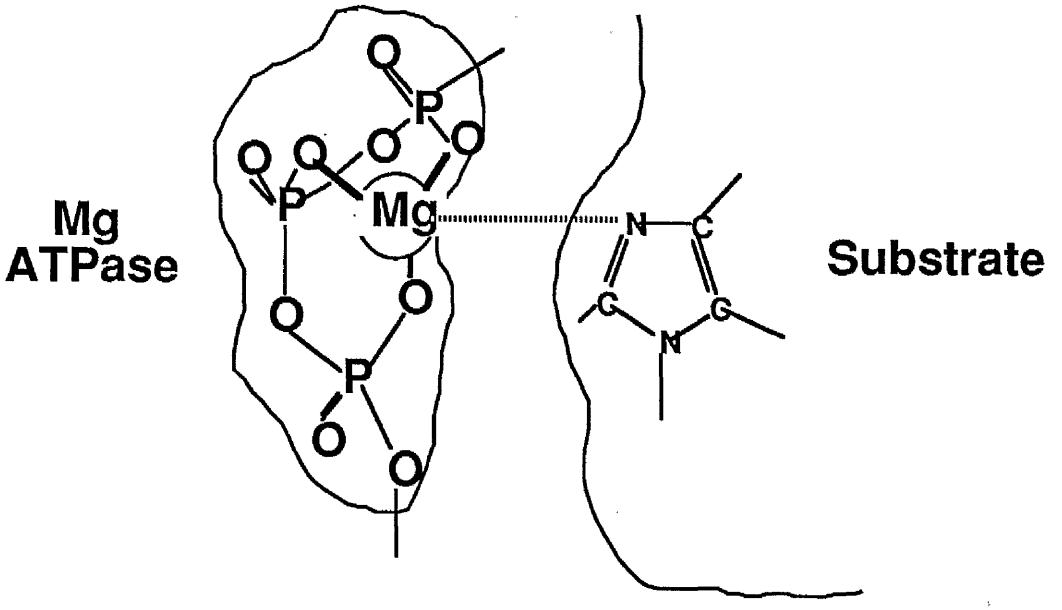
MAGNESIUM AND THE HEART

Introduction

Magnesium is the sixth most abundant cation on Earth. The unique configuration of the electron cloud surrounding the nucleus of the magnesium atom facilitates the formation of coordinate chemical bonds with a variety of substrates. Over the millenia, magnesium has become vital to hundreds of enzyme systems throughout Nature. The magnesium atom is at the centre of the chlorophyll molecule, and is an integral part of the active site of the ATPase molecule (Figure 1.1). In man, magnesium is a cofactor in at least three hundred enzymatic reactions. It is integrally linked to the process of oxidative phosphorylation via the electron transport chain within the mitochondria, and hence to intracellular energy production.

Figure 1.1

Magnesium and ATPase



Of all cell types, mitochondria are found in greatest numbers in cardiac myocytes. The requirement for energy, and hence magnesium, within the myocyte, over and above that necessary for basic cellular function, is enormous, including repeated activation of the actin-myosin complexes (about one hundred thousand times per day), the control of intracellular calcium concentration and the maintenance of baseline negative membrane potentials. Quite apart from the energy required to maintain intracellular calcium homeostasis, intracellular magnesium ion is an important determinant of intracellular calcium concentration. The chemical similarity of magnesium and calcium has lead to the development of a symbiotic, antagonistic relationship between these two ions, which has lead some workers to dubb magnesium as "nature's calcium antagonist".

A deficiency of intramyocardial magnesium might be expected to produce diverse adverse effects. In animal studies, significant cardiomyopathy and vasculopathy associated with magnesium deficiency are described, but there are few data available in man. Such data as there are, rely heavily on postmortem studies which suggest a reciprocal role for magnesium and calcium, in myocyte protection, and injury, respectively.

Historical review

"The presence of a substance in the body is purposive and constitutes prima facie evidence not only of its usefulness but of its indispensability."

Such was popular medical opinion in the early part of this century[1]. However, as the human body was found to contain an increasing number of substances, there began to be some doubt as to the validity of this statement. Magnesium occurs in such vast quantities in all species that few questioned the

proposition that this element must be essential to animal life. It was not until the 1930s, however, that the trend toward objective verification of all scientific hypotheses led to the examination of this telelogical doctrine by specific experimentation.

Osbourne and Mendel are credited with the first report of the effects of a diet low in magnesium[2]. Their paper published in 1918, established the necessity for calcium in the normal diet, but failed to show an absolute requirement for magnesium. The authors point out that they were unable to reduce the magnesium content of the diet sufficiently to comment upon the potential role of magnesium (purification techniques were not sufficiently advanced at that time). However, they did chart the progress of a single rat, the diet of which was rendered low in magnesium; growth proceeded normally until maturity, after which the animal lost weight. The animal was then fed a normal diet resulting in a rapid reversal of the weight loss. This work was repeated by Leroy (1926) using a diet that was significantly more magnesium deplete[3]. Given to two white mice within three weeks of birth, growth and development was arrested within 9-13 days with death occurring in 24-35 days. A mouse fed a control diet did not exhibit these features. A second group of animals were fed a magnesium deficient diet, and changed to control rations after 10-14 days. These animals initially followed the same course as the magnesium-deficient rat, but rapidly gained weight on reinstituting magnesium replete rations, running a subsequent course identical to that of the control animals.

This work was not repeated, nor was it extended to other species, and the subject remained unexplored until the index work of Kruse and colleagues at John Hopkins University, Baltimore, in 1932[1]. These workers sought to answer two questions (i) Is magnesium an indispensable element for body function? (ii) if it is essential, what effect has its deprivation upon the body?

A diet free in magnesium is impossible to attain, since many protein and vitamin complexes contain magnesium. However, by strict attention to detail Kruse reduced the magnesium content of an otherwise normal diet to 1.8 parts per million. Using more than 200 rats these workers demonstrated in a controlled trial that magnesium deficiency is characterised by cutaneous vasodilatation followed after a few days by vasoconstriction, hyperirritability of the central nervous system, cardiac arrhythmia, tetany and epileptiform convulsions. The term "cardiac arrhythmia" is used in the summary paragraph by the authors to describe a sinus tachycardia secondary to vasodilatation, and to encompass bradycardia at the time of convulsions. Death occurred within a few minutes of the terminal convulsion. Respiration suddenly ceased but sinus rhythm was maintained until the heart stopped in what is described as "ventricular systole". There was no circumstance to suggest cardiac death or specific ventricular arrhythmia.

In 1937 Tufts and Greenberg investigated serum and tissue content of magnesium and calcium in magnesium deficient rats[4] and found that tissue levels of magnesium fell 10-20%, whilst serum and red cell levels fell by almost 50%. Tissue calcium content rose 60-200%, with changes particularly marked in heart, skeletal muscle and kidney. In animals fed a magnesium-deficient diet additionally high in calcium, the severity of the associated magnesium deficiency syndrome was increased.

As yet magnesium deficiency syndromes had not been described in humans, but a condition known as grass tetany (or grass staggers) in cattle had a striking similarity to the experimentally-induced magnesium deficiency syndrome. Serum analysis from animals with grass tetany revealed hypocalcaemia, normo-phosphataemia and severe hypomagnesaemia. In work by Duncan et al (1935) prompted by the observations of Kruse and

colleagues discussed above, the syndrome was reproduced in calves fed a magnesium deficient diet to suggest that the primary cause of grass staggers was hypomagnesaemia [5].

Whilst magnesium had been established as vital to animal life and the characteristics of magnesium deficiency described, it was not until 1950 that a determined attempt to identify the histological lesions associated with magnesium deficiency was made. Lowenhaupt and colleagues described the pathological findings in rats fed magnesium deficient rations[6]. The basic lesion consisted of an inflammatory reaction involving polymorphs and eosinophils, occurring around arterioles and capillaries, resulting in fibroblastic proliferation and fibrous healing. Cardiac involvement was specifically described, documenting a vasculitis around small coronary arteries in both the subendocardium and myocardium, the pattern following that seen in many other tissues with tissue necrosis and fibrosis associated with the vascular abnormalities.

Vitale and colleagues (1957) investigated the biochemical abnormality induced by magnesium deficiency[7]. Vitale's work was based upon three observations: (i) cold stress had been shown to increase requirements for magnesium. (ii) cold was also known to increase thyroid activity; (iii) thyroxine uncouples oxidative phosphorylation whilst administration of magnesium prevents such uncoupling. ie. magnesium requirements are increased in the face of an excess of thyroxine, and a relative shortfall of magnesium results in uncoupling of oxidative phosphorylation. It was therefore suggested that magnesium deficiency per se might result in abnormalities of oxidative phosphorylation, and an experiment was designed to test this hypothesis using rat-heart mitochondria, since cardiac mitochondria were known to be particularly sensitive to the effects of thyroxine administration and magnesium

deficiency. Uncoupling of oxidative phosphorylation was seen within four days of the administration of a magnesium deficient diet, coinciding with the clinical syndrome of hyperaemia and convulsions. Hepatic and renal mitochondria were much more resistant to the effects of magnesium depletion than were those from the heart[8].

It was against this backdrop that Wener (1964) reported the cardiovascular effects of hypomagnesaemia in dogs[9]. The same clinical syndrome of hyperaemia, growth retardation, hyperexcitability and convulsions was noted. Histologically there were foci of calcification in the large arteries and also in the liver and kidney but not in the myocardium. However, a perivascular inflammatory infiltrate affecting small and medium sized arteries was described similar to that seen in Lowenhaupt's study, with myocardial necrosis and fibrosis. As in the original study by Kruse, no abnormality of cardiac rhythm was reported; in addition, no changes in the electrocardiogram were noted.

Mishra (1960) and Heggtveit (1965) went on to report light and electron microscopic changes in the myocardium of magnesium deplete rats[10,11]. Fibroplastic proliferation associated with scarring, with and without calcification, was seen in the myocardium, with destruction of the nuclei following extensive sarcoplasmic damage. Ultrastructural changes were most marked in the mitochondria with distortion of the membrane and compression of the cristae. Progressive deposition of calcium within the mitochondria occurred accompanied by disruption of the myofibrils[12]. The tissue damage insuing resulted in focal necrosis and eventual fibrosis. Magnesium-deficient animals exposed to cold stress exhibited similar myocardial abnormalities; these changes tended to be more severe in the cold-stressed animals providing a histological counterpart of Vitale's biochemical observations

regarding exacerbation of the disruption of oxidative phosphorylation induced by cold stress and magnesium deficiency.

The first inkling that magnesium may be important in ischaemic heart disease came from Rigo(1963), who demonstrated that the area of infarcted muscle was larger and myocardial calcium content higher, in rats rendered magnesium deficient as compared with normal controls[13].

There are few data in the literature relating to myocardial function in magnesium deficiency. There is one report suggesting that exercise tolerance is reduced in mice, but this may well relate to invariable concomitant potassium depletion in skeletal muscle[14]. The symptomatology of severe magnesium depletion is predominantly related to the nervous system, which makes the specific study of ventricular function rather difficult. In general the animals have died early in their clinical course, and signs and symptoms of magnesium deficiency cardiomyopathy might only become apparent over time. There are no long term studies of the effect of mild to moderate magnesium depletion.

In man, severe magnesium depletion occurring in clinical practice is invariably accompanied by depletion of other nutrients, and conclusions as to the effects of specific deficiencies are not possible. With reference to the heart, observation is largely confined to analysis of material obtained post mortem. The same general pattern of myocardial magnesium depletion associated with calcium overload is seen in many biochemical studies. There are few in vivo studies; these are limited to the measurement of magnesium in the myocardium, and have not been designed to assess histological changes or myocardial calcium content.

Magnesium in Man

Magnesium is the second most common intracellular ion in man (after potassium). 95% of total body magnesium is intracellular, and 30% of that found in serum is protein bound. Assessment of total body magnesium status from knowledge of serum levels alone is generally not possible[15]; the problems involved in the measurement of magnesium in serum and tissue are discussed below. Physiologically, magnesium exists bound to a variety of ligands, most commonly phosphates (complexed with RNA), and in an ionic free state in the cytosol. It is present in all body tissues, particularly bone, which forms the largest body store of magnesium.

Physiological control of total body magnesium

The average daily intake of magnesium is 5-12 mmol/day; approximately 30% of this is absorbed in the small bowel, under the influence of vitamin D[16]. Urinary excretion is 3-5 mmol/day in magnesium balance; the kidney will avidly conserve magnesium in states of magnesium depletion excreting less than 1 mmol/day under these circumstances[17]. Renal magnesium balance is a complex interaction of tubular sodium, potassium and calcium concentration, influenced by parathyroid hormone and the rate of glomerular filtration. Western diet provides barely adequate magnesium; foods high in magnesium - legumes and dark green vegetables, fish (including shellfish), whole nuts and grains - do not form a large part of average fare. Furthermore, fat, sugar, salt and fibre together with inorganic phosphates, decrease intestinal absorption. It has been suggested that magnesium depletion is commoner in soft water areas as compared with comparable hard water areas, where the magnesium content of tap water is significantly higher[18,19].

Magnesium deficiency

As described above, most studies of magnesium depletion have been performed in animal models fed a magnesium deficient diet. In man, skeletal muscle and neural tissue are particularly dependent on magnesium, and tissues with high energy requirements of rapid tissue turnover, such as skin and gut, are particularly sensitive to magnesium depletion.

Table 1.1 Causes of Magnesium Depletion in Man

1. Inadequate nutrition : Protein-calorie malnutrition Inflammatory bowel disease Parenteral nutrition Anorexia nervosa Alcohol abuse Critical illness	3. Renal toxicity Aminoglycoside antibiotics Cytotoxic agents Cyclosporin A Solvent abuse
2. Renal losses Diuretics Diabetes Mellitus Alcohol abuse	4. Excess faecal losses Inflammatory bowel disease Cathartic abuse

Biochemically, hypomagnesaemia quickly develops, accompanied by severe hypokalaemia which, due to secondary abnormalities of renal potassium handling, is resistant to treatment with potassium supplementation, producing attendant features of potassium depletion[20]. Lethargy, behaviour disturbances, and cardiac arrhythmia due to severe potassium depletion are well documented [21,22]. There are a few isolated case reports in which magnesium deficiency is attributed as the primary cause of heart failure[23,24].

Lipids are adversely effected by magnesium depletion, with a rise in LDL and a fall in HDL fractions, although the mechanism is unknown[25,26]. Atherogenesis is accelerated if associated with magnesium deficiency[27].

The causes of magnesium depletion are listed in Table 1.1. Uncontrolled diabetes mellitus (particularly during episodes of ketoacidosis [28]), alcohol abuse and prolonged episodes of critical illness, are the most common associations of hypomagnesaemia and magnesium depletion.

Drugs, Magnesium and the Kidney

Unlike the majority of metal ions, the main site of renal magnesium reabsorption and/or secretion is situated in the thick ascending limb of the loop of Henle (see Chapter Four). The concentration of sodium delivered to the loop of Henle is a major determinant of subsequent magnesium handling and thus diuretics have the potential to disrupt the fine control of magnesium balance. There appears to be a specific mechanism by which magnesium is reabsorbed in the tubule, but the precise pathways have yet to be elucidated[17]. Drug-induced renal magnesium wasting is a relatively common cause of magnesium depletion in hospital practice - often exacerbated by a concomitant catabolic state. The mechanism by which renal magnesium wasting occurs is unknown in most instances, but is well recognized to be an accompanying feature of aminoglycoside therapy[21], chemotherapy (particularly cisplatinum)[22,29] and of immunosuppressive therapy with cyclosporin[30]. The neurological effects of magnesium deficiency predominate in these circumstances with tremor a prominent symptom; convulsions are common in children[31]. Cardiac arrhythmia is not described, but has not been specifically studied.

Magnesium Deficiency After Cardiac Transplantation

Cardiac transplant recipients are treated with cyclosporin A, a powerful immunosuppressive agent. Among many adverse effects, cyclosporin A induces significant renal magnesium loss, the mechanism of which is as yet undetermined. The potential for magnesium deficiency is thus high in this population in whom, due to the necessity for frequent endomyocardial biopsy (for the detection of rejection), it is possible to monitor serially the potential biochemical and histological changes induced in the myocardium by magnesium depletion. Despite the relative abundance of information as to the significant adverse effect of magnesium depletion on the animal heart, there are few data available in man. There is no serial study of the temporal relationship between serum and tissue magnesium and no serial study to determine the ongoing effects of magnesium depletion in the cardiac myocyte.

Magnesium Toxicity

Magnesium toxicity is rare, and is usually iatrogenic. In renal failure, the capacity for magnesium excretion is greatly reduced, and inadvertant administration of magnesium salts - as antacids in the form of magnesium hydroxide or as enemata as magnesium sulphate - can result in hypermagnesaemia, although serious effects are seen only when serum levels are elevated beyond 4 mmol/l (i.e nearly four times normal)[32]. Symptoms include drowsiness, hypotonia with loss of deep tendon reflexes and respiratory depression [32]. When serum levels reach 6 mmol/l, coma and death are invariable.

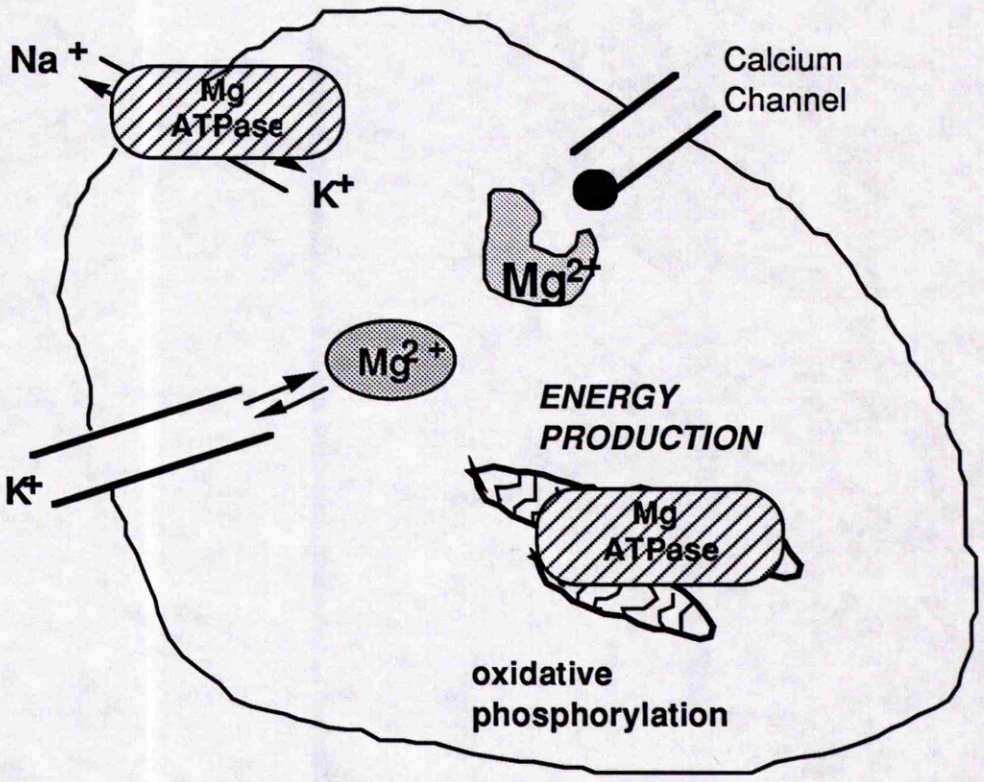
The Physiological Action of Intracellular Free Magnesium

Intracellular free myocardial magnesium ion concentration has been estimated by $^{31}P/^{19}F$ magnetic resonance imaging (NMR) to be 0.5-1.2 mM[33, 34]. Using the rat heart (which has a magnesium content w/w similar to the human heart), workers have demonstrated that cellular magnesium is totally exchangeable over a relatively short period of time (20 hours); a dependence of magnesium efflux on extracellular magnesium concentration was also demonstrated[35]. The physiological action of intracellular free magnesium in man is not known, but in isolated cell studies, progressive increases in magnesium ion concentration have been shown to block inward calcium current [36], and to prolong the action potential by an effect on three or more potassium channels [37-39]; the nett effect of magnesium depletion will vary from cell to cell dependent on membrane potassium channel characteristics, but in general, would be expected to result in shortening of the action potential[40].

Trans-membrane electrochemical gradients, maintained in part by energy-dependent active transport mechanisms, are also regulated by absolute ionic concentration. Quite apart from the dependence of Na-K ATPase on covalent magnesium as a cofactor, intracellular ionic magnesium is an important independent determinant of sodium, potassium, and calcium concentration. Hence, normal cardiac conduction is indirectly heavily reliant on the magnesium ion [41]. These varying effects of magnesium are depicted in Figure 1.2, and are discussed further in Chapters Seven, Eight and Nine.

Figure 1.2

Magnesium and the Cardiac Myocyte

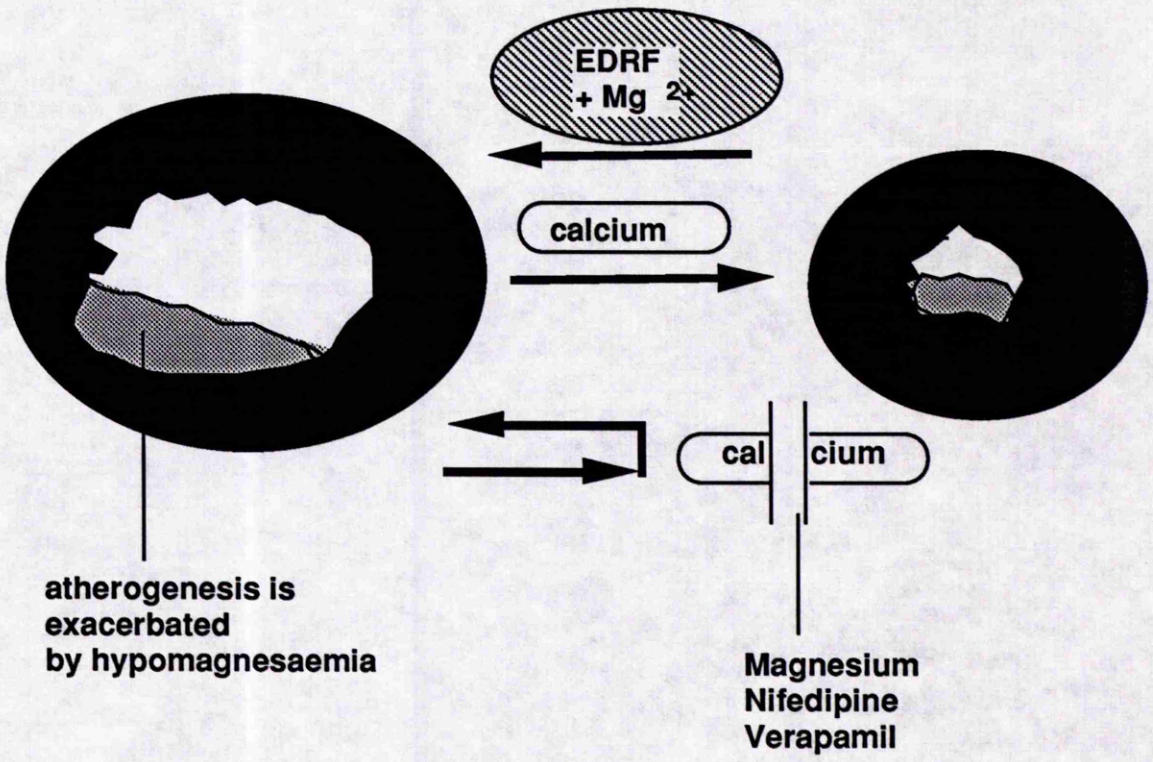


Magnesium and Smooth Muscle Tone

Endothelium derived relaxing factor (EDRF - now known to be nitric oxide) is magnesium-dependent [42] (Figure 1.3). The reciprocal effects of magnesium and calcium on smooth muscle tone have led some authors to label magnesium as nature's "calcium antagonist"[43]. Magnesium deficiency can produce spasm of the coronary artery, possibly contributing to the excess death from ischaemic heart disease seen in magnesium-deplete populations (discussed further below)[44]. Intravenous magnesium is effective pharmacologically in the prevention and relief of coronary artery spasm [45,46].

Figure 1.3

Magnesium and Coronary Artery Tone



The severe vasoconstriction associated with the eclamptic syndrome is very sensitive to pharmacological doses of magnesium sulphate, which is also useful as a tocolytic agent[47]. There is no evidence that magnesium deficiency is aetiological factor in eclampsia.

Outside the field of cardiovascular medicine, intravenous magnesium is effective as a bronchodilator in acute severe asthma[48] and has been used in nebulised form to attenuate exercise-induced asthma in children[49].

Magnesium and Calcium

Magnesium is generally regarded as a calcium antagonist and there is increasing evidence of this phenomenon at cellular level[50], with possible stereotaxic effects of the magnesium ion at the cytosolic entrance to the calcium channel[51]. There is also evidence from post mortem studies for a reciprocal relationship of calcium with magnesium, in that myocardial calcium content is increased, and magnesium decreased, in subjects dying from ischaemic heart disease compared to normal controls[52-56], and that this difference is greater in subjects in whom cardiac death is sudden[53,57,58]. Magnesium has been shown to decrease calcium overload in experimental models of ischaemia and to limit infarct size[59-62]. Catecholamine injury is also attenuated by magnesium[63]. Massive calcium influx occurs during ischaemia, with the deposition of characteristic electron dense material composed of calcium within the mitochondria, with consequent cellular dysfunction[64,65]. An unexpected finding in a placebo-controlled study of intravenous magnesium after myocardial infarction (discussed in detail below) was a reduction in the incidence of cardiogenic shock in the magnesium treated patients, suggesting that magnesium is acting to limit myocardial injury [66]. Provisional data from LIMIT 2 (Leicester Myocardial Infarction Trial), a placebo controlled trial of the use of magnesium in the peri-infarction period in some 2000 patients, indicate a reduction in mortality in the magnesium treated group of 24% (K. Woods, personal communication); preservation of myocardial function was greater in the magnesium-treated group ($p<0.004$). Such data strongly support the notion that ischaemic injury is attenuated by magnesium in man. Magnesium is known to be an important constituent of cardioplegia solution, with faster post-ischaemic recovery times reported with magnesium-containing solutions [67,68], although over-enthusiastic use can be detrimental[69]. The search for better myocardial protectants is ongoing[70];

it may be that magnesium will be useful in other ischaemic scenaria - eg unstable angina and high risk coronary angioplasty.

Magnesium Depletion and Cardiac Arrhythmia

Literature reports of hypomagnesaemia-induced tachyarrhythmia usually refer to situations in which serum magnesium has fallen acutely, in association with prescribed drug therapy or severe alcohol abuse[22,71,72]. There are also reports of ventricular tachycardia and magnesium depletion occurring in patients undergoing total parenteral nutrition[73]. However, in all these circumstances significant hypokalaemia was coexistent, and would certainly have contributed to arrhythmogenic potential. Arrhythmia unequivocally due purely to acute hypomagnesaemia has not been reported.

In the three published in vivo studies investigating the relationship between arrhythmia and myocardial magnesium content, ventricular arrhythmia (most commonly ventricular ectopy) was noted to occur more commonly in patients with lower myocardial magnesium content, regardless of serum magnesium levels[74-76]. No overall consensus regarding the concomitant effects of potential potassium depletion could be drawn from these studies. The question of the arrhythmogenicity of magnesium depletion is addressed in detail in Chapter Eight.

Magnesium as a Pharmacological Agent

A number of case reports and a series of extensive reviews suggest that magnesium may be an effective anti-arrhythmic agent in the acute control of ventricular and supraventricular arrhythmia, effective in normo- and hypomagnesaemic patients.

As discussed earlier, intravenous magnesium has been shown to reduce mortality following acute myocardial infarction. These placebo controlled

studies of intravenous magnesium administered within the first 24 hours of acute myocardial infarction have also demonstrated a reduction in the risk of ventricular arrhythmia by up to 50%[77]. Other postulated mechanisms for the beneficial action of magnesium in acute myocardial infarction include modification of coronary artery tone (see above), and a decrease in platelet aggregation, probably by acting as a calcium antagonist via prostacyclin[78]; prolongation of the prothrombin time by magnesium infusion has also been reported[79]. The role of magnesium in the attenuation of calcium-induced ischaemic injury has been discussed earlier in this Chapter.

Whilst the mechanism by which magnesium acts to attenuate calcium-induced injury is partly established, its mode of action as a possible antiarrhythmic agent remains a mystery. Acute changes in myocardial perfusion or extracellular fluid composition might be expected to induce rapid modification of myocyte electrolyte concentrations. Romani and Scarpa, using an isolated perfused rat heart, have shown that up to 20% of total intracellular magnesium may be mobilised in under two minutes in response to noradrenergic stimulation[80]. One might postulate that such rapid changes in myocyte electrolytes would produce an early loss of free magnesium ion with a consequent fall in intracellular free magnesium ion concentration, followed by later replenishment of free ion from intracellular stores. Such a fall in intracellular magnesium may alter the permeability of membrane channels and induce abnormalities of the action potential.

The Measurement of Magnesium in Serum and Tissue

Until recently, technological problems have prevented satisfactory automated measurement of magnesium in serum. The advent of atomic absorption spectroscopy (AAS) was an important milestone, since for the first time, both tissue and serum magnesium could be measured with some accuracy.

However, AAS is expensive and time consuming, making it an unattractive proposition for routine use in most centres. A sensitive and accurate colorimetric technique involving xylidyl blue is now widely available (previous colorimetric assays using titan yellow were too insensitive), enabling clinicians access to more that the occasional estimation of serum magnesium[81].

A mere 5% of total body magnesium is contained in serum, 30% of which is protein bound. Several studies have identified a poor correlation of serum with tissue levels, and despite the use of lymphocyte magnesium or red cell magnesium, correlation is still not adequate to reliably extrapolate the information gained from blood analysis to infer the state of tissue magnesium content [82,83]. Total body magnesium deficiency may be diagnosed by using a magnesium loading test in which a quantity of magnesium is administered by mouth (or intravenously, if absorption is impaired) and renal excretion of magnesium is assessed to infer a state of negative or positive magnesium balance. Such a test is unreliable in the presence of renal disease, and its use is largely limited to research. At present, the investigation of magnesium deficiency in a particular tissue requires direct assay of the tissue under study (see Chapter Two).

The Next Horizon

Little is known of the relationship between serum and tissue magnesium; furthermore, the long term effects of selective magnesium deficiency on the myocardium in man have not been investigated. In view of the potentially serious effect of magnesium depletion on the myocardium identified in animal studies, a model of myocardial magnesium deficiency in man would be of great interest.

Cardiac transplant recipients are at high risk of hypomagnesaemia secondary to treatment with cyclosporin A. Regular myocardial biopsy forms part of

clinical follow up for these patients and they are therefore an ideal group in which to study the possible effects of magnesium depletion on the myocardium in man.

The following Chapters describe a prospective, non-interventional study of the incidence, effect and associations, of myocardial magnesium depletion after cardiac transplantation. At the outset, it rapidly became obvious that there was no satisfactory method for the analysis of magnesium in the small samples obtained from modern in vivo endomyocardial biopsy, requiring the development of a new micro assay which is described in an opening chapter. The new assay was then used (i) to establish the prevalence of myocardial magnesium depletion in cyclosporin-treated cardiac transplant recipients, and (ii) to investigate the relationship between serum and myocardial magnesium. Disturbances in myocardial calcium are predicted from the animal and post-mortem studies, and were sought in a biochemical and histological study sub-study.

Should myocardial magnesium deficiency result from treatment with cyclosporin A, this would provide a unique model for electrophysiological study of the effects of hypomagnesaemia and /or myocardial magnesium depletion. Studies to address (a) the possible arrhythmogenicity of hypomagnesaemia and (b) the mechanisms of the observed anti-arrhythmic action of intravenous magnesium, are reported, together with a discussion incorporating the (potential) findings with recent advances by cellular electrophysiologists in this field.

Summary

Magnesium is ubiquitous throughout nature and is vital to normal cardiac function. There is a close interaction with magnesium of both calcium and potassium, resulting in a dependence on magnesium of mechanical and

electrical cardiac function. Magnesium depletion induces a cardiomyopathy with significant mitochondrial dysfunction, whilst magnesium used therapeutically may have a beneficial action as a myocardial protectant and an anti-arrhythmic agent. Serum levels are a poor guide to total body magnesium status and sensitive detection of magnesium deficiency requires direct tissue analysis. Few data are available in man, and none in cardiac transplant recipients - a group at high risk of magnesium depletion secondary to treatment with cyclosporin A. This group form the basis of a study of the effects of magnesium depletion on the myocardium in man.

CHAPTER TWO

A NEW MICRO METHOD FOR THE ANALYSIS OF MAGNESIUM IN MYOCARDIAL BIOPSIES

Introduction

There has been growing interest over the last twenty years in the metal content of the myocardium in ischaemic heart disease, prompting several analytical studies, many of which measured magnesium content, establishing a link between low myocardial magnesium content and death from ischaemic heart disease[52,57,84,85]. Most studies involving analysis of tissue metal ion content have been performed using material obtained at post mortem[53,55,56, 58,85-88]. The mass of tissue available for analysis was rarely a limiting factor in a discussion of the analytical techniques used. One of the main difficulties in assaying ions in biological tissue is to achieve a quantitative aqueous extraction of the ion in question, a problem compounded should there be availability of only a small quantity of tissue for analysis.

Tissue Solubilisation

Traditionally, tissue is obtained from the cadaver heart and the weight of tissue measured (expressed in terms of wet weight of myocardium), unless the tissue is to be dry-ashed before solubilisation, in which case, weight is expressed as dry weight of myocardium. Tissue solubilisation is achieved either by acid digestion of the Kjeldhal type[18,57,84] or by dry-ashing and subsequent suspension in strong nitric or hydrochloric acid.

Acid digestion in Kjeldhal flasks is slow and several volumetric transfers make conservation of tissue difficult. Extraction by glacial acetic acid and trichloroacetic acid described by Sparrow and Johnstone(1964), with or

without tissue homogenisation, is quicker and easier, but this method also requires tissue transfer in several stages[87]. This method has been recently refined by Dorup (1988) and extended to allow quantification of membrane pumps, but requires 10mg of tissue for analysis[89]. A complicated method involving ashing of tissue in a muffle oven at 800^{0}C and extraction in 5M hydrochloric acid was later described by Hunt[90]. These methods are unsuitable when the tissue mass available for analysis is less than 5 mg dry weight.

None of the extraction techniques noted above is ideally suited to the small samples obtained from the in vivo heart with modern biopsy forceps. Recent studies involving the analysis of tissue obtained during in vivo right heart catheterisation have tended to use variations of acid extraction techniques, but, to achieve sufficient sensitivity, several biopsy samples have been pooled together and assayed as a single sample; reproducibility is poor and consequently the quoted standard error is large[88,91,92]. Ventricular biopsy is not without the risk of ventricular perforation and tamponade, the risk increasing with each biopsy obtained; an accurate technique suitable for the analysis of a single biopsy would be of considerable practical advantage.

Subtilisin, a proteolytic enzyme, is used to solubilise meat protein on a industrial scale[93]; there had been some experience in the Analytical Unit of the Department of Cardiological Sciences at St George's Hospital Medical School, London, in using subtilisin to solubilise myocardium for the measurement of drug levels (David W Holt, personal communication). In a search for an alternative method of tissue solubilisation for metal ion analysis, preliminary work was performed using subtilisin to solubilise myocardium for the eventual assay of magnesium ion. Unfortunately, the alkaline conditions required for the optimal efficacy of subtilisin were not compatible with subsequent quantitative analytical techniques (see below). Attention was

drawn to a paper by Jimenez (1978) in which pepsin, another proteolytic enzyme, had been used to solubilise heart valve collagen, a tissue particularly resistant to conventional methods of solubilisation [94]. Pepsin is effective over a wide pH range (pH 1.8-4.4), and is active in a 0.1M solution of acetic acid [95]. Preliminary results with pepsin using cadaver hearts were very encouraging, and led to this study investigating the use of pepsin in a one stage procedure to extract magnesium from myocardial biopsy samples with an average dry weight per biopsy of less than one milligram.

Quantitative Analysis

In common with nearly all the published data, atomic absorption spectrometry (AAS) is used to assess magnesium content quantitatively. This technique is highly specific and very sensitive[96]. It is extremely well suited to the quantification of micromolar concentrations of metal ions [97]. The optimum conditions for the accurate quantification of tissue magnesium by AAS have been extensively investigated [90]. Lanthanum salts (usually lanthanum chloride) are used to suppress interference by phosphate and calcium ions.
It was at this stage of the assay that the use of subtilisin had to be abandoned, since the addition of lanthanum to the alkaline subtilisin-tissue solution resulted in the variable precipitation of magnesium salts. The optimal pH for the physiological action of pepsin is in the range of pH 2-4, a range compatible with the use of lanthanum for atomic absorption spectrometry.

Development of the Pepsin Assay for Clinical Use

The pepsin assay was to be specifically designed to analyse minute myocardial samples obtained by endomyocardial biopsy in vivo. Therefore, to recreate clinical conditions as precisely as possible, samples of right ventricular interventricular septum obtained from fresh post mortem

specimens were used as the study material. In order that the technique might be compared with those reported in the literature, samples of left ventricular muscle were also assayed and the results expressed in terms of both wet weight and dry weight of myocardium.

The details of both the experimental procedure and a comparison of this assay with an established technique of metal ion extraction are presented. The clinical application of the new pepsin method is demonstrated using myocardium obtained serially from two patients by in vivo endomyocardial biopsy.

Materials and Methods

The Pepsin Study

Materials

•Deionised water ($\Omega\infty$) from an Elgastat deioniser (Elga Ltd, High Wycombe, Buckinghamshire, England) was used throughout.

•Analytical grade acetic acid (17.5M), lanthanum chloride (10%w/v) and analytical grade 70% nitric acid were purchased from BDH Chemicals Ltd., Poole, England. Dilutions of 0.1M acetic acid, 0.2M lanthanum chloride and 0.1M nitric acid were made with deionised water.

•Lyophilised pepsin (EC3.4.23.1.) was purchased from Sigma, Poole, England. Pepsin solution (0.1mg/ml) was prepared in 0.1M acetic acid (final pH 3.1). This ensured that adequate pepsin would be present after the addition of 1ml of pepsin solution to biopsy samples up to 10 mg wet weight (requirement: 1mg pepsin per 100mg wet weight of tissue[94]). The pepsin solution contained no detectable magnesium ions.

•Calibrator solution (759 350) from Boehringer-Mannheim GmbH, Mannheim, West Germany was used to prepare magnesium standards for atomic

absorption spectrometry. Standard dilutions of 2.6 μmol/l, 13.2 μmol/l and 20.3 μmol/l were made with 0.2M lanthanum chloride.

Sample collection and storage

Pieces of interventricular septal myocardium (approximately 1 $cm^{3)}$ from 21 patients who had died from various causes were provided by the Department of Pathology, St. George's Hospital. The samples were collected into polyethylene bags and immediately transported to the laboratory. After washing sparingly with deionised water to remove blood clot etc., biopsies were taken using biopsy forceps identical to those available for clinical practice in this hospital (Cordis 7F, Cordis Corporation, Miami, Florida, USA). Areas of macroscopic infarction, fibrosis or haemorrhage were avoided.

Each biopsy was placed on a clean microscope slide and stored in a Falcon tube at -20^{0}C until analysis. Normal left ventricular myocardium (from the left ventricular free wall) was obtained at post mortem from three other cadavers, and similarly stored for analysis.

To demonstrate the clinical application of the assay, a single biopsy from the interventricular septum of each of two patients undergoing in vivo myocardial biopsy were collected onto a microscope slide on four separate occasions using the biopsy forceps described. The biopsies were immediately stored as described above until analysis.

Tissue preparation

The biopsies were transferred to 5ml polystyrene tubes and lyophilised overnight to constant weight, at -50^{0}C and 0.3 kPa (Modulyo, Edwards, Crawley, England). An analytical balance (AE240, readability 0.01mg, Mettler, Greifensee, Switzerland) was used to weigh the biopsy on a tared plastic crucible; the biopsy was then transferred back into its container. To facilitate a

comparison with the literature, additional biopsies from normal left ventricular myocardium were weighed without prior lyophilisation (the results to be expressed in terms of wet weight of myocardium)

Tissue digestion

1 ml of pepsin solution was added to the biopsy sample. After incubation at room temperature (20^{0}C) for 18 hours and subsequent centrifugation at 3500 rpm for 10 minutes at 5^{0}C, 0.5ml of the supernatant was pipetted into a conical polypropylene tube and diluted 1:1 with 0.2M lanthanum chloride. The solution was then recentrifuged prior to magnesium analysis by atomic absorption spectrometry.

Plastic laboratory ware was used throughout to avoid loss of magnesium by absorption onto silica glass[90]. All reusable vessels had been thoroughly cleansed and rinsed twice with deionised water.

To investigate the effect of temperature and incubation time on analytical result, 32 biopsy samples from a single cadaver were incubated in four groups (n=8) at 4, 20, 37 and 50^{0}C for 18 hours. 40 biopsy samples from a further cadaver were similarly incubated at room temperature(20^{0}C) for 2, 6, 12, 18 and 24 hours.

Samples of left ventricular myocardium were analysed with and without prior lyophilisation, to obtain results for comparison with those previously reported.

The Nitric Acid Comparison Study

1 ml of 1M nitric acid was added to the biopsy sample and incubated at room temperature for 24 hours, using a protocol reported by other workers using this method[52,88,91]. Centrifugation, separation and treatment of the supernatant was then performed as for pepsin digestion in preparation for atomic absorption spectrometry.

Since there are no reports in the literature of nitric acid extraction being used in the analysis of biopsies of less than 1 mg dry weight, three groups of biopsies were prepared. Group I: large weight 1-3 mg; (nitric acid extraction); Group II: small weight <1mg; (nitric acid extraction); Group III: small weight <1mg; (pepsin digestion). 24-30 biopsy samples of differing weight were taken from a single cadaver, and analysed in three groups of 8-10 biopsies. This was repeated using a total of four cadaver hearts (A-D).

The results of the three methods of analysis were compared using two-way analysis of variance (ANOVA). The factors in the analysis were cadavers (A-D) and the method of analysis (large sample, nitric acid; small sample, nitric acid; small sample, pepsin). The pairwise comparison between methods was carried out using the method of least significant difference [98].

Atomic Absorption Spectrometry

A Perkin Elmer 1100B atomic absorption spectrophotometer (Perkin Elmer, Beaconsfield, Buckinghamshire, England) was used for magnesium analysis. The quoted linear analytical range for magnesium using this meter is 0.1- 20 μmol/l. The final volumes of pepsin solution and nitric acid added to the biopsy had been calculated such that, after biopsy digestion and necessary dilution with lanthanum chloride, the resultant solution would contain magnesium ions in the optimal concentration range for atomic absorption spectrometry.

The magnesium concentration of the lanthanum-treated supernatant was measured using an air-acetylene flame (Acetylene: 2.0 l/min; Air: 8.0 l/min). Atomic absorption was measured in duplicate at 285.3nm with a spectral band width of 0.7nm. Deionised water was used as the zero reference point, and a three point calibration curve derived from the standards detailed above. After allowing for dilution, myocardial magnesium content was expressed in micromols magnesium per gram myocardium (μmol/g).

Results

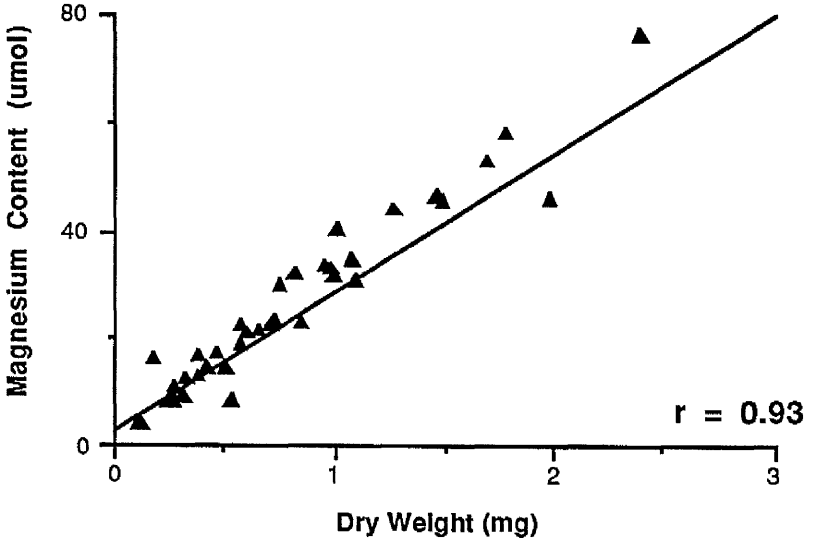
Analysis of Magnesium Content of the Myocardium using the Pepsin Method

The magnesium content of right ventricular septum

The median dry weight of biopsies from the interventricular septum of 21 cadavers with various pathology was 0.74 mg (range 0.11-2.56; n=224). Analyses of the magnesium content of these biopsies yielded a range of 16.7-48.8 (median 30.5) µmol/g dry weight myocardium (mean 30.6; SD 5.2). Myocardial magnesium content correlated linearly with dry weight of biopsy (r=0.96) for a given cadaver. This correlation between the magnesium content and the dry weight of 21 biopsies from a single cadaver is illustrated in Figure 2.1.

Figure 2.1

Correlation of Biopsy Weight and Magnesium Content



The magnesium content of the left ventricular free wall (expressed in terms of wet weight of myocardium)

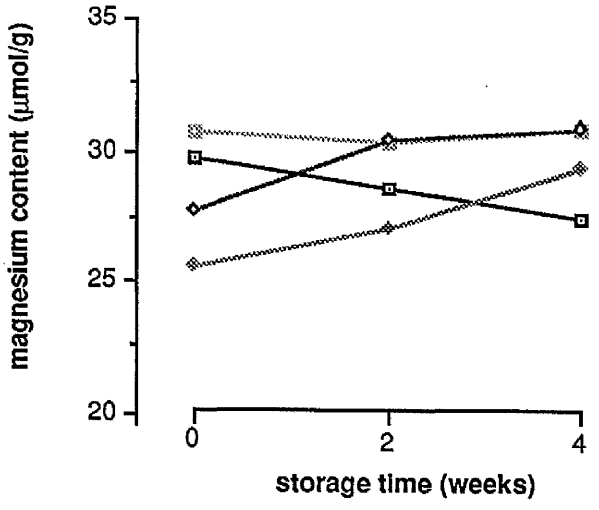
For the purposes of a literature comparison, pepsin digestion studies were performed using left ventricular myocardium from three normal cadaver hearts, before and after lyophilisation, to obtain values for magnesium content expressed in terms of "dry" and "wet" weight. The median wet weight of left ventricular biopsies was 11.0mg (range 8-14; n=10); median dry weight was 0.55 mg (range 0.31-1.02; n=29). The mean magnesium content (SD) of normal left ventricle was 9.7 (1.5) µmol/g wet weight myocardium, and 37.3 (8.2) µmol/g dry weight.

Storage, incubation time and temperature

Biopsies from four samples were assayed at the time of collection, and biopsies from the same samples stored and assayed on three later occasions.

Figure 2.2

Serial Analysis of Stored Biopsies: Lack of Effect of Storage



Storage did not affect analytical result (Figure 2.2). Incubation times from 2-24 hours were found to be equally effective. Incubation temperatures from 20-50^{0}C were equally effective, but an incubation temperature of 4^{0}C was found to be significantly suboptimal (temp 4^{0}C v 20-50^{0}C: 36.6 v 40.3 μmol/g; $p< 0.05$; Fisher's exact test).

Reproducibility

The within-assay coefficient of variation (CV) for the technique was calculated using the results from the simultaneous analysis of 4 or more biopsies from the same cadaver. The median within-assay CV for the analysis of 98 biopsies from 17 cadavers was 8.8% (range 4.5-15.1)

The between-assay CV of the technique was calculated using a total of 90 biopsies from 5 cadavers. 2-10 (median 5) biopsies from each of the five cadavers were assayed on five different days. The median between-assay CV was 9.4% (range 7.9-10.6).

Pepsin digestion - completeness of magnesium extraction

To ascertain the completeness of the initial digestion, 26 of 224 pellets of interventricular septum remaining after post-digestion centrifugation were selected randomly for redigestion. The residue was suspended in 0.5ml of deionised water, washed three times and then collected in a filter paper. The residue was transferred to a clean container and lyophilised as above.

Digestion of the residues and subsequent analysis yielded, on average, less than 1% of the magnesium extracted at the initial digestion. Median extraction at first digestion was 99.3% (range 92.9-100.0).

Comparison of Pepsin Digestion with Nitric Acid Extraction Method

To establish the correlation between magnesium content measured after pepsin digestion and magnesium content measured after a more traditional method of metal ion extraction, a direct comparison of nitric acid extraction and pepsin digestion was made (Table 2.1).

The results of the ANOVA showed that there were very highly significant differences between the methods of analysis ($p<0.001$; Fig 2.3). The yield from the large nitric acid samples was significantly smaller than both the small nitric acid samples ($p<0.05$) and following the use of pepsin ($p<0.01$). Similarly, the yield of pepsin digestion was significantly greater than nitric acid digestion of small samples ($p<0.001$; Figure 2.3).

Table 2.1

A Comparison Between the Extraction of Magnesium from Myocardial Biopsies by Nitric Acid and by Pepsin, for Large and Small Biopsy Size.

analytical sub-group	**MYOCARDIAL MAGNESIUM CONTENT mean (SD) umol/l** SUBJECTS			
	A	**B**	**C**	**D**
GROUP I (nitric acid)	34.5 (6.0) *n=8*	29.4 (0.7) *n=8*	38.2 (3.1) *n=8*	36.7 (1.9) *n=10*
GROUP II (nitric acid)	31.8 (5.6) *n=8*	33.2 (2.1) *n=8*	36.7 (2.0) *n=8*	39.4 (3.6) *n=10*
GROUP III (pepsin)	40.0 (2.3) *n=8*	33.1 (2.0) *n=8*	38.9 (3.2) *n=8*	40.3 (1.7) *n=10*

Figure 2.3

Myocardial Magnesium Analysis:

A Comparison of Pepsin Digestion with Nitric Acid Extraction

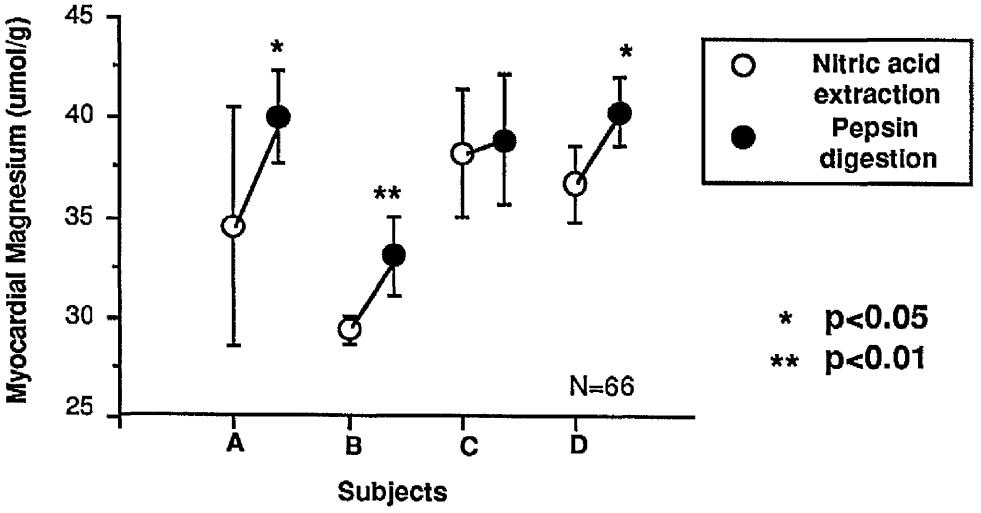
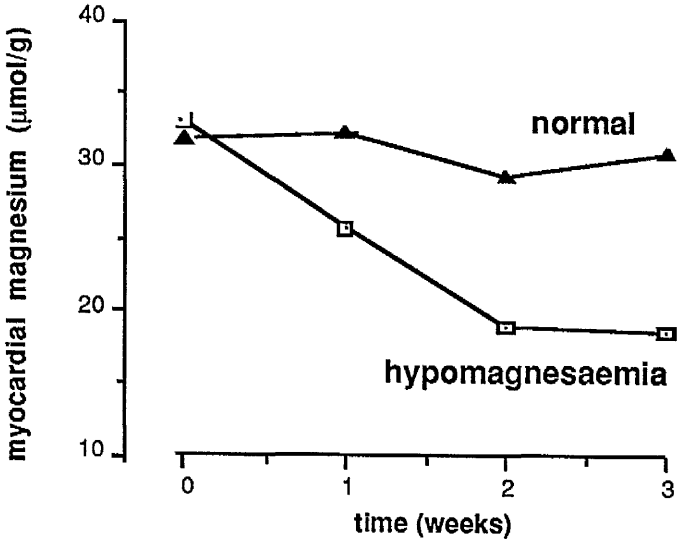


Figure 2.4

Application of the Pepsin Assay in Clinical Practice



The Pepsin Assay in Clinical Practice

To demonstrate the application of the technique in clinical practice, the new method was used to analyse repeated single interventricular septal biopsies (dry weight 0.21-0.97 mg) from two subjects over a period of four weeks. One subject remained normomagnesaemic throughout and the other developed persistent hypomagnesaemia within one week of the first biopsy. The results are illustrated in Figure 2.4.

Discussion

In the present study, a new method was employed for the measurement of tissue magnesium in which tissue solubilisation was achieved utilising a proteolytic enzyme. The study was designed to look specifically at the assay of myocardial magnesium from small biopsy samples using this technique. The assay is rapid, and reproducible over a wide range of laboratory conditions. Poor results obtained at an assay temperature of 4^0C were probably due to the decreased activity of pepsin at a considerably sub-physiological temperature. The technique is equally applicable to fresh and stored specimens. Very small biopsies (<0.5 mg dry weight) have been analysed successfully. It has been demonstrated that the technique can be applied in clinical practice (Figure 2.4). In direct comparison studies, pepsin digestion was superior to nitric acid extraction in the extraction of metal ions from tissue under these circumstances.

These results compare favourably with those reported in the literature, although direct comparisons are difficult. Different anatomical sites within the heart are known to contain differing amounts of magnesium [86]. Most of the reported studies have used left ventricular myocardium or have not stated which anatomical area has been studied. Furthermore, many studies were

performed using samples uncorrected for water content, with the results expressed in terms of wet weight. Table 2.2 shows the mean results of pepsin digestion of "wet" left ventricular myocardium, in comparison with those reported by other workers using similar tissue and variations of acid extraction to solubilise the tissue. 95% confidence intervals of these mean values (Figure 2.5) indicate that the pepsin enzymatic method yielded significantly more magnesium than in two of the other studies and is not significantly different from the third[53,55,58]. (This statistic cannot be calculated for the Valiathan study [88]).

Other direct comparisons are not possible from the literature, but is possible to make indirect comparisons. Speich analysed biopsy samples from right and left ventricular myocardium; the method used produced results in μmol magnesium per gram of tissue protein[56]. The mean (SD) ratio between right and left ventricular myocardial magnesium content in the Speich study was 0.90 (±0.26); this study using pepsin digestion produced a ratio of 0.89 (±0.20).

Previous studies have not quoted the coefficient of variation for the assay used. However, despite the use of substantially larger tissue samples, the calculated CVs of the studies quoted in Table 2.2 would be similar to those reported for the new technique (utilising much smaller tissue samples).

Lyophilisation to obtain constant dry weight has not been previously described in this circumstance, but has been found to be effective in this study. The specimens are easy to handle, and lyophilisation is a clean, convenient alternative to the muffle oven or drying chamber.

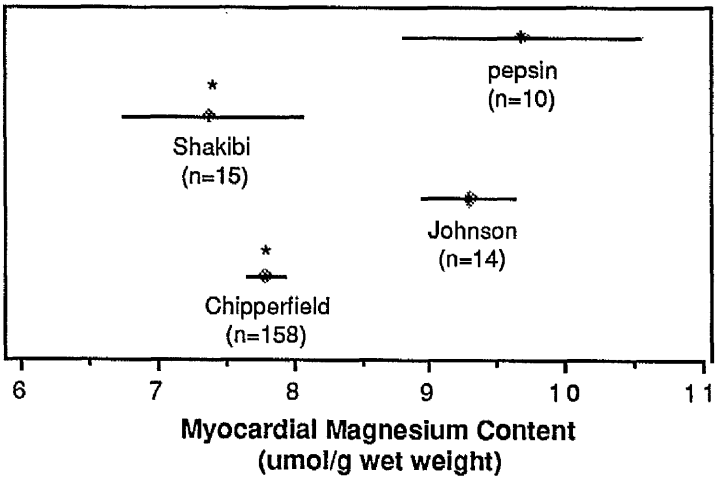
Table 2.2

Comparison of Results from the Pepsin Study with Published Literature of Analysis of the Magnesium Content of Left Ventricular Myocardium (results expressed in terms of wet weight of myocardium)

STUDY (year)	METHOD	Mg CONTENT umol/g wet weight (1SD)
Chipperfield [58] (1978)	dry ashing and acid extraction	7.8 (1.0)
Johnson [53] (1979)	acetic acid extraction	9.3 (0.7)
Shakibi [55] (1981)	dry ashing and acid extraction	7.4 (1.4)
Valiathan [88] (1986)	acid extraction	6.3 (n/a)
Pepsin study (1991)	enzyme digestion	9.7 (1.5)

Figure 2.5

95% Confidence Intervals for the Measurement of Mean Myocardial Magnesium Content of the Left Ventricle: Comparison of the Pepsin Digestion Method with Published Reports (myocardial magnesium is expressed in terms of wet weight of myocardium)



Conclusions

None of the methods of metal ion extraction from tissue quoted in the literature has addressed the accuracy of assaying very small tissue samples resulting from in vivo myocardial biopsy in current clinical practice. In this new assay, it has been shown that pepsin digestion is superior to nitric acid extraction in this circumstance. Although this study has been limited to magnesium assay in the myocardium, it is probable that the technique could be adapted for the measurement of other metal ions in a variety of tissues.

Appendix

Appendix A describes the use of the Pepsin assay to produce a normal range of myocardial magnesium content; the normal range obtained was used in a serial study of myocardial magnesium content in the transplanted heart described in Chapter Three.

Appendix B details the modification of the assay for the measurement of myocardial calcium, the application of which is the subject of Chapter Five.

CHAPTER THREE

THE MAGNESIUM CONTENT OF THE TRANSPLANTED HUMAN HEART IN NORMO- AND HYPO-MAGNESAEMIA: AN IN VIVO, LONGITUDINAL STUDY

Introduction

Iatrogenic hypomagnesaemia secondary to renal magnesium wasting is associated with the use of several nephrotoxic agents (see Chapter One). The effect of the resultant magnesium depletion on tissue magnesium is not known, and in particular, the longterm relationship between serum and myocardial magnesium content has not been described.

In animal models magnesium deficiency produces a rapidly progressive cardiomyopathy secondary to calcium overload, with mitochondrial calcification[12,14,99]; similar changes occur in ischaemia in man[100]. Accelerated vasculopathy limits the survival of many transplant recipients, and exhibits some of the histological features of magnesium deficiency cardiomyopathy.

In man, myocardial magnesium depletion is aetiologically linked with sudden cardiac death[58,101], is associated with arrhythmia following cardiac surgery[75], and exacerbates arrhythmia secondary to digoxin toxicity[102].

Cardiac transplant recipients are at high risk of drug-induced hypomagnesaemia; loop diuretics are used extensively pre-transplantation, and are frequently required post-operatively, and cyclosporin, used as an immunosuppressive agent, is used in relatively high doses (10mg/kg/day) in the early period post-transplantation.

In view of potential adverse effects of magnesium imbalance in this previously uninvestigated high risk group, a prospective, observational study to

investigate the incidence of hypomagnesaemia post-transplantation, and its possible effect on myocardial magnesium content, was performed. As secondary aims, the clinical sequelae of magnesium depletion were sought, and the possible contribution of loop diuretics and cyclosporin A to any observed disturbance of magnesium balance investigated.

Methods

All patients entering the cardiac transplantation programme in St George's Hospital, London, between January and October 1990, and surviving at least one month post-transplantation were eligible for the study. All patients received standard immunosuppressive therapy with cyclosporin A, azathioprine, and steroid taper over twelve weeks. Endomyocardial biopsy was performed weekly for two months post-transplantation, twice-monthly for a further month, and at six and nine months, in accordance with an established protocol for the detection of rejection. A symptom review and a careful drug history were taken at each biopsy visit, including direct questioning about the use of proprietary medication. Serum magnesium, potassium and creatinine were measured pre-transplantation and at the time of each subsequent endomyocardial biopsy. Serum magnesium was measured by a colorimetric method previously validated against atomic absorption spectroscopy. Serum potassium and creatinine were assayed using standard automated techniques.

Myocardial magnesium was measured from a single myocardial biopsy obtained at each histological assessment of rejection, and analysed using the pepsin micromethod previously described (see Chapter Two). The lower limit of normal of myocardial magnesium content was defined as 30.5 μmol/g dry weight of myocardium; this figure is derived from age-matched cadaver control

hearts analysed using the same technique (see Appendix 1). Four additional biopsies were taken from each patient on each occasion and submitted for histological assessment of myocardial muscle content.

Blood cyclosporin was measured at trough level (pre-dose), analysed using the Cyclo-Trac SP-whole blood radioimmunoassay (Incstar Corporation, Stillwater, Minnesota). In addition, a profile of blood cyclosporin levels was performed during the first month post-transplantation, constructed from multiple sampling over a 24 hour period; the highest recorded value was designated "peak cyclosporin level".

Statistical analyses were performed using a two-tailed paired student t-test for within-patient indices, appropriate regression analysis, and ANOVA for multivariate group analysis[98].

Results

Demography

Of twenty-five consecutive patients transplanted within the study period, nineteen were eligible for the study. Subsequent follow-up was complete to nine months in 18/19 patients. Mean age was 50.4 (range 43-61) years. Transplantation was performed for intractable ventricular tachycardia in one patient and end stage heart failure in eighteen patients (ischaemic heart disease:14, dilated cardiomyopathy:4).

Serum Magnesium

Serum magnesium was normal at the time of transplantation in 15/19 patients (79%); by three months, all 19 patients exhibited hypomagnesaemia (less than 0.7mmol/l) on a least one occasion. The prevalence of hypomagnesaemia over the study period is illustrated in Figure 3.1(a). Prolonged hypomagnesaemia (persisting for more than six weeks) occurred in

all but one patient. Serum magnesium was severely depressed (less than 0.6 mmol/l) in 13 patients (68%) within the study period, persisting for up to six weeks in 4 patients. Mean serum magnesium at baseline, three, six and nine months post-transplantation is shown in Figure 3.2(a). There was a pronounced fall in mean serum magnesium to hypomagnesaemic levels in the first three months post-transplantation (mean (SD): 0.80 (0.16) v 0.64 (0.07) mmol/l; $p<0.002$), which had only partially recovered at six months (0.70 (0.06); $p<0.01$), despite a significant rise in serum magnesium between three and six months ($p<0.05$). By nine months, mean serum magnesium was within normal limits, but was still significantly depressed over baseline at 0.72 (0.06) mmol/l ($p<0.04$). Minimum mean serum levels occurred at three months.

Myocardial Magnesium

The magnesium content of the donor heart was normal in 17/19 patients (89%). By six months, magnesium depletion had occurred in 18/19 patients (94%). Figure 3.1(b) illustrates the prevalence of myocardial magnesium depletion during the study period. Persistent myocardial depletion occurred in 10/19 patients (53%). Myocardial magnesium content failed to rise in two donor hearts deplete at transplantation (as assessed from the biopsy taken seven days post-transplantation). Overall, the changes in myocardial magnesium were more variable than those in serum, as seen in Figure 3.2(b). Mean myocardial magnesium content fell significantly over the first three months (mean(SD): baseline v 3 months: 33.6 (4.3) v 30.6 (4.3) µmol/g; $p=0.018$). In the 17 patients with normal myocardial magnesium content at transplantation, mean levels were still significantly depressed over baseline at six months (30.1 (5.9); $p=0.04$). Prolonged myocardial magnesium depletion (over six weeks) occurred in 9 patients (50%). In those patients with normal

serum and myocardial magnesium levels at baseline, minimum mean myocardial levels occurred at six months.

Inter-relationship between serum and myocardial magnesium

Little relationship existed between serum and myocardial magnesium levels sampled simultaneously. The within-patient change in serum and myocardial magnesium over time is exemplified in Figure 3.3(i-iii) in which the two variables are plotted against time in weeks. Data from three patients are presented as examples. (i) Normomagnesaemia and normal myocardial magnesium content at baseline (15/19 patients); (ii) Myocardial magnesium depletion at baseline (2 patients); (iii) Late development of hypomagnesaemia with early preservation of myocardial levels (2 patients). It can be seen that, in general, changes in serum magnesium are mirrored two to six weeks later by similar alterations in myocardial magnesium content.

Mild hypomagnesaemia (0.65-0.69 mmol/l) induced mild myocardial depletion (28-30 μmol/g) in 7/8 patients; in only one patient were myocardial magnesium levels normal throughout the study. More significant hypomagnesaemia was followed by greater, more prolonged myocardial depletion in 8/9 patients, with only mild depletion occurring in the ninth patient. Severe myocardial magnesium depletion present in two patients at transplantation, did not resolve until serum levels had been persistently normal for nine and twelve weeks respectively.

Figure 3.1

Prevalence of (a) Hypomagnesaemia and (b) Myocardial Magnesium Depletion in the First Nine Months after Cardiac Transplantation

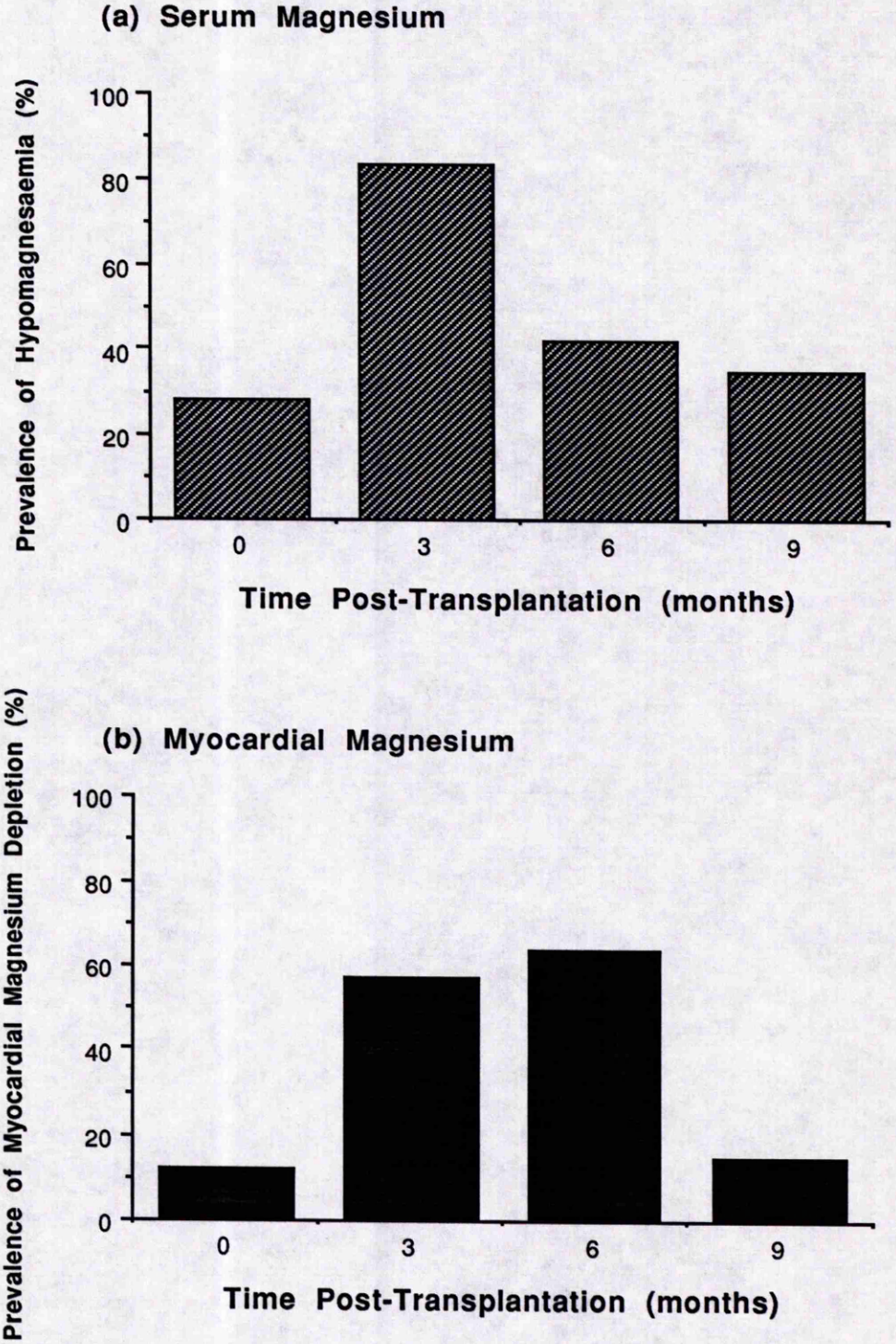


Fig 3.2 (a)

Mean Serum Magnesium
at Baseline, Three, Six and Nine Months Post-Transplantation

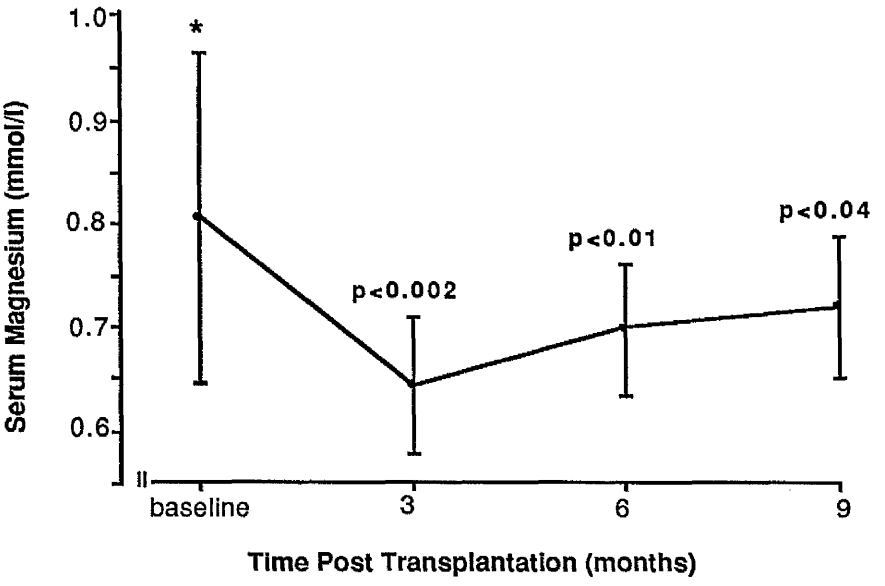


Figure 3.2 (b)

Mean myocardial magnesium
at Baseline, Three, Six and Nine Months Post-Transplantation

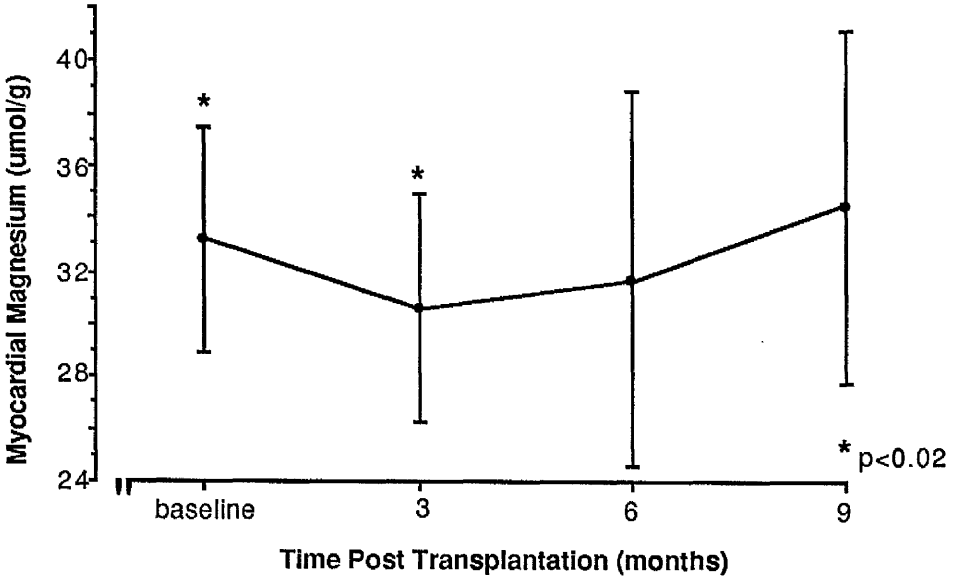
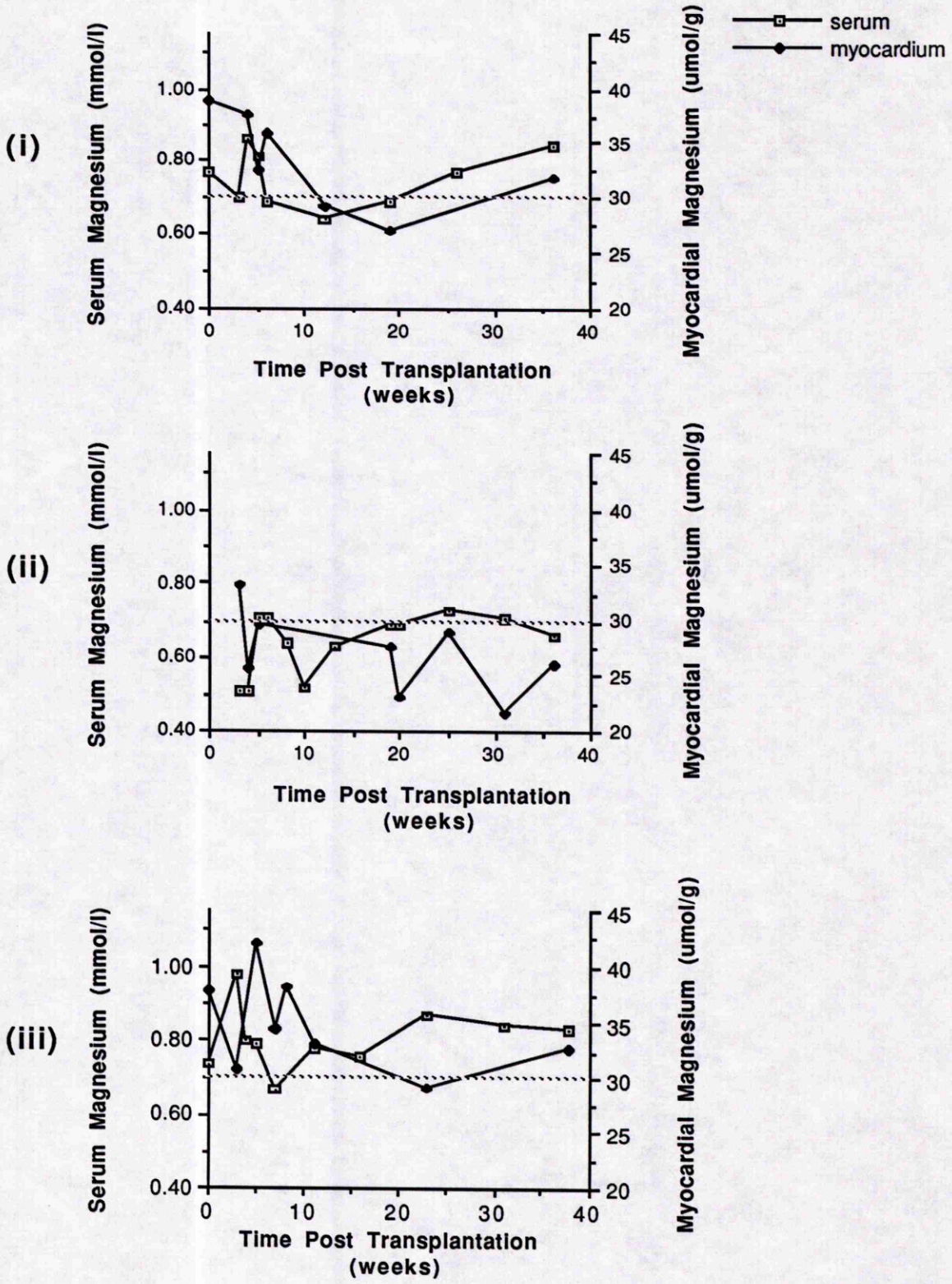


Fig 3.3 (i-iii)

Within-patient Variation of Serum and Myocardial Magnesium



Other Biochemical Indices.

Severe renal impairment was not seen in 18/19 patients (maximum serum creatinine 240 μmol/l). The remaining patient died at sixty-four days having developed acute rejection and renal failure requiring haemodialysis. No significant changes in serum creatinine were seen over the study period. Mean serum potassium was 4.7 mmol/l pre-transplant, 4.3 mmol/l at three months (baseline v 3 months; $p<0.05$) and 4.5 mmol/l at six months (p=ns).

Diuretics

Pre-transplant therapy included loop diuretics in 18/19 patients. Post-transplant, diuretics were continued beyond one week after operation in 10/18 patients. There was no significant difference at three or six months between the diuretic-treated group and the untreated group, in serum magnesium, myocardial magnesium, serum potassium or serum creatinine (Table 3.1).

Table 3.1

Effect of Concurrent Diuretic Treatment on Serum and Myocardial Magnesium

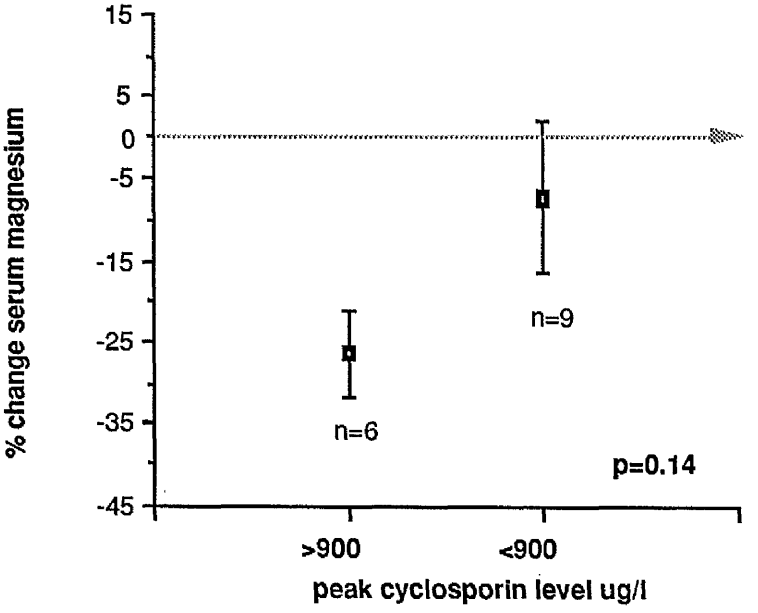
	FRUSEMIDE n=10	NO FRUSEMIDE n=8	p value
serum Magnesium (mmol/l)	0.65	0.63	ns
myocardial Magnesium (umol/g)	30.2	29.3	ns
serum Potassium (mmol/l)	4.3	4.1	ns
serum Creatinine (umol/l)	135	126	ns

Cyclosporin

Mean trough cyclosporin level correlated with neither serum nor myocardial magnesium level within the study period, nor with the percentage change from baseline of either variable. There was similarly little direct relationship with

peak cyclosporin values (available in 15 patients). Patients were divided into two groups; peak cyclosporin greater than 900µg/l (A; n=6) and less than 900 µg/l (B; n=9). There was a strong trend toward a greater fall in serum magnesium over the three month high dose period in patients with higher peak cyclosporin levels (Figure 3.4). A much weaker trend was evident for myocardial magnesium ($p<0.25$).

Figure 3.4
Relationship between Peak Cyclosporin Level at One Month and Serum Magnesium at Three Months



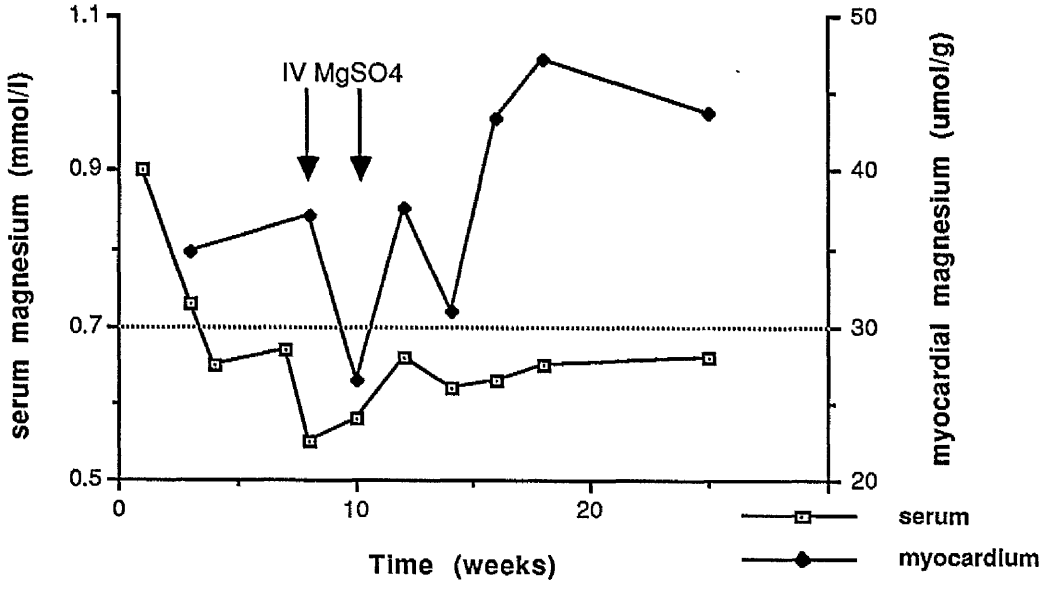
Other Drugs

No patient received prescribed intravenous or oral magnesium therapy (except for the single patient detailed below), and antacid use was limited to magnesium-free preparations. The use of corticosteroids, either longterm or in high doses during rejection episodes, was not related to magnesium status. Azathioprine, aspirin, nifedipine and amiloride were similarly unrelated.

Clinical Events

Two patients complained of tremor within three months of transplantation. In one patient the tremor was such that he was unable to hold a cup; serum magnesium was 0.51 mmol/l with therapeutic blood cyclosporin levels. The tremor was abolished immediately following an intravenous bolus of magnesium sulphate (8mmol), and was followed by an infusion of 48 mmol over twenty four hours. The subsequent course is illustrated in Figure 3.5; the arrows indicate this and another episode requiring intravenous magnesium. Attention is drawn to the delayed timing of the change in myocardial magnesium as relative to changes in serum magnesium.

Figure 3.5
Serum and Myocardial Magnesium in a Patient
with Symptomatic Hypomagnesaemia



The second patient had a complicated polyneuropathy and tremor. Serum magnesium was 0.61 mmol/l. In view of the experience with the first patient, a

trial of intravenous magnesium was given. No symptomatic benefit was conferred. The neurological signs were subsequently demonstrated to be due to aminoglycoside toxicity.

The incidence of symptomatic arrhythmia was very low in this population. One patient developed exercise-induced atrial flutter that did not require treatment soon after transplantation. Moderate ventricular ectopic activity was present on 24 hour ECG monitoring; no ventricular tachycardia was observed. This occurred in the patient described above with recurrent tremor secondary to hypomagnesaemia; myocardial magnesium levels are depicted in Figure 3.4. A normal exercise test and 24 hour ECG recording were completed at nine months, at which time serum and myocardial magnesium were within normal limits.

Discussion

It has been demonstrated that hypomagnesaemia is very common after cardiac transplantation managed with current immunosuppressive regimens and that subsequent myocardial magnesium depletion is almost invariable. Serum and tissue magnesium fell over the first few months post-transplantation, serum levels reaching a nadir at about three months, with myocardial content being lowest at six months. Serum magnesium then rose, followed by a later, but more rapid rise in myocardial levels. In the majority of cases, the degree of hypomagnesaemia was matched by a similar depletion of myocardial magnesium. Significant myocardial magnesium depletion did not develop when serum levels were maintained within normal limits. The cause of magnesium depletion would appear to relate to treatment with cyclosporin A; this suggestion is explored in more detail in the following chapter.

Simultaneous measurement of serum and tissue magnesium are well known to correlate poorly, and our findings are in keeping with this observation[15,83]. Such a relationship between the two variables would be expected physiologically, but has remained elusive in the absence of previous longitudinal studies. The data presented suggest that such a relationship does exist - at least in respect of denervated myocardium. Serum magnesium returned to normal well before tissue levels were seen to rise. This may explain the curious discrepancy noted in the literature between measured serum and tissue magnesium content. It is probable that the magnesium depletion evident in the cardiac transplant recipients represents loss of myocyte magnesium stores (previously complexed to phosphates), rather than loss of metabolically active magnesium ion as occurs in the sympathetic response to ischaemia[80].

Clinical effects

Symptomatic cardiac arrhythmia is rare post-transplantation [103,104], and a correspondingly low incidence in this study was noted (albeit only observational by design), despite profound and persistent hypomagnesaemia. Throughout the literature, pluricausal hypomagnesaemia is associated with a resistant hypokalaemia[20,71], and although a parallel relationship with a statistically significant fall in serum potassium occurring during the time of rapid magnesium flux has been demonstrated in the transplant group, the fall was not clinically significant and hypokalaemia did not occur. Literature reports of hypomagnesaemia-induced tachyarrhythmia in the absence of recent hypokalaemia are lacking and arrhythmia unequivocally due purely to hypomagnesaemia has not been reported.

Neurological symptoms were prominent in two patients, one of whom responded dramatically to intravenous magnesium. The neurological effects of

magnesium depletion were by far the most prominent in a report of hypomagnesaemia in bone marrow transplant recipients treated with cyclosporin A; no cardiac effects were reported[31]. Given the frequency of hypomagnesaemia noted in this study, and the fact that the symptoms of cyclosporin neurotoxicity are very similar to those of hypomagnesaemia[105,106], it is not improbable that the cause of neurological symptomatology is sometimes misdiagnosed, since serum magnesium is not usually routinely measured in these patients[107].

Limitations of the study

Serial measurement of myocardial electrolyte content has not been previously reported. Methods of tissue analysis described in the literature are cumbersome and designed for use with large samples[52,53,56,87,90]; it was necessary to develop a new technique specifically developed for the assay of the tiny tissue fragments produced by current in vivo myocardial biopsy practice (Chapter 2). Inaccuracies in such measurement are limited by the assay design. Results outside clearly pre-defined limits are discounted, based on a biphasic distribution curve and normal limits obtained from cadaver studies involving the assay of over 400 biopsies (Appendix A), thus addressing the difficult problem of the 'fibrous' biopsy (usually obtained after inadvertent sampling of an old biopsy site); there is an excellent correlation with histological evaluation using this technique (Appendix A). The varying patterns of serum and myocardial magnesium content seen in Figure 3.3, suggest that the trends demonstrated are real and that the techniques used are appropriately sensitive. Furthermore, despite the well-documented diminution in biopsy quality with time post-transplantation[108], the initial fall in myocardial magnesium content was followed by a *rise* over the latter six to nine month period of the study.

Potential long term cardiac effects

In animal models, prolonged magnesium depletion leads to a rapidly progressive cardiomyopathy characterised by intracellular calcium deposition and mitochondrial disarray[9,11,65]. The inter-relationship between magnesium and calcium has been the subject of much debate[43]. Several post mortem studies have demonstrated a reciprocal relationship between myocardial magnesium and myocardial calcium levels[56,58,109] and animal studies have demonstrated the accumulation of significant calcium deposits within the myocytes of magnesium-deplete hearts[12]. Myocardial magnesium content is lower in subjects dying of ischaemic heart disease when compared to subjects dying from non-cardiac causes - a discovery prompted by the observation that death from IHD is commoner in areas supplied by soft water, than that in corresponding hard water areas[18]. Myocardial potassium content is also lower, secondary to the renal effects of diuretic therapy on potassium handling - effects which are greatly exacerbated by abnormalities of magnesium balance[110,111]. One study has linked sudden cardiac death with myocardial magnesium depletion, noting that myocardial magnesium content in patients with chronic stable angina dying from non-cardiac causes did not differ from controls[53]. Should persistent hypomagnesaemia result in myocardial magnesium depletion in the native innervated heart, as has been demonstrated to occur in the transplanted denervated heart, such depletion may contribute to the high incidence of arrhythmic events observed in patients with class III-IV heart failure, in whom hypomagnesaemia and hypokalaemia are common; hypomagnesaemia has been suggested as an independent risk factor for mortality in this group[112].

Accelerated coronary disease with or without hyperchoiesterolaemia is increasingly recognized in the "ageing" transplant population. Hypomagnesaemia is independently associated with adverse changes in

serum lipids[113], and whilst the mechanism of accelerated graft atherosclerosis is thought to be due, at least in part, to a chronic rejection process, it is interesting to note the similar obliterative arteriopathy that complicates the cardiomyopathy of myocardial magnesium depletion in animal models[114]. The question of calcium-induced myocyte injury secondary to magnesium depletion and calcium overload is explored more fully in Chapter Five.

Conclusions

Hypomagnesaemia occurs frequently in cardiac transplant recipients, commonly accompanied by depletion of myocardial magnesium. The early clinical effects of such depletion are mild and treatable; the long term sequelae are unknown. The cause of magnesium depletion is independent of diuretic use, but appears to be related to treatment with cyclosporin A. In the transplanted heart, magnesium deficiency may exacerbate accelerated coronary disease, and, if the findings of this study extrapolate to the native heart, the effects of magnesium deficiency on potassium balance may adversely influence the arrhythmic profile of the ischaemic myocardium.

CHAPTER FOUR

CYCLOSPORIN AND RENAL MAGNESIUM LOSS: THE CAUSE OF MAGNESIUM DEPLETION POST CARDIAC TRANSPLANTATION?

Introduction

The use of frusemide induces hypomagnesaemia by increasing sodium delivery to the loop of Henle [110]. In the study described in the previous chapter, only 21% of patients were hypomagnesaemic at the time of transplantation despite treatment with large doses of loop diuretics. Concomitant use of potassium-sparing drugs such as amiloride and angiotensin-converting enzyme inhibitors possibly compensate for the expected magnesium loss[110]. Within a few weeks of transplantation 100% of patients were hypomagnesaemic regardless of diuretic usage, suggesting that frusemide alone cannot be the mechanism of magnesium loss . Furthermore, no difference in magnesium status was detected between diuretic-treated and untreated patients (Table 4). In contrast to most previous reports of hypomagnesaemia, in this study serum potassium was maintained within normal limits despite profound falls in serum magnesium. This suggests a separate mechanism of magnesium imbalance to be the basis of the magnesium depletion observed in the transplant population.

That this may be related to the effect of cyclosporin A, is demonstrated in a relationship between peak cyclosporin levels occurring early post-transplantation and the subsequent fall in serum magnesium seen in the Transplant Study (Chapter Three - Figure 3.4). Although it is generally accepted that the trough (pre-dose) cyclosporin level is the more sensitive indicator of cyclosporin toxicity, and that careful manipulation of such levels

reduces the incidence and severity of toxicity[115], no correlation of magnesium with trough levels was demonstrated in the Transplant study. Recently, peak levels measured early post-transplantation have been shown to better relate to later renal function[116]; and these data are in accordance with this observation. It is known that cyclosporin selectively affects the tubular cells of the thick ascending loop of Henle to produce its toxic, magnesuric effect, although the precise mechanism is unclear[117]. It is possible to conclude that high *peak* levels of cyclosporin are responsible for at least one of the toxic renal effects of cyclosporin. It is interesting to note that hypomagnesaemia also occurs secondary to aminoglycoside renal toxicity, the avoidance of which requires monitoring of both trough *and* peak levels.

To clarify the extent of renal magnesium loss induced by cyclosporin A, an unselected group of transplant recipients were followed prospectively with serial analyses of serum and urinary magnesium.

Patients and Methods

Urinary magnesium excretion was assessed in 25 transplant recipients on a total of 47 occasions (range 1-5 per patient; median 1) over a median follow-up of 22 weeks (range 2-76). Urinary magnesium excretion was calculated from 24 hour urine samples collected into acidified containers and assayed by atomic absorption spectrometry. Serum magnesium was measured concurrently by standard colorImetric techniques. Renal function was assessed by calculation of creatinine clearance.

Cyclosporin A was prescribed to each recipient to maintain blood cyclosporin levels within previously defined limits in accordance with an established protocol. 0 to 6 weeks post-transplantation: 300-350 µg/l; 6 weeks to 6 months: 200-250 µg/l. 6 months to 1 year 150-200 µg/l. Peak blood cyclosporin levels

were established from the cyclosporin profiles analysed within one month of transplantation, assayed as described previously (Chapter Three).

In a small sub-group of patients for whom cyclosporin profile data were available retrospectively, serum magnesium was assayed at one year.

Results

Urinary excretion of magnesium and serum magnesium

Figure 4.1 depicts the range of urinary magnesium excretion as assessed in mmol/24 hours as a function of the serum magnesium measured concurrently; the vertical dotted line marks the lower limit of normal of serum magnesium. The renal excretion of magnesium in normo- and hypomagnesaemia is depicted in Figure 4.2(a) and (b); the dotted line signifies the upper limit of normal for renal magnesium excretion when hypomagnesaemia is present. Significant excretion of magnesium (up to 6 mmol/24 hours) occurred even in the presence of marked hypomagnesaemia.

Figure 4.1
Urinary Magnesium Excretion and Serum Magnesium

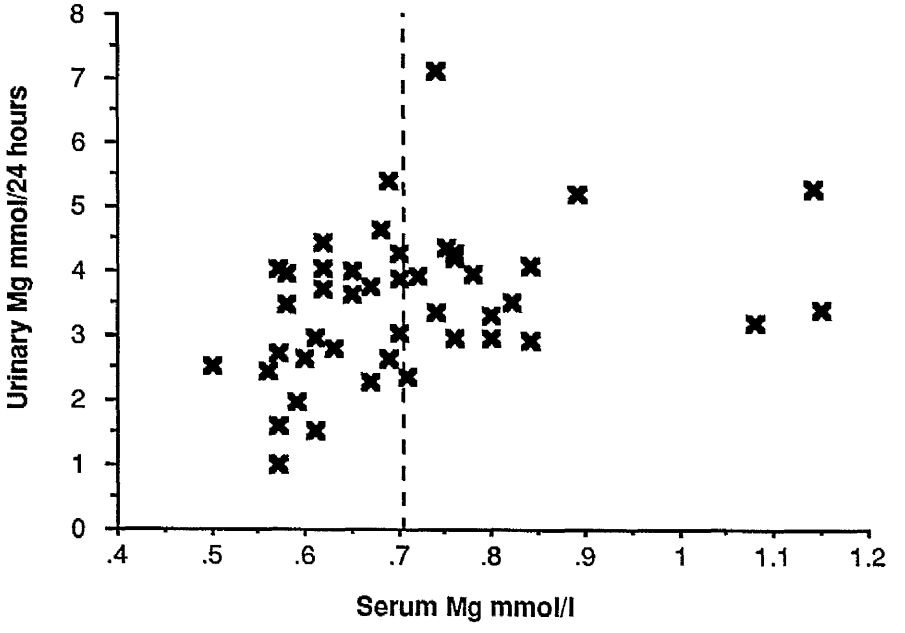
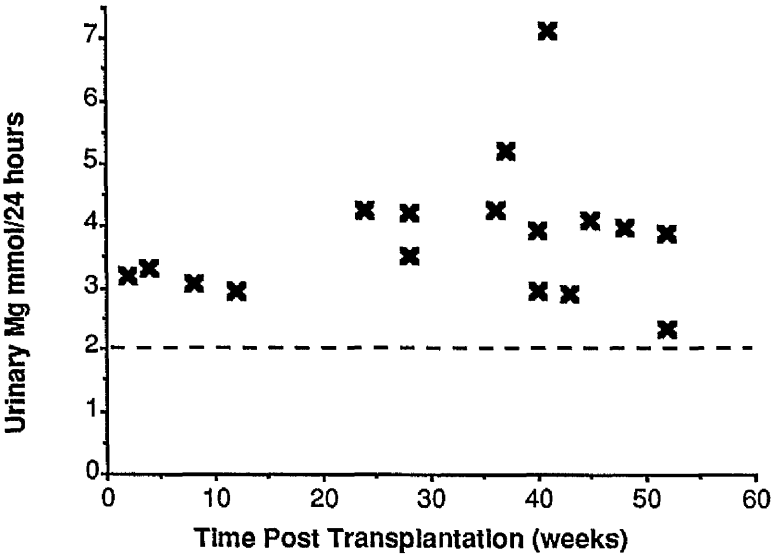
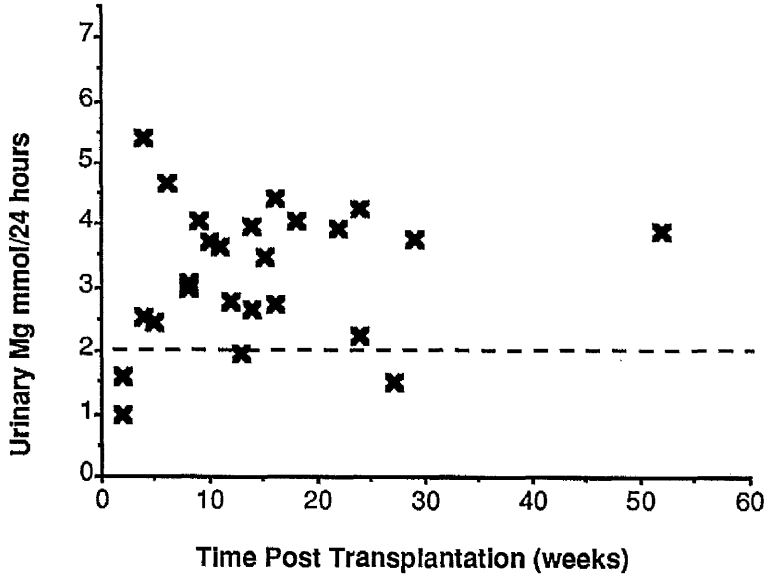


Figure 4.2
Renal Excretion of Magnesium in (a) Normomagnesaemia and (b) Hypomagnesaemia

(a) Normomagnesaemia



(b) Hypomagnesaemia

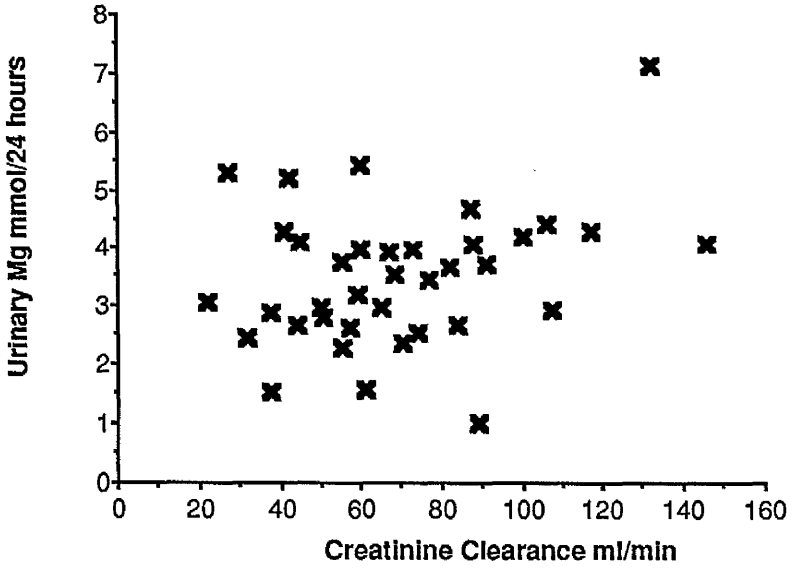


Urinary magnesium and renal function

Magnesium excretion was independent of renal function (assessed by creatinine clearance) measured at the time of magnesium analysis as shown in Figure 4.3. The use of diuretics and renal magnesium excretion was not correlated; the lack of correlation of serum magnesium and diuretic usage was discussed in Chapter Three (Figure 3.4).

Figure 4.3

Urinary Magnesium Excretion and Renal Function



Cyclosporin and urinary magnesium excretion

Figure 4.4 demonstrates the relationship between high peak cyclosporin levels and magnesium excretion as a function of serum magnesium. The normal renal response to hypomagnesaemia is almost complete cessation of renal magnesium loss, and certainly magnesium excretion should fall to below 2 mmol/24 hours. It can be seen from Figure 4.4 that in the majority of cases, urinary magnesium excretion is inappropriately high, particularly in those patients with high peak cyclosporin levels.

Figure 4.4

Peak Cyclosporin and Urinary Magnesium Excretion

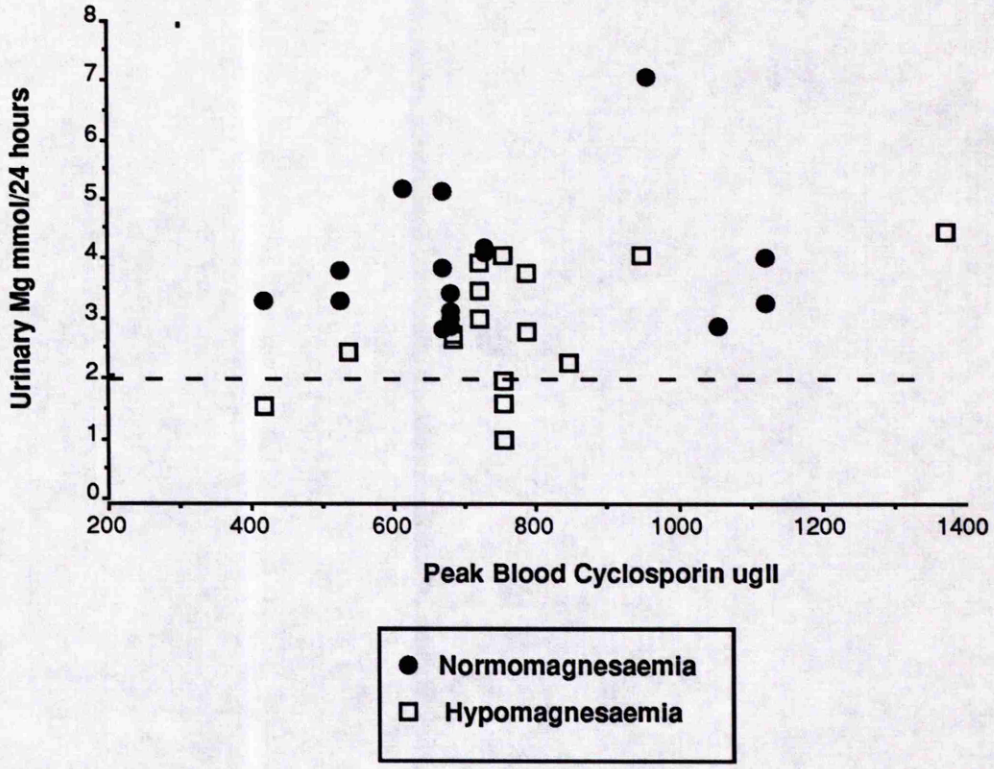


Figure 4.5

Peak Cyclosporin Level Early Post-Transplantation
and Serum Magnesium at One Year

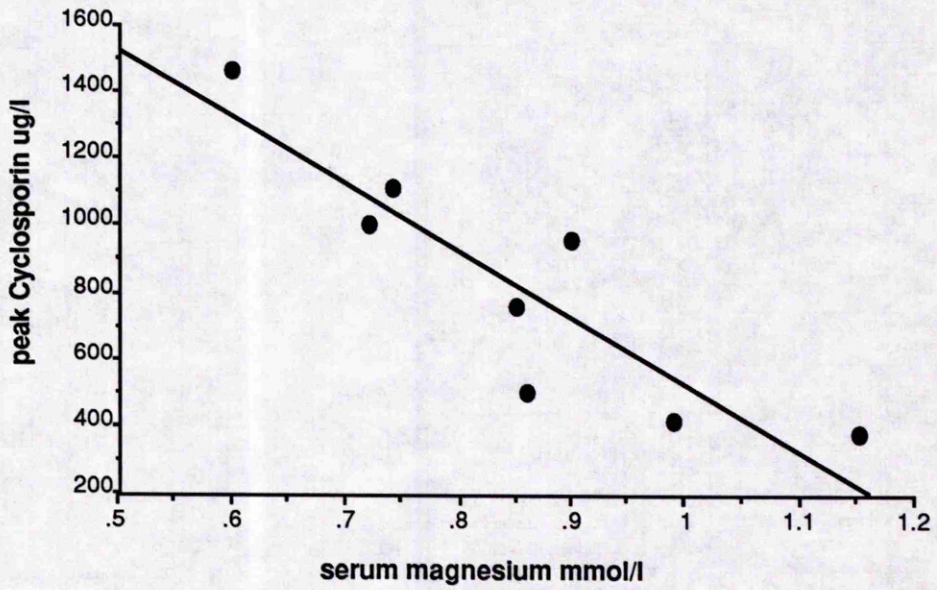


Figure 4.5 depicts a negative correlation of high peak cyclosporin levels early in the post-transplant period with serum magnesium at one year, suggesting that renal magnesium wasting may not be entirely reversible.

Discussion

The normal kidney will avidly retain magnesium in states of magnesium deficiency, reducing excretion to almost zero[17,118,119]. In this study, daily excretion of magnesium was 1-6 mmol despite significant hypomagnesaemia, suggesting that the normal control mechanisms were significantly impaired in the transplant recipients, even in patients with apparently normal renal function. There is still a trend toward renal magnesium conservation in the magnesium deplete patients, but the whole system appears to have been "downregulated" (Figure 4.1) i.e. the renal mechanism is acting as if "normomagnesaemia" has been re-defined at a lower level of about 0.5 mmol/l.

The temporal relationship of hypomagnesaemia with highest cyclosporin doses (i.e around the third month post-transplantation) together with the correlation of early peak cyclosporin levels, renal excretion of magnesium and subsequent serum magnesium, strongly implicate cyclosporin A as the major cause of post-transplantation hypomagnesaemia.

There are several other studies that have reported similar findings of renal magnesium wasting despite persistent hypomagnesaemia; cisplatinum and gentamicin are frequently associated with such a syndrome[21,22,120,121]. In renal transplant recipients, an increase in fractional, but not total, renal magnesium excretion has been noted[122]; the average daily dose of cyclosporin A after renal transplantation is considerably lower than that after cardiac transplantation (3 mg/Kg versus 10 mg/Kg).

Adverse effects of the combination of hypomagnesaemia and cyclosporin are also reported. Magnesium deficiency has been shown to exacerbate renal arteriolar constriction secondary to cyclosporin[115], whilst the administration of magnesium appears to protect against such generalised vasoconstriction [123-125]. Although magnesium is generally ineffective in the treatment of essential hypertension[126,127], it is possible that hypomagnesaemia may contribute toward the cyclosporin-induced hypertension that occurs in a significant minority of transplant recipients. In a point that will be taken up in Chapter Five, cyclosporin A has been reported to induce myocardial and mitochondrial calcification in mice, a finding that may relate to unrecognised magnesium depletion [30]. Hypomagnesaemia is also thought to contribute to cyclosporin-induced neurotoxicity[31].

Conclusions

Magnesuria persists despite significant hypomagnesaemia in cardiac transplant recipients treated with cyclosporin A, an effect independent of renal function or diuretic use. The evidence strongly implicates cyclosporin as the major cause of magnesium depletion post cardiac transplantation. Hypomagnesaemia potentiates cyclosporin neurotoxicity, and may potentiate some of the serious adverse haemodynamic effects of cyclosporin therapy.

CHAPTER FIVE

THE EFFECT OF MAGNESIUM DEPLETION ON THE CALCIUM CONTENT OF THE MYOCARDIUM

Introduction

The control of intracellular calcium ion concentration is largely effected by magnesium as (i)intracellular free magnesium ion, (ii) by action on membrane calcium channels and (iii) by interaction with intracellular sodium and hydrogen ion [40]. Non-ionised calcium is found bound to intracellular organelles and in precipitate form as calcium phosphate.

The inotropic effect of calcium ion within the myocardium is well recognized and until relatively recently was widely exploited during cardiopulmonary resuscitation[128]. However, the adverse effects of calcium "overload" are now equally recognized, and the use of calcium antagonists as anti-ischaemic agents is under investigation [59,63,129-131].

Calcium deposition within mitochondria occurs during ischaemia with adverse effects on mitochondrial function, precipitating cell death[65,Chapter 1]. Magnesium depletion is also associated with mitochondrial calcium deposition, with calcification occurring in a very similar manner to that seen in the ischaemic models. These same mitochondrial abnormalities occur in catecholamine-induced myocardial injury. These latter changes are exacerbated by magnesium depletion [65,132] and attenuated by pre-treatment with magnesium [63,65]. In animal models of myocardial infarction, infarct size is increased by administration of calcium, and reduced by treatment with magnesium and other calcium antagonists such as verapamil [132-135].

Studies have indicated that low myocardial magnesium content is associated with high levels of myocardial calcium in both normal and ischaemic

hearts[56,58,136]. Magnesium depletion is associated with a higher incidence of ischaemic heart disease per se, in conjunction with raised myocardial calcium[58,64]; no mitochondrial studies have been reported.

There is a high incidence of myocardial magnesium depletion in the year following cardiac transplantation (Chapter 3). In view of the damaging effects of potential calcium overload, a prospective biochemical and histological study was performed to investigate the effect of magnesium depletion on the calcium content of the transplanted heart.

Patients and Methods

All patients entering the cardiac transplantation programme at St George's Hospital, London and surviving at least three months post-transplantation were eligible for the study. Demographic data, including details of the donor heart and transplantation procedure, were recorded at trial entry. All patients received standard immunosuppressive therapy with cyclosporin, azathioprine, and steroid therapy tapered over twelve weeks. Other therapy was prescribed as necessary.

Endomyocardial biopsy was performed weekly for two months post-transplantation, twice-monthly for a further month, and at six, nine and twelve months, in accordance with an established protocol for the detection of rejection. Serum magnesium, calcium, potassium and creatinine were measured pre-transplantation and at the time of each subsequent endomyocardial biopsy. Serum analyses were performed using standard automated techniques.

Myocardial magnesium was measured from a single myocardial biopsy obtained at each histological assessment of rejection, and analysed as previously described. Using the same biopsy specimen and a modification of the assay for magnesium, myocardial calcium was also analysed (Appendix

B). Specimens from the cadaver hearts utilised to develop the magnesium assay (described in Chapter 2) were stored and later analysed for calcium to be used as controls (see Appendix B).

Statistical methods

Statistical analyses were performed using a two-tailed paired student's t-test for within-patient indices, appropriate regression analysis, and ANOVA for multivariate group analysis[98].

Electron microscopy

Electron microscopy was utilised to evaluate the presence or absence of mitochondrial calcium deposition graded as absent, moderate or severe in an unselected sub-group of patients. A second endomyocardial biopsy was taken and placed directly into 3% glutaraldehyde in cacodylate buffer prior to processing in osmium (standard procedure). Preliminary work had established alizarin red and potassium pyroantimonate as suitable calcium stains. Confirmatory qualitative analysis was performed using electron energy loss spectral analysis (EELS), courtesy of RAF Halton.

Results

Biochemical Study

Data was available from 15 recipients over a median follow-up period of 38 weeks (range 11-52).

Myocardial calcium content

Myocardial calcium analysis yielded a range of 5-112 μmol/g dry weight of myocardium, considerably higher ($p<0.001$) than that obtained in the control series (6-20 μmol/g; see Appendix B and Fig B.3), as shown in Figure 5.1.

Figure 5.1

Wide Range of Myocardial Calcium Content in the Transplanted Heart
(dotted line represents upper limit of normal)

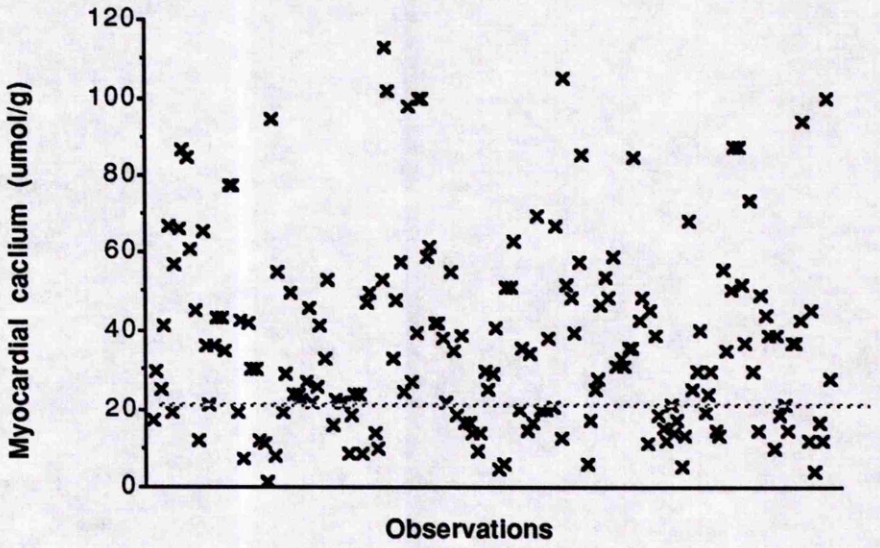
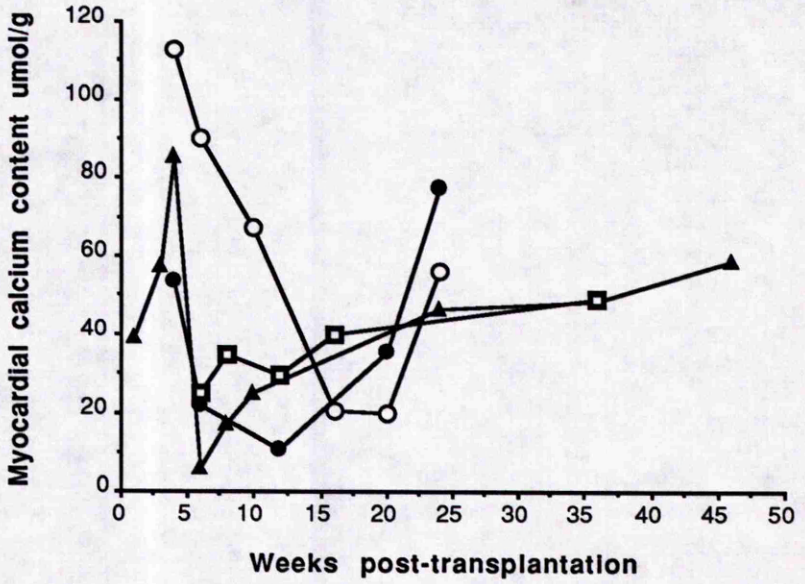


Figure 5.2

Myocardial Calcium Content : Inter- and Intra-patient Variability



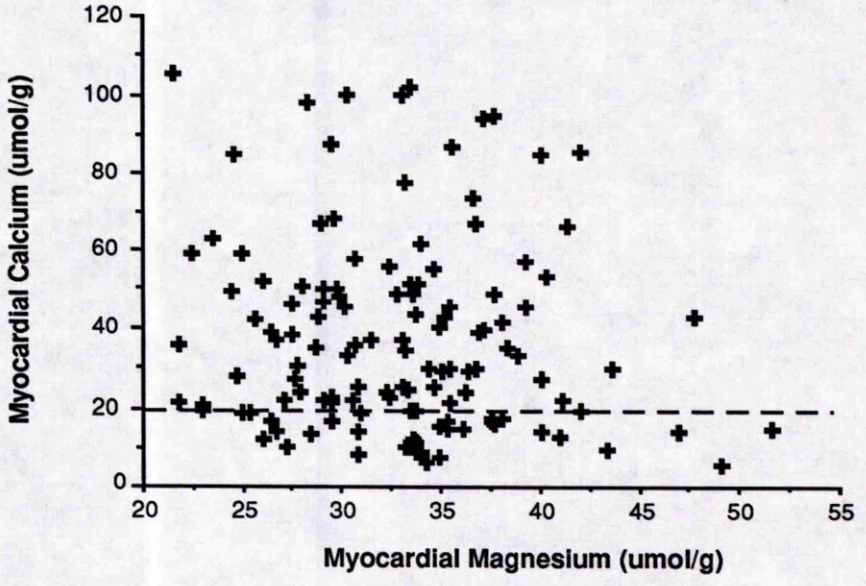
Myocardial calcium was consistently at least twice that of the upper control limit in 10 patients (67%). Baseline calcium was within control range in 8 patients, but in only 2 patients did myocardial calcium remain persistently within the control range throughout the study period. Inter- and intra-patient variation of myocardial calcium is illustrated in Figure 5.2, in which serial analysis of myocardial calcium in four patients is presented. Myocardial calcium was within the control range at three months in 10 patients; by six months, myocardial calcium content had increased significantly (mean(SD) three v six: 11.7 (6.1) v 47.2 (21.6); $p<0.001$).

Myocardial calcium and magnesium

Figure 5.3 demonstrates the relationship between myocardial calcium and magnesium as measured simultaneously in each biopsy sample.

Figure 5.3

The Relationship Between Myocardial Magnesium and Myocardial Calcium



In only a few instances were myocardial calcium levels within the normal range associated with a normal myocardial magnesium content

(>30.5 μmol/g). There was a trend toward higher myocardial calcium content at three and six months in patients with myocardial magnesium depletion (normal v deplete: three months 25.9 v 43.2; p=0.14; six months 27.4 v 45.2; p=0.33).

Other indices

There was no correlation of baseline myocardial calcium with perioperative ischaemic time, or of subsequent myocardial calcium with episodes of rejection or the use of calcium antagonists. Serum calcium was normal throughout.

Electronmicroscopy

Electronmicroscopy was performed in serial biopsies from seven patients. Electron- dense intramitochondrial deposits were largely absent from biopsies taken within two weeks of transplantation. Thereafter, the size and number of electron-dense deposits increased over nine months in six of the seven patients (moderate deposition), with particularly marked deposition occurring in three patients (severe deposition). Few deposits occurred in mitochondria from the seventh patient, even at nine months. Qualitative analysis (by EELS) confirmed the deposits to be composed largely of calcium phosphate, although magnesium phosphate was also detected within some of the deposits. The typical distribution of deposits are demonstrated in the electronmicrographs shown in Plates 1a and 1b, which are taken from myocardial biopsies from the same patient at three months (Plate 1a - moderate deposition), and nine months (Plate 1b - severe deposition), after transplantation. The micrographs are illustrated schematically in Figure 5.4.

Figure 5.4
Schematic view of electronmicrograph

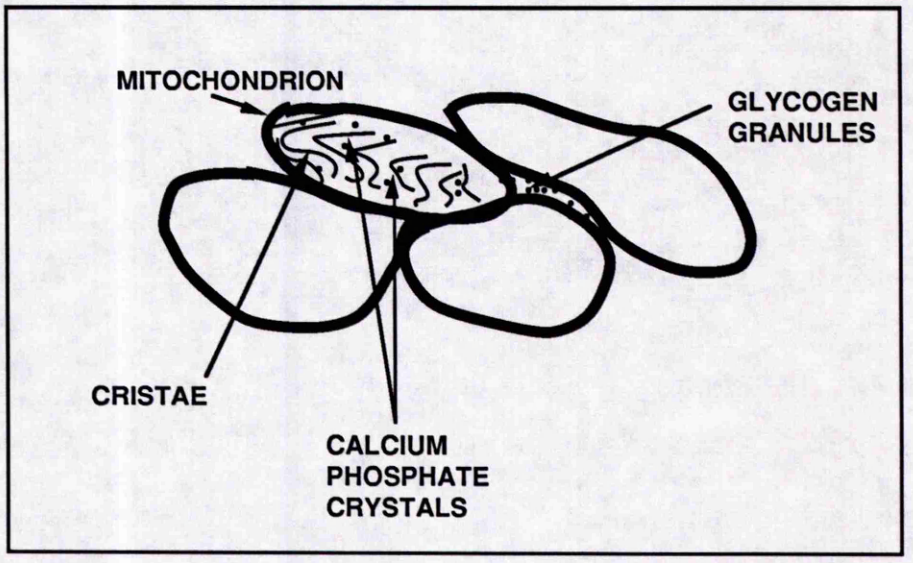


Plate 1(a)
Electronmicrograph of Myocardial Biopsy taken Three Months Post-Transplantation Showing Mild Calcium Deposition (x 30,000)
(overleaf - page 85)

Plate 1(b)
Electronmicrograph of Myocardial Biopsy Taken from the Same Patient Nine Months Post-Transplantation now Showing Severe Calcium Deposition
(overleaf - page 85)

Plate 1(a) Mild Calcium Deposition at Three Months (x30,000)

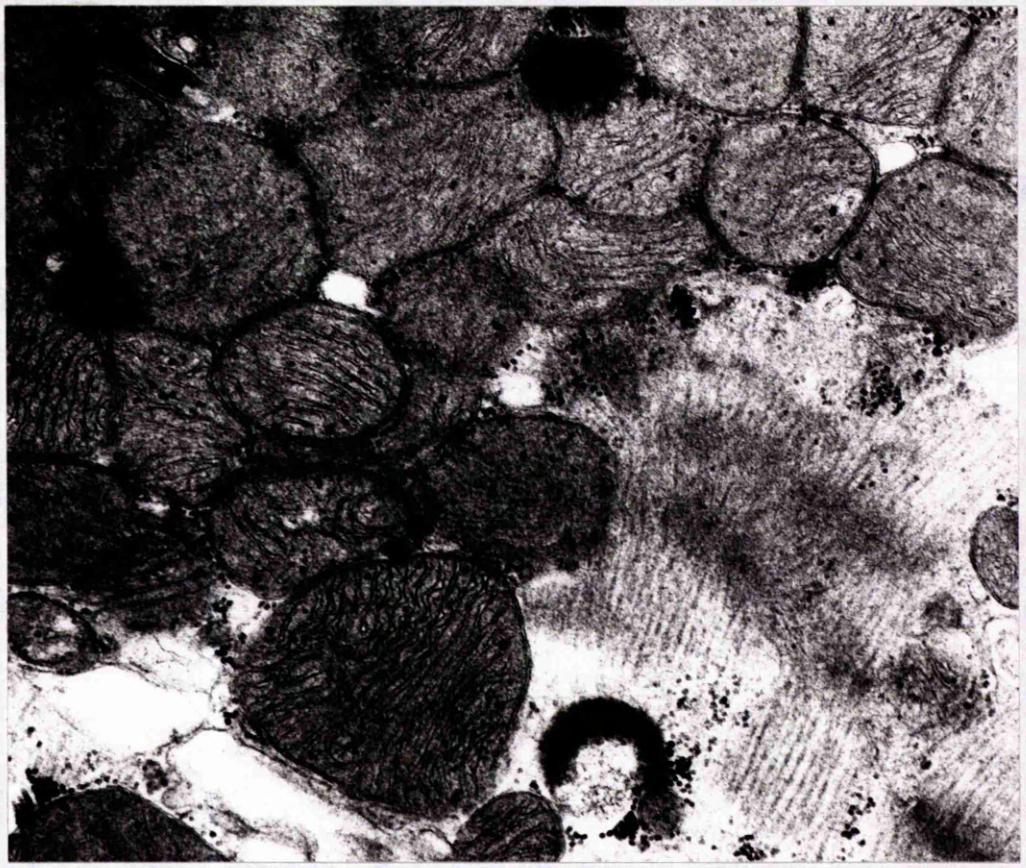
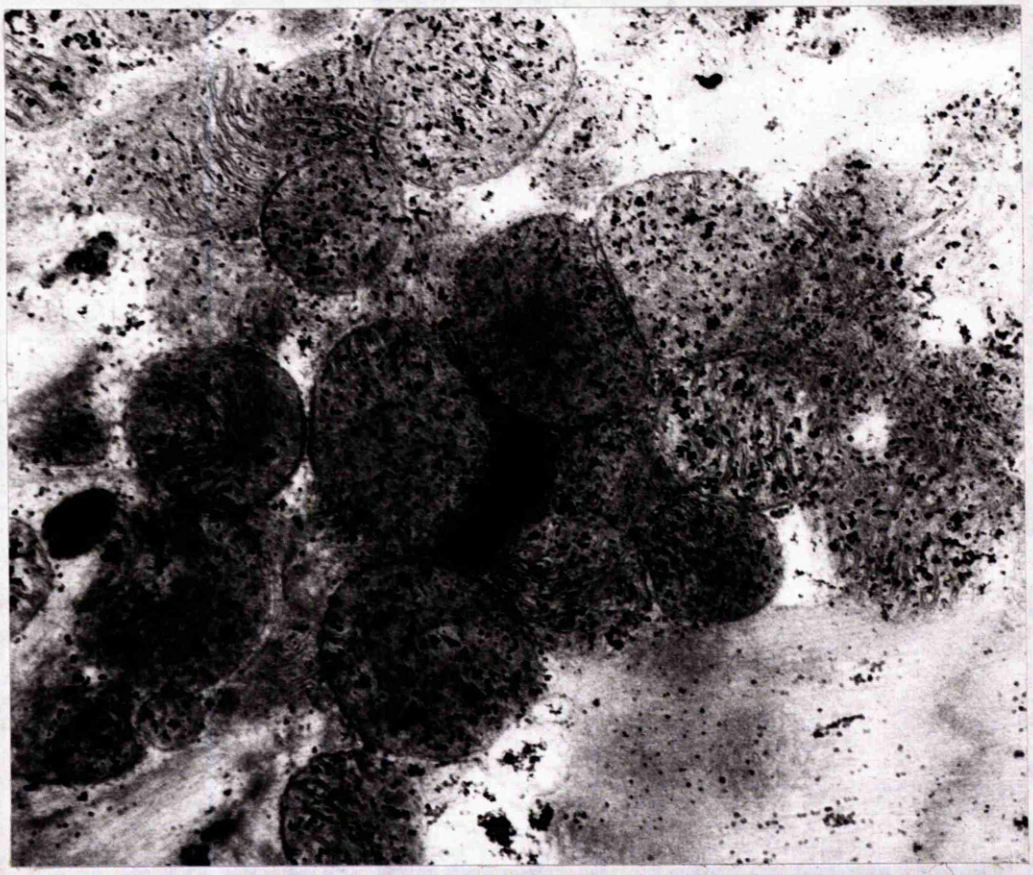


Plate 1(b) Severe Calcium Deposition at Nine Months (x30,000)



Correlation of the Biochemistry with Electronmicroscopy

There was no consistent relationship between biochemical calcium analysis and mitochondrial calcium deposition - the most marked deposition occurring in a patient with moderately raised calcium levels (20-45 μmol/g). However, this patient was hypomagnesaemic at the time of transplantation and remained profoundly magnesium-deplete until thirty weeks later. Myocardial magnesium fell from 48.7 μmol/g at baseline to 29.8 μmol/g three months post-transplantation. The patient in whom mitochondrial calcium deposition did not occur was hypomagnesaemic for only a short period around the three month nadir and myocardial magnesium was maintained accordingly. In the remaining patients with moderate calcium deposition, hypomagnesaemia and myocardial magnesium depletion developed over three months, persisting for a further three to five months - the pattern seen in the majority of transplant recipients (see Chapter Three).

Discussion

A striking finding in this study was the enormous range of myocardial calcium content in cardiac transplant recipients as compared with a cadaver control group, the results from which are as expected from the literature[18,56,58]. Although the obvious explanation that this may be due to the difference between in vivo and in vitro sampling must be considered, the following points suggest that the findings of this study are representative:

(i) In each individual patient, the results obtained are largely consistent (Figure 5.2); and in four patients, myocardial calcium remained at or just above the control range; (ii) Many cardiac transplant recipients are profoundly hypomagnesaemic for a period of a least six weeks; there are no previous in vivo or in vitro studies in man of myocardial calcium in this circumstance. In the

animal studies, significant changes in myocardial calcium content in association with magnesium depletion are reported[86,137], findings mirrored in the present study in transplant recipients.

The large range of myocardial calcium observed, both at baseline and subsequently, made statistical analysis of the results difficult to interpret. However, a distinct trend toward a higher calcium content in magnesium-deplete grafts could be discerned. Furthermore, a significant rise in myocardial calcium was observed in those patients with myocardial calcium levels initially within the control range level, corresponding with a fall in myocardial magnesium.

The use of calcium antagonists had no measurable effect on subsequent myocardial calcium levels. Clinical trials investigating the use of calcium antagonists in the reduction of calcium-induced injury have produced mixed results[129,134,135]. This suggests that there is more than one mechanism by which calcium overload occurs, perhaps reflecting varying calcium channel behaviour.

Changes in cytosolic magnesium result in modification of cytosolic calcium levels in vitro, not only by modification of calcium channel characteristics, but additionally by effecting a change in intracellular calcium binding[40,59,64]. Magnesium, oft dubbed "nature's calcium antagonist"[43], has been shown to protect against catecholamine-induced calcium overload[63,65], and has reduced the incidence of cardiogenic shock secondary to acute myocardial infarction[66,138]. As demonstrated in the study of magnesium content (Chapter Three), rejection (and the additional drugs used at that time) appeared to little effect myocardial calcium.

The data from electron microscopy must be interpreted with caution in view of the few patients studied. It is however interesting to note that the patient with severe hypomagnesaemia developed very significant mitochondrial calcium

deposition in distinct contrast to the absence of such deposition in a patient with very mild hypomagnesaemia, with the moderate magnesium depletion of the remaining patients reflected in moderate mitochondrial calcium deposition. Calcium-induced myocardial damage with mitochondrial calcification was reported by Russo in 1981[132], whilst Fruscati (1987) demonstrated supercontraction of sarcomeres in response to calcium overload associated with magnesium deficiency, in biopsies from patients with idiopathic dilated cardiomyopathy [91].

The control of intramitochondrial calcium content is effected by three independent mechanisms; a sodium dependent efflux antiporter is inhibited by high cytosolic calcium concentration, whilst the influx of calcium is inhibited by cytosolic magnesium ion[139]. The ratio of total intracellular calcium to magnesium is about 1:4; within the mitochondria the ratio is reversed (4:1) [140,141]. The tendency of the mitochondrion (in particular) to calcium overload would tend therefore, mole for mole, to be more sensitive to changes in intracellular magnesium content rather than to alterations in calcium content. These latter points may explain the observed trend toward more significant calcium deposition within the mitochondria of the magnesium-deplete heart as compared with magnesium-replete heart with a similar calcium content.

Summary

The calcium content of the transplanted myocardium is significantly higher than has been documented in the native heart and there does appear to be an inverse relationship between total myocardial calcium content and myocardial magnesium content. Electron microscopy reveals that mitochondrial calcium deposition does occur, and in this preliminary study at least, appears to be related to magnesium depletion rather than to absolute myocardial calcium

content. Studies to investigate the effect of mitochondrial calcium deposition on mitochondrial function and on myocyte contractility would further improve our understanding of the role of magnesium in the myocardium and identify areas that may be potentially exploited to the therapeutic advantage of both the transplanted homograft and the native ischaemic heart.

CHAPTER SIX

THE INCIDENCE OF CARDIAC ARRHYTHMIA IN CARDIAC TRANSPLANT RECIPIENTS: EFFECT OF SERUM AND MYOCARDIAL MAGNESIUM CONTENT

Introduction

Hypomagnesaemia is considered by many to predispose to cardiac arrhythmia, and there is a myriad of reports in the literature, indicting low serum magnesium as the cause of ventricular and supraventricular arrhythmia. Some studies have measured myocardial or skeletal muscle magnesium and found a positive correlation of arrhythmia and low muscle magnesium[75,76]. In all cases, serum and/or tissue potassium was simultaneously significantly deranged, and it is possible that disorders of magnesium ion concentration predispose to cardiac arrhythmia only in the presence of potassium depletion. This hypothesis led to the following study, and will be explored in detail in Chapter Eight.

Hypomagnesaemia and myocardial magnesium depletion are common after cardiac transplantation (Chapter 3). Cardiac arrhythmia after orthotopic heart transplantation is generally rare, unless associated with episodes of rejection[103,104]. Atrial arrhythmias do sometimes occur in the first month, particularly in the immediate post-operative period, but these are rarely persistent or troublesome[142]. Potassium depletion is not seen in these patients, who thus represent an unusual group in whom hypomagnesaemia is not accompanied by potassium depletion and in whom the effects of isolated disorders of magnesium balance on the incidence of cardiac arrhythmia in the denervated heart may be studied.

Patients and Methods

A prospective study in unselected cardiac transplant recipients within the first year of transplantation was undertaken. Patients attending for endomyocardial biopsy (for the detection of rejection) underwent ambulatory electrocardiography in the twenty-four hours following the biopsy. Serum and myocardial magnesium and serum potassium were measured at the time of the biopsy, and the time from transplantation recorded. Patients receiving antiarrhythmic medication, and those who had received magnesium as part of another protocol, were excluded from the study.

Ambulatory electrocardiography was undertaken, recorded using a two channel recorder (Marquette Electronics, GB Ltd. Manchester, UK) and analysed using a standard computer algorithm by an experienced observer blinded to the results of biochemical analyses. Results were printed in a standard format to allow total ventricular and supraventricular ectopic count to be evaluated. Complex forms were reported as follows: bigemminal cycles, couplets, and runs (including longest and fastest run). Significant ventricular ectopic activity was defined as more than 10 ventricular ectopics per hour. Recordings were not entered into data analysis if there were technical difficulties in either obtaining or analysing the signals. Single channel recording was accepted, providing that the recording was continuous.

Results

Ambulatory electrocardiograms were obtained from 21 patients on 27 occasions, recorded 4-52 weeks (median 22) post-transplantation. No patient had been excluded from the study because of concurrent anti-arrhythmic therapy; recruitment was limited by recorder availability.

Concurrent hypomagnesaemia (serum magnesium <0.7 mmol/l) was present in 14 of 27 records (group S), myocardial magnesium depletion (myocardial

magnesium <30.5 μmol/g) in 11 of 27 (group M), occurring simultaneously in 6 patients (group SM). Serum and myocardial magnesium were normal in 6 patients (group N).

Mean serum potassium was 4.1 mmol/l; hypokalaemia did not occur.

Symptomatic arrhythmia was not reported and the incidence of recorded arrhythmia was very low. Non-sustained ventricular tachycardia and frequent ventricular ectopics were recorded in only one patient (group N). Significant ventricular ectopic activity occurred in one other patient (gp SM); supraventricular ectopics occurred in one patient (gp S). Median twenty-four hour ventricular and supraventricular ectopic counts were not significantly different among the groups (Table 6.1).

Table 6.1

Incidence of Ventricular and Supraventricular Arrhythmia Post Cardiac Transplantation: Influence of Magnesium Status

EctopicActivity median (range)	Serum Magnesium		Myocardial Magnesium	
	normal	low	normal	low
Ventricular	6 (1-6199)	4 (0-335)	6 (0-6199)	3 (1-334)
Supraventricular	3 0-20	0 (0-1058)	3 (0-1057)	0 (0-11)

Discussion

The finding of a low incidence of cardiac arrhythmia after cardiac transplantation is in keeping with other published studies; this, despite profound, prolonged hypomagnesaemia. This numbers in this study are small, but it is possible to expand the potential data pool in that the vast majority of recipients in the other post-transplantation arrhythmia studies were receiving

treatment with cyclosporin A using broadly similar regimes; it is not improbable that hypomagnesaemia also occurred in these patients in much the same way. Taken as a whole therefore, hypomagnesaemia is unlikely to be significantly arrhythmogenic in the denervated heart.

The validity of the original suggestion that hypomagnesaemia is arrhythmogenic is discussed in detail in Chapter Eight; the discussion below will be limited to the possible extrapolation of these results to the native heart.

1. The denervated heart is particularly sensitive to several agents - both endogenous and exogenous - and the effective dose of drugs such as adenosine are up to 50% less than those necessary in the native heart [143]; sensitivity to circulating catecholamines has also been reported[144]. Catecholamines shorten the action potential and predispose to ventricular arrhythmia in the native heart; the effect in the sensitised denervated heart would be expected to be greater. The denervated myocardium might therefore be expected to be particularly sensitive to any pro-arrhythmic effect of hypomagnesaemia.

2. The causative factors in ventricular arrhythmia in the native heart are complex. Recently the role of the autonomic system has been highlighted with the finding that lack of diurnal heart rate variability (HRV) is associated with an increased risk of arrhythmia and sudden cardiac death following myocardial infarction; poor HRV is thought to represent autonomic imbalance with parasympathetic predominance [144].

Orthotopic cardiac grafts are completely denervated and there is no myocardial autonomic tone (at least in the first year following transplantation). The arrhythmogenic potential of low HRV may well relate to precipitate *changes* in autonomic tone which are poorly tolerated, rather than to low HRV per se; the complete lack of such tone in cardiac transplant recipients is possibly protective.

It is not possible to directly extrapolate data obtained in the denervated, but otherwise normal transplanted heart, to the ischaemic, innervated native heart, but one may conclude that hypomagnesaemia and/or myocardial magnesium depletion are not *primarily* arrhythmogenic.

Conclusion

Hypomagnesaemia and/or myocardial magnesium depletion, in the presence of a persistently normal serum potassium, is not associated with significant cardiac arrhythmia in the denervated heart. There are many factors governing the instigation and spread of an arrhythmic focus in the native heart. At present it is not possible to state whether the denervated heart would be more or less susceptible than the native heart to arrhythmia induced by hypomagnesaemia. Further identification of the role of the autonomic system in the genesis of cardiac arrhythmia is required before this question can be confidently addressed. On balance, it is probable that the findings of this study are broadly applicable both to the transplanted, and non-transplanted, human heart.

CHAPTER SEVEN

THE EFFECT OF MAGNESIUM ON MONOPHASIC ACTION POTENTIAL DURATION IN THE DENERVATED HEART

Introduction

The empiric use of magnesium (Mg) as an anti-arrhythmic agent has been reported for over fifty years, but the mechanism of such beneficial effect remains obscure. Previous reports of the electrophysiological effects of intravenous magnesium have failed to identify any changes in ventricular electrophysiology that might explain the observed anti-arrhythmic effect of magnesium[145,146].

The transplanted denervated heart is known to be super-sensitive to catecholamines and adenosine[143,147,148], and is free of "interference" from extracardiac haemodynamic reflexes; myocardial autonomic tone is absent. A "cleaner" recording of the effects of intervention are thus possible. It was therefore postulated that subtle changes in ventricular electrophysiology in response to intravenous magnesium would be more apparent in the transplanted myocardium.

In literature reports, hypomagnesaemia is associated with torsade de pointes, a rare chaotic ventricular arrhythmia akin to ventricular fibrillation[73]. Small deflections ("humps") in the repolarisation phase of the ventricular monophasic action potential have been described in association with torsade, both in vivo and in vitro[149,150]. The small deflection or hump is thought to represent an early depolarisation of the myocyte membrane occurring in the final stages of the main action potential. Such early after depolarisations may

reach threshold to trigger an early action potential and thus an ectopic beat; "triggered activity" is thought to be the basis of some ventricular dysrhythmias. The depressant effect of exogenous magnesium on such after depolarisations is well documented[151]. In vitro, susceptible myocardium will exhibit the same early after depolarisation abnormalities of the action potential during pacing protocols introducing two extrastimuli, the first at a long period after the preceding beat, the second following in quick succession (Figure 7.1)[152]. The early after depolarisation is seen on the end of the second extrastimulus (Figure 7.2).

Figure 7.1
Pacing Train for Study of After Depolarisations as seen on the Surface ECG

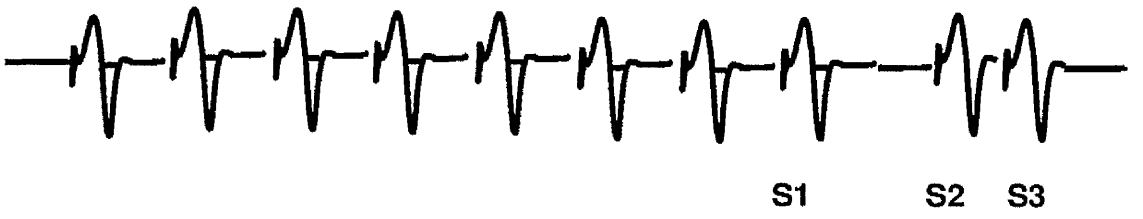
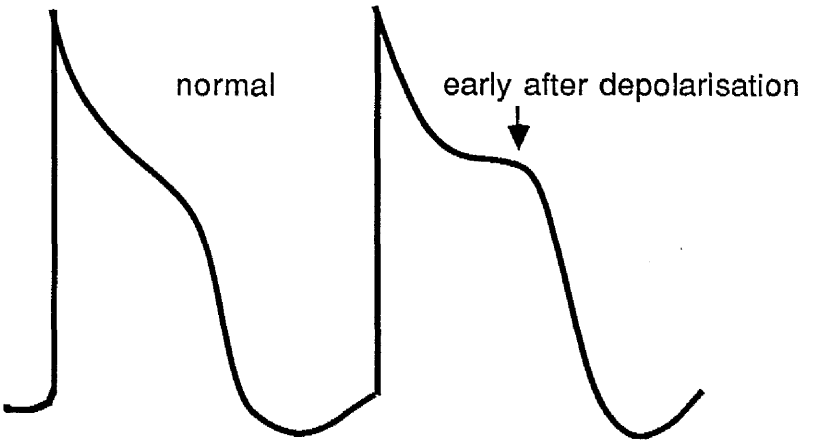


Figure 7.2
The Distortion of the Monophasic Action Potential
by Early After Depolarisation



Should hypomagnesaemia be associated with a tendency toward torsade, then it may be possible, using similar pacing protocols, to induce early after depolarisations in hypomagnesaemic cardiac transplant recipients. The depressant effect of pharmacological magnesium on such early after depolarisations would be sought by the administration of an intravenous bolus of magnesium should such abnormalities become apparent.

Patients and Methods

Cardiac transplant recipients undergoing routine right ventricular biopsy (as part a protocol for the detection of rejection in the first year following transplantation) were invited to enter the study which had been approved by the Wandsworth District Ethics Committee.

An 8F Cordis introducer (Cordis, Miami, Florida) was sited in the right subclavian or internal jugular vein, and endomyocardial biopsies obtained using a Cordis Bioptome; one biopsy was subsequently analysed for magnesium as previously described.

On completion of the biopsy procedure, 10 mls of venous blood were taken for magnesium and potassium estimation, and a twelve lead electrocardiogram was recorded at a paper speed of 50 mm/s. With the patient supine, and requiring no sedation, a Franz-Josef quadripolar MAP/pacing catheter (EP Technologies Ltd, California, USA) was placed under radiographic control to the right ventricular apex. A stable position for ventricular monophasic action potential recording (MAP) was sought, and suitable pacing thresholds confirmed.

Plate 2(a)

Franz-Josef Quadripolar MAP/ Pacing Catheter

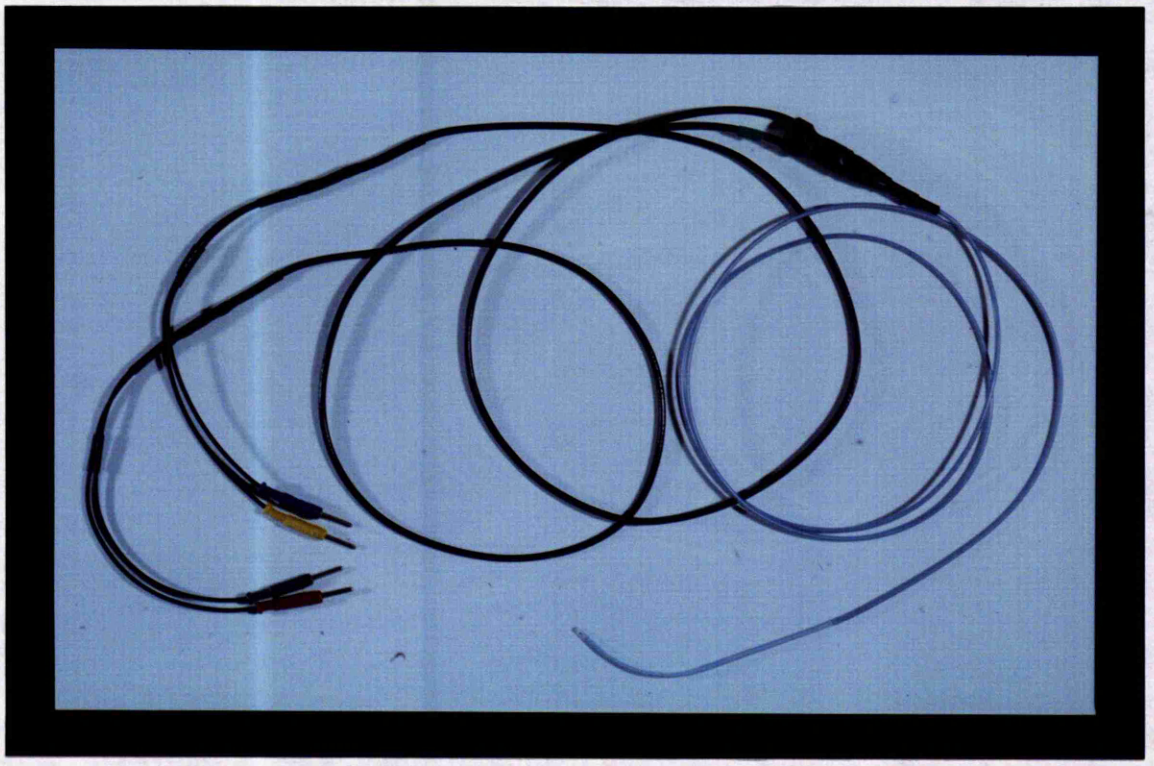
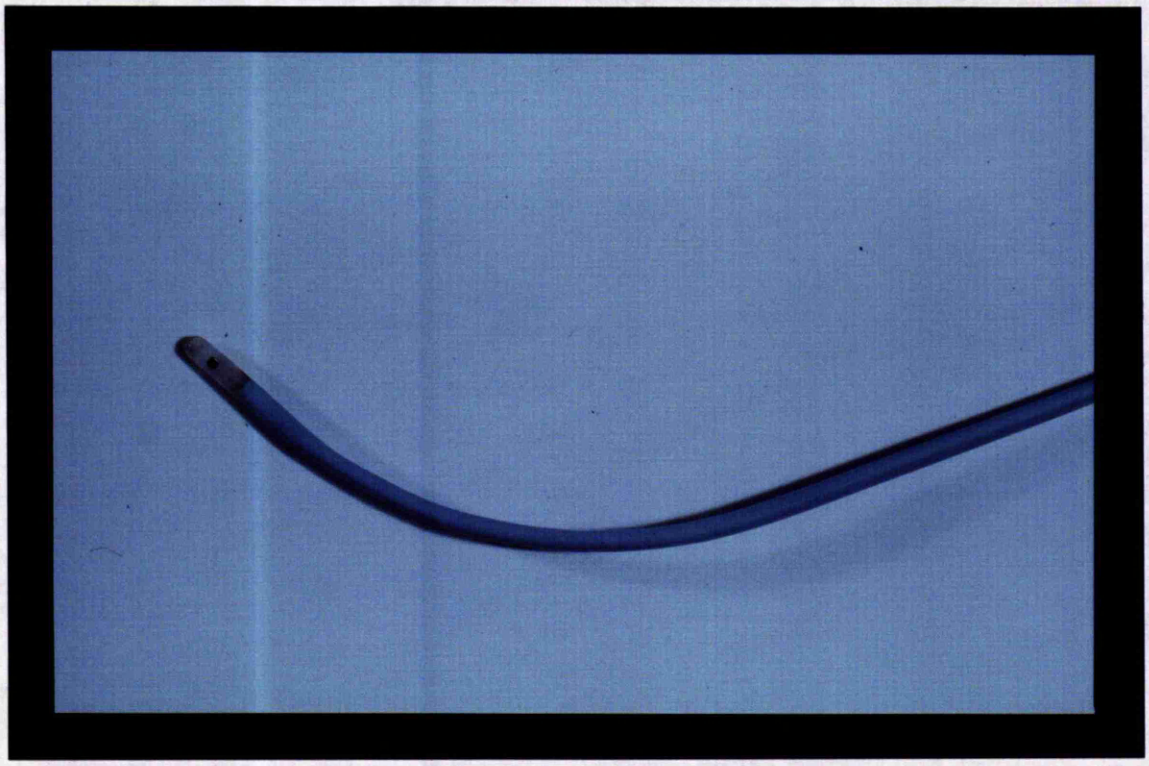


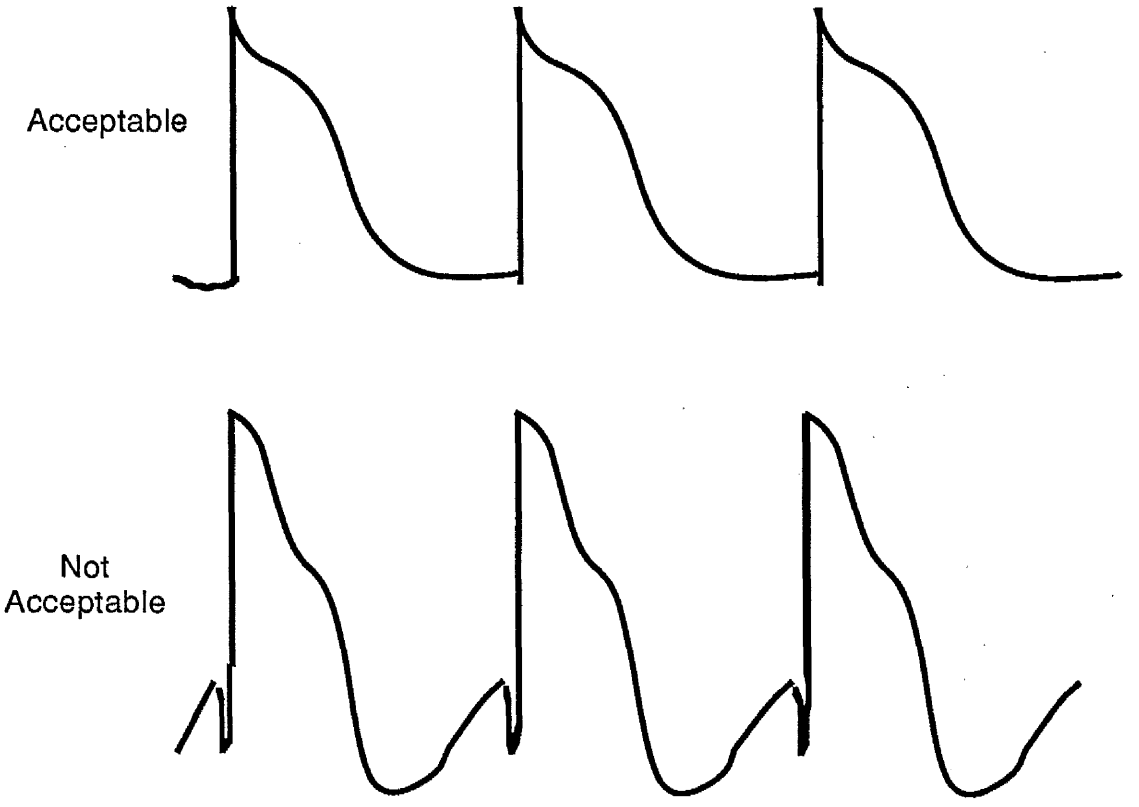
Plate 2(b)

Specialised Catheter Tip Allowing Simultaneous
Recording of Monophasic Action Potentials and Same Chamber Pacing



The Franz-Josef catheter allows simultaneous recording of monophasic action potentials and bipolar same chamber pacing (Plate 2(a) and (b)). The pacing electrodes of the MAP catheter were connected to a stimulator (Medtronic, Indianapolis, Indiana, USA); the recording electrodes were connected via an amplifier to a paper recorder. Three channels of the surface electrocardiogram were continuously displayed on an oscilloscope, and could be recorded simultaneously on hard copy.

Figure 7.3
Monophasic Action Potential Recordings



A stable recording of the ventricular MAP obtained, ventricular pacing at a cycle length of 600 milliseconds (100 bpm) was established and continued for at least three minutes before baseline MAPs were recorded at a paper speed

of 100mm/sec (protocol 1). This protocol has been demonstrated to produce baseline stability[153]. The stability of the MAP was determined by a constant, identifiable baseline potential followed by an action potential of sufficient amplitude to facilitate accurate measurement, returning to baseline with a minimum of "far-field" interference (Figure 7.3).

Two pacing protocols were performed in each patient with a resting heart rate of less than 95 beats per minute. If the resting heart rate was greater than 95 per minute, the second protocol only was performed.

Ventricular effective refractory period (VERP) was determined to the nearest 5 ms using the following regime: Baseline pacing at 600 ms for a train of eight cycles with an extrastimulus introduced at 20ms decrements until refractoriness was encountered i.e. S1-S2 = 580, 560, 540 ms etc. At refractoriness (R), S1-S2 was increased to R+20, and then decreased in 5 ms stages. VERP was defined as the largest interval (S1-S2) at which the ventricle failed to capture.

The second pacing protocol was identical to the first in all but the starting cycle length of 400ms, with the first extrastimulus introduced at 380 ms etc.

At the completion of the VERP protocols, the ventricle was paced at 600ms for a further three minutes before beginning the protocol for the induction of early after depolarisations. This protocol was performed at a basic cycle length of 600ms (500ms if resting heart rate greater than 95 bpm) with a train of eight cycles at 600 (500) ms (S1) followed by the first extrastimulus at 900 (800) ms (S2) and then a third at a starting cycle length of 580 (480) ms (S3), reducing S3 at 20 ms intervals until refractoriness was encountered. Continuous hard copy recording was obtained throughout.

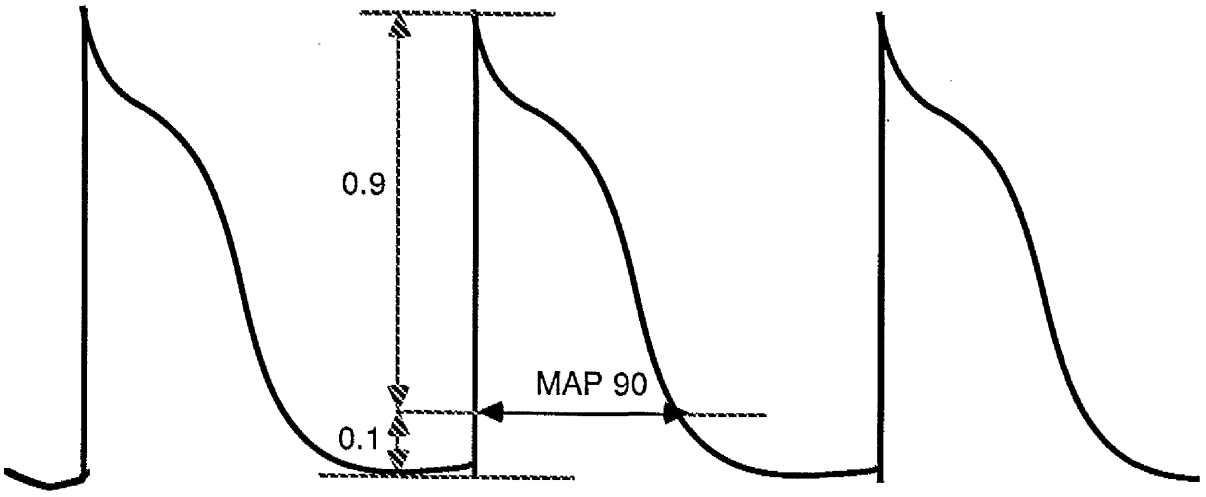
The entire protocol was then repeated, starting five minutes after the administration of an 8 mmol bolus of magnesium sulphate followed by an infusion of 2.5 mmol per hour. At the end of the procedure, a twelve lead

electrocardiogram was recorded and a further 10 mls of venous blood obtained for magnesium analysis.

Analysis

PR and QTc intervals, together with QRS duration were measured from the surface electrocardiogram recorded before and after the administration of magnesium. The duration of the monophasic action potential at 90% repolarisation was measured from the paper recordings taken during baseline steady-state pacing as depicted in Figure 7.4. The average of at least three potentials was recorded, taken before and after administration of magnesium. The presence or absence of abnormal patterns of repolarisation were determined by scrutinisation of the hard copy recorded during the specific pacing protocol.

Figure 7.4
The Measurement of MAP 90



Results

Ventricular monophasic action potentials (MAPs) were recorded from the right ventricular apex during ventricular pacing in 10 of 12 unselected cardiac transplant recipients (age 37-61 years). Median time from transplantation was 15 weeks (range 1-35). All subjects were receiving standard immunosuppressive therapy and none was treated with antiarrhythmic drugs. Serum potassium and calcium were within normal limits. Mean (SD) serum magnesium rose two-fold after the administration of magnesium; 0.69 (0.08) v 1.31 (0.23) mmol/l ($p<0.001$).

Action potential duration: There was a strong trend toward prolongation of the ventricular action potential in response to Mg (95% confidence intervals (CI) of difference: -1.3 to 12.0 msecs; $p=0.11$).

Effective refractory period: No consistent effect on ventricular effective refractory period was noted.

QRS duration and QTc interval: : QRS duration was prolonged in 8 out of 11 subjects ($p<0.07$ (paired two-tailed T-test); 90% CI of diff : 0.8-13.6 msecs), revealing or exacerbating a right bundle branch block pattern. There was no significant difference in the QTc interval.

Magnesium status: Baseline hypomagnesaemia (serum Mg <0.70) was concurrent in 5 subjects; there was no difference in action potential duration at baseline pacing between hypo- and normo-magnesaemic recipients (Table 7.1). However, mean VERP during both pacing protocols was significantly shorter both pre and post Mg in the hypomagnesaemic group ($p<0.05$; ANOVA).

Table 7.1

Electrophysiological Variables and Magnesium Status
(please see text for abbreviations)

Variable Tested (ms) (mean value)	Serum Magnesium			Myocardial Magnesium		
	Normal	Low	p value	Normal	Low	p value
QTc Interval	14.4	15.1	ns	13.4	15.1	ns
MAP90 600	286	271	ns	205	251	0.06
MAP90 400	247	228	ns	263	284	ns
VERP 600	269	231	0.05	248	246	ns
VERP 400	245	214	0.15	240	224	ns

After depolarisations: No abnormalities of repolarisation were detected either at baseline or during long-short interval pacing; early after depolarisations were not seen in either normo- or hypomagnesaemic recipients.

Discussion

The response of the monophasic action potential to magnesium in man has not been previously reported. In some animal studies, there would appear to be little effect of magnesium on action potential duration in the absence of a significant reduction in extracellular calcium [154]. From the data presented above, a prolongation of the action potential is noted in response to a doubling of serum magnesium concentration. This effect is predicted from isolated cell studies and from in vitro animal work. These data are combined with recent electrophysiological studies by basic scientists and discussed in

Chapter Nine in an attempt to provide an electrophysiological basis for the observed beneficial action of magnesium in ventricular tachycardia in vivo.

The electrophysiological effects of intravenous magnesium on the surface electrocardiogram and intracardiac intervals have been previously described, and are discussed in detail in Chapter Nine.

The absence of abnormal repolarisation patterns in response to pacing provocation suggests that isolated hypomagnesaemia and/or myocardial magnesium depletion in the denervated heart are not associated with the underlying electrophysiological abnormalities demonstrable in heart preparations prone to torsade, or those found in patients with torsade associated with congenital long QT syndromes. Torsade is sometimes responsive to beta-blocker therapy, suggesting that sympathetic nervous system activity is important in the genesis and/or maintenance of torsade; furthermore, interruption of the left stellate ganglion may substantially reduce the occurrence of torsade in experimental preparations (see Chapter Nine). The findings of this study in the denervated heart devoid of sympathetic activity must be interpreted accordingly and extrapolation to the native heart in this instance may not be justified.

The changes in action potential duration after magnesium administration were independent of baseline magnesium status. Whilst there was no change in ventricular effective refractory period pre- and post-magnesium, a shorter VERP was noted in the hypomagnesaemic recipients, unaltered by intravenous magnesium. From the ambulatory study described in the previous chapter, this would not appear to have any clinical arrhythmogenic effect. However, were other mechanisms to further interfere with normal ventricular conduction - eg. hypokalaemia or ischaemia - this small fall in VERP might become clinically relevant. This will be discussed in more detail in Chapter Eight.

Conclusions

Intravenous magnesium prolongs the ventricular action potential in the denervated human heart. This effect is independent of baseline magnesium status. Such effects are predicted from single cell studies, but have not been previously demonstrated in man. Abnormal repolarisation in response to pacing provocation was not identified in normo- or hypomagnesaemia, although these data from denervated hearts should be extrapolated to the native heart with caution. Effective refractory period is shorter in hypomagnesaemic recipients and may be clinically relevant in association with other adverse events.

CHAPTER EIGHT

HYPOMAGNESAEMIA AND CARDIAC ARRHYTHMIA

Introduction

Interest in magnesium and cardiac arrhythmia dates back to the 1930s when Zwillinger reported a beneficial action of magnesium administered to patients with paroxysmal tachycardia and ventricular extrasystoles associated with digoxin and strobanthine toxicity[155]. Other workers confirmed a remarkable reversal of arrhythmias secondary to digoxin toxicity, a common cause of ventricular arrhythmia at that time [156-158]. An increase in atrioventricular conduction time was noted, occasionally sufficient to effect reversion of atrial flutter to sinus rhythm. Magnesium had been known to have significant cardiac effects but the mechanisms of such actions were unknown [32]. Sixty years later, understanding of the actions of magnesium has progressed little, and is the subject of much controversy.

The availability of automated serum analysis has rekindled interest in magnesium which had previously been dubbed the "cinderella ion"[159]. Advances in nuclear magnetic resonance imaging have led to the resolution of the long-standing methodological impasse relating to intracellular magnesium concentration [34], and there is increasing interest in this ion among basic scientists, research physicians and clinicians alike.

Hypokalaemia is known to be arrhythmogenic, and administration of potassium salts suppresses such arrhythmia. The attenuation of cardiac arrhythmia by magnesium in hypomagnesaemic patients is assumed by some workers to be mediated by a similar redress of a physiological imbalance [72]. However, raising serum potassium above the physiological range results in significant abnormalities of cardiac conduction, and potassium is not effective

in the suppression of cardiac arrhythmia occurring in the presence of normal serum potassium. In contrast, magnesium can attenuate cardiac arrhythmia regardless of serum magnesium concentration, and has not been shown to have any adverse effect on cardiac conduction until serum concentration is raised at least four-fold i.e. well above that seen in clinical practice[160,161]. The "redress of magnesium depletion" theory cannot therefore be the only mechanism by which cardiac conduction is modified. The situation is further complicated by the intracellular nature of magnesium - only 5% of total body magnesium is found in serum, hence serum levels are a poor reflection of myocyte magnesium (and by implication, of intracellular free magnesium); such factors have confounded investigators and led to confusion amongst clinicians and researchers alike [15].

Points for Discussion

1. What is the role of magnesium ion in the maintenance of normal cardiac conduction?
2. What is the relationship between serum magnesium, myocardial and intracellular free magnesium?
3. Does cardiac arrhythmia occur as a direct result of disturbances in magnesium balance?
4. Are the observed anti-arrhythmic actions of intravenous magnesium a result of correction of a co-existent magnesium deficiency (possibly occult), or is magnesium acting pharmacologically as an antiarrhythmic agent?

The Actions of Cytosolic Magnesium

The physiological actions of cytosolic (intracellular, free) magnesium in cardiac muscle are not known, but there are interesting data produced by cellular electrophysiologists that may facilitate applied physiological interpretation.

Progressive increases in cytosolic magnesium ion concentration have been shown to block inward calcium current [40,50,162,163], to reduce outward (slow) sodium current[164], and to modify the action potential by an effect on three or more potassium channels [36-38,51,165-167]. Inward rectification plays an important role in the maintenance of the resting potential in ventricular cells [168] and increasing cytosolic magnesium enhances such rectification by blocking the open potassium channels. The change in cytosolic magnesium ion concentration necessary to produce these effects in vivo is very small and well within the physiological range for mammalian ventriculocytes. Repolarisation is prolonged by an additional effect of cytosolic magnesium on delayed outward rectifying potassium current [168], and on phase four calcium current [163]. The effects on the outward rectifier are unlikely to be seen physiologically in vivo, but may may be important pharmacologically. However, there is considerable inter-species variation, and ion channel composition varies even between atrium and ventricle, causing difficulty in prediction of an overall effect in the intact human heart [169].

Does Modulation of Ion Channel Activity by Cytosolic Magnesium Occur in Vivo?

At present this question cannot be answered directly, but it is possible to infer that this might be so, by postulating a mechanism for reperfusion arrhythmia based on studies in the intact heart. If total myocyte magnesium content (@ 40 μmol/g) were in the cytosol in its free state, cytosolic concentrations would be

about 12 mM; the actual concentration is nearer 1 mM, as measured by 31-P NMR [34,170,171]. Magnesium is bound to intracellular proteins, particularly ATP, and is additionally associated with the sarcoplasmic reticulum and mitochondria, usually complexed with phosphates[172]. Changes in intracellular pH, calcium or sodium concentration can lead to significant changes in cytosolic magnesium, which are not due to influx or efflux of magnesium from the cell and are presumed to be due to changes in intracellular magnesium binding [172]. Several workers have demonstrated a rise in cytosolic magnesium, in response to falling ATP levels occurring as a result of ischaemia [170,173-175]. The rise in cytosolic magnesium is less than that calculated from observed ATP degradation, and suggests either binding of magnesium by other organelles, or nett loss of magnesium from the cell. Under normal circumstances the myocyte membrane is only minimally permeable to magnesium despite a strong electrochemical gradient from outside in, but several workers have demonstrated a beta-receptor activated, cyclic AMP-mediated loss of magnesium from cardiac myocytes during reperfusion following ischaemia, suggesting that the latter rather than the former explanation is likely to predominate [35,80]. With regeneration of ATP during reperfusion and consequent organelle reuptake of free magnesium, cytosolic magnesium falls rapidly to a level below the pre-ischaemic state and total intracellular magnesium is reduced. As discussed above, even micromolar changes in cytosolic magnesium may have a profound effect on potassium channel activity and hence action potential characteristics. Such changes - a six-fold increase in cytosolic magnesium followed by a rapid fall below baseline - would not occur uniformly throughout ischaemic myocardium producing spatial and temporal dispersion of conduction and repolarisation - the substrate of ventricular arrhythmia.

Hypomagnesaemia and Myocardial Magnesium Depletion in Relation to Cytosolic Magnesium.

Under normal conditions the myocyte membrane is virtually impermeable to magnesium [35] and at one time it was thought that a decrease in extracellular magnesium had no effect on cytosolic magnesium [176,177]. These data were limited to short perfusion times (less than 20 minutes) and studies performed using a longer low-magnesium perfusion time (in excess of sixteen hours) demonstrated a substantial reduction in cytosolic magnesium. The mobility of intracellular magnesium was investigated by Poliemi and Page, who demonstrated that over 95% of intracellular magnesium is exchangeable over 30 hours [136]; Quamme, using embryonic chick myocytes cultured in a low magnesium medium, demonstrated that extracellular magnesium can have a profound effect on cytosolic concentration [166]. In these studies extracellular magnesium was reduced from 0.85 mM to 0.12 mM producing substantial falls in cytosolic magnesium concentration; the effects of less drastic reductions (i.e to that encountered in clinical practice) have yet to be reported.

There is a poor correlation between levels of magnesium measured simultaneously in serum and in tissue [83]; data from the Transplant Study described in Chapter Three suggest that this may in part be due to redistribution of magnesium stores resulting in a delayed reaction of tissue magnesium to falling serum levels. Persistent hypomagnesaemia (in excess of four weeks) results in significant myocardial magnesium depletion, which may take several weeks to return to baseline after normalisation of serum level. Cytosolic magnesium has not been measured in this circumstance, but evidence from the intact perfused heart suggests that a fall in total intracellular magnesium may indeed result in a lowered cytosolic magnesium [172]. The situation is complicated by competitive binding of calcium and magnesium within the cell (as discussed earlier), and much more work is required before

substantiation of the suggestion that a fall in total cellular magnesium is reflected by a fall in cytosolic magnesium ion concentration in vivo.

The Interdependence of Magnesium and Potassium

Cytosolic magnesium has been shown to indirectly effect the action potential by modification of potassium channel behaviour. Disturbances in potassium balance alone are potent precipitators of arrhythmic catastrophe. A combination of potassium *and* magnesium imbalance might therefore be expected to be of particular significance, especially in the presence of other factors known to precipitate arrhythmia (ischaemia, digoxin toxicity etc). This interdependence of potassium and magnesium has been recognized clinically for some years, although the mechanisms have remained obscure [20].

The literature abounds with case reports of cardiac arrhythmia associated with hypomagnesaemia occurring in both normal and abnormal hearts.[71,74,102, 178,179] However, *in every report of cardiac arrhythmia associated with hypomagnesaemia cited in the literature, hypokalaemia is an accompanying feature.*

There are no reports of cardiac arrhythmia occurring as a result of *isolated* hypomagnesaemia; in all reports, hypokalaemia was either co-existent or sufficiently recent to suggest significant tissue potassium depletion. In general, hypomagnesaemia and hypokalaemia are coexistent, since causal factors are very similar (see Table 1.1). The situation is exacerbated by a disturbance in renal potassium homeostasis induced by hypomagnesaemia resulting in renal potassium wasting [180].

Several studies have highlighted the difficulty in diagnosing magnesium deficiency in such circumstances, since serum magnesium may be normal despite significant tissue depletion [76,159]. Magnesium deficiency is known to greatly increase the risk of potassium-mediated arrhythmia due to digoxin

toxicity [20,157,159], the contribution of magnesium imbalance to hypokalaemic arrhythmia is probably largely unappreciated.

Doubt that isolated hypomagnesaemia is arrhythmogenic is suggested by the experience in cardiac transplant recipients among whom the overall incidence of arrhythmia is very low [103,181,182]. As described in Chapter Six, despite documented prolonged hypomagnesaemia and severe myocardial magnesium depletion (secondary to cyclosporin), ventricular arrhythmia is rare in cardiac transplant recipients. Hypokalaemia did not occur in these patients. Extrapolation of the results of this study to the innervated heart must obviously be undertaken with caution, but suggests that disturbances in magnesium homeostasis associated with normal potassium balance are not intrinsically arrhythmogenic.

Diuretic treatment is known to induce hypomagnesaemia and hypokalaemia [84,110], and in general, serum magnesium levels are lower in this population, which may promote further tissue depletion of potassium [111,183]. Ventricular ectopic activity is increased in normomagnesaemic, myocardial magnesium-deplete patients with congestive cardiac failure treated with digoxin as compared with magnesium-replete controls [76]. Potassium homeostasis is a major problem in this circumstance and the pro-arrhythmic effect of magnesium depletion might be expected to be particularly evident in patients undergoing treatment for chronic heart failure - as was recently demonstrated by Gottlieb [112]. Myocardial magnesium depletion is demonstrable in subjects dying from ischaemic heart disease [52, 56] and is particularly marked in such subjects dying suddenly (presumed arrhythmic death)[53]; myocardial potassium is also lower as compared with controls. Reinhart (1991) reported that arrhythmia occurring immediately following coronary artery surgery was commoner in a magnesium-deplete group as compared with those with normal myocardial magnesium content; there was no correlation with serum

magnesium [75]. Information regarding myocardial potassium was not reported in this study, (which is complicated by the proximity of cardio-pulmonary bypass) but it is unlikely that magnesium would have been the only ion disturbed in patients with ischaemic heart disease, some treated with diuretics.

Electrophysiology

Isolated hypomagnesaemia is not associated with changes in the electrocardiogram; contrary data from the early literature are explained by calcium and potassium deficiency presumed from the information given to have been coexistent [184,185]. One author comments that the *absence* of ECG changes in a child with tetany should suggest hypomagnesaemia rather than hypocalcaemia[161]. In animal studies, despite profound hypomagnesaemia, ECG changes were lacking until bradycardia secondary to respiratory depression had supervened [114].

A shortening of the action potential by low cytosolic magnesium is predicted by the isolated cell studies, but there are no invasive electrophysiological studies in hypomagnesaemia in man reported. The preliminary data discussed in Chapter Seven suggest that there is no difference in action potential duration in the denervated heart measured in hypo- and normo-magnesaemic cardiac transplant recipients. The ventricular effective refractory period was significantly shorter in the hypomagnesaemic group and may reflect a particular sensitivity of one or more rectifying potassium channel to changes in intracellular magnesium. As noted above, shortening of the VERP was not reflected in an increased incidence of arrhythmia in these normokalaemic subjects.

Further insight may be gleaned from a study of torsade de pointes - an arrhythmia particularly evident in severe hypokalaemia associated with hypomagnesaemia (and can occur in the normal heart). Torsade de pointes is

thought to arise from early after depolarisations (EADs) occurring in susceptible myocardium or as a result of electrolyte disturbance. In animal models of torsade (usually cesium-induced) and in patients with long QT syndromes, EADs can be precipitated by specific pacing protocols. Utilising the same pacing techniques, EADs were not demonstrated during ventricular MAP studies in hypomagnesaemic cardiac transplant recipients as described in Chapter Six. An effect on calcium channels was investigated by Hoffmann and Suckling in 1956 using isolated dog papillary muscle, and Surawicz in 1961 using isolated rabbit heart, showing little effect of magnesium on the ventricular action potential until perfusion calcium concentration was reduced markedly, at which time action potential duration was significantly *prolonged* by *low* magnesium perfusate, gradually *shortening* as magnesium concentration rose [154,186]. This observation may explain the extraordinary efficacy of magnesium in the termination of torsade de pointes, an arrhythmia thought to be calcium-channel dependent [41,187].

Treatment of Cardiac Arrhythmia Associated with Magnesium Deficiency

Most of the case studies of hypomagnesaemia-associated arrhythmia report a dramatic favourable response to treatment with intravenous magnesium (whilst efforts are made to correct potassium imbalance)[16,20,102,156,179,188]. Overall control of the arrhythmia is only attained when tissue magnesium and potassium levels approach normal [189]. *Acute* control of the arrhythmia can be attained with a bolus of intravenous magnesium (with serum levels rising in excess of 2 mmol/l) regardless of the potassium concentration, the pharmacodynamics suggesting that magnesium is acting *pharmacologically* at supraphysiological concentrations. Arrhythmia arising in normomagnesaemic, normokalaemic subjects can be controlled acutely in exactly the same way [190,191]. The suggestion that such an action is due to repletion of occult

depletion is unsubstantiated. This infers a separate, pharmacological action of magnesium on cardiac conduction, a suggestion that is explored in detail in the next chapter.

Summary

There is no evidence that isolated hypomagnesaemia is pro-arrhythmic and little evidence that myocardial magnesium depletion precipitates arrhythmia. There is however evidence from single cell studies that low cytosolic magnesium, secondary to prolonged hypomagnesaemia and/or myocardial magnesium depletion, would be expected to exacerbate potassium-mediated arrhythmia, by a complex interaction with potassium, sodium and calcium channels to modify the action potential. It is concluded that *magnesium deficiency accompanying potassium depletion* has an electrophysiological basis in vitro for its observed pro-arrhythmic effect in vivo; hypomagnesaemia will reduce the threshold for the occurrence of cardiac arrhythmia secondary to hypokalaemia (particularly if related to digoxin). It is recommended therefore that magnesium be administered simultaneously with potassium for more rapid control of arrhythmia occurring in this circumstance.

CHAPTER NINE

MAGNESIUM AS AN ANTIARRHYTHMIC AGENT

Introduction

The use of magnesium as a pharmacological agent to control cardiac arrhythmia was first reported in 1935, soon after which some of the largest series of the beneficial therapeutic effect of magnesium in clinical practice were described [155,156]. There are a number of case reports and short studies in which magnesium has been used with varying success to modify or terminate a wide range of cardiac arrhythmia, in both normal and abnormal hearts, with and without hypomagnesaemia [157,179,187,190,192].

There is debate as to the nature of the observed anti-arrhythmic action. Is the administered magnesium merely repleting a state of magnesium depletion or is magnesium, in pharmacological doses, independently antiarrhythmic? The following discussion will examine the hypothesis that magnesium is acting pharmacologically rather than physiologically in this circumstance, and will investigate the possible electrophysiological mechanisms of such an action.

Isolated cell studies have examined the effects of extracellular magnesium on potassium currents using magnesium in supraphysiological concentrations; significant modification of potassium and calcium channel activity in these circumstances has been documented. Modification of the action potential forms the basis of action for many anti-arrhythmic drugs [193]. Magnesium could be considered to act in a similar pharmacological fashion in its modification of potassium and calcium channel behaviour.

Electrophysiology

Basic Intervals

The observed electrophysiological effects of intravenous magnesium in man vary amongst authors, but prolongation of the PR interval is a common finding at higher doses; changes in the surface QRS are not observed. Intracardiac recordings reveal an increase in the corrected sinus node recovery time and the AH interval. There is little effect on the HV interval, or on atrial or ventricular refractoriness after bolus administration of conventional doses (8-12 mmol) of magnesium sulphate [145,146,194-196]. The maximum effect demonstrable is at the level of the atrio-ventricular node where prolongation of conduction occurs, suggesting an effect on calcium channels.

An increase in QRS duration during rapid ventricular pacing in response to intravenous magnesium was noted by DiCarlo in a heterogeneous group of patients undergoing invasive clinical electrophysiological studies (mean serum magnesium 2.7±0.04 mM); no change in ventricular refractoriness was found [194]. Using isolated, perfused animal hearts, Stark and colleagues demonstrated a rate-dependent increase in ventricular refractoriness at serum concentrations of 4.6 mM, in addition to prolongation of SA nodal, AV nodal and His bundle conduction [197]; these observations may be of relevance to the effect of magnesium in ventricular tachycardia.

Membrane Channels, the Action Potential and Magnesium

In vitro voltage clamp studies pose difficulties in interpretation since there are no less than six different potassium channels in mammalian cardiac tissue [41]; whilst the effect of any given intervention on individual potassium channels can be established in vitro, the resultant effect on the action potential in vivo is much more difficult to demonstrate [37,38,165-167]. To further confound the issue, recent evidence suggests that there is considerable

interspecies variation in ventriculocyte response to magnesium, reflecting wide variation in potassium channel characteristics [38].

Changes in extracellular magnesium concentration: Agus (1989) reported a shortening of the action potential in single cells as extracellular magnesium rose from 0-9.4 mM [40]. Such concentrations are not possible in vivo; skeletal muscle paralysis occur at levels in excess of 4 mM [198]. Studies of isolated dog papillary muscle and rabbit ventriculocytes failed to show any effect of increasing perfusate magnesium concentration on the action potential [154,186]; the shortening of action potential duration with increased magnesium was noted by Surawicz only when extracellular calcium was substantially reduced below normal [154].

Changes in cytosolic (intracellular) magnesium concentration: The inward rectifying potassium current determines the duration of the plateau phase of the action potential [41]; this channel is blocked by increasing cytosolic magnesium thus prolonging action potential duration [199,200]. The outward delayed rectifying current is also sensitive to increases in cytosolic magnesium; inward calcium current is simultaneously blocked. Prolongation of repolarisation and a reduction in the slope of phase 4 of the action potential results [36,201].

In contrast to the studies of extracellular magnesium, the quantitative changes in cytosolic magnesium concentration necessary to produce an effect on the action potential are well within the physiological range and suggest a possible role for cytosolic magnesium in vivo.

Atrium versus ventricle: The alterations of ventricular refractoriness induced by magnesium described by DiCarlo were not detected by Stark in his study of atrial refractoriness, a negative feature of magnesium action that has been consistently reported since its first therapeutic use in 1935. A relative paucity of inwardly rectifying potassium channels in atrial tissue (responsible for the

final rapid depolarisation phase of the ventricular action potential) suggest that potassium channel modification may be a mechanism by which magnesium exerts it effect in the ventricle.

Clinical studies: Early data investigating the effect of intravenous magnesium on ventricular electrophysiology in cardiac transplant recipients (as described in Chapter Seven), suggest that monophasic action potential duration in the denervated heart is increased after bolus magnesium administration (an effect independent of baseline magnesium status). These observations are not entirely in keeping with those described above, since changes in action potential duration resulting from relatively small increases of *extracellular* magnesium in vitro have not been described (up to 2.8mM). However, should a bolus of intravenous magnesium result in even a small change in *cytosolic* magnesium concentration, then the findings of Chapter Seven are entirely consistent with the data from the single cell studies which report a prolongation of the action potential in response to an increase in cytosolic magnesium (see above).

It is not known whether rapid fluxes in serum magnesium are transmitted intracellularly equally rapidly. The data presented in Chapter Three suggest that *falls* in extracellular magnesium are *not* reflected immediately by a fall in *total* intracellular magnesium, but the effect on *cytosolic* magnesium in this circumstance is completely unknown. Even less is known about the movement of magnesium ion across the myocyte membrane after an intravenous bolus of magnesium; work by Romani and Scarpa suggests that more than 90% of total intracellular magnesium is rendered extracellular in under two minutes in response to noradrenaline receptor activation (see Chapter One)[80] indicating that there is at least the potential for rapid movement of magnesium across the myocyte membrane.

Whilst the data from Chapter Seven are generally consistent with the in vitro basic electrophysiology, several questions remain to be answered. The effects of magnesium on the calcium channels of the AV node are well recognized, but the possible modification of potassium channel activity in vivo has yet to be proven. The prolongation of the action potential by magnesium described in Chapter Seven could explain the observed slowing of cycle length of established tachycardia - perhaps enough to terminate it - and would serve to render the myocardium more resistant to propagation of abnormal impulses. A recent preliminary study of ventricular conduction velocity in normal hearts has demonstrated a slowing in conduction velocity in response to a rapid rise in serum magnesium[202]; this same study failed to show any change in action potential duration. A second recent study suggested that the mechanism of anti-arrhythmic action of magnesium in ischaemia was a *prevention* of conduction slowing[203]. It can be seen from this and the ensuing discussion, that we are a long way from explaining all the clinical effects of magnesium in cardiac arrhythmia.

The Use of Magnesium in Clinical Practice

Ventricular Fibrillation (VF)

From the above discussion, it would be predicted that an increase in extracellular magnesium would increase fibrillation threshold. This has been demonstrated in animal studies where thresholds for ventricular fibrillation (induced both by pro-arrhythmic drugs and ischaemia) are raised by prior administration of magnesium [69,133,204,205]. This effect is well documented and has been studied with particular reference to drugs used in anaesthesia[206,207]. The modification of risk of ventricular fibrillation in acute

myocardial infarction in man by therapeutic doses of magnesium is under investigation in the ongoing ISIS 4 trial(see below).

Torsade de Pointes

Torsade de pointes, a rare ventricular tachyarrhythmia, has been described in patients with severe hypomagnesaemia, with and without previous evidence of conduction abnormality or structural heart disease [73]. Administration of intravenous magnesium abolishes the torsade, which recurs almost immediately unless correction of the underlying magnesium and potassium depletion is simultaneously achieved [187]. (Concomitant hypokalaemia is an invariable accompanying feature in all such reports). Torsade can also occur spontaneously in association with congenital or drug-induced long QT syndromes, regardless of serum or tissue magnesium levels, or serum potassium [208]. Intravenous magnesium is highly effective in the termination of such an arrhythmia, and is repeatedly effective should the arrhythmia recur. The mechanisms of torsade is poorly understood; Fucher(1989) undertook intra-cavity electrophysiological studies in patients with torsade secondary to long QT syndrome undergoing open heart surgery; no change in measurable variables to account for the action of magnesium in this circumstance was detected[209]. Calcium antagonists and vagal stimulation are also effective, suggesting that magnesium is acting via modification of calcium channels [41,210]. Data from animal studies indicating that calcium-channel mediated early after depolarisations are related to the maintenance of torsade, would tend to endorse that view [151,211,212].

Beta-blockers are also useful in the control of torsade, suggesting a neurohumoral basis for the maintenance of the tachycardia either through an action on the stellate ganglia, or directly by G-protein induced modification of potassium channel behaviour [213]. Data from Stanbury (1948) and Hutter

and Kostial (1956) demonstrate that excess magnesium blocks synaptic transmission in the sympathetic nervous system [214,215]; Somjen and Baskerville (1968) went on to demonstrate a reduction in vagal inhibition by magnesium[216], and recent data in ischaemic models suggest a rapid change in cytosolic magnesium effected by noradrenaline [80]. The particular efficacy of magnesium in the acute management of torsade may well relate to a combination of its calcium channel and neurohumoral effects, justifying its increasing use as the agent of choice for this particularly malignant form of ventricular arrhythmia[217].

Ventricular Tachycardia (VT)

There are no controlled studies of magnesium versus other agents in the termination of ventricular tachycardia. There are however, a profusion of case reports suggesting its efficacy.

Of the larger "series" Allen et al (1989) reported the reversion from sustained monomorphic VT to sinus rhythm in 7 of 11 patients, after treatment with 8-12 mmol of magnesium [190]. Iseri (1989) collected 10 patients with VT/VF (of varying aetiology) resistant to conventional drug therapy and cardioversion[189]. 9 of the 10 patients converted to sinus rhythm within 5 minutes of an 8 mmol magnesium bolus. The literature abounds with similar heterogenous accounts; it is interesting that in many cases magnesium is used as " a last resort" with surprisingly results. It might be that by a direct effect on potassium channels (a major component of the plateau phase of the action potential) magnesium is counteracting the pro-arrhythmic affects of the polypharmacy that has preceded it use.

As with torsade, the particular efficacy of magnesium in the management of VT secondary to digoxin toxicity first noted in 1935 is widely reported and merits further discussion. Personal experience typifies that of the literature [102,180].

A 76 year old man with ischaemic heart disease developed intractable, unstable, monomorphic VT, unresponsive to lignocaine or amiodarone, 24 hours after intravenous loading with digoxin (for rapid atrial fibrillation developing five days after complicated thoracic surgery). Sinus rhythm was established ten seconds after the administration of 8 mmol magnesium, which was followed immediately by a magnesium infusion (2.5 mmol/hour); VT did not recur.

Part of the efficacy of magnesium in this circumstance has been shown to relate to an altered responsiveness of the myocyte membrane to digoxin. In isolated cell studies, cytosolic sodium (and hence potassium) is related to extracellular magnesium; this effect is blocked by ouabaln and is overcome by excess magnesium [36,218]. There is no direct effect of excess magnesium on the sodium-potassium pump.

"Normal heart "VT

Brooks (1991) described 6 patients with inducible VT in structurally normal hearts [192]. All six patients were non-inducible after intravenous magnesium administered during VT stimulation studies. The patients were treated with calcium antagonists and all remained non-inducible and arrhythmia-free during one year follow up.

VT mapped to the right ventricular outflow tract (RVOT) has been found to be responsive to calcium antagonists, particularly verapamil [219]. This is the usual site of tachycardia in normal heart VT and strongly suggests an abnormality in calcium channel behaviour. Magnesium has been used to predict response to calcium channel blockers in this situation.

Abnormalities in the terminal portion of the ventricular action potential have been recorded from patients with normal heart VT whilst in sinus rhythm[220]. These abnormalities are very similar to the after depolarisations seen in

patients with long QT syndromes in whom subsequent torsade is very sensitive to magnesium administration.

Non-sustained VT and Ventricular Ectopy

Ventricular ectopy does not lend itself easily to investigation, as the frequency of such arrhythmias vary on daily basis. Secondly, it is in a discussion as to the possible effects of magnesium on ventricular ectopy that the grey area between (i) the pharmacological action of magnesium and (ii) the effects of physiological repletion of magnesium on potassium homeostasis is most apparent [16]. Thirdly, oral supplements do not raise serum magnesium concentration (see below). As with oral administration, the pharmacokinetics of low dose intravenous doses, administered without a preceding bolus, does not result in a rise of serum magnesium levels outside the normal range, and a direct therapeutic action cannot be inferred.

Pharmacological action: Perticone (1990) treated 10 patients with dilated cardiomyopathy with therapeutic doses of intravenous magnesium/placebo [221]. VT was abolished, and single and couplet ectopic frequency decreased significantly ($p<0.001$) *during* the infusion in the magnesium-treated group. In contrast, ventricular ectopic activity was unaffected in a group of patients with congestive heart failure monitored *after* a short infusion of magnesium [222]. These complimentary studies may be interpreted as suggesting that, in the short term, persistent ventricular arrhythmia is modified only in the presence of therapeutic levels of magnesium.

Pharmacological action or repletion of a deficiency - the role of potassium: A small study by Fruscati of 8 patients to investigate the differential effect of a 24 hour intravenous infusion of magnesium on ventricular ectopy secondary to long standing cardiomyopathy as compared to that due to myocarditis

reported that intravenous magnesium abolished ectopy in the cardiomyopathy group but had no effect in the myocarditis group[91]. Myocardial magnesium content was substantially lower in the cardiomyopathy group than in the myocarditis group. Although myocardial potassium was not measured in the Fruscati study, in most studies of myocardial magnesium depletion in ischaemic heart disease (IHD), myocardial potassium is similarly depressed; both these variables are further depressed in patients dying suddenly (presumed arrhythmic death) [52-56, 58]. Since, diuretic-induced potassium and magnesium depletion is well recognized in patients with IHD[110], these data would strongly suggest that arrhythmia, primarily due to potassium depletion is exacerbated by magnesium deficiency; repletion of magnesium would attenuate the pro-arrhythmic effect of potassium depletion, and would also facilitate regulation of potassium homeostasis by the kidney.

A report from 1944 that magnesium attenuated the "cardiotoxic effects" of mercurial diuretics would certainly suggest that potassium depletion plays a major role in the generation of magnesium-sensitive arrhythmia[223]. The patients with myocarditis in Fruscati's study had not been treated longterm with diuretics and were not magnesium-deplete; significant potassium depletion would also be unlikely. It is therefore not unexpected that any arrhythmia occurring was resistant to treatment with low dose magnesium.

Repletion: Several studies of the effect of oral magnesium salts in the suppression of ventricular ectopic activity are now available. Bashir (1991) performed a randomised cross-over placebo controlled trial of oral magnesium chloride in 12 patients with congestive cardiac failure (NYHA II-IV) and frequent ventricular ectopics (>10/hour)[224]. All patients were receiving treatment with diuretics. Episodes of VT, couplets and ventricular premature complexes (VPCs) were significantly reduced in the treated group. As predicted, a rise in serum magnesium did not occur and none of the

haemodynamic effects associated with such a rise were documented (fall in blood pressure and systemic resistance, alteration of plasma catecholamines). Serum potassium rose, but just failed to reach statistical significance ($p<0.06$). Singh (1990) reported a randomised, controlled trial of magnesium-rich diet against a "normal" diet. 400 high risk subjects were entered into the trial and followed for 10 years [101]. The incidence of sudden death and deaths due to ischaemic heart disease was 10.2% in the treated group and 15.2% in the normal diet group ($p<0.02$). An uncontrolled study in Germany produced data to suggest that the prevalence of ventricular ectopy is reduced with magnesium supplementation [225]. However, the recent results of Cardiac Arrhythmia Suppression Trial (CAST) suggest that studies using ventricular ectopy as a sole measure of outcome must be viewed with caution[226].

In summary, the data pertaining to intravenous and oral magnesium in the treatment of ventricular ectopy suggest that a repletion of occult magnesium depletion is probably the main mechanism of action, facilitating secondary correction of tissue potassium depletion, although some anecdotal reports indicate that therapeutic doses may be effective pharmacologically in the acute control of such arrhythmia, particularly in the setting of acute myocardial infarction (discussed further below).

Supraventricular Tachycardia

Table 9.1 summarises the literature experience of treatment of AV nodal re-entrant tachycardia with magnesium. As would be expected from the well-recognised calcium channel blocking effect of magnesium, resultant delayed conduction within the AV node may have an effect on AV re-entrant tachycardias. There is additional evidence that magnesium may modify retrograde conduction in some accessory pathways [229].

Table 9.1
Magnesium in Supraventricular Tachycardia

Author	no pts	SR	Dose $MgSO_4$	Mode of Termination
Sager[227]	11	2	10mmol/10 mins	Antegrade AVB
Wesley[228]	10	7	8mmol/5secs	VPC 4/7 pts
Viskin[229]	9	5	8 mmol/1-2 mins	Antegrade AVB 3/7 Retrograde block in accessory pathway

SR = sinus rhythm established ; AVB=atrioventricular block

Multifocal atrial tachycardia (MAT) is associated with severe electrolyte imbalance, and has been described in normal hearts [230]. Iseri (1986) reported eight cases of MAT, seven of which reverted to sinus rhythm following a bolus of intravenous magnesium [72]. Potassium depletion is the commonest associated electrolyte imbalance in this group, often accompanied by hypomagnesaemia. There are no studies of magnesium used to control atrial fibrillation, although the ventricular rate may be transiently reduced by AV nodal blockade [156].

Arrhythmia and Acute Myocardial Infarction (AMI)

Many workers have studied the changes in serum magnesium that accompany acute infarction and have found little correlation of serum magnesium with outcome or with subsequent cardiac arrhythmia[231-235]. Serum magnesium falls very slightly in the first twenty-four hours, presumed secondary to catecholamine release[236], but rapidly returns to normal.

However, there is increasing evidence that intravenous magnesium infused during the first 24 hours following AMI reduces early mortality [77], and that the benefit conferred is still present at one year[237]. The mechanism of such an action is undoubtably complex and probably relates at least in part to a reduction in calcium-related injury. However, several of the small trials that form the basis of the experience in this circumstance have demonstrated a reduction in all forms of cardiac arrhythmia during the magnesium infusion as indicated in Table 9.2 .

Table 9.2

The Antiarrhythmic Effect of Magnesium in Acute Myocardial Infarction

Author	Nature of Arrhythmia	% of Patients with Arrhythmia		p value
		MAGNESIUM	CONTROL	
Abraham[238]	Serious Arrhythmias	14.6	16	<0.05
Rasmussen[66]	Arrhythmias requiring Rx	21	47	0.004
Shecter[138]	All Arrhythmias	32	45	0.19
Ceremuzynski[239]	VT (on Holter)	28	78	0.001
Smith[241]	VT	7	14	ns
Morton[240]	Use of Lignocaine	magnesium < control		0.05
	Ventricular Ectopy	not available	not available	ns

Acute changes in cytosolic magnesium are known to occur during ischaemia, whereby pathological alteration of rectifying current would result not only in increased membrane excitability, but in an accumulation of potassium ions in the immediate extracellular environment [170,173-175]. Such accumulation would be exacerbated by poor blood flow and would result in spatial and temporal dispersion of repolarisation, forming a patchy arrhythmogenic substrate. The therapeutic use of magnesium in the immediate period post acute myocardial infarction is under study in the large, multi-centre ISIS 4 trial (40,000 patients), due to report in 1993 [70] and in the Leicester LIMIT 2 study

(2000 patients). This latter trial is completed, but the results are not yet published. Early data suggest a 25% reduction in mortality in the treated group (K.Woods, personnel communication); the full data are eagerly awaited.

Pharmacokinetics

Serum magnesium levels rise sharply to twice baseline after the administration of an intravenous bolus of 8 mmol magnesium sulphate over 2 to 5 minutes, and then fall away quickly to normal over 15 to 20 minutes i.e the half-life is in the order of a few minutes [242,243]. Clearance is rapidly effected by the kidney; redistribution occurs only if there is magnesium depletion (see below). Theoretically, the myocyte membrane would be exposed to supraphysiological concentrations of magnesium for only a short time and any anti-arrhythmic effect would be expected to be short lived. It would be necessary to administer an intravenous infusion of magnesium to keep serum levels above the physiological range (i.e. in the "therapeutic range") should there be a need for continuing antiarrhythmic therapy.

Intravenous magnesium salts are usually administered as the sulphate, although magnesium chloride and magnesium aspartate hydrochloride are available. There are no large scale comparative trials, and at present personal physician preference determines which salt is used. Most experience has been gained with magnesium sulphate, and for the purposes of further discussion "intravenous magnesium" will refer to the sulphate salt.

Intravenous magnesium is irritant to peripheral veins and should be diluted before use, although bolus doses are comfortably administered by flushing the vein with normal saline between bolus aliquots. Cutaneous flushing secondary to vasodilatation is almost invariable with bolus administration, but is short lived and rarely gives rise to problems. With boluses in excess of 12 mmol, nausea and vomiting can occur. A small drop in mean arterial pressure

secondary to a fall in systemic vascular resistance occurs but is rarely symptomatic, and is rapidly reversible if the bolus is halted, or if necessary, by the administration of calcium as a specific magnesium antagonist [244,245]. There is no effect on cardiac contractility[246].

Oral magnesium salts, initially in the form of magnesium oxide, are seriously limited in their use by side-effects, most notably gastric irritation and diarrhoea. The recent development of enteric-coated magnesium chloride has overcome the former, and the aspartate hydrochloride is now remarkably free of both adverse effects. Both agents must be prescribed in at least three divided doses, to limit side effects and maximise absorption. Slow release preparations are under development; at the time of writing, oral magnesium salts were not available in the UK unless combined (in minimal doses) with other salts as over-the-counter preparations. About 15 mmol per day are required for adequate supplementation [16].

The therapeutic use of oral magnesium is limited by the rapid regulation of serum magnesium by the kidney [17]. A magnesium load is excreted by the kidney in its entirety within 24 hours in magnesium-replete subjects. Such is the reliability of the kidney in this regard, that the intravenous magnesium-load test is used by research scientists to establish magnesium status [15,247]. An oral load is part excreted unchanged in the faeces, since magnesium absorption from the gut is hormonally controlled by vitamin D and stoichiochemically modulated by calcium[159]. The rapid renal clearance of magnesium from the serum prevents magnesium administered orally from raising serum concentrations significantly. The potential use of magnesium as an antiarrhythmic agent is thus limited to the intravenous route.

Dose of Intravenous Magnesium

Studies vary widely in the dose of magnesium used (Table 9.3). In our own experience a starting dose of 5 mmol administered over 1-2 minutes, can be repeated within 5 minutes if necessary. Should 8-10 mmol have been administered as a bolus, an infusion of 2.5 mmol per hour (in 5% dextrose or 0.9% saline) will maintain serum levels at 1.5-2.0 mmol/l. Should the patient have compromised renal function (serum creatinine in excess of 240 μmol/l), the bolus is administered as described and the infusion rate reduced according to serum magnesium levels.

Table 9.3
The Use of Magnesium in
Ventricular and Supraventricular Tachycardia

Principal Trialist	Initial Bolus Dose	Subsequent Infusion Rate	Peak serum Mg (mmol/l)
Abraham [238] (AMI)	10 mmol stat	315mmol/36hrs	1.5 @36 hrs
Woods[243] (AMI)	8 mmol in 5 mins	65 mmol/24 hrs	1.5 @ 5 mins 1.36 @ 6 hrs 1.49 @12 hrs 1.51 @ 18 hrs
Iseri[179] (VT)	8 mmol in 20 mins	138 mmol/36 hrs	2.1 @ 4 hrs 1.85 @ 8 hrs
Wesley[228] (SVT)	4 mmol in 5 secs	8 mmol/5 mins	1.81 @ 5 mins
DiCarlo[194] (EPS)	12 mmol in 6 min	20mmol/2hrs	2.25 @ 30 mins
Sager [227] (SVT)	11 mmol in 10 mins	12mmol/30 mins	3.8 @ 5&10 mins

AMI = acute myocardial infarction; VT = ventricular tachycardia; SVT = supraventricular tachycardia; EPS = electrophysiological studies

Summary

Intravenous magnesium in therapeutic doses:

(a) modifies the ventricular action potential in vivo

(a) increases tachycardia cycle length in a rate-dependent fashion at high rates, suggesting a role for modification of ventricular tachycardia

(b) has been shown to be effective in the termination of ventricular tachycardia of various aetiologies, but no controlled trials of its use have been reported

(c) by an action on the AV node, can be useful in the termination of AV re-entrant tachycardia.

There is an electrophysiological basis for and clinical experience of the use of magnesium in:

(d) the acute control of torsade de points (regardless of aetiology)

(e) the treatment of ventricular arrhythmia secondary to digoxin toxicity

(f) RVOT ("normal heart")VT

(g) the modification of arrhythmic potential post acute myocardial infarction

Mechanism of action:

(h) almost certainly involves modification of calcium channel activity, particularly in the AV node and in phase 4 of the action potential

(i) may involve complex modification of potassium channel behaviour to effect changes in phase 2 and 3 of the action potential.

Conclusions

Magnesium is increasingly recognized as an important ion in the maintenance of normal cardiac conduction, active physiologically by complex modifications of potassium and calcium channel behaviour. Further modifications of the action potential by pharmacological doses of magnesium demonstrated in

vitro, suggest a mechanism by which this ubiquitous ion might effect its antiarrhythmic action, and provisional electrophysiological data in man appear to be consistent with these observations. It will be necessary to study the transmembrane kinetics of magnesium in vivo that further progress in this field may be made. It is possible that this may be facilitated by studies utilising new magnetic resonance imaging techniques.

Magnesium is a useful therapeutic agent for the acute control of a variety of cardiac arrhythmias particularly torsade de pointes and those due to digoxin toxicity. There is evidence that use in the immediate post infarction period may reduce the incidence of fatal and non-fatal ventricular arrhythmia. The latter point is already a subject of a major, international study. The time has come to perform proper, controlled trials of magnesium as an antiarrhythmic agent in clinical practice.

CHAPTER TEN

SUMMARY
MAGNESIUM - THE OMNIPRESENT ION

Measurement of Myocardial Magnesium

The preceding chapters have documented the development, optimisation and validation of an analytical method for the measurement of magnesium and calcium in single myocardial biopsy specimens. The relationship between serum and myocardial magnesium was described in a serial study, indicating that persistent, but not short-term hypomagnesaemia, resulted in a significant reduction in myocardial magnesium content. Literature opinion has remained undecided as to the usefulness of serum magnesium as a marker of tissue magnesium depletion; the data from the Transplant Study suggest that *serial* measurements of serum magnesium will provide valuable information about tissue magnesium stores. Myocardial magnesium depletion after cardiac transplantation was shown to relate to hypomagnesaemia occurring secondary to cyclosporin-induced renal magnesium wasting. The predicted rise in myocardial calcium content secondary to myocardial magnesium depletion was demonstrated, and resultant mitochondrial calcium deposition observed.

Magnesium Depletion and the Transplanted Heart

The consequences of myocardial magnesium depletion for the transplanted heart have yet to be fully elucidated. In animal models, prolonged magnesium depletion leads to pathological changes both in the myocyte and in the myocardial vasculature. Mitochondrial calcium deposition results in myocyte dysfunction and cell death, with resultant myocardial fibrosis. The changes in

the vasculature are most marked in small and medium size arteries with proliferation of connective tissue in the media and loss of intimal lining. A defect in oxidative phosphorylation would seem to underly the abnormalities of mitochondrial function.

It is recognized that accelerated coronary disease is a major problem in the transplanted human heart. Large arteries are not affected, making coronary angiography a very insensitive tool for detection. The histological changes in the small and medium sized arteries are limited almost entirely to the intima, which undergoes massive proliferation resulting in obliteration of the vessel lumen. A variable lymphocyte presence is noted, an observation generally considered insufficient to account for the remarkable changes seen. This process is probably a manifestation of graft rejection, but this has yet to be confirmed and remains a puzzling problem.

Cyclosporin is a vasoconstrictor and hypomagnesaemia is known to result in coronary artery spasm both in small and large vessels. Whilst each of these effects alone may be insufficient to result in clinical detectable events, the consequences of their combination with intimai proliferation, taken with the effects of myocardial magnesium depletion discussed above, may prove catastrophic, with widespread small vessel disease in a myocardium in which myocyte function is already rendered suboptimal by mitochondrial dysfunction and calcium overload. Ischaemia and myocardial fibrosis would result - in a manner reminiscent of diabetic ischaemic cardiomyopathy - with the painless, but relentless, deterioration of myocardial function seen all to often in our transplant population. Graft vasculopathy is not limited to cardiac allografts, but that seen in renal grafts is much less severe. The reasons for this are not clear, but is it is tempting to attribute at least some of the difference to the lower dose of cyclosporin used (and hence a lower incidence of adverse effects) after renal transplantation.

Magnesium and Acute Myocardial Infarction

Reperfusion injury, ever more important in the race for effecting a patent infarct-related artery, with faster and better thrombolytics and/or salvage coronary angioplasty, is known to relate to intracellular calcium overload, with serious repercussions for subsequent myocardial function. Calcium, once administered freely as an inotrope, is now treated with circumspection as the more sinister actions of this redoubtable cation are revealed. Calcium-related injury is also important at the fringe of the ischaemic zone, an anatomical site where subsequent function is jeopardised whether or not reperfusion has been attempted.

The use of pharmacological calcium antagonists to limit ischaemic injury has met with mixed success, whilst the use of magnesium in the modification of such ischaemic injury has a long positive history (although most of the studies are in animal models). Magnesium was first used in man in the setting of acute myocardial infarction in the 1980s and since then several small trials have been performed, a recent meta-analysis indicating a significant reduction in mortality in magnesium-treated patients (see Table 9.2).

The mechanism of action was initially assumed to be a reduction in peri-infarct arrhythmia; surprisingly there appeared to be an additional effect to reduce the incidence of cardiogenic shock. In the LIMIT 2 study, the positive effect of magnesium on mortality extended to the survivors, who were shown to have a greater ejection fraction post-infarction than did the controls. This finding was consistent with earlier animal models of ischaemia, where magnesium significantly reduced intracellular calcium overload and limited myocardial damage, substantially reducing infarct size. The increase in ejection fraction in the LIMIT 2 study occurred both in thrombolysed and non-thrombolysed patients, suggesting that the beneficial effect of magnesium is additive to, and distinct from, that of thrombolysis.

The mechanism of reduction in post-infarction mortality by magnesium is probably the result of a combination of its known therapeutic actions :

- reduction in calcium influx into the myocyte with preservation of myocardium and reduction in infarct size
- an anti-arrhythmic action
- reduction in coronary artery tone
- anti-platelet action
- anti-thrombotic action

Such is the interest in this previously neglected ion that 40,000 patients in four continents are in the process of recruitment in the latest of the international studies for infarct survival, ISIS 4, which is to investigate the effects of captopril, isosorbide-5-mononitrate and magnesium in acute myocardial infarction[70].

Magnesium and Chronic Ischaemic Heart Disease

Post-mortem studies indicate higher levels of calcium and lower levels of magnesium in hearts from subjects with ischaemic heart disease and animal data suggest that infarct size is greater in magnesium-deplete myocardium. The effects of magnesium deficiency on myocyte function have not been specifically studied, but would be expected to interfere with mitochondrial energy production, with significant consequences for myocyte function and integrity. Many of these patients were treated with magnesuric drugs (particularly frusemide) and were also found to have lower levels of myocardial potassium.

As discussed, tissue magnesium depletion occurring in association with potassium depletion would pose the additional risk of cardiac arrhythmia. This risk is taken with or without those of overt hypomagnesaemia - ie an increased risk of coronary artery spasm and adverse effects on myocardial autonomic

tone - in patients with depressed myocardial function secondary to calcium overload. The only study that differentiated "sudden" from "non-sudden" cardiac death reported a significant reduction in myocardial magnesium and potassium in the victims of sudden cardiac death, perhaps supporting the conclusions outlined above.

The use of angiotensin-converting enzyme (ACE) inhibitors in heart failure (New York Heart Association class III-IV) results in improved survival, although the precise mechanisms remains unclear. These drugs reduce renal excretion of potassium; magnesium is similarly spared. After introduction of ACE inhibitors, it is usually possible to reduce the dose of loop diuretics, further sparing magnesium and potassium. From the above discussion, is possible that the resulting maintenance of near normal intra- and extra-cellular electrolyte composition plays a significant part in the mode of action of these intriguing drugs. Further developments are awaited.

Magnesium and Cardiac Arrhythmia

The genesis of cardiac arrhythmia is multi-faceted, with local electrophysiological substrate being modified by autonomic tone and intra- and extracellular electrolyte composition; magnesium is physiologically involved at all such levels. Confusion over the physiological and pharmacological actions of the magnesium ion in the genesis and treatment of cardiac arrhythmia has resulted in the establishment of a folklore. In the preceding chapters, this has been thoroughly examined in the light of recent new developments, incorporating findings from the electrophysiology studies in the denervated heart. Hypomagnesaemia is generally considered to be arrhythmogenic, based on the observation that magnesium administered to hypomagnesaemic subjects with cardiac arrhythmia results in modification of

that arrhythmia. The fallacy of this argument has been discussed at length, concluding that hypomagnesaemia is not arrhythmogenic in the absence of potassium depletion.

The evidence reviewed in Chapter Nine reports an electrophysiological basis for the observed role of intravenous magnesium in the acute control of a variety of arrhythmias, although good, placebo controlled trials in clinical practice are sadly lacking. The empiric use of magnesium established by Zwillinger in 1935, is still very much in evidence in 1992 - nearly sixty years on. In the wake of now-dampened enthusiasm for treating each and every ventricular ectopic, a wiser cardiological fraternity is seeking newer and safer methods of managing troublesome cardiac arrhythmia. This, the final decade of the twentieth century, is seeing something of a "green revival". What better time to stage the successful comeback of nature's own antiarrhythmic agent?

The Future

Magnesium depletion and calcium overload after cardiac transplantation together with the adverse effect of treatment with cyclosporin may play an important role in the events surrounding cardiac allograft failure. Investigations directed toward mitochondrial function in magnesium depletion and repletion are indicated, together with studies to investigate the adjuvant effects of hypomagnesaemia on the coronary vasculature in the hypersensitive, denervated transplanted heart.

For the native heart, the ISIS 4 trial will provide data on the possible protective effects of magnesium in acute myocardial infarction. Investigation of the use of magnesium during coronary angioplasty would be interesting, for its role in protection from ischaemia, in coronary vasodilatation, and as a anti-platelet agent.

Appreciation of magnesium as an anti-ischaemic agent and as a calcium antagonist are augmented by an increasing realisation of the effectiveness of magnesium as an antiarrhythmic agent, particularly in the acute control of ventricular dysrhythmias. Controlled trials of magnesium in clinical electrophysiological practice are required, along with further investigation to establish the mechanism of anti-arrhythmic action.

Magnesium, once dubbed the "cinderella ion", is now generating extraordinary interest across the world of cardiology. The next few years will see a wealth of data produced on all aspects of the physiology and pharmacology of this fascinating cation.

APPENDIX A

NORMAL VALUES FOR MYOCARDIAL MAGNESIUM ANALYSIS

Introduction

In most reports of myocardial magnesium content, conclusions have been drawn as to changes in magnesium content by comparing the study group with a control group, and thus the reported changes are *relative to the control group* (since no attempt was made to standardise the assays used) and the absolute values for myocardial magnesium content are not directly referable to other studies. During the development of the new assay described in Chapter Two, it was therefore necessary to establish a normal range for the magnesium content of the right ventricular septum.

Pitfalls in the Establishment of a "Normal Control"

There are several areas of concern when discussing the measurement of magnesium in the myocardium, some of which only became evident during analysis.

1. Magnesium content is not uniform throughout the myocardium, hence specification and standardisation of the site of biopsy is necessary.
2. Magnesium is largely intracellular, and should significant autolysis occur prior to the processing of cadaver material, a falsely low magnesium content would be recorded, thus all cadaver material was collected within twelve hours of death.
3. A significant difference in the magnesium content of the ischaemic and non-ischaemic heart is reported in the literature[55,56,58], and thus the cause

of death and any evidence to suggest accompanying ischaemic heart disease in each control case was established from the pathologist's report.

4. The magnesium content of the heart has been reported to remain stable throughout adult life[54]. One author has reported a slight rise in serum magnesium with age (thought to be related to the fall in glomerular filtration rate with increasing age)[81], whilst lymphocyte magnesium is said to fall in later decades[248]. It became obvious during analysis of the 21 cadaver hearts described in Chapter 2, that there appeared to be a *significant fall in myocardial magnesium content with age*. To confirm or refute this suspicion, a further 12 cadaver hearts of younger subjects were analysed to bring the total number of subjects to 33. Table A.1 details the demography and cause of death of each subject in chronological order of specimen receipt.

Table A.1

Demography and Cause of Death : Control Subjects

SUBJECT	AGE	SEX	CAUSE OF DEATH
1	62	M	hypertension
2	86	F	coronary artery disease
3	82	F	carcinoma bronchus
4	82	M	coronary artery disease
5	75	F	chronic bronchitis
6	52	M	asphyxia
7	80	F	coronary artery disease
8	42	F	drug poisoning
9	63	M	coronary artery disease
10	76	F	pulmonary embolus
11	15	F	drug poisoning
12	42	M	coronary artery disease
13	64	M	coronary artery disease
14	73	M	coronary artery disease
15	52	M	coronary artery disease
16	63	M	coronary artery disease
17	19	F	intracranial haemorrhage
18	50	M	asphyxia
19	40	F	coronary artery disease
20	70	M	coronary artery disease
21	52	F	carcinoma bronchus
22	30	M	trauma
23	26	M	intracranial haemorrhage
24	24	M	trauma
25	53	F	drug poisoning
26	19	F	trauma
27	21	F	trauma
28	36	F	trauma
29	47	M	coronary artery disease
30	27	M	trauma
31	9	M	trauma
32	57	M	coronary artery disease
33	50	M	coronary artery disease

Control group - influence of age and diagnosis

In the control population taken as a whole, mean (SD) myocardial magnesium content was 35.5 (6.5) µmol/g dry weight myocardium. This is in keeping with that reported in other published series[53,55,58,88].

The mean (SD) myocardial magnesium content for four age groupings (less than 31 years, 31-50, 51-70, greater than 70 years) are depicted in Figure A.1. A significant fall with increasing age is observed; the raw data are presented in Figure A.2. There was no sex difference when corrected for age.

Figure A.1

Mean Myocardial Magnesium Content

in Various Age Groupings in Normal Subjects

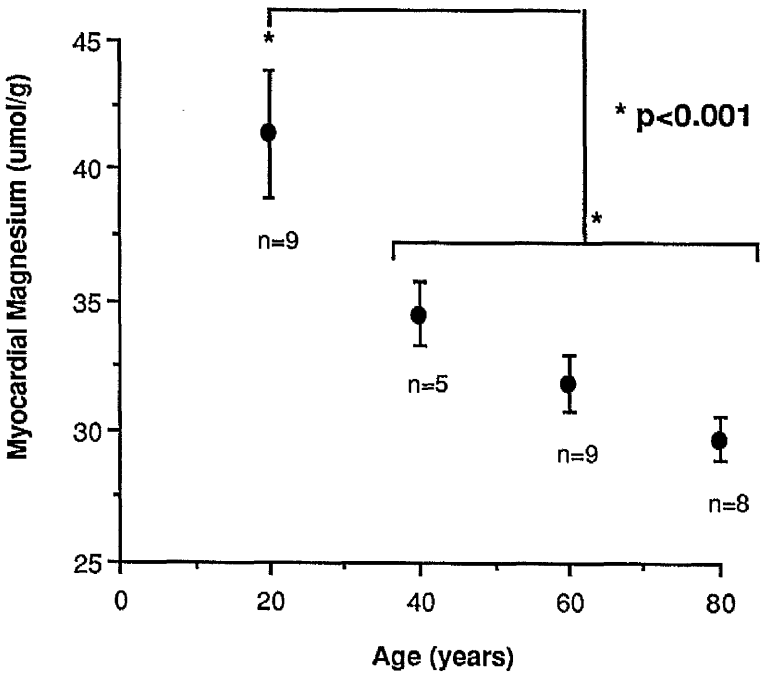
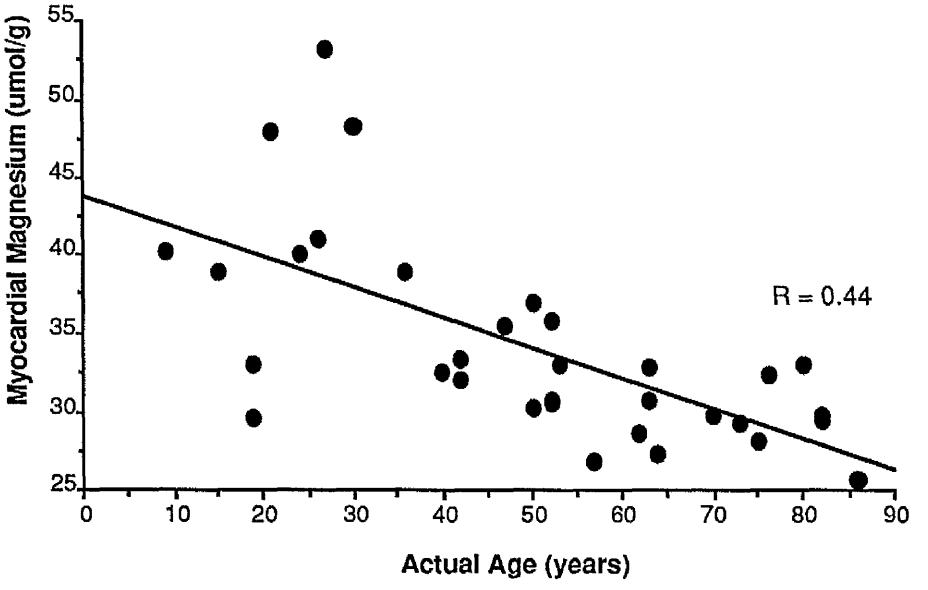


Figure A.2

The Inverse Relationship Between Myocardial Magnesium Content and Age in Normal Subjects



In the group as a whole, there was a significant difference in myocardial magnesium content between the subjects with (IHD; n=15) and without (N; n=18) ischaemic heart disease (mean (SD) IHD v N : 30.2 (2.4) v 37.4 (7.0) μmol/g; p=0.0006). When these figures were corrected for age, the difference remained significant only at the 90% level (29.8 (2.4) v 32.4 (3.3); p=0.09).

Few studies of myocardial ion content in ischaemic heart disease have used age-matched controls; in most, young trauma victims formed the bulk of the control figures. Whilst there does appear to be a reduction in myocardial magnesium content in subjects with ischaemic heart disease, the actual reduction may not be as great as has been reported.

The Derivation of Normal Range for the Transplant Study

The donor heart population is less than 60 years of age, and has no history of Ischaemic heart disease. Any subject in the main control group without these characteristics was excluded from inclusion in a subgroup that subsequently formed the Transplant Study Controls. The range of myocardial magnesium in the Transplant Study Control group was 30.5-53.2 μmol/g (n=12).

A number of biopsies taken in clinical practice are composed largely of fibrous tissue and would therefore yield an erroneously low value for myocardial magnesium. A value of 25.5 μmol/g (two standard deviations below the mean of the age-matched control population) was ascribed to the lower limit of values to be accepted as representative of myocardial magnesium; an upper limit of 53.5 was similarly devised. All values in the entire control group were within these limits.

Retrospective confirmation of normal values

The validity of the lower limit of myocardial magnesium content was confirmed by histological examination (H) of 806 consecutive biopsies obtained during the Transplant Study (Chapter 3); 603 biopsies (75%) were composed of myocardial tissue. Of 157 biopsies taken simultaneously and submitted for magnesium analysis (A), 71% fitted the prospective criteria detailed above (%H v %A; p=ns).

Summary

By attention to all the factors known to affect the quantitative result obtained from analysis of cadaver myocardium for magnesium, a normal range for the magnesium content of the right ventricular septum was established to be used for the Transplant Study described in Chapter Three. To allow for the

possibility of inadvertant biopsy of an old biopsy site etc, a lower quantitative limit was set, the validity of which was subsequently demonstrated by histological examination.

APPENDIX B

MICRO-METHOD FOR THE ANALYSIS OF MAGNESIUM IN MYOCARDIAL BIOPSIES: MODIFICATION FOR THE ANALYSIS OF CALCIUM

Introduction

From post-mortem studies of myocardial magnesium and calcium, the expected calcium content of the myocardium is 2-10 μmol/g - i.e. a molar ratio of 1:4 calcium to magnesium[52,54-56,58,86].

Chapter Two details the development of a new assay for the analysis of myocardial magnesium from single myocardial biopsies. The nature of the assay was such as to allow adaptation for the analysis of similar ions.

The magnesium content of the myocardium can be analysed by atomic absorption spectrometry (AAS) after enzymatic digestion of the biopsy specimen (as described in Chapter Two). In this method, the concentration of extracted magnesium in the supernatant after digestion (1 ml by volume) requires that the solution be diluted two-fold to render the resultant concentration of magnesium in solution within the linear range for magnesium analysis by AAS using the Perkin Elmer 1100B spectrometer (Perkin Elmer, Beaconsfield, Buckinghamshire, England). The calculated calcium concentration in the original supernatant would be 4-20 μmol/l - i.e. at the lower end of the linear range of calcium analysis by AAS (3-100 μmol/l).

Manoeuvres to Increase Sensitivity of a Modified Assay

To improve the sensitivity of calcium analysis, it was suggested that the undiluted supernatant be lyophilised and resuspended in a smaller volume of lanthanum chloride prior to analysis by AAS.

Method

Calcium solution (1M) was formulated using calcium chloride secahydrate and deionised water. Aliquots of varying dilutions of this solution were lyophilised overnight and resuspended in 5% lanthanum chloride prior to analysis for calcium by AAS. Controls solutions of known concentration were also prepared but were not lyophilised prior to quantitative analysis.

Results

Figure B.1

Mean (SD) Calcium Content of Lyophilised and Control Solutions

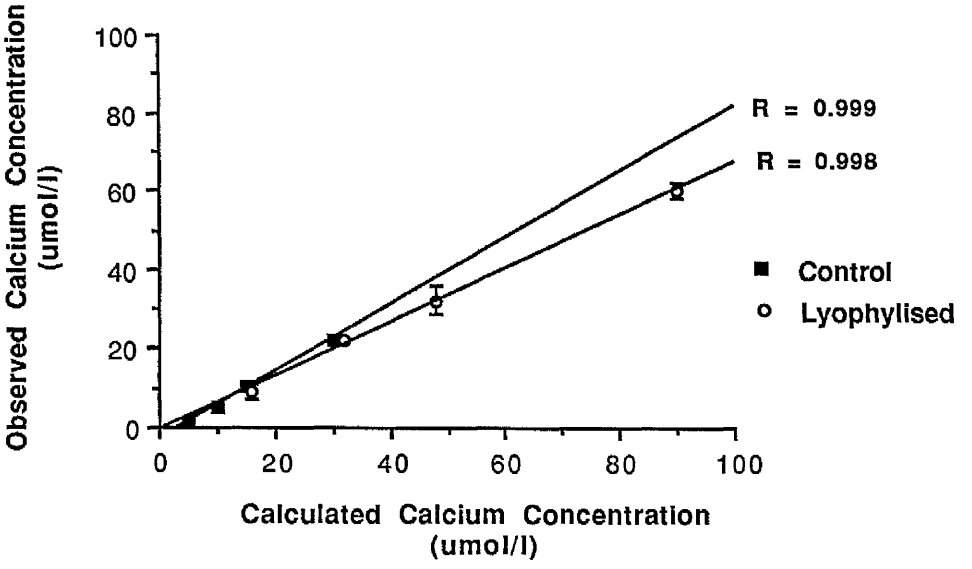


Figure B.1 depicts the actual calcium concentration against that expected for samples analysed with and without prior lyophilisation; it can be seen that although lyophilisation yielded slightly lower values for calcium analysis, a linear relationship is maintained throughout, particularly at the lower calcium concentrations expected in clinical practice (correlation coefficient of 0.998).

The experiment was repeated using a calcium solution formulated with pepsin solution (0.1g/l) rather than deionised water. Unfortunately, although the non-lyophilised samples produced reliable results, those from the lyophilised samples were grossly inaccurate.

Lyophilisation of pepsin solution forms a fine "snow" with significant static charge which coats the receptacle and more importantly, the receptacle cover. Despite modification of the apparatus, complete resuspension of the lyophisillate was technically impossible in a substantial minority of cases.

Conclusion

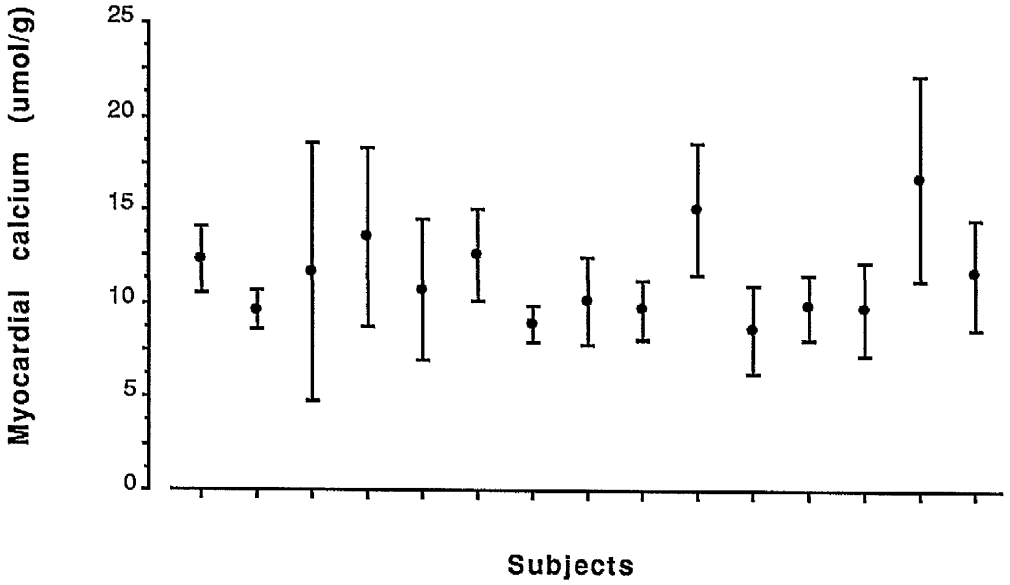
It was concluded that the original methodology should be modified such that only 0.5ml of pepsin solution (rather than 1ml) be added to the biopsy sample, should calcium analysis be planned, in order to maximise the concentration of calcium. This would not effect the measurement of magnesium.

The Control Range for Myocardial Calcium

Measurement of myocardial calcium

Using 15 fresh cadaver hearts, samples of interventricular septum were analysed using the modified technique described above, producing a range of 6-20 µmol/g dry weight of myocardium (median 12). The coefficients of variation ranged from 10-59% (median 25%) with a median of three biopsies per subject (range 3-6) as demonstrated in Figure B.2 At first sight, the coefficients of variation are very high; the explanation for this has not been fully established, but in part relates to the small amounts of calcium present. If these results are multiplied 10 fold i.e to bring them in to the range of the magnesium assay, the calculated coefficients of variation are comparable.

Figure B.2
Mean(SD) of Myocardial Calcium Analysis of Cadaver Hearts



Sensitivity of the Modified Assay

Control v Transplant Study

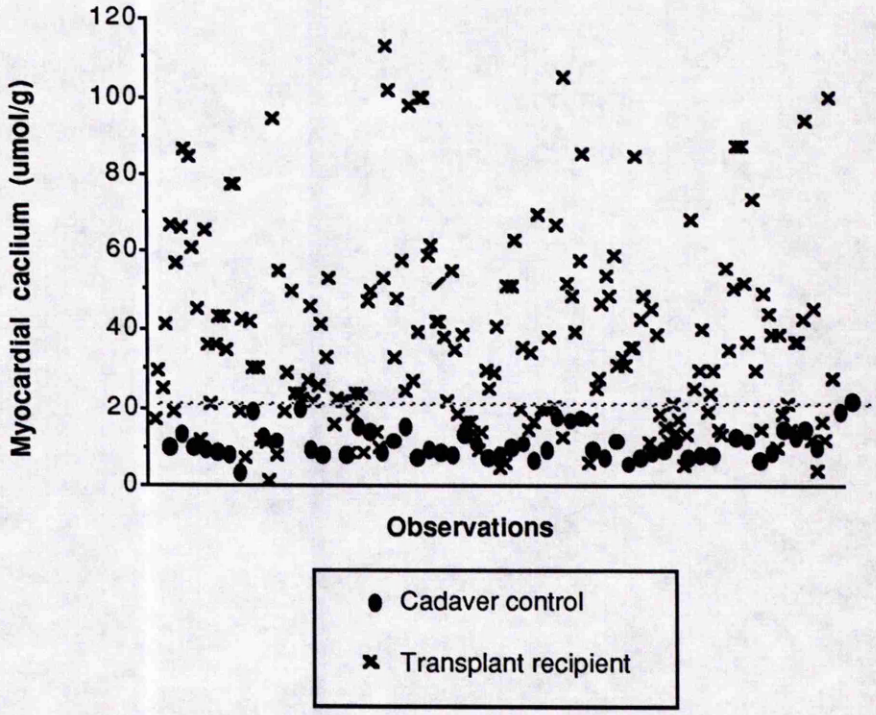
The range of myocardial calcium estimations from the early part of the Transplant study were in general much higher than expected, although a small number of results did fall within the control range described above. If the results from the cadaver study are expressed graphically on the scale of the results from the Transplant Study (Figure B.3), it can be seen that although there is considerable numerical variation in the cadaver group, the discriminatory power of the assay (ie between high and low calcium content) is maintained.

Myocardial Calcium and Magnesium - Control Group

As predicted from the literature studies, myocardial calcium in the control group was inversely related to myocardial magnesium when myocardial magnesium content was low (Figure B.4). Myocardial magnesium content was

reduced (<30.5 μmol/g) in 8 cadaver hearts (group D) as compared with the remainder (group N); calcium content was significantly higher in the magnesium deplete group ($p<0.005$; Figure B.5).

Figure B.3
Myocardial Calcium Analysis in the Cadaver Control Group and in the Transplant Recipients



These results are wholly in keeping with those reported in the literature and support the ability of the assay to discriminate between normal and abnormal myocardial calcium content.

Figure B.4

The Rise in Myocardial Calcium in Magnesium Deficiency:

Cadaver Control Group

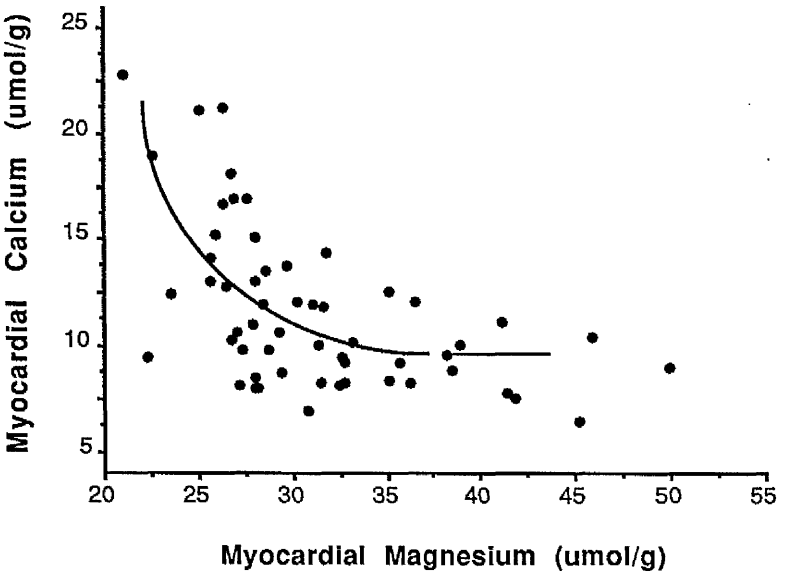
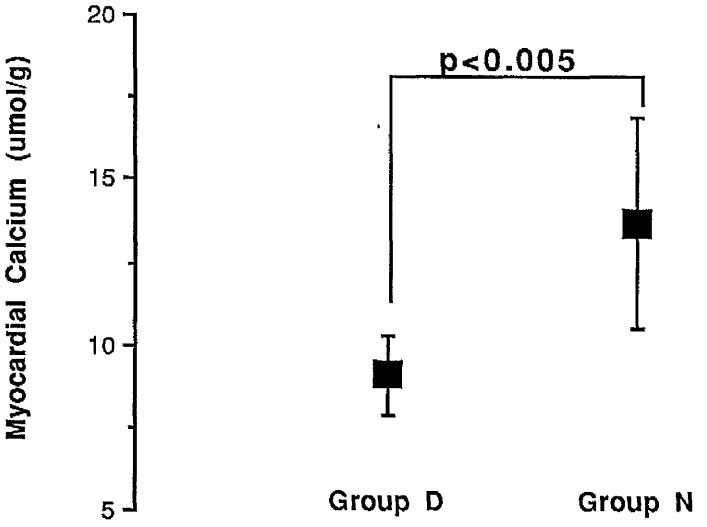


Figure B.5

Higher Myocardial Calcium Content in Magnesium-Deplete Subjects (D)

Compared with Normal Controls(N)



Summary

Modification of the validated magnesium assay described in Chapter Two for calcium analysis was performed by analysis of cadaver hearts using a minor modification of the original technique invoked to improve the sensitivity of the technique. There was excellent correlation with values reported in the literature.The assay was capable of discriminating between normal and raised myocardial calcium content and of detecting the rise in myocardial calcium content that occurs in magnesium-deplete hearts.

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