# Chronic Electrophysiological changes in Adriamycin Cardiomyopathy in the rabbit.

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## ABSTRACT

Sudden cardiac death accounts for 35-45% of all deaths in heart failure, presumably from malignant ventricular arrhythmias. The aetiology of sudden arrhythmic death has not been fully elucidated, although a number of mechanisms exist which could potentially be arrhythmogenic. These include activation of the renin-angiotensin system, an increase in sympathetic nervous activity, electrolyte depletion and the use of inotropes and diuretics. It is likely however, that these mechanisms are secondary to the underlying electrophysiological changes in the myocardium resulting from the processes of myocyte damage and necrosis, compensatory hypertrophy and fibrosis which occur in the failing heart. In order to understand the basic mechanisms responsible for sudden death in heart failure, there is a need for animal models in which the electrophysiological changes produced by myocardial damage and the interrelationship between the myocardial electrophysiological substrate, neurohormonal activity and inducibility of arrhythmias can be studied in detail.

In this study cardiac failure was induced in rabbits by injection of the cardiotoxin adriamycin 1mg/Kg twice weekly for 8 weeks. Electrophysiological recordings were made in conscious animals using bipolar pacing electrodes,

implanted in the right ventricular apex. Recordings were made of the effective refractory period and the Stimulus-T interval of the paced evoked response. Neurohormonal assays and measurement of plasma electrolytes were undertaken durimg the study, and pathological analysis when the animals died.

Progressive shortening in the stimulus-T interval and effective refractory period occurred in the adriamycin treated animals. No significant changes were seen in controls.

Right ventricular papillary muscles from uninstrumented animals were used for in vitro electrophysiological studies.

Action potential duration and effective refractory period were significantly shorter in the adriamycin group compared to controls.

The changes in these electrophysiological parameters were independent of changes in plasma electrolytes and neurohormones. Captopril did not reverse the electrophysiological changes, but did cause a reduction in heart and liver weight, and ventricular ectopic activity.

A cohort of animals treated with adriamycin and frusemide did not have a progressive shortening in effective refractory period and stimulus-T interval, and this questions the reproducibility of the electrophysiological changes in the study.

# CHAPTER 1

# INTRODUCTION AND HISTORICAL REVIEW

## 1.1 Prognosis in congestive heart failure.

Management of patients with congestive cardiac failure has tended to concentrate on alleviation of symptoms associated with this condition. Consequently, research undertaken in this area has mainly focussed on the pathophysiological mechanisms which are associated with the haemodynamic abnormalities found in patients with heart failure. Although death from progressive ventricular dysfunction is a significant feature of the condition, sudden unexpected death, presumably from ventricular tachyarrhythmias, accounts for 35% - 45% of all deaths in heart failure (Burggraf, G.W. & Parker, J.O.(1975); Lee, W. & Packer, M.(1984); Cohn, J.N. et al(1984)). Although some authorities differ in their viewpoint, a useful working definition of sudden death is death from circulatory failure within 1 hour of the onset of symptoms in a patient with advanced left ventricular dysfunction whose heart failure symptoms have remained stable or improved over the previous 2 to 4 weeks and in whom another cause for circulatory collapse cannot be identified clinically (Packer, M. (1985)). Excluded from such a diagnosis therfore are the 10% - 15% of patients

whose deaths follow the typical presentation of acute myocardial infarction or pulmonary embolus. As a result of research undertaken into haemodynamic abnormalities associated with heart failure, clinicians are now able to improve cardiac performance by pharmacological means. Subsequently, it is likely that sudden unexpected death from ventricular tachyarrhythmias will become the most frequent cause of death in patients with congestive heart failure.

## 1.2 Relationship of ventricular arrhythmias to prognosis in congestive heart failure.

To identify a subset of patients with heart failure who are at risk for subsequent sudden unexpected death, we must first try to understand the pathophysiological mechanisms involved. Sudden unexpected death in heart failure is most likely the result of a terminal ventricular arrhythmia, either ventricular tachycardia or ventricular fibrillation. 24 hour ambulatory monitoring studies indicate that 60% - 90% of patients with congestive cardiac failure have frequent or complex ventricular ectopic beats, and that 40%-60% of patients have spontaneous non-sustained ventricular tachycardia (Huang, S.K., Masser, J.V. & Denes, P.(1983) ; Meinertz, T. et al(1984); Wilson, J.R. et al(1983); Holmes, J. et

al(1985); Cleland, J.G.F., Dargie, H.J. & Ford, I.(1987); Von Olshausen, K. et al(1984)). (table 1). Data from these studies would suggest that although the occurence of ventricular tachycardia would seem to predict total mortality (Huang, S.K. et al(1983); Meinertz, T. et al(1984); Wilson, J.R. et al(1983) and Holmes, J. et al (1985)), the relationship between pre-existing spontaneous ventricular tachycardia and sudden death is not so well defined. This suggests that the presence of such malignant ventricular ectopic activity merely reflects the severity of the functional and haemodynamic abnormalities which are present, and is not an independent risk factor for sudden death. Although Meinertz, T. et al(1984) did show a relationship between the frequency of ventricular tachycardia and sudden death, the extremely high arrhythmia rate, > 20 episodes of Ventricular tachycardia in 24 hours, proposed by the authors for the identification of high risk patients is extremely rare in patients with congestive heart failure. Huang, S.K. et al(1983); Wilson, J.R. et al(1983); Holmes, J. et al(1985); Cleland, J.G.F. et al(1987) and Von Olshaushen, K. et al(1984), found like Meinertz, T. et al(1984) no relationship between the occurence of ventricular tachycardia and subsequent sudden death, (table 1). Current evidence therefore suggests that spontaneous ventricular tachycardia does not necessarily precede

Table 1.

Relationship of ventricular tachycardia to sudden death in heart failure.

| Study | Number | Age | FU | Aetiology | %VT | %SD | Relation |
|---|---|---|---|---|---|---|---|
| Huang | 35 | 51±12 | 34±17 | IDC | 60 | 50 | No |
| Meinertz | 74 | 51±10 | 11±3 | IDC | 49 | 63 | Frequency |
| Wilson | 77 | 61±11 | 12±11 | CAD:52%<br>IDC:36%<br>VHD:12% | 51 | 43 | No |
| Von Olshausen | 60 | 45±9 | 12±5 | IDC | 42 | 43 | No |
| Cleland | 152 | 59±9 | 21±12 | CAD:61%<br>IDC:16%<br>VHD:10%<br>HBP:6% | 61 | 75 | No |
| Holmes | 31 | 56 | 14 | CAD:52%<br>IDC:48% | 39 | 86 | No |
| Unverf. | 69 | 45 | 12 | IDC | 25 | 92 | No |

IDC=Idiopathic dilated cardiomyopathy.

CAD=Coronary artery disease.

VHD=Valvular heart disease.

HBP=High blood pressure.

VT=Ventricular tachycardia.

FU=Follow-up.

SD=Sudden death. Relation=Relation of VT to sudden death.

sudden unexpected death, and that its presence on 24 hour ECG monitoring does not identify patients who are at high risk and require medical intervention. Although the current evidence does not permit any association to be made between ventricular tachycardia and sudden arrhythmic death, this does not necessarily mean that the relationship does not exist. One reason for this could be inherent problems with the design of the studies which have addressed this problem. If we analyse the studies cited above it is possible to detect certain areas which could lead to problems with interpretation of the data. Firstly, the number of patients in each study was small. The largest study group was only 152 patients (Cleland, J.G.F. et al(1987)). None of the other studies included more than 77 patients. Small numbers of patients could make it difficult to detect any significant relationship between spontaneous ventricular tachycardia and sudden death. Secondly, the mean duration of follow-up in each of the studies was short. The studies by Meinertz, T. et al(1984); Wilson, J.R. et al(1983); Von Olshausen, K. et al(1984) and Holmes, J. et al(1985) had a mean duration of follow-up which was less than 15 months, and only the studies by Cleland, J.G.F. et al(1987) and Huang, S.K. et al(1983) were of longer duration, 21 and 34 months respectively. If the patients in these studies had been followed for a greater period of time, a relationship

between ventricular tachycardia and sudden death might have been exposed.

Another important observation is that the incidence of sudden death does not increase with increasing severity of heart failure, as defined by progressively more limited functional class and greater 1 year total mortality (Burgraff, G.W. and Parker, J.O.(1975) ; Massie, B. et al(1981); Cohn, J.N. et al(1984) and Wilson, J.R. et al(1983)). The fact that patients with more severe heart failure and greater impairment of left ventricular function, who theoretically at least would have a greater degree of activation of both the sympathetic and renin-angiotensin systems, are not at a higher risk of sudden death would tend to exclude these potentially arrhythmogenic mechanisms as a cause of sudden death. One possible reason why no relationship has been identified between sudden death and the severity of heart failure is that the clinical picture in patients with severe heart failure is so dominated by marked dyspnoea at rest, that the onset of a terminal ventricular tachyarrhythmia could be difficult to identify clinically.

The failure to identify patients who are at high risk of sudden death on the basis of pre-existing spontaneous ventricular tachycardia and the degree of left ventricular impairment, leads us to examine other potentially arrhythmogenic mechanisms.

## 1.3 The role of structural factors in arrhythmogenesis in congestive heart failure.

Previous reports have validly emphasised the importance of structural factors such as myocardial fibrosis in predisposing to the re-entry phenomena that are critical to the development of ventricular ectopic rhythms (El-Sherif, N. et al(1983) and El-Sherif, N., Sherlag, B.J & Lazzara, R.(1975)). Re-entrant phenomena involve a disturbance of impulse conduction and require two electrophysiologically distinct pathways, with unidirectional block in one and slowed conduction in the other. Under these conditions, the impulse blocked in the first pathway may be conducted sufficiently slowly down the second such that it reaches the distal point of the first pathway at a time when it is no longer refractory, thus permitting reciprocation of the impulse and the establishment of a sustained arrhythmia. This mechanism is considered to account for the majority of chronic recurrent ventricular tachyarrhythmias particularly in patients with previous myocardial infarction where the necessary electrophysiological substrate can be provided by the presence of ischaemically damaged or infarcted tissue.

## 1.4 The clinical role of ventricular wall stress in causing cardiac arrhythmias.

Excessive ventricular wall stress has also been implicated as a potential mechanism predisposing to sudden death in heart failure. Changes in myocardial fibre stretch mediated by changes in ventricular pressures and volumes may alter the electrophysiological properties of the left ventricle and predispose to the occurence of serious ventricular arrhythmias (Bigger, J.T. Jr(1973)). Such changes may be greatly exaggerated in areas of ventricular asynergy or aneurysm formation, which frequently contain the initiating site of re-entry circuits that lead to the development of sustained ventricular rhythm disturbances (Cohn, M., Packer, M. & Gorlin, R.(1983)). There is little evidence however that interventions directed at reducing ventricular wall stress can effectively reduce the frequency or complexity of ventricular arrhythmias. One study has shown however that the long term administration of oral hydralazine and oral isosorbide dinitrate significantly reduced mortality when compared with placebo in patients with heart failure,although no evidence is available as yet with regard to any reduction in ventricular arrhythmias or sudden death (Cohn, J.N., Archibald, D.G. & Ziesche, S.(1986)). The Consensus Trial Study Group 1987, examined the effect of the angiotensin

converting enzyme inhibitor enalapril, on mortality in patients with severe heart failure. Although overall mortality was reduced, there was no reduction in the incidence of sudden arrhythmic death.

A more recent study which investigated the effect of enalapril on mortality in heart failure reached similar conclusions to the Consensus study. The authors found that enalapril reduced overall mortality in patients with heart failure when compared to placebo, although there was no reduction in the incidence of sudden death (The SOLVD Investigators(1991)).

In contrast to this study, Cohn, J.N. et al(1991), found that enalapril significantly reduced the incidence of sudden death in patients with heart failure in a trial comparing enalapril with the combination of hydralazine and isosorbide dinitrate.

## 1.5 Experimental electrophysiological changes in relation to myocardial hypertrophy and failure, which may be arrhythmogenic.

A number of studies have attempted to assess the relationship between ventricular wall stress and the potential for cardiac arrhythmias. Gelband, H. & Basset, A.L.(1973) described the in vitro electrophysiological changes in the right ventricular papillary muscles from cats with chronic partial pulmonary arterial occlusion who developed right ventricular hypertrophy and thence right heart failure. The authors found that compared to control animals, the average resting potential and the action potential overshoot were significantly decreased in the animals with heart failure. The rate of rise of the action potential was also markedly reduced by 24%. The authors also reported that action potential duration at 100% repolarisation was significantly increased when compared to controls. The same authors have also described the effects of chronic partial occlusion of the pulmonary artery and subsequent right ventricular hypertrophy, on cardiac electrophysiology and found no significant changes in action potential amplitude, rate of rise and duration (Basset, A.L. & Gelband, H.(1973)). This model has certain limitations. Firstly, no increase in heart rate was observed as the cats developed heart

failure. This differs from the clinical situation where a reflex-mediated increase in heart rate is commonly observed as a compensatory mechanism to maintain cardiac output as the contractile function of the heart begins to fail. A second limitation of this particular model is that 3 of the cats in the study with apparently overt right ventricular failure had relatively normal electromechanical properties with respect to the right ventricular papillary muscles. Another problem with the study was that the definition of heart failure was made initially on clinical grounds which were rather arbitrary and then only subsequently by a marked elevation in right ventricular end diastolic pressure. Large variations were also present in the degree of pulmonary banding which was used within the group of animals which subsequently developed heart failure, and no assessment was made of the intrinsic contractile state prior to pulmonary artery banding.

More recent studies have examined the effect of acute increases in ventricular volume on the electrophysiological properties of the isolated perfused rabbit heart (Reiter, M.J., Synhorst, D.P. & Mann, D.E.(1988)). The hypothesis proposed in this study was that one potential cause of ventricular tachyarrhythmias in heart failure in the clinical setting is ventricular dilatation. As ventricular volume was increased by

inflating a balloon in the left ventricle, a progressive decrease in left ventricular effective refractory period was noted. The change in refractory period was unrelated to changes in pacing threshold. No biochemical evidence of ischaemia was found which could account for the shortening in refractory period. Another important feature of this model is that the acute dilatation of the balloon was associated with a marked heterogeneity of refractoriness, both intra and interventricular. Heterogeneity of refractoriness is known to predispose to re-entrant ventricular tachyarrhythmias and indeed in this model, ventricular tachycardia and fibrillation were both frequently induced by the use of the single extra-stimulus technique in measuring effective refractory period.
The effects of mechanical stress on the electrophysiology of isolated muscle preparations has also been studied by Lab, M.J.(1982). The author observed that changes in muscle length and tension may result in changes in resting membrane potential, action potential duration and amplitude. High muscle tension in the form of isovolumetric contraction is associated with a shortening in action potential duration in both isolated muscle preparations and the intact frog heart. This process whereby changes in mechanical function may precede and alter action potential morphology, and therefore result in

cardiac arrhythnias is known as contraction-excitation feedback.

Another study in isolated, perfused canine ventricles, in dogs with chronic myocardial infarction, investigated the effects of volume load on ventricular refractoriness (Calkins, H. et al(1989)). It was found that increasing volume load resulted in a significant reduction in the absolute refractory period, in areas of control myocardium. A greater reduction was seen however, in both the absolute and relative refractory periods in areas of infarcted myocardium. It was also demonstrated in this study that the induction of tachyarrhythmias was associated with conditions of high volume load and reduction in refractoriness.

A number of clinical studies have also demonstrated the relationship between changes in myocardial stress and the electrical properties of the heart.

Ford, E.L. & Campbell, N.P.(1980) demonstrated that a reduction in afterload induced by the vasodilator amylnitrate, resulted in prolongation of the surface QT interval in normal volunteers.

Taggart, P., Sutton, P.M.I., Treasure, T. et al(1988), have shown that action potential duration is reduced in patients withdrawn from cardio-pulmonary bypass as myocardial wall stress is increased.

Further clinical evidence for contraction-excitation feedback is found in a study which demonstrated changes in action potential duration in patients undergoing balloon valvuloplasty for congenital pulmonary stenosis (Levine, J.H.(1988)).

Other workers have investigated the electrophysiological changes associated with the cardiomyopathies which occur in a number of animal species. "Round Heart Disease" in turkeys is a naturally occuring cardiomyopathy model (Einzig, S. et al(1980)). The aetiology of the cardiomyopathy is thought to be viral in origin as C-type viral particles have been observed in myocardial cells from turkeys by electronmicroscopy (Einzig, S., Jankus, E.F. & Moller, J.H.(1972)). The natural disease process progresses to a cardiomyopathy which is similar in its end-stage to endocardial fibroelastosis (Einzig, S. et al(1972)). The cardiomyopathy is associated with arrhythmias and also it has been noted that sudden death occurs frequently. Electrophysiological studies in the work by Einzig, S. et al (1981) were performed on ventricular endocardial muscle cells from both the right and left ventricles, in myopathic and non-myopathic animals. The authors found that action potential duration was significantly reduced at 50% repolarisation in cells from both ventricles and at 90% repolarisation in cells

When the animals were subdivided on the basis of ventricular weight/body weight, the maximum upstroke velocity was also reduced in animals with ventricular hypertrophy. One possible mechanism for the shortening which the authors demonstrated in action potential duration was thought to be an increase in intracellular calcium concentration. This could result from disruption of the sarcolemmal membrane structure during viral infection. It has been previously demonstrated that an increase in intracellular calcium concentration, independent of the aetiology, shortens the action potential duration (Carmeliet, E.(1978); McDonald, T.F. & McLeod, D.P.(1973); Isenberg, G.(1975); Reuter, H. (1974)). The mechanism by which an increase in intracellular calcium concentration shortens action potential duration may be mediated by inhibition of the slow inward calcium current or by an increase in potassium conductance. Although the principal finding in the study by Einzig, S. et al(1981) was of a shortening in action potential duration, most experimental evidence would suggest that ventricular hypertrophy is associated with prolongation of the action potential duration.
Rossner, K.L. & Sachs, H.G.(1978) investigated the electrophysiological changes associated with Syrian hamster cardiomyopathy. Bajusz, E. et al(1969), first described a mutant strain of Syrian hamster which

spontaneously developed a cardiomyopathy which was similar in many respects to human cardiomyopathies. The cardiomyopathy is transmitted by an autosomal recessive mechanism. The initial myopathic process is manifest as small focal lesions which heal with subsequent compensatory hypertrophy. The animals eventually die from cardiac failure although the clinical picture varies with some animals developing gross generalised oedema and others apparently unaffected. Electrophysiological experiments in the study by Rossner, K.L. & Sachs, H.G.(1978) were performed on isolated left ventricular papillary muscles from the myopathic hamster hearts. Action potential duration was found to be significantly increased in cardiomyopathic hamsters when compared to controls at 20%, 50% and 95% repolarisation. Action potential overshoot was also found to be significantly greater when compared to controls. The electrophysiological data expressed in this study is not consistent with some aspects of the findings of other workers who have studied cardiac muscle in either spontaneous or induced ventricular hypertrophy. Coltart, D.J. & Meldrum, S.J.(1970), have shown that left ventricular obstructive cardiomyopathy in man is associated with a decrease in upstroke velocity as well as a prolongation of action potential duration. Tritthart, H. et al(1975) also found a decrease in upstroke velocity

following the induction of right ventricular hypertrophy in cats. Basset, A.L. & Gelband, H.(1973) found no such change in a similar study in cats. The fact that the upstroke velocity was not reduced in the study by Rossner, K.L. & Sachs, H.G.(1978) probably reflects an unaltered sodium channel. The mechanism by which action potential duration is prolonged in cells from the cardiomyopathic hamsters may be related to either an enhanced slow inward current and/or a decreased late outward current. Previous studies have suggested that isotopic calcium uptake by left ventricular myocytes from a related strain of myopathic hamsters is increased when compared to controls. This may suggest that there is an increased trans-sarcolemmal calcium ion flux associated with the cardiomyopathy (Lossnitzer, K. et al(1975)). This would be consistent with an increased slow inward calcium current, and would be likely to result in a prolongation of the action potential duration (Zipes, D.P., Besch, H.R. Jr. & Watanabe, A.M.(1975)).

Uhley, H.N.(1961) measured action potential amplitude in transmembrane potentials recorded from myocytes in hypertensive rats. No change was observed in this parameter in animals who had been hypertensive for 5-8 months. Studies performed in spontaneously and renal hypertensive rats with ventricular hypertrophy, have reported increased action potential durations with no

other abnormalities in action potential morphology (Gulch, R.W., Baumann, R. & Jacob, R.(1979); Aronson, R.S.(1980)). The apparent heterogeneity in animal models of cardiomyopathy may be the result of 2 processes. Firstly, a cytopathic effect which could result in shortening of action potential duration, and secondly compensatory hypertrophy which in most models results in action potential prolongation.

## 1.6 Clinical and experimental effects of potassium depletion on cardiac electrophysiology.

Although structural factors are undoubtably important in the genesis of ventricular arrythmias and sudden death, more recently 3 potentially reversible mechanisms have been proposed. These include electrolyte depletion, activation of neurohormonal mechanisms and drug therapy in heart failure.

Patients with congestive heart failure have marked deficits of total body and intracellular potassium, which may or may not be reflected by a measurable decrease in serum potassium concentration. The administration of diuretic drugs may further deplete body stores of potassium (as well as magnesium) by promoting the renal excretion of these predominantly intracellular cations, an effect potentiated by co-existing hyperaldosteronism and

metabolic alkalosis. Furthermore, the high levels of circulating catecholamines in patients with heart failure may enhance the movement of potassium into cells by a $beta_2$-receptor mediated mechanism, thereby exacerbating the hypokalaemic state and potentiating its arrythmogenic effects (Brown, M.J., Brown, D.C. & Murphy, M.B.(1983)). Recent studies indicate that malignant ventricular ectopic rhythms in some patients with congestive heart failure may entirely be the result of potassium and magnesium depletion. Both short and long term repletion may abolish these arrhythmias and reduce the risk of sudden death without long-term antiarrhythmic drug therapy (Bertuso, J.R. et al(1984); Chadda, K., Ballas, M. & Bodenheimer, M.M. (1984)).

Potassium and magnesium deficits commonly coexist and under such circumstances, repletion of magnesium appears to be critical to the success of treatment. Therapy with potassium salts alone may fail to restore normal serum levels of potassium or normal sinus rhythm, whereas magnesium administration corrects not only the hypomagnesaemia and hypokalaemia but abolishes the accompaning tachyarrhythmias as well (Chadda, K. et al (1984)).

Having described hypokalaemia as being important in the genesis of ventricular arrhythmias, it is important to assess the effects of potassium depletion on isolated

cardiac tissue and changes in action potential morphology which may predispose to cardiac arrhythmias.

Abnormalities of potassium homeostasis can affect the electrophysiology of cardiac cells in a number of different ways. Effects have been noted previously on depolarisation, repolarisation and also on normal pacemaker activity in a number of tissues, and these effects may potentially be arrhythmogenic. The effect on the resting membrane potential of lowering the extracellular potassium concentration in isolated muscle preparations, is to make the potential more negative (Vassalle, M.(1965); Gettes, L.S., Surawicz, B. & Shiue, J.C. (1962)). This hyperpolarisation may negate the effect of antiarrhythmic drugs which inhibit the inward sodium current during depolarisation.

Another mechanism whereby lowering extracellular potassium concentration may be arrhythmogenic, is by causing prolongation of action potential duration. This effect has been previously described (Surawicz, B. et al(1959); Roden, D.M. & Hoffman, B.F.(1985); Gettes, L.S. et al(1962); Vassalle, M.(1965)). The mechanism whereby hypokalaemia-induced prolongation of the action potential duration may be arrhythmogenic, is that ventricular ectopic beats, arising during the terminal portions of prolonged action potentials will have a reduced sodium current and will therefore be propagated slowly. This

slow conduction may predispose to re-entrant tachyarrhythmias. These changes may be particularly important in arrhythmogenesis when hypokalaemia coexists in patients with bradycardias, and also with antiarrhythmic drugs that prolong action potential duration such as quinidine (Roden, D.M. et al(1986)).
A lowered extracellular potassium concentration also increases the slope of phase 4 in the Purkinje network, thereby causing or increasing the rate of normal automaticity (Gettes, L. & Surawicz, B.(1968); Gettes, L.S. et al(1962); Vassalle, M.(1965)). Increased automaticity is one of the mechanisms predisposing to ventricular tachyarrhythmias.

The major electrocardioghraphic features of hypokalaemia are a decrease in T-wave amplitude and an increase in U wave amplitude, with the eventual development of marked QT prolongation (Surawicz, B.(1967)).
Hypokalaemia may also predispose to arrhythmias by interacting with a number of cardioactive drugs. In particular effects have been demonstrated with the cardiac glycosides and also with class 1 antiarrhythmic agents. One possible mechanism by which hypokalaemia may predispose to digoxin toxicity is by causing an increase in cardiac binding of the drug. Cohn, K.E., Kleiger, R.E. & Harrison, D.C.(1967) demonstrated that in mice who were

made hypokalaemic by a low potassium diet, increased concentrations of cardiac digoxin were found when compared to controls. The effect of the interaction between hypokalaemia and drugs such as quinidine which not only block sodium channels but prolong action potential duration has also been well described and may lead to the ventricular tachyarrhythmia Torsades de pointes in 0.5% to 7% of patients in whom quinidine is prescribed (Roden, D.M., Woosley, R.L. & Primm, R.K.(1986)).

## 1.7 Clinical and experimental effects of magnesium depletion on cardiac electrophsiology.

Electrophysiological effects of changes in extracellular magnesium levels on isolated cardiac tissue, have been studied by a number of authors. Watanabe, Y. & Dreifus, L.S.(1972) conducted experiments on isolated, perfused rabbit hearts. They found that lowering the extracellular magnesium concentration resulted in a reduction in the effective refractory period, action potential amplitude, membrane resting potential and the maximal rate of depolarisation. The effects of increasing the magnesium concentration on the other hand produced the opposite electrophysiological effects. Data from the above study also demonstrated that these effects were maximal in the

presence of high extracellular potassium concentrations, and reduced in the presence of low potassium concentrations.

Other workers have found conflicting results however. Roden, D.M. & Iansmith, D.H.S.(1987) found no significant changes in action potential morphology when magnesium alone was removed from the perfusate of canine Purkinje fibres. The authors did find that when both extracellular calcium and potassium levels were reduced, significant changes did occur. These included membrane arrest, multiple early afterdepolarisations and delayed afterdepolarisations.

Other workers have assessed the direct chronotropic effect of changes in magnesium concentration on the cells in the canine sinus node. Woods, W.T. et al(1979) described an increase in sinus rate on removing magnesium from the experimental perfusate, and increasing magnesium resulted in a decrease in the sinus rate. The ability of a low magnesium concentration to result in a tachycardia is one possible explanation for the proarrhythmic effect clinically of hypomagnesaemia.

In addition to the in vitro data there is clinical evidence that changes in plasma magnesium concentration may predispose to cardiac arrhythmias.

Studies have shown that a reduction in plasma magnesium concentration is associated with an increase in ventricular ectopic activity. The standard use of thiazide diuretics in the treatment of hypertension, and the ability of these diuretics to lower magnesium levels (Hollifield, J.W.(1986)), has led to a number of studies documenting the relationship between hypomagnesaemia and ventricular ectopic activity (Hollifield, J.W.(1984); Hollifield, J.W.(1986)).

In addition to magnesium depletion resulting in an increase in ventricular ectopy, other studies have demonstrated that normalisation of hypomagnesaemia may terminate ventricular tachyarrhythmias. Ramee, S.T. et al(1985) described 2 cases of patients with Torsades de pointes associated with hypomagnesaemia, whose arrhythmias were abolished by repletion of plasma magnesium. Chadda, K. et al(1984) also described the efficacy of magnesium replacement in the treatment of ventricular arrhythmias in patients with hypomagnesaemia.

Another study which examined the relationship between plasma magnesium levels and atrial fibrillation found that patients with hypomagnesaemia required significantly greater amounts of intravenous digoxin to control the ventricular rate when compared to patients with normal magnesium levels (De Carli, C., Sprouse G. & LaRosa, J.C.(1986)).

Considerable evidence therefore exists both in vivo and in vitro that magnesium depletion is associated with cardiac arrhythmias, and that magnesium repletion may result in the restoration of sinus rhythm.

## 1.8 The potential arrhythmogenic effect of neurohormonal mechanisms in congestive heart failure.

The clinical syndrome of congestive heart failure is associated with activation of a number of homeostatic mechanisms which modify cardiac output in order to maintain blood pressure and perfusion of vital organs. One of the most important of these mechanisms is the renin-angiotensin system. The decrease in cardiac output and the resultant increase in sympathetic tone which accompany heart failure result in a reduction in renal perfusion. This in turn results in an increase in renin secretion by the macula densa in the kidney. Circulating renin converts angiotensinogen to angiotensin I which in turn is converted to angiotensin II by angiotensin converting enzyme. Others factors such as the use of diuretics may also contribute to activation of the renin-angiotensin system by sodium depletion (Brown, J.J. et al(1970)). One of the effects of this increase in angiotensin II is to stimulate secretion of aldosterone which results in an increase in sodium reabsorption in the

kidney and a concomitant increase in potassium excretion. The effect of the increase in sodium reabsorption is to expand the vascular volume in an attempt to maintain tissue perfusion. Angiotensin II is also a potent vasoconstrictor and in this capacity causes an increase in systemic vascular resistance and blood pressure. Another major effect of angiotensin II is to act directly on the efferent renal arteriole to increase glomerular filtration pressure and solute clearance (Packer, M., Lee, W.H. & Kessler, P.D.(1986)). The argument as to which of these functions of angiotensin II is the most important is probably unresolved.

In a dissertation on the subject Packer, M.(1987) eloquently discusses the problem. The premise which is proposed by Packer is that the prime function of activation of the renin-angiotensin system is to maintain renal function.

It has previously been held that in two different clinical situations where cardiac output and blood pressure is reduced, namely hypovolaemia and heart failure, the renin-angiotensin system is activated in order to maintain blood pressure. The author states that the body is able to differentiate between the two situations however. In the former, atrial natriuretic peptide(ANP) secretion is decreased while in heart failure it is increased. The effect of ANP is to reduce the release of renin from the

kidney and negate the effect of angiotensin II on the peripheral vasculature (Kleinert, H.D. et al(1984); DeLean, A. et al(1984)). If the primary function of angiotensin II in heart failure was the maintanance of blood pressure then it would seem unlikely that the actions of ANP and angiotensin II would be antagonistic. Another potent argument against its role in the preservation of circulatory volume is the site of action of angiotensin II in the kidney. If volume preservation were its prime function, then it would be more logical that the hormone would act on the afferent arteriole and thus reduce sodium excretion, rather than cause constriction of the efferent arteriole, the sole purpose of which is to preserve renal function.

Whether the primary function of the renin-angiotensin system is to maintain circulatory homeostasis or preserve renal function, there is no doubt that this neuroendocrine system is activated in patients with heart failure. Despite the obvious advantages to the circulation, activation of this system may be potentially arrhythmogenic.

The systemic vasoconstriction caused by angiotensin II limits cardiac function by increasing afterload (Curtiss, C. et al(1978)). Furthermore the increase in sodium and water retention which results from the increase in circulating aldosterone levels results in an increase in

ventricular wall stress and myocardial oxygen consumption (Massie, B. et al(1982)). Excessive ventricular wall stress has been implicated as a potential mechanism predisposing to sudden arrhythmic death in patients with heart failure, as discussed in chapter 1.3. This premise has been further substantiated by in vitro studies which have demonstrated that an increase in afterload may result in a decrease in effective refractory period and action potential duration (Lermann, B.B. et al(1985); Reiter, M.J. et al(1988)). This decrease in refractory period may lead to the conduction of premature extra-stimuli and the initiation of tachyarrhythmias. The decrease in plasma potassium and magnesium which accompany the increase in aldosterone may also be potentially proarrhythmic as discussed in chapters 1.6 and 1.7.

Although the proposal that neurohormonal activation has a role to play in the pathogenesis of sudden death in heart failure would seem attractive, at least on theoretical grounds, the reality of the situation is that there is little clinical evidence avalible to confirm this hypothesis. The relative incidence of sudden arrhythmic death does not increase with increasing severity of heart failure (Burgraff, G.W. & Parker, J.O.(1975); Massie, B. et al(1981); Cohn, J.N. et al (1984) and Wilson, J.R. et al(1983)). The fact that patients with more severe heart

failure and greater impairment of left ventricular function also have the greatest level of activation of the renin-angiotensin system, would tend to exclude this as a mechanism for sudden death in heart failure.

If the hypothesis that activation of the renin-angiotensin system was important in sudden death, then it should follow that inhibition of this system should theoretically reduce the incidence of those deaths in cardiac failure which could be classified as sudden. Perhaps the best study so far which has addressed the problem is the Cooperative North Scandanavian Enalapril Survival Study (Consensus): The Consensus trial study group, (1987). The Consensus trial was designed as a randomised, double-blind, placebo controlled, parallel-group trial in patients with severe congestive heart failure (New York Heart Association functional class IV). The study involved two treatment groups, one treated with enalapril the angiotensin converting enzyme inhibitor, and the other with an identical placebo. At the end of 6 months, the crude mortality was 26% in the enalapril-treated group and 44% in the placebo group, a reduction of 40%(p= 0.002). At the end of 12 months, mortality was reduced by 31%. By the end of the study there had been 68 deaths in the placebo group and only 50 in the enalapril group which represents a reduction of 27% (p= 0.003). Perhaps the

most interesting aspect of the Consensus study however, lies in the fact that the entire reduction in mortality was found to be among patients with progressive heart failure, whereas no difference was observed in the incidence of sudden unexpected death. It would seem therefore that inhibition of the renin- angiotensis system does not have any effect on the incidence of sudden death in heart failure, and the proposal on theoretical grounds at least, that activation of this system is important in the pathogenesis of sudden death, may not be relevant in the clinical setting.

Other studies have confirmed the beneficial effects of angiotensin converting enzyme inhibition on overall prognosis in heart failure (Furberg, C. & Yusef, S.(1985); Creager, M.A., Faxon, D.P. Halperin, J.L.(1982)), but like the Consensus study failed to show any change in the incidence of sudden death.

As discussed in chapter 1.4, two more recent studies have reached conflicting conclusions with regard to the effect of angiotensin converting enzyme inhibition on overall mortality and sudden death in heart failure. The SOLVD Investigators,(1991) found that enalapril reduced overall mortality in patients with mild to moderate heart failure but had no effect on the incidence of sudden death. The study by Cohn, J.N. et al(1991) on the other hand found that the reduction in mortality associated with the use of enalapril, in comparison with hydrallazine and nitrates, was mainly related to a reduction in the incidence of sudden death.

The weight of published data would seem at present to support the view that inhibition of the renin-angiotensin system does not result in a reduction in sudden death in heat failure.

The other important neurohormonal mechanism which is activated in heart failure is the sympathetic nervous system.

The reduction in cardiac output which occurs in heart failure not only results in activation of the renin-angiotensin system, but also the sympathetic nervous system, in an attempt to maintain circulatory homeostasis (Francis, G.S. et al(1984); Francis, G.S.(1985)). This increase in circulating catecholamines may be potentially arrhythmogenic either directly, or by the depletion of potassium and magnesium (Brown, M.J. et al(1983)). It is also possible that by increasing afterload secondary to systemic vasoconstriction, cardiac action potential duration will be decreased as previously discussed. Other evidence for the role of increased sympathetic activity in predisposing to arrhythmias and sudden death is found in the Beta Blocker Heart Attack Trial,(1984). In this study, the use of propanolol in post-infarct patients with heart failure was associated with a reduction in mortality.

## 1.9 Vasodilator mechanisms of captopril.

Although the principle mode of action of angiotensin converting enzyme(ACE) inhibition in producing a hypotensive response is the inhibition of the conversion of angiotensin I to angiotensin II(a potent vasoconstrictor), a number of other vasoactive mechanisms have been proposed. This would imply that the effects of

ACE inhibition in improving prognosis in patients with heart failure may be independent of the degree of activation of the renin-angiotensin system, and would therefore be as pronounced in both mild and severe heart failure. In this section therefore the evidence for a non angiotensin II mediated hypotensive response of captopril is discussed.

The role of prostaglandins in the hypotensive effect of captopril was examined in a clinical study by Moore, T.J. et al(1981). In this study captopril was given to sodium restricted patients with essential hypertension. After a single oral dose of captopril, the blood pressure and levels of angiotensin II decreased, while the levels of the metabolite of $PGE_2$(PGE-M) increased. Prostaglandin synthesis was then blocked by the administration of either indomethacin or aspirin before repeating the captopril dose. The authors found that the PGE-M response was effectively blocked and as expected the levels of angiotensin II were decreased. The significant finding in this study however was that the hypotensive effect of captopril was significantly blunted, thus implying a role for prostaglandins in the hypotensive effect of captopril. Another clinical study reached similar conclusions after assessing the effects of indomethacin on the acute hypotensive effect of captopril (Silberbauer, K., Stanek, B. & Templ, H.(1982)). DeBruyn, J.H.B. et al(1980),

examined the haemodynamic effects of a single dose of 100mg of captopril in 3 different patient subgroups; patients with renovascular hypertension, essential hypertension, and anephric patients. The pre-treatment concentrations of active renin in plasma were significantly higher in the subgroup of patients with renovascular hypertension when compared to the 2 other groups, while in turn the renin concentration was higher in the essential hypertension group when compared to the anephric group of patients. The authors found that the administration of captopril resulted in a similar hypotensive response in all 3 groups. They concluded from this that the hypotensive response to captopril would seem to be independent of circulating renin levels.

Further evidence for the role of non-angiotensin mediated vasoactive mechanisms can be found in animal studies. Marks, E.S. et al(1980) found that captopril produced a fall in blood pressure in nephrectomised rats despite the absence of renin from the peripheral circulation. It was also found that in animals made hypertensive by the administration of deoxycorticosterone, a potent salt retaining steroid, captopril again caused a significant fall in blood pressure. The rats in these experiments had markedly suppressed plasma renin activity. Another study in dogs found that the infusion of angiotensin II into sodium depleted animals produced an increase in both blood

pressure and levels of angiotensin II, and that these parameters were related in a dose response curve. Following an infusion of captopril, it was found that the levels of angiotensin II which were required to produce the same blood pressure as a group of animals which were not given captopril was significantly higher. This is further evidence that the acute hypotensive effect of captopril is not entirely dependent on a reduction in angiotensin II.

## 1.10 Possible effects of drug therapy on mortality in heart failure.

The role of converting enzyme inhibition in improving prognosis in heart failure has been previously discussed in chapter 1.8. The increased sympathetic tone which is found in patients with heart failure and its arrhythmogenic potential would imply that pharmacologically blocking the sympathetic adrenergic receptors could lead to a reduction in cardiac arrhythmias. Limited evidence does exist that direct antagonism of the proarrhythmic effects of catecholamines may be achieved by the administration of beta adrenergic blocking agents. Data from the Beta Blocker Heart Attack Trial(1987) indicate that treatment with beta blockers substantially reduces the incidence of sudden death in

patients following an acute myocardial infarction that was complicated by congestive heart failure (Furberg, C.D. et al(1984)). These observations support the findings of uncontrolled studies which indicate that long-term, low-dose beta blockade may reduce mortality in patients with congestive cardiomyopathy (Swedberg. K. et al(1979)). Inherent difficulties exist however with the use of beta-blocker therapy in patients with heart failure. The negative inotropic effect of these drugs may suppress myocardial contractility and precipitate acute pulmonary oedema and possibly death in patients whose myocardial function is already severely compromised. It is important therefore to balance the possible benefits in terms of a reduction in the frequency of arrhythmias against the probable deterioration in cardiac function.

Most of the drugs which are used to improve cardiac performance and relieve the clinical symptoms in patients with congestive failure can exacerbate ventricular tachyarrhythmias. Of all the available treatment modalities, diuretic drugs may prove to be the most arrhythmogenic. These agents activate both the sympathetic nervous and renin-angiotensin systems and promote the renal loss of both potassium and magnesium. Although activation of the renin-angiotensin system has been proposed as a possible mechanism for sudden

arrhythmic death in heart failure, a number of previous reports have suggested that in patients with mild to moderate cardiac failure who are not on concurrent treatment, plasma levels of renin may be normal, and may only increase following the administration of diuretics (Brown, J.J. et al(1970); Bayliss, J. et al(1987)). All of these factors acting alone or in concert may contribute to the frequency and complexicity of malignant veutricular rhythms in patients with congestive heart failure. The mechanisms and evidence for the arrhythmogenic potential of hypokalaemia and hypomagnesaemia have been previously discussed in sections 1.6 and 1.7.

A similar sequence of arrhythmic events has been postulated to occur during the administration of diuretics in hypertensive patients (Medical Research Council working party on mild to moderate hypertension,(1983); Holland, O.B., Nixon, J.V. & Kohnert, L.(1981); Papademetriou, V. et al(1983)), and may explain the failure of diuretic therapy to reduce overall cardiovascular mortality in these patients in large-scale trials (Hollifield, J.W.(1984)).

Another important factor which may contribute to ventricular arrhythmogenesis in patients with cardiac failure is the use of positive inotropic therapy. The most commonly used drug available for oral therapy is

digoxin, a cardiac glycoside. Although digoxin is effective as an agent which increases cardiac contractility, it has the disturbing side effect of also increasing cardiac irritability. This drug has a narrow toxic-therapeutic ratio and in the presence of hypokalaemia and hypercalcaemia may provoke serious ventricular arrhythmias. Such malignant arrhythmias may contribute to the increased cardiovascular mortality noted in patients with heart failure and complex ventricular ectopy who receive long-term digitalis therapy after an acute myocardial infarction (Bigger, J.T. Jr. et al(1985)).

Recently developed inotropic agents enhance myocardial contractility by increasing intra-myocellular levels of cyclic AMP, either by promoting its synthesis (catecholamines) or by inhibiting its degradation (phosphodiesterase inhibitors): both mechanisms are potentially arrhythmogenic. Although intravenously administered catacholamines can increase ventricular ectopy in patients with congestive heart failure, neither dopamine or dobutamine appear to have increased the risk for sudden death, since these drugs are not generally administered over a long term. Orally active phosphodiesterase inhibitors (such as amrinone, milrinone and enoximone) are being developed for long-term use and appear to have a similar arrhythmogenic potential. This

may explain the high incidence of sudden death in patients with cardiac failure treated with these agents (Packer, M., Medina, N. & Yusak, M.(1984)). The Promise Study Research Group reported in 1991 on a trial comparing oral milrinone with placebo, in patients with grade IV heart failure. After a mean follow-up period of 6.1 months, mortality was 28% higher in the milrinone group.

## 1.11 Antiarrhythmic drug therapy in congestive heart failure.

In attempting to design a therapeutic approach to the prevention of sudden unexpected death in patients with congestive heart failure, two central questions need to be considered. Firstly which patients with congestive heart failure are at a high risk of sudden death and should receive prophylactic therapy, and secondly, are conventional anti-arrhythmic drugs succesful in preventing sudden death in these patients? Although there is general agreement that patients with symptomatic ventricular tachycardia or ventricular fibrillation should be treated to prevent future sudden unexpected death, only a small proportion of patients with heart failure who die suddenly experience premonitory symptoms. The clinical picture in most patients is so dominated by dyspnoea and fatigue that the first manifestation of a clinically important

arrhythmia may be sudden death itself. Some workers have suggested that the finding of complex ventricular rhythms, in particular non-sustained ventricular tachycardia, during ambulatory electrocardiographic monitoring may predict future fatal ventricular arrhythmias. However, a number of reports indicate that pre-existing spontaneous ventricular tachycardia is only a predictor of total mortality and not sudden cardiac death (Meinertz, T. et al(1984); Huang, S.K. et al(1983); Wilson, J.R. et al (1983); Holmes, J. et al(1985)).

Other observers have suggested that the finding of sustained ventricular tachycardia during programmed ventricular stimulation can identify a high risk population (Kaul, U. et al(1987)). However the arrhythmias in patients with non-ischaemic cardiac failure may be particularly resistant to electrical provocation (Naccarelli, G.V. et al(1982); Wellens, H.J.J., Duren, D.R. & Lie, K.I.(1976)). The lack of success in inducing ventricular tachycardia in these studies was in contrast to Poll, D.S. et al(1984), who found that in eleven patients with idiopathic dilated cardiomyopathy and sustained ventricular tachycardia, programmed stimulation reproducably induced venticular tachycardia in all patients.

The high incidence of ventricular arrhythmias and sudden death in patients with heart failure has encouraged many

clinicians to treat patients with anti-arrhythmic agents in the hope of prolonging survival. This has been made on the assumption that conventional anti-arrhythmic therapy will reduce or abolish ventricular arrhythmias and that the suppression of these arrhythmias will reduce the likelihood of sudden death.

Whether or not this approach is appropriate depends on two important factors. Firstly, does the use of anti-arrhythmic drugs prolong survival of patients with heart failure? Secondly do the adverse effects of these drugs outweigh any potential benifits?

The hypothesis that administration of anti-arrhythmic therapy to patients with heart failure reduces the incidence of lethal ventricular arrhythmias and thereby prolongs survival is an attractive one. Consequently, several investigators have attempted to test this proposal by assessing the effect of anti-arrhythmic therapy on survival in patients with heart failure, although no large-scale placebo-controlled trials have been published. Two basic types of study have been conducted in patients with heart failure. Firstly there are those in which the patients are non-randomly assigned to anti-arrhythmic therapy, and secondly there are studies in which the anti-arrhythmic therapy is directed by electrophysiological testing prior to treatment, (table 2).

Table 2.

The effect of anti-arrhythmic treatment on prognosis in congestive heart failure.

| Study | EPS | Sudden death | Overall mortality |
|---|---|---|---|
| Parmley | No | - | Reduced |
| Cleland | No | Reduced | Reduced |
| Neri | No | Reduced | No effect |
| Chakko | No | No effect | No effect |
| Gomez | Yes | Increased | Increased |
| Poll | Yes | - | - |
| Kaul | Yes | Increased | No effect |

EPS = Electrophysiological study.

Parmley, W.W. & Chatterjee, K.(1986), analyzed the incidence of sudden death in a sub group of patients with heart failure and complex ventricular arrythmias who were treated with class one agents (procainamide or quinide) n=26, or amiodarone n=13. This subgroup had a cumulative survival of 90-95% at 6 months compared to 65% in patients not receiving antarrythmic therapy ($p<0.05$). It was therefore proposed that antiarrythic therapy may be of benefit in patients with heart failure. Cleland, J.G.F. et al(1987) studied clinical variables of prognostic significance in 152 patients with heart failure. Within this study group 41 patients were treated with amiodarone and the rest were not. The authors found that overall mortality was reduced in the group of patients treated with amiodarone, and in particular the incidence of sudden death was reduced. Interestingly, in contrast to the Consensus Trial Study Group,(1987), the effect of angiotensin converting enzyme inhibition was also to reduce the incidence of sudden death, but to have no effect on overall mortality. This ambiguity highlights the problems in retrospective studies with small numbers of patients which are not blinded or randomised, and which try to predict clinical features of prognostic significance. Another study by Neri, R. et al(1987), studied 65 patients with dilated cardiomyopathy. Fifty two patients in the study were found to have evidence of

complex ventricular ectopy as detected by Holter ambulatory ECG monitoring. Forty one of these patients were treated with amiodarone. The authors found that the incidence of sudden death was significantly reduced in the group which received anti-arrhythmic therapy. The small number of events in this study(only 4 sudden deaths occured) would make it unlikely that the statistical significance in the reduction in the incidence of sudden death was clinically significant.

In contrast to the above studies, Chakko, C.S. & Gheorghiade, M.(1985), followed 43 patients with chronic heart failure due to dilated cardiomyopathy (28 ischaemic, 15 idiopathic). On Holter monitoring 88% of the patients were noted to have complex venticular arrythmias and non-sustained ventricular tachycardia was seen in 51 %. 23 of the patients were placed on long-term therapy with procainamide (n=20) or quinidine (n=3). The remaining 20 patients did not receive antiarrythic therapy. Both groups were clinically comparable. At a mean follow-up period of 16 months, 16 deaths had occurred, 62% of which were caterogized as sudden. No significant difference was noted in the survival of patients receiving antiarryhthmic therapy compared with those not receiving therapy.

The result of studies using electrophysiological testing are also conflicting and methodologically limited. Gomes,

J.A.C. et al(1984) studied 73 patients with asymptomatic complex ventricular arrhythmias. 77% of these patients had ischaemic heart disease and had had a myocardial infarction at least 6 months previously. 14% had cardiomyopathy or valvular heart disease. Electrophysiological testing was performed with single or double extrastimuli at 2 paced cycle lengths at the right ventricular apex and outflow tract. Electrophysiological testing identified 20 patients (27%) in whom ventricular tachycardia or fibrillation was induced. 17 of the 20 patients with inducible arrhythmias were placed on antiarrhythmic therapy as directed by electrophysiological testing, whereas the other patients were randomly allocated to antiarrhythmic or no antiarrhythmic therapy. The incidence of sustained ventricular tachycardia and/or sudden death was 31% in the former group despite directed therapy, and only 2% in the latter group. The results from this study therefore suggest that electrophysiological testing may identify a high risk group, however little definitive information is yielded about the value of anti-arrhythmic therapy. Poll, D.S. et al(1984) have suggested that conventional anti-arrhythmic therapy may be relatively ineffective in patients with heart failure. They studied 11 consecutive patients with idiopathic dilated cardiomyopathy and spontaneous sustained ventricular tachycardia. Electrophysiological

testing induced the arrhythmias in all 11 patients. Despite evaluation of 3.7 $\pm$ 2.4 antiarrhythmic drugs per patient, including amiodarone in 8, 9 of the 11 patients continued to have inducible ventricular tachycardia on all drugs. During follow-up 6 patients had sudden death or recurrent ventricular tachycardia. Another study assessed the effect of anti-arrhythmic therapy in patients with complex ventricular ectopy and ventricular tachycardia which was induced by programmed electrical stimulation, and compared this study group to other patients with complex ventricular ectopy but no inducible ventricular tachycardia. The latter group were given no anti-arrhythmic treatment (Kaul, U. et al(1987)). The authors found that the incidence of sudden death was significantly higher in the group of patients treated with anti-arrhythmic therapy compared to those who were not. One significant problem with this study is that the patient population was heterogeneous and included 21 patients with no structural heart disease. None of these patients had inducible ventricular tachycardia and would therefore have biased the results as the mortality in these patients would presumably be lower than in patients with structural heart disease.

In summary therefore, current information is inadequate but does not support the administration of conventional antiarrhythmic drugs to patients with chronic heart

failure either directed by electrophysiological studies or given empirically.

The most successful approaches to the control of arrhythmias in the future for patients with congestive heart failure may be endomyocardial resection of the re-entry pathway(guided by electrophysiological mapping) or the use of an automatic implantable defibrillator (Poll, D.S. et al(1984)). The use of surgery may be limited however as patients often have poor left ventricular function. Although the use of such aggressive and expensive therapies may be indicated for patients with symptomatic ventricular tachyarrhythmias, they are not practical solutions to the problem of sudden death in most patients with heart failure. With the advent of pharmacological agents which can improve pump performance, sudden unexpected death from ventricular tachyarrhythmias may soon become the most common cause of death in patients with congestive cardiac failure. In addition to haemodynamic and neurohormonal factors, and the commonly used inotropes and diuretics in cardiac failure, there may also be an underlying abnormality in cardiac electrophysiology which either independently or in conjunction with neurohormonal activation may predispose to sudden unexpected death. Clinical investigation by cardiac electrophysiology has been confined to studies

which are concerned with the inducibility of ventricular tachyarrhythmias in patients with cardiac failure. These studies are normally conducted only once in any given patient, and are unlikely to involve a control group of patients. The measurement of electrophysiological parameters such as ventricular repolarisation and refractoriness(abnormalities of which may predispose to sudden death) in a "one-off" situation would therefore be meaningless. Repeated electrophysiological studies in patients with cardiac failure or in suitable controls would be unethical as cardiac catheterisation is an invasive technique not without morbidity and mortality, and repeated exposure to such an investigation would further compound the risks involved.

There is a need therefore for animal models of chronic congestive cardiac failure in which the basic electrophysiological mechanisms underlying the predisposition to sudden death, and the interrelationship between the myocardial electrophysiological substrate, neurohormonal activity and inducibility of arrhythmias can be studied in detail. Previous publications have discussed the electrophysiological changes and inducibility of arrhythmias after experimental myocardial infarction (Cobbe, S.M. et al(1985); Spear, J.F., Michelson, E.L. & Moore, E.N. (1983); Donaldson, R.M. et

al(1983)), but have not studied animals showing evidence of congestive heart failure.

## 1.12 Animals models of congestive heart failure(table 3)

A number of methods are available to induce heart failure experimentally in animals. Most of these models are limited in their usefulness however, as they more readily lend themselves to the study of specific problems rather than the clinical syndrome of heart failure which is dominated by dyspnoea and fatigue. The techniques which have been employed to induce heart failure experimentally include pressure overload, volume overload and conditions in which the myocardium itself is directly damaged,(Smith, H.J. & Nuttall, A.)

Pressure loading has been used in a number of species to induce ventricular hypertrophy and thence failure. Gelband, H. & Bassett, A.L.(1973) produced right ventricular hypertrophy and subsequently failure in cats by partial occlusion of the pulmonary artery. The extent to which heart failure occured varied considerably between animals in the experiments described, and also developed over a variable timecourse. Welham, K.C., Silove, E.D. & Wyse, R.K.H.(1978) described similar experiments in the pig. The authors employed a similar technique to that used in cats, and again found that the degree of heart failure which occured was variable, with only about two thirds of the animals demonstratimg signs of heart failure, although all of the animals had evidence of right

Table 3.

Animals models of congestive heart failure.

| | Author | Species |
|---|---|---|
| Pressure load | Gelband and Basset | Cat |
| | Wellham et al | Pig |
| | Mercadier et al | Rat |
| | Alexander et al | Rabbit |
| | Sordahl et al | Rabbit |
| Volume load | Flaim et al | Rat |
| | Carabello et al | Rabbit |
| | Natarajan et al | Cat |
| | Forester et al | Dog |
| Myocardial infarction/ Cardiomyopathy | | |
| | Miller et al | Dog |
| | Kirk et al | Dog |
| | Brooks et al | Pig |
| | Pfeffer et al | Rat |
| | Bajuze | Hamster |
| | Einzig et al | Turkey |
| | Laks et al | Dog |
| | Arnolda et al | Rabbit |

ventricular hypertrophy. An inherent problem with the above studies is the degree of technical difficulty which is involved. The use of general anaesthesia and surgical thoracotomy inevitably lead to a high mortality.
A number of studies have attempted to induce left ventricular hypertrophy by constriction of the aorta with similar results to those above (Mercadier, J.J. et al(1981)1; Alexander, N., Hinshaw, L.B. & Drury, D.R.(1957); Sordahl, L.A., Wood, W.G. & Scharwz, A.(1970)).
The animals models of pressure loading would seem therefore more suited to the study of ventricular hypertrophy than heart failure.

Volume overload may be induced by the surgical creation of aortic to vena-caval fistulae (Flaim, S.F. et al(1979)), aortic valve incompetence (Cerabello, B.A. et al(1981)), and atrial septal defects (Natarajan, G. et al(1979)). Although these techniques may induce heart failure, and be of use in the study of the specific models which they reproduce, they are not directly relevant to the syndrome of heart failure which occurs in the majority of patients.

The model of experimental heart failure which would seem to be the most attractive on the basis of clinical

relevance is that of heart failure occuring after myocardial infarction.
Several workers have employed coronary artery occlusion to produce myocardial infarction and thence failure (Forester, J.S. et al(1972); Miller, R.R., Awan, N.A.& Mason, D.T.(1978); Kirk, E.S. et al(1978); Brooks, M., Holland, R., AL-Sadir, J.(1977); Pfeffer, J.M., Pfeffer, M.A. & Braunwald, E.(1987)). Although this model of heart failure is clinically relevant, the heart failure which occurs is usually acute and cardiogenic shock often supervenes (Lluch, S. et al(1969)).
Another intrinsic problem with such studies is the initial mortality rates which may be as high as 50% (Pfeffer, J.M. et al(1987)). It would seem therefore that animal models of heart failure induced by myocardial infarction are unsuitable for the study of chronic heart failure.

The final type of model of experimental heart failure is that of the cardiomyopathies. A cardiomyopathy is known to occur spontaneously in a specific species of Syrian hamster, first described by Bajusz, E.(1969). A naturally occuring cardiomyopathy also exists in turkeys (Einzig, S. et al(1980)). Other workers have employed acute or chronic administration of catecholamines to induce myocardial infarction, in particular isoprenaline (Kahn, D.S., Roden, G. & Chappel, C.I.(1969)) and noradrenaline

(Laks, M.M., Morady, B.A. & Swan, M.J.C.(1973)). The use of catecholamines in animals has also been found to produce myocardial necrosis (Reichenbach, D.D. & Benditt, E.P.(1970)). The clinical relevance of such models is unclear but there have been reports of a clinical cardiomyopathy associated with chronic amphetamine useage (Smith, H.J. et al(1976)).

## 1.13 Clinical and experimental aspects of adriamycin cardiomyopathy.

Perhaps the myocardial toxin which has been most extensively studied in animals is adriamycin. Adriamycin is a tetracyclic aglycone to which an amino sugar is attached through a glycosidic linkage. This drug is a potent broad spectrum chemotherapeutic agent which is effective against human malignancy such as leukaemias, lymphomas and many solid tumors (Blum, R.H. & Carter, S.K.(1984)). The cardiotoxic effects of adriamycin can be subdivided into acute and chronic depending on their temporal relationship to the administration of the drug. The acute cardiovascular effects of the drug develop within minutes or hours after its intravenous injection, and are characterised by hypotension, tachycardia and arrhythmias (Singal, P.K., Deally, C.M.R. & Weinberg, L.E.(1987)). Acute cardiac decompensation in patients

however is not commonly observed during administration of the drug (Appelfield, M.M. & Egorin, M.J.(1984)). Although the acute administration of adriamycin has been associated with sudden death in a small number of patients (Wortman, J.E. et al(1979)), a number of other studies have reported a lack of arrythmias associated with the drug. Friers, G.G. et al(1985) investigated a total number of 30 patients by continuous ECG monitoring, 24 hours before, during and 24 hours after adriamycin administration. Nine of the 30 patients experienced ventricular ectopy prior to adriamycin administration. Five of the these patients had a decline in the severity of their arrhythmias with treatment, 3 patients arrhythmia was unchanged and 1 patients arrythmia increased in severity. Conversely 6 patients had ventricular ectopy observed after treatment where none was seen before treatment. No patient experienced cardiac symptoms in conjunction with ectopy on the monitor tracing. In the remaining 15 patients in this study, no ventricular ectopy was seen before or after treatment. There were no instances in any patients of ventricular ectopic beats occuring in the form of triplets or salvoes, ventricular tachycardia or ventricular fibrillation.
Although Wortmann, J.E. et al(1979) described 3 cases of sudden death associated with acute adriamycin cardiotoxicity as described previously, the 3 cases were

amoung approximately 550 individual patient treatments with adriamycin over a 3 month period and therefore represent an uncommon clinical occurence.

Chronic administration of adriamycin to patients is associated with the development of a cardiomyopathy which often leads to congestive cardiac failure (Buja, L.M. et al(1973); Lefrak, E.A. et al(1973); Von Hoff, D.D. et al(1979)). Patients treated by repeated injections of the drug may eventually show marked hypotension, tachycardia, cardiac dilatation and ventricular failure which is refractory to positive inotropic drugs and mechanical circulatory assistance (Lefrak, E.A. et al(1973)). Increases in serum lactate dehydrogenase and creatinine phosphokinase have also been reported in patients developing heart failure: Lefrak, E.A. et al(1973).

The incidence of adriamycin-induced cardiomyopathy is directly related to the cumulative dose of the drug. The incidence of the cardiomyopathy increases with increasing total dose to 3.5% of patients given 400mg/$m^2$, 7% of those given 550mg/$m^2$ and 15% of those given 700mg/$m^2$ (Appelbaum, F.R. et al(1976); Von Hoff, D.D. et al(1979)). The observation of this dose-dependent cardiomyopathy has led to the recommendation that the total dose not exceed 550mg/$m^2$ (Blum, R.H. & Carter, S.K.(1974); Gottlieb, J.A. et al(1973)).

The morphological changes associated with chronic adriamycin cardiotoxicity in humans are characterised by myofibrillar loss and a marked cytoplasmic vacuolization (Billingham, M.E.(1979); Lefrak, E.A. et al(1973)), due to swelling of the sarcotubular system. Other changes which are known to occur include structural abnormalities in mitochondria, increased numbers of lysosomes and a accumulation of lipids (Ferrans, V.J.(1978); Singal, P.K. et al(1985)). A number of common features of adriamycin-induced structural change have been found in the myocardium of a variety of species other than man. These include rabbits: Jaenke, R.A. (1974); Olson, H.M. et al(1974), mice: Lambertenghi-Delliers, G., Zanon, P.L. & Pozzoli, E.F.(1976); Myers, C.E. et al(1977), and rats: Callcroft, S.C.W., Gavin, J.B. & Herdon, P.B.(1973); Singal, P.K. et al(1985).

Although the morphological changes induced by adriamycin can be detected by light microscopy, electron microscopy is used for more accurate and specific scoring of the degree of cardiotoxicity. A point scoring system for assessment of the degree of cardiac damage has been described by Bristow, M.R. et al(1978). The cardiac biopsies in the above study were scored as follows: 0 was allocated to normal biopsies, 1 = scanty cells showing early myofibrillar dropout or swelling of the sarcoplasmic reticulum, or both, 2 = more widespread changes, with

groups of cells showing definite myofibrillar dropout or definite cytoplasmic vacuolization, or both, and 3 = diffuse myocyte damage with more marked cellular changes and frank necrosis.

The exact aetiology of adriamycin-induced cardiotoxicity is undefined, but two possible mechanisms have beeen proposed. Firstly, an increase in intracellular calcium: Olson, H.M. et al(1974); Revis, N. & Marusic, N.(1979), and secondly damage to cellular and organellar membranes by the production of free radicals: Myers, C.E. et al(1977); Milei, J. et al(1986); Bachur, N.R., Gordon, S.L. & Gee, M.V.(1977).

Although the importance of morphological and subcellular abnormalities are important in the assessment of animal models of heart failure, the syndrome itself is not a pathological diagnosis. It is therefore necessary to study such models from a haemodynamic aspect and to ascertain whether or not there are functional abnormalities which would be consistent with a diagnosis of chronic heart failure.

## 1.14 Experimental haemodynamic aspects of adriamycin cardiomyopathy in the rabbit.

Two recent publications have extensively studied adriamycin cardiomyopathy in the rabbit and have reached similar conclusions with regard to central haemodynamics and regional blood flow. Arnolda, L. et al(1985), injected New Zealand White rabbits with intravenous adriamycin $1mg.kg^{-1}$ twice weekly for 8 weeks. This was a specific dosage regimen designed to limit marrow toxicity: Arnolda, L. et al(1985). The authors measured cardiac output by the thermodilution technique using a chronically inplanted aortic thermister catheter which was inserted via the illio-lumbar artery under halothane anaesthesia, 2 weeks prior to the commencement of adriamycin therapy. Blood pressure was measured via a catheter in the ear artery and right atrial pressure via an external jugular catheter. Both catheters were inserted on the day of haemodynamic study under local anaesthetic. Renal blood flow in this study was measured by the clearance of $^{125}I$ labelled ortho-iodohippurate using a single injection method. The haemodynamic studies were performed on 6 rabbits at weeks 0 and 8 of the study protocol. Plasma renin activity, noradrenaline and vasopressin levels were also measured at weeks 0,4 and 8. At the end of the study protocol the animals were sacrificed and the hearts were

blotted dry, weighed and samples fixed for histological assesment. As well as plasma neurohormonal analysis, samples were also taken for $Na^+$, $K^+$ and creatinine. After 8 weeks treatment with adriamycin, the authors found that cardiac output had decreased significantly from $799\pm61$ $ml.min^{-1}$ to $624\pm44ml.min^{-1}$, representing a fall of 22%. They also observed a parallel fall in mean blood pressure and an increase in total peripheral resistance in 4 out of 6 animals. Renal blood flow also fell significantly over an eight week period and renal vascular resistance increased in all animals. No change was observed in plasma vasopressin levels over an 8 week period but plasma renin activity increased significantly by week 4 and plasma noradrenaline levels increased significantly by week 8 of the study. No significant changes were found in either plasma $Na^+$,$K^+$ or creatinine over an 8 week period. Marked pathological changes were also found at post-mortem, with evidence in all of the adriamycin treated animals of serous effusions, left ventricular hypertrophy and fibrosis. The heart weights of the test group were significantly increased when compared to controls and this difference was accentuated when the heart weights were expressed as a ratio of the body weight which tended to be less in the adriamycin group. Microscopic examination also revealed the classic morphological changes associated with adriamycin therapy

which include widespread atrophy and lysis of cardiac myocytes, associated interstitial fibrosis, extensive myocyte vacuolation and a generalised reduction in myofilament numbers.

The second study which has examined the central haemodynamics and regional blood flow in adriamycin cardiomyopathy in the rabbit is that by (Wanless, R.B. et al(1987)). The injections of adriamycin in this study were according to the same protocol as Arnolda et al 1985 ie $1mg.kg^{-1}$ intravenously twice weekly. The duration of the injection protocol was altered between different study groups. The different groups were injected for periods of 6,8 and 10 weeks, with a further group injected for 8 weeks with a 2 week period then allowed before making haemodynamic measurements to allow for recovery from the unwanted systemic side-effects of the drug. Two further groups of animals were also studied, the first on arrival in the animal unit and the second after 8 weeks of identical conditions to the study groups. The insertion of the catheters for haemodynamic study was carried out under sodium pentobarbitone anaesthesia at the end of each injection protocol. This aspect of the Wanless study was different to the Arnolda report in that Arnolda had chronically implanted catheters. Right atrial pressure was measured with a catheter inserted into this chamber via the right external jugular vein. A catheter was also

inserted into the carotid artery and thence into the left ventricle and ascending aorta. A further catheter bearing a thermister probe was also inserted into the descending aorta via the femoral artery. The haemodynamic measurements in this study were performed 48 hours after surgery in conscious unrestrained animals.

Another additional aspect of the Wanless study was an attempt to assess the response of the heart to increased stress. Because it is not possible to induce rabbits to exercise, the authors attempted to assess the cardiac response to stress by volume expansion with an infusion of 50ml of isotonic dextrose intravenously. During the 15-30 minutes following the intravenous infusion, the cardiac output was repeatedly measured as the right atrial pressure fell, thus allowing the construction of Frank-Starling curves.

When the central haemodynamic measurements were stable, cardiac output was measured by thermodilution, regional blood flow by the radioactive microsphere technique, and right atrial and system blood pressure were also continuously monitored. Venous blood samples were also taken for measurement of plasma $Na^+$, urea , protein content, haemoglobin and white cell count.

Wanless et al 1987 found that after 6 weeks treatment with adriamycin, cardiac output fell by 27%, after 8 weeks by 31% and 2 weeks after 8 weeks treatment by 52%. The

greater reduction in cardiac output in this study when compared to Arnolda et al 1985, could be explained by the more accurate measurment of a low cardiac output using a left ventricular injection of cold saline. Total peripheral resistance increased significantly when compared to controls at week 8 although there was no change in the mean systolic blood pressure. Right atrial blood pressure also increased as treatment progressed, becoming significant when compared to controls 2 weeks after 8 weeks of treatment. Renal tissue vascular resistance increased in parallel with total systemic resistance and myocardial and cerebral tissue vascular resistance were also found to have increased but again only 2 weeks after 8 weeks of treatment. The Frank-Starling curves in the cardiomyopathic rabbits were clearly distinguishable from the control animals with a flattened response seen as a right atrial pressure increased. No haemodynamic changes were observed in this study between the animals in the control group who were studied immediately and those who were studied after 8 weeks in the animal unit.

Haemoglobin levels decreased significantly after 6 weeks of treatment and the white cell count tended to decrease although these changes did not reach statistical significance. Both of thes parameters returned to control values in those animals treated for 8 weeks and then

allowed to recover for 2 weeks. These results would tend to support the premise that 8 weeks of treatment followed by a two week recovery period, allows for minimisation of the toxic systemic side-effects of the drug.

An interesting difference between the Arnolda and Wanless studies is the presence in the former of ascites and pleural and pericardial effusions and their absence in the study by Wanless et al. It was noted however by Wanless et al, that chronic implantation of catheters gave rise to endocarditis and severe nephrotic syndrome. Vegetations could be seen on the catheter tips and the animals developed proteinuria, hypoproteinaemia and serous effusions. When the catheters for haemodynamic study were implanted only two days prior to the measurments being made, such complications were avoided.

From the 2 studies we can see therefore that the model of chronic heart failure in the rabbit, induced by the cardiotoxin adriamycin has been extensively studied haemodynamically and has been shown to produce chronic stable, low-output heart failure over a 10 week period. It would seem therefore that this model has the advantage over some of the others which have been previously described. Cardiogenic shock does not appear following the initial treatment with the drug, (a situation often seen following acute coronary ligation) and secondly the haemodynamic features of this model and its chronicity

have many features in common with the broader clinical aspects of chronic heart failure.

## 1.15 An experimental model for chronic measurement of ventricular repolarisation and refractoriness in vivo.

To study in detail the basic electrophysological mechanisms underlying the predisposition to sudden death in chronic heart failure, a technique is required which allows chronic measurement of basic myocardial electrophysiological parameters such as repolarisation and refractoriness in conscious, unsedated, unrestrained animals. Such a technique has been previosly described by Manley, B.S. et al(1989). They described a method whereby both ventricular repolarisation and refractoriness can be measured in vivoby virtue of a chronically implanted conventional bipolar pacing electrode. Ventricular repolarisation time was measured by recording the ventricular paced evoked response by means of an interface depolariser (Walton, C., Economides, A.P. & Gergely, S.(1985)). The paced evoked response is the local unipolar electrogram recorded from the right ventricular apex while the animals are being paced. The function of the interface depolariser is to counteract the capacitive charge stored at the electrode endocardial interface as a result of the delivery of the pacing stimulus, and it allows accurate recordings of the local electrogram to be made as early as 5 ms following delivery of the pacing stimulus. The stimulus-T interval of the paced evoked

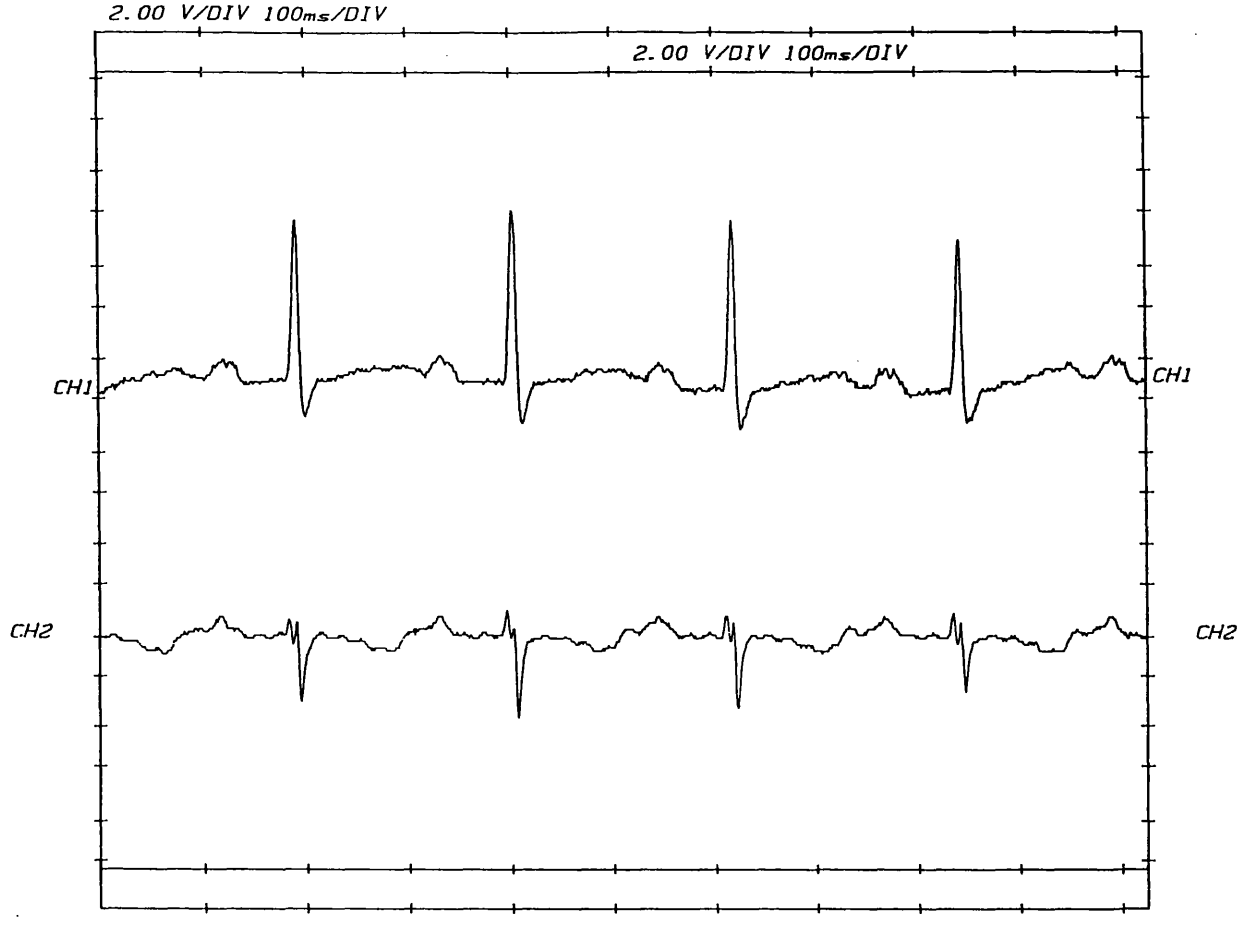


Figure 1.

Graph illustrating in the upper panel 4 cycles of the unpaced surface ECG, and in the lower panel 4 cycles of the unpaced right ventricular electrogram.

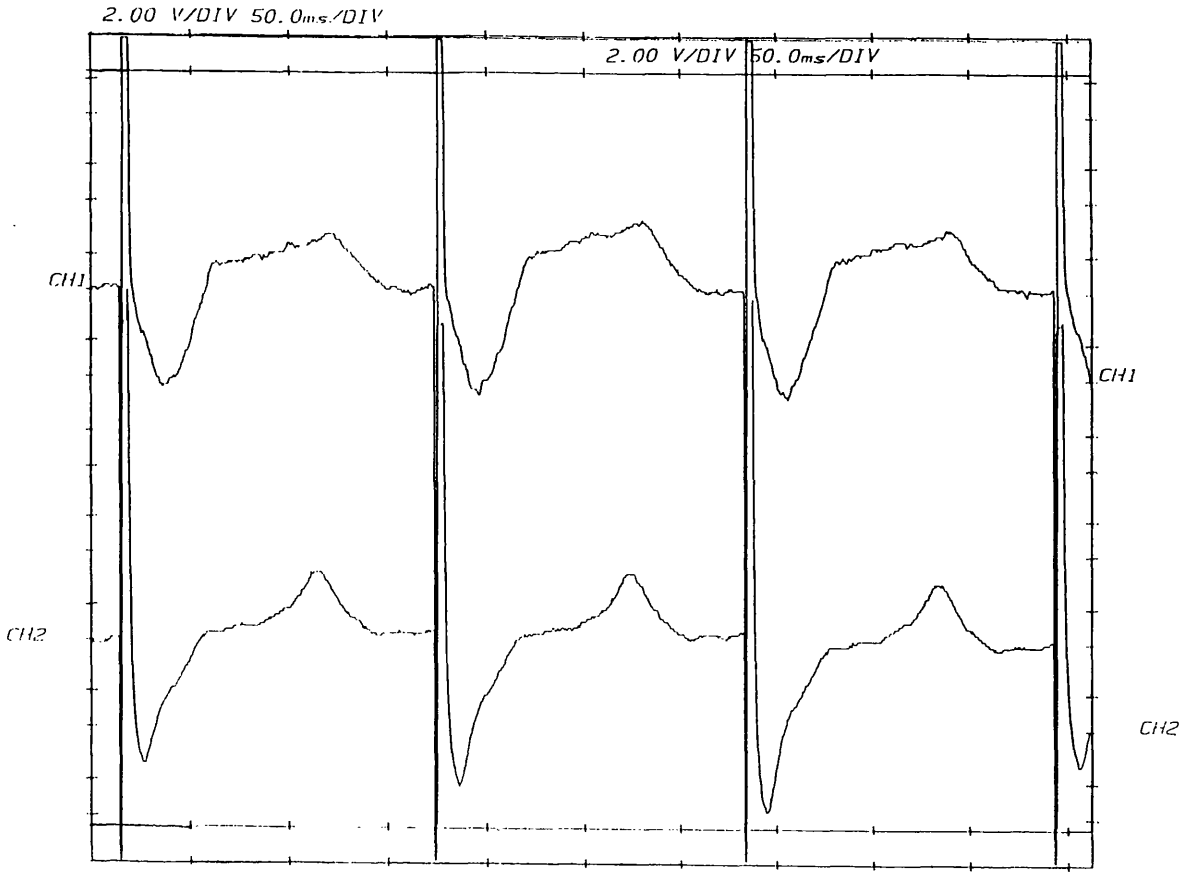


**Figure 2.**

Simultaneous recording of surface ECG(upper panel) and right ventricular paced evoked response(lower panel), during right ventricular pacing at cycle length 180ms. The larger amplitude and clearer peak of the T wave can be seen in the paced evoked response.

made as early as 5 ms following delivery of the pacing stimulus. The stimulus-T interval of the paced evoked response is the measurement of ventricular repolarisation time. The advantages of this method of measuring repolarisation time over the more conventional surface QT interval are that the peak of the T wave is well defined in comparison to the surface ECG, and that with the animals in this study being paced at a constant cycle length, no corrective measures are required for changes in heart rate, (Fig. 1 and 2). Unpaced QT intervals are normally corrected for changes in heart rate by the formula described by Bazett HC in 1920. However, recent evidence has shown that such corrections are unreliable since they do not take into account factors which alter repolarisation independently of changes in heart rate (Brown, K.F. et al(1982); Butrous, G.S.(1986)). Manley, B.S. (1989) has also shown that the stimulus-T interval of the paced evoked response corresponds closely to the cardiac intracellular action potential duration. To prove this hypothesis they studied the electrophysiology of the isolated perfused rabbit interventricular septum, and by recording from both the pacing electrode and a floating glass intracellular microelectrode, the paced evoked response and cardiac action potential duration could be measured simultaneously. To allow assesment of the relationship over as wide a range of values of action

included the addition of d-sotalol to prolong action potential duration and exposure to hypoxia to shorten action potential duration. The result of these experiments was that the stimulus-T interval of the paced evoked response and action potential duration were found to be strongly correlated (r=0.96).

Effective refractory period was also measured chronically by recording the signal from subcutaneous stainless steel ECG electrodes and pacing the animals by means of an isolated stimulator, driven by a microcomputer based programmable pulse genarator. The important conclusion from the study by Manley, B.S. et al(1989), is that the technique which they have described allows for accurate reproducible measurements of both ventricular repolarisation time and refractorness for up to 150 days following implantation of the pacing electrode, after allowing for a 3 week period during which time the electrophysiological measurements were allowed to stabilise.

The development of a haemodynamically stable model of chronic heart failure in the rabbit by virtue of the injection of the cardiotoxic adriamycin as described by Arnolda, L. et al (1985) and Wanless, R.B. et al(1987), and the development of a technique whereby ventricular repolarisation and refractoriness can be measured chronically in the same species, would seem therfore to

provide the required means for assessing the myocardial electrophysiological substrate which may provide a clue to the pathogenisis of sudden death in heart failure. Technical difficulties, in particular the problems associated with the development of chronic heart failure experimentally (as previously outlined), have meant that there is little information available from animal studies with regard to chronic in vivo electrophysiological changes in heart failure. Similar inherent problems exist with attempts to define changes in action potential morphology in vitro as previously discussed in chapter 1.5.

# CHAPTER 2

## AIMS OF THE STUDY

The principal aim of this study was to investigate the electrophysiological changes in adriamycin cardiomyopathy in the rabbit, by measuring ventricular repolarisation and refractoriness.

The study was designed to investigate the basic electrophysiological changes which occur in heart failure as a result of myocardial damage, and the interrelationship between the myocardial electrophysiological substrate, neurohormonal activity and the inducibility of arrhythmias.

The hypothesis proposed is that changes in ventricular repolarisation and refractoriness may occur in heart failure and predispose to arrhythmias and sudden death.

# CHAPTER 3

# GENERAL METHODOLOGY

## 3.1 In vivo electrophysiological studies.

Adult New Zealand white rabbits 2.5-3.5 kg in weight were premedicated with fentanyl/fluanisone (Hypnorm,Janssen) 0.3ml.$Kg^{-1}$ intramuscularly. Anaesthesia was maintained on an oxygen/nitrous oxide/halothane mixture, administered via a mask. Using aseptic technique, a conventional, tined, bipolar permanent pacing electrode(Pacesetter 818) was introduced into the right internal jugular vein via a neck incision. The tip of the pacing electrode was positioned in the apex of the right ventricle using x-ray visualisation. The suitability of the electrode position was confirmed by recording the intrinsic ventricular electrogram and determining the ventricular pacing threshold. 4 stainless steel ECG electrodes (25$mm^2$) were implanted subcutaneously and all leads were exteriorised via a mid-line dorsal incision. The animals wore jackets to protect the external leads from interference by the animal, while affording the operator easy access for electrophysiological recordings.

Following a 3 week period during which all electrophysiological measurements were allowed to stabilise (Manley, B.S. et al(1989)), animals were randomly allocated to either adriamycin-treated or control groups. Those animals in the treated group received adriamycin (Farmitalia Carlo Erba) 1mg.$Kg^{-1}$ intravenously, twice weekly for 8 weeks, and those in the control group 0.9% saline in equivolumetric doses. After 8 weeks therapy, animals were left for a further 2 weeks and electively killed. For the duration of the study the animals were housed separately 1 per cage. They were fed a standard laboratory diet and were allowed to drink water ad libitum. The electrical lights in the laboratory were switched off at night to observe a normal light/dark cycle.

Animals were withdrawn from the study before the end of the 10 week protocol if they lost more than 10% of their initial body weight. Any other sign of distress or pain was also deemed a reason for withdrawal. All of the experiments in this study were conducted in observance with the Animals(Scientific Procedures) Act 1986.

## 3.2 Measurements.

Electrophysiological recordings were made in conscious, unrestrained, unsedated animals at weekly intervals for

up to 10 weeks. The right ventricular paced evoked response (PER) was recorded from the pacing electrode by means of an interface depolariser (Walton, C. et al(1985)). This device counteracts the capacitative charge stored at the electrode-endocardial interface as a result of the delivery of the pacing stimulus. This allows accurate recordings of the local endocardial electrogram to be made from as early as 5ms after the pacing impulse. The amplifier settings on the interface depolariser were left unchanged during all of the electrophsiological studies. The subcutaneous ECG was recorded using a standard amplifier (model 4615-65, ECG Biotach,Gould Electronics). All signals were displayed on a digital storage oscilloscope(4125/125 Gould Electronics) which allowed PER waveforms to be averaged digitally and time and voltage measurements to be obtained by means of on-screen cursors.

Measurements were made of the Stimulus-R and Stimulus-T interval of the paced evoked response, defined as the time interval between the leading edge of the stimulus pulse and the nadir of the R wave (Stim-R), and the peak of the T wave (Stim-T) respectively (Fig.3). All measurements were made at paced cycle lengths of 200ms, 180ms and 160ms using averages of 8 beats. A minimum of 2 minutes was allowed after changing the pacing cycle lengths before making recordings to allow for electrical restitution

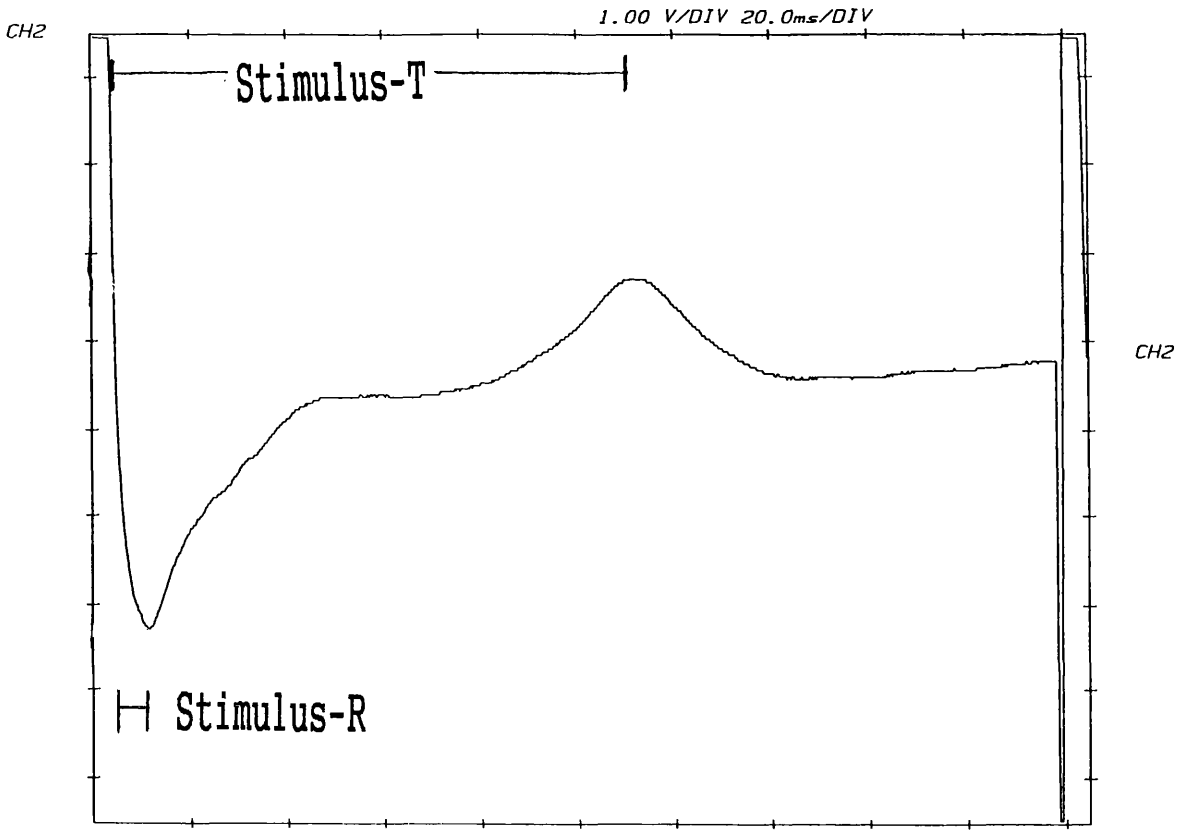


Figure 3.

Graph illustrating 1 cycle of the paced evoked response from the right ventricle and demonstrating the Stimulus-R and Stimulus-T intervals.

(Morgan, J.M., Cunningham, A.D. & Rowland, E.(1990)). The ECG signal was used for the determination of the effective refractory period by means of extra-stimuli delivered by a microcomputer-based programmable pulse generator, driving an isolated stimulator operating at twice diastolic threshold. Extra-stimuli($S_2$) were delivered after 8 beats of the basic cycle length, initially 80ms after the pacing impulse($S_1$) and subsequently at 2ms increments. The ERP was defined as the longest $S_1$-$S_2$ interval which failed to produce a response. The ERP was measured at the same cycle lengths as the PER.

Spontaneous sinus cycle length was measured by calculating the unpaced R-R interval from the right ventricular electrogram. The endocardial QT interval and the surface QT intervals were also measured from the beginning of the Q wave to the end of the T wave, at the point were the tangent to the T wave crosses the isoelectric line.

Attempts were also made to determine ventricular fibrillation threshold by delivering a single pulse of 4ms duration after 8 ventricular paced beats at a cycle length of 180ms. The onset of each single pulse was adjusted to begin 80ms after the onset of the QRS complex and subsequently to scan diastole at 5ms increments. The strength of the pulse was increased from 1mA to a maximum of 50mA in 1mA increments( Inoue, H. et al(1987)).

Continuous ambulatory ECG monitoring was undertaken using the signal from the right ventricular permanent pacing electrode. Armoured cable was used to protect the wire connecting the pacing electrode to the tape recorder. The signals were recorded onto standard C-60 magnetic tapes by means of Oxford MR 14 tape recorders. The heart rates was too rapid to permit analysis by convential Holter recording analysis systems. Tapes were therefore replayed and printed onto paper by means of total disclosure mode, and the presence of ventricular arrhythmias was noted by visual assessment.

Venous blood sampling from the marginal ear vein was undertaken at weeks 1, and 8 for plasma $Na^{+}$, $K^{+}$, $Cl^{-}$, $Mg^{2+}$, total protein, albumin and urea. Samples were also collected in EDTA and plain test-tubes which were kept in ice, for subsequent assay of plasma renin, atrial natriuretic peptide(ANP) and noradrenaline respectively. The venous blood was centrifuged at 2000 revolutions.$min^{-1}$ at a temperature of 4°C. The plasma was removed and stored in a freezer at -50° C. The assay methods employed for each hormone have been previously described (Millar, J.A., Leckie, B.J. & Morton, J.J.(1980); Richards, A.M. et al(1987); Prada, M. & Zurcher, G.(1976)).

After each animal died or when they were electively killed at the termination of the study, the hearts were excised and dissected free from the great vessels. They were then

blotted dry and weighed. Samples were taken from either ventricle for assessment of the wet/dry weight ratio. For this purpose the weighed aliquots were placed in an oven at 60°C for 5-7 days, the dry weight being taken as the weight which was stable on 3 consecutive days. The remainder of the heart was placed in formalin for histological examination. The liver, kidneys and lungs were also removed, blotted dry and weighed, with samples taken for histology. Total body weight was recorded at the beginning and the end of the study.

## 3.3 Intracellular electrode studies.

As previously described, New Zealand white rabbits were injected with either adriamycin or 0.9% saline for 8 weeks. Venous blood sampling was undertaken at weeks 1 and 8 for plasma electrolytes, protein and neurohormonal assay. 2 weeks after the end of treatment, the animals were killed by an overdose of sodium pentobarbitone intravenously. The hearts were excised and the right ventricular papillary muscles removed and mounted in a tissue bath, volume 0.4ml. The remainder of the heart was blotted dry and weighed, with samples taken for histology from either ventricle. The liver, kidneys and lungs were

also removed, blotted dry and weighed with samples stored in formalin for histological examination. Total body weight was recorded at the beginning and the end of the study.

The papillary muscles were superfused at 4ml.min$^{-1}$ with a solution containing(in mmol.l$^{-1}$) $Na^+$ 142, $K^+$ 4.0, $Ca^{2+}$ 1.8, $Mg^{2+}$ 1.0, $Cl^-$ 121, $HCO3^-$ 28, $H_2PO4^-$ 0.4 and glucose 11.0. The fluid was equilibrated with 95% $O_2$ and 5% $CO_2$. The muscles were field stimulated at 1 Hz using square wave stimuli of 1ms duration at twice diastolic threshold. The papillary muscles were maintained at 32° C and allowed to equilibrate for 3 hours before action potentials and ERP's were recorded. Floating glass microelectrodes filled with 4 Molar KCl were used to record transmembrane action potentials and the signals were amplified(model M710, WP instruments), displayed on a digital storage oscilloscope and recorded onto magnetic tape(storage-7DS, Racal) for later analysis. ERP's were determined by stimulating the papillary muscles at twice diastolic threshold at a cycle length of 1000ms. Extra-stimuli were introduced with an increasing $S_1$-$S_2$ interval duration until a second action potential was recorded. Action potentials were measured to 90% repolarisation $APD_{90}$ and 50% repolarisation $APD_{50}$. The onset of the action potential was taken at the beginning of phase 0 depolarisation. Action potential amplitudes were measured

from the diastolic resting membrane potential to the spike of the overshoot. For each muscle, numerous impalements of cells were made to sample the population of cells.

## 3.4 Statistical methods.

All data in this study is expressed as means(standard error of mean). Comparison of data was performed using the paired or unpaired Student's t test as appropriate. Data which were significantly different at baseline were compared by normalising the initial data and expressing subsequent data as the percentage change from baseline.

# CHAPTER 4

## IN VIVO ELECTROPHYSIOLOGICAL CHANGES IN ADRIAMYCIN-INDUCED CHRONIC HEART FAILURE IN THE RABBIT.

This chapter details an investigation into the effects of experimental adriamycin cardiomyopathy on ventricular repolarisation and refractoriness, neurohormonal activation, electrolyte depletion and spontaneous and induced ventricular arrhythmias, in order to characterise possible mechanisms for the predisposition to sudden death in heart failure.

## 4.1 Survival data(table 4).

A total of 16 animals entered the study in the adriamycin group and 11 animals in the saline control group. Of the adriamycin-treated animals, 2 died with evidence of subacute bacterial endocarditis(diagnosed by the presence of catheter tip vegetations, renal emboli, hypoproteinaemia and proteinuria). 1 animal developed ventricular fibrillation during rapid ventricular pacing at a cycle length of 140ms(no further animals were subsequently paced at this cycle length), 3 animals were found dead with no apparent cause(the animals were previously eating and drinking normally and no gross abnormalities were found at post-mortem examination), and 10 animals survived to the end of the study protocol.

One rabbit died in the saline control group with evidence of subacute bacterial endocarditis, 2 animals died during radio-isotope scanning(as a result of inadequate isolation of recording apparatus) and 8 survived 10 weeks.

Table 4.

Survival data in the adriamycin and saline control groups.

| | Initial number | OM | SD | SBE | Other |
|---|---|---|---|---|---|
| Adriamycin | 16 | 6 | 3 | 2 | 1 |
| Saline control | 9 | 3 | 0 | 1 | 2 |

Initial number = Initial number of animals in study.

OM = Overall mortality.

SD = Sudden death.

SBE = Subacute bacterial endocarditis.

## 4.2 Electrophysiology.

Acute effects of adriamycin(Figures 4 and 5)

The stimulus-T interval and effective refractory period data were collected at cycle lengths ranging from 160ms-200ms, but the trends and results were similar at all cycle lengths. For simplicity therefore, only the data obtained at a cycle length of 180ms are presented. The stimulus-T interval and ERP were measured for 5 consecutive days in 3 adriamycin and 3 saline control animals, following the first injection in week 1 of the study, to assess any acute effect of the drug on cardiac electrophysiology. No change was observed in either of these parameters over this period of time.

Chronic effects of adriamycin.

Prior to entering animals into the study protocol for weekly electrophysiological testing, the stimulus-T interval and the diastolic pacing threshold were measured twice weekly. Stable measurements of both these parameters were required before the animals entered the study. A total of 34 animals had permanent pacing electrodes implanted. Of these 27 eventually entered the study. The 7 animals which did not enter the study all died. The principal cause of death was infection. One of

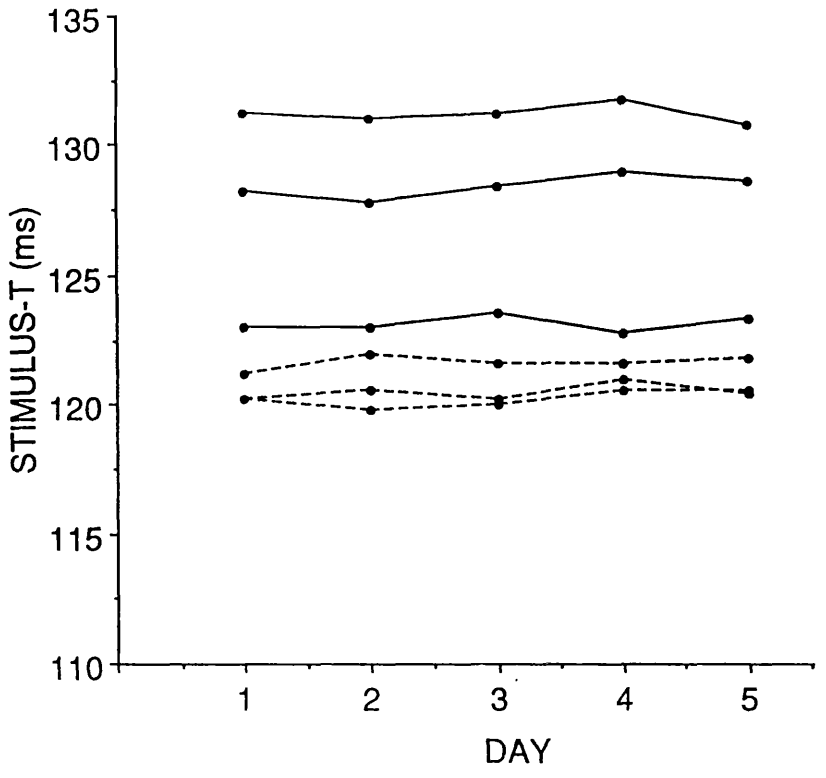


Figure 4.

Graph demonstrating the lack of any acute effect of adriamycin on the stimulus-T interval. The adriamycin treated animals are represented by the broken lines and the control animals by the continous lines.

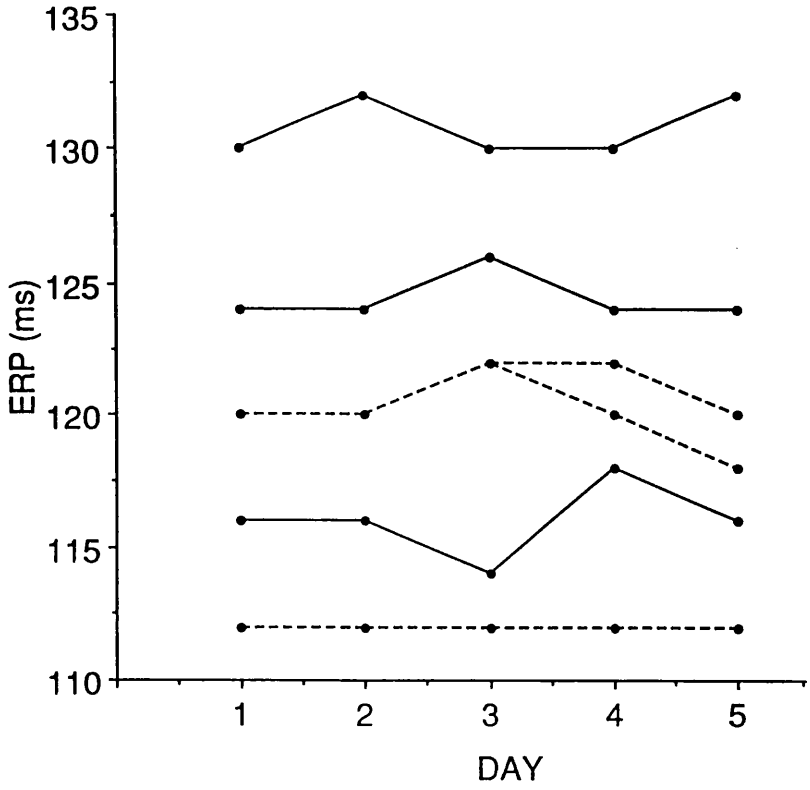


Figure 5.

Graph demonstrating the lack of any acute effect of adriamycin on the right ventricular effective refractory period. The adriamycin treated animals are represented by the broken lines and the control animals by the continous lines.

the animals had an infection of the neck wound where the pacing wire had been inserted. Five of the remaining animals were non specifically unwell and were anorexic with weight loss of more than 10% of their initial weight. The cause of death in these animals was presumed to be infection. One other animal died from a broken back after struggling to free itself from an animal technician.

Diastolic pacing threshold

The diastolic pacing threshold showed a slight decrease in the adriamycin group falling from 4.44 ± 0.54V at week 0 to 4.05 ± 0.56V at week 10. A similar pattern was observed in the saline control group with the threshold falling from 3.85 ± 0.61V to 3.06 ± 0.23V. These changes were not statistically significant and no difference was observed between the groups,(table 5).

Analysis of the data for those animals which survived to the end of the study, using the paired Student's t-test, revealed a similar pattern to above.

The mean diastolic pacing threshold was 4.1±0.5V at week0, and 4.1±0.6V at week 10 in the adriamycin group, n=9.

In the saline control group the threshold decreased from 4.2±0.8V to 3.1±0.2V, n=7.

Table 5.

Changes in diastolic pacing threshold in adriamycin and saline control groups.

| | Diastolic pacing threshold | |
|---|---|---|
| | Week0 | Week10 |
| Adriamycin | 4.44±0.5V(16) | 4.05±0.6V(10) |
| Saline control | 3.85±0.6V(11) | 3.06±0.2(8) |

The numbers in parenthesis represent the numbers of animals.

There were no significant differences between groups or within the groups between weeks 0 and 10.

Stimulus-T interval(Fig.6 and table 6)

The stimulus-T interval progressively shortened over a 10 week period in those animals treated with adriamycin, this change becoming statistically significant by week 4 ($p < 0.05$). At week 0 stim-T was 116±1.5ms vs 108±2.0ms at week 4, n = 16 and 14 respectively. By week 10 the stim-T interval was 102±1.9ms, a decrease of 12% when compared to week 0($p< 0.001$). There was no significant difference between the adriamycin and saline control groups at week 0, and no change was observed in the saline control group over the ten week period. When the adriamycin group was compared to the saline control group, the stimulus-T interval was significantly reduced by week 4, and by week 10 stim-T was 102±1.9ms vs 120±3.8ms, a difference of 15%($p<0.001$).

Comparison of paired data in animals which survived to the end of the study revealed similar results to those above. In the adriamycin group the stimulus-t interval decreased from 116±2ms at week0 to 102±2ms at week 10, $p<0.001$ and $n=10$. The values in the saline control group were 122±4ms at week0 and 120±4ms at week10, $n=8$.

Table 6

Changes in stim-T and ERP in adriamycin and saline control groups.

| Week | 0 | 5 | 10 |
|---|---|---|---|
| Adriamycin:n | 16 | 12 | 10 |
| ERP(ms) | 115 ± 2.0 | 101 ± 3.8 | 98 ± 2.9 |
| STIM-T(ms) | 116 ± 1.5 | 108 ± 2.0 | 102 ± 1.9 |
| Control: n | 11 | 11 | 8 |
| ERP(ms) | 113 ± 4.2 | 114 ± 4.0 | 120 ± 3.2 |
| STIM-T(ms) | 120 ± 2.9 | 118 ± 2.9 | 120 ± 3.8 |

The stim-T interval and ERP significantly decreased in the adriamycin group, between weeks 0 and 10($p<0.001$).
There were no significant changes in the control group.

ERP = Effective refractory period.
Stim-T = Stimulus-T interval

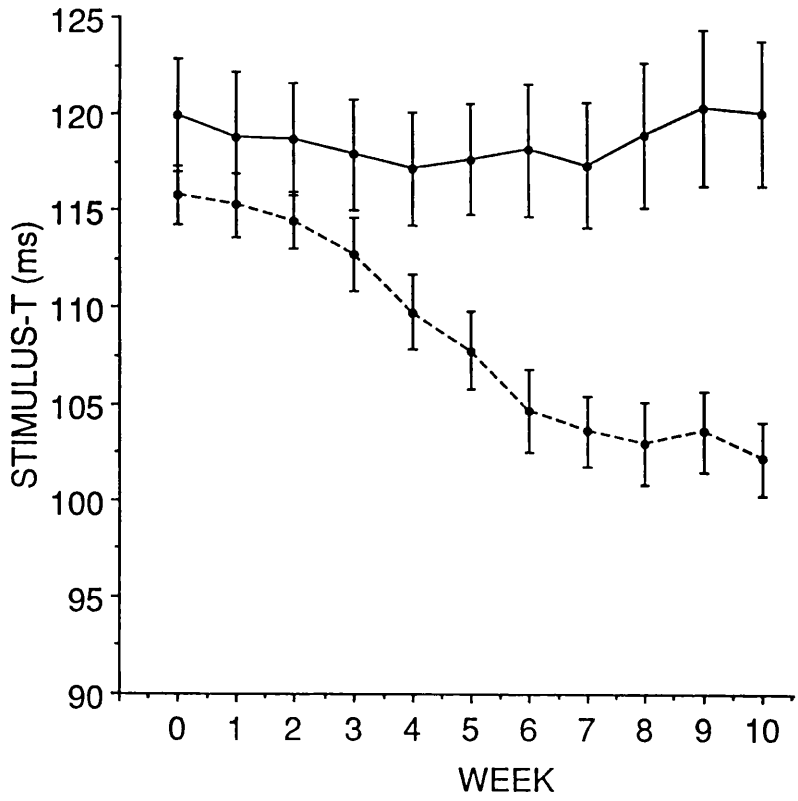


Figure 6.

Graph demonstrating the chronic effects of adriamycin on the stimulus-T interval. The adriamycin treated animals are represented by the broken line and the control animals by the continous line. The stimulus-T interval progressively shortens in the adriamycin group.

Effective refractory period(Fig.7 and table 6)

There was a progressive shortening in ERP over the 10 week period in the adriamycin-treated group,from 115±2.0ms at week 0 to 101±3.8ms at week 5($p<0.05$). At week 10 the ERP was 98±2.9ms, a 15% decrease when compared to week 0($p<0.001$). No difference was observed between the adriamycin and saline control groups at week 0, and no significant change in ERP occured in the saline control group over the ten week period. A significant decrease in ERP between the adriamycin and saline control groups developed by week 5 $p<0.05$. At week 10 the ERP was 98±2.9ms in the adriamycin group vs 120±3.2ms in the saline control group $p<0.001$.
Analysis of paired data in animals surviving to the end of the study demonstrated a significant decrease in the adriamycin group from 116±2ms at week0 to 98±3ms at week 10, $p<0.001$ and n=9. There was no significant change in the saline control group, 121±4ms at week0 and 120±4ms at week 10, n=7.

Sinus cycle length(table 7)

Sinus cycle length tended to decrease in the adriamycin group over a 10 week period from 238±6.7ms at week 0 to 221 ±7.9ms at week 10, while the saline control group

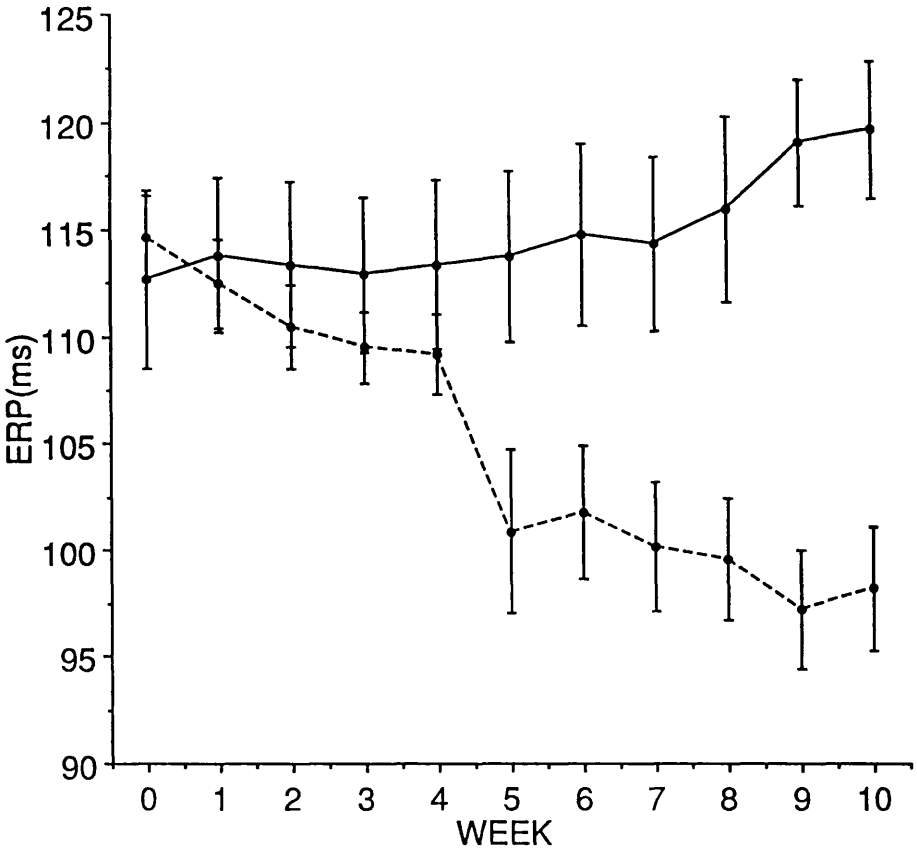


Figure 7.

Graph demonstrating the chronic effects of adriamycin on the effective refractory period. The adriamycin treated animals are represented by the broken line and the control animals by the continuous line. The ERP progressively shortens in the adriamycin group.

Table 7.

Changes in sinus cycle length in adriamycin and saline control groups.

| | Sinus cycle length | |
|---|---|---|
| | Week0 | Week10 |
| Adriamycin | 238±7(16)ms | 221±8(10)ms |
| Saline control | 230±4(11)ms | 238±3(8)ms |

The numbers in parenthesis represent the numbers of animals.

There were no significant differences between the groups or within the groups between weeks 0 and 10.

demonstrated a non-significant increase from 230±3.8ms to 238±2.8ms. No significant difference was observed between the groups at week 0 or week 10.
Analysis of paired data from animals which survived to the end of the study revealed a non-significant decrease in the adriamycin group from 240±7ms at week0 to 221±8ms at week10, n=10, and a non-significant increase in the saline control group from 232±4ms to 238±3ms, n=8.

Ambulatory ECG recording

A total of 52 ambulatory ECG recordings were attempted, although only 12 complete tapes were successfully recorded. The low rate of successful recordings was caused by animals chewing through the armoured protective cable which surrounded the connecting wires. In the adriamycin group of animals, 1 episode of ventricular bigemini was detected and in the majority of the others infrequent ventricular ectopic activity ie less than 10 beats.Hour$^{-1}$ was seen. There was no evidence of spontaneous ventricular tachycardia or ventricular fibrillation. No arrhythmias were recorded in the control group.

Arrhythmia induction

Attempts were made to determine the ventricular fibrillation threshold using the protocol described in chapter 3.2. Three animals were studied using the protocol described by Inoue, H. et al(1985). No arrhythmias were induced in any of the animals and further attempts were therefore abandoned. It should also be noted that no arrhythmias were induced by the single extra-stimulus technique when the ERP was measured in each animal during the weekly electrophysiological study. Attempts to initiate ventricular tachycardia using up to 3 extra-stimuli were made in 3 adriamycin treated rabbits. Because of the unusual electrical restitution properties of the rabbit heart, it was observed that addition of a second or third extra-stimulus did not result in any shortening of the effective refractory period compared with the first extra-stimulus. Possibly as a result of this, no arrhythmia could be induced. It was considered that arrhythmia induction by rapid ventricular pacing(see above) was too non-specific and thus systematic attempts at arrhythmia induction were not pursued.

## 4.3 Biochemistry.

Neurohormonal data(table 8).

The mean levels of plasma noradrenaline, renin and atrial natriuretic peptide were measured at week 0 in the instrumented and non-instrumented animals prior to adriamycin/saline injection. Mean values for noradrenaline were 6.04 $\pm$ 1.05 vs 4.91 $\pm$ 1.14 ($nmol.l^{-1}$), plasma renin concentration 69.6 $\pm$ 8.04 vs 83.7 $\pm$ 6.8 $uU.ml.^{-1}$ and ANP 98.0 $\pm$ 6.5 vs 128.7 $\pm$ 17.03 ($pg.ml^{-1}$) in the instrumented and non-instrumented groups respectively. None of these differences were statistically significant, and thus the results for the instrumented and non-instrumented animals have been pooled.

Plasma noradrenaline levels showed no significant change in either the adriamycin or saline control groups when samples were compared between week one and eight, nor was there any significant change between the groups.

Similarily no change was observed in the levels of ANP in either group, over an 8 week period nor between the groups. Plasma renin concentration decreased significantly in the saline control group between weeks 1 and 8, but there were no significant differences between the groups at weeks 1 or 8. There was no significant

Table 8.

Neurohormonal changes in adriamycin and saline control groups.

| Week | 1 | | 8 |
|---|---|---|---|
| Adriamycin: | | | |
| Renin | 74.2 ± 6.8(11) | | 66.6 ± 8.5(18) |
| ANP | 137.7 ± 24.9(13) | | 94.3 ± 10.7(17) |
| NA | 5.1 ± 1.3(19) | | 5.4 ± 0.8(19) |
| Saline control: | | | |
| Renin | 72.6 ± 7.2(12) | * | 56.1 ± 4.2(17) |
| ANP | 118.7 ± 12.5(10) | | 118.8 ± 14.4(14) |
| NA | 3.5 ± 0.5(18) | | 5.2 ± 1.2(11 |

Numbers in parenthesis represent numbers of animals.

* = $p<0.05$

Renin = Plasma renin concentration ($uU.ml^{-1}$)
ANP = Atrial natriuretic peptide concentration($pg.ml^{-1}$)
NA = Plasma noradrenaline concentration($nmol.l^{-1}$)

change in the level of plasma renin in the adriamycin group between weeks 1 and 8.

Analysis of paired data from animals which survived to the end of the study demonstrated a non-significant increase in plasma noradrenaline levels in both the adriamycin and saline control groups between weeks 1 and 8. The mean values were $3.8\pm0.6 nmol.l^{-1}$ at week1 and $5.8\pm1.2 nmol.l^{-1}$ at week8 in the adriamycin group, n=12, and $3.3\pm0.5 nmol.l^{-1}$ vs $5.2\pm1.4 nmol.l^{-1}$ in the saline control group.

The mean levels of plasma ANP decreased in the adriamycin group and increased in the saline control group although these changes were non-significant. The levels in survivors were $112.6\pm16 pg.ml^{-1}$ at week1 vs $84.2\pm14.7 pg.ml^{-1}$ in the adriamycin group, n=11, and $114\pm14 pg.ml^{-1}$ vs $134.2\pm15 pg.ml^{-1}$ in the saline control group, n=11.

Plasma electrolytes

As with the neurohormonal results, the data for the instrumented and uninstrumented animals was pooled with regards to plasma electrolytes. The mean value of plasma $K^+$ fell slightly in the adriamycin group from $4.9\pm0.12$ $mmol.l^{-1}$ at week 1 to $4.6\pm0.08$ $mmol.l^{-1}$ at week 8, and showed no change in the saline control group over an 8 week period $4.8\pm0.11$ $mmol.l^{-1}$ vs $4.8\pm0.12$ $mmol.l^{-1}$ There

were no significant differences between the groups at weeks 1 or 8, (table 9).

Comparison of paired data from animals which survived to the end of the study revealed similar results to those above. There was a non-significant decrease in the adriamycin group from $4.9\pm0.1 mmol.l^{-1}$ to $4.6\pm0.1 mmol.l^{-1}$, n=18 and no change in the saline control group, $4.8\pm0.1 mmol.l^{-1}$ vs $4.8\pm0.1 mmol.l^{-1}$, n=16.

Plasma $Mg^{2+}$ significantly decreased between weeks 1 and 8 in the adriamycin group of animals, falling from $0.95 \pm 0.05 mmol.l^{-1}$ to $0.70 \pm 0.02 mmol.l^{-1}$. There was a tendency for plasma $Mg^{2+}$ levels to fall in the saline control group over the 8 week period, although this was less pronounced than in the adriamycin group, such that when the groups were compared, plasma $Mg^{2+}$ levels were significantly less in the adriamycin group when compared to the saline control group at weeks 8. There were no differences between the groups at week 1 (table 9).

Paired analysis of the data in animals which survived to the end of the study again demonstrated a significant decrease in the adriamycin group from $0.95\pm0.05 mmol.l^{-1}$ to $0.69\pm0.03$ $mmol.l^{-1}$, $p<0.01$ and n=16, and a non-significant decrease in the saline control group from $0.95\pm0.05 mmol.l^{-1}$ to $0.85\pm0.03 mmol.l^{-1}$, n=16.

Table 9.

Changes in plasma magnesium and potassium concentrations in the adriamycin and saline control groups.

| | Adriamycin | | Saline controls | p |
|---|---|---|---|---|
| $K^+$Week1 | 4.87 ± 0.12(23) | | 4.79 ± 0.11(17) | NS |
| $K^+$Week8 | 4.59 ± 0.08(18) | | 4.8 ± 0.12(16) | NS |
| $Mg^{2+}$Week1 | 0.95 ± 0.05(16) | | 0.95 ± 0.05(16) | NS |
| | *** | | | |
| $Mg^{2+}$Week8 | 0.70 ± 0.02(20) | *** | 0.85 ± 0.03(16) | 0.001 |

NS = not significant.

*** = $p<0.001$.

Numbers in parenthesis represent numbers of animals.

$K^+$ : Plasma potassium ($mmol.l^{-1}$)

$Mg^{2+}$ : Plasma magnesium ($mmol.l^{-1}$)

No significant changes were seen in plasma sodium concentration over an 8 week period in the adriamycin group of animals. There was a small decrease in the saline control group over an 8 week period. Although this was statistically significant, there were no differences between the groups at weeks 1 or 8, (table 10).
Analysis of paired data revealed no significant change in the plasma sodium concentration in either group between weeks 1 and 8. The values were $143\pm0.8 mmol.l^{-1}$ at week1 vs $143\pm0.5 mmol.l^{-1}$ at week8 in the adriamycin group, n=20, and $143\pm0.5 mmol.l^{-1}$ vs $142\pm0.4 mmol.l^{-1}$ in the saline control group, n=16.
Plasma urea levels showed a non-significant increase in the adriamycin group and a non-significant decrease in the saline control group over the 8 week period, and there were no significant differences when the groups were compared (table 11). Analysis of paired data revealed similar results to those above. The mean plasma urea concentration increased from $6.5\pm0.3 mmol.l^{-1}$ to $7.0\pm0.3 mmol.l^{-1}$ in the adriamycin group, n=20, and decreased from 6.8±0.3mmol.l-1 to $6.7\pm0.3 mmol.l^{-1}$ in the saline control group, n=16.

Table 10.

Changes in plasma sodium concentration in adriamycin and saline control groups.

| | Plasma sodium($mmol.l^{-1}$) | | |
|---|---|---|---|
| Week | 1 | 8 | p |
| Adriamycin | 142.8±0.7(24) | 143.0±0.5(20) | NS |
| Saline control | 143.4±0.5(18) | 142.0±0.4(16) | 0.05 |

There were no differences between the groups at week 1 or 8.

The numbers in parenthesis represent the nunbers of animals.

Table 11.

Changes in plasma urea concentration in adriamycin and saline control groups.

| | Plasma Urea($mmol.l^{-1}$) | |
|---|---|---|
| Week | 1 | 8 |
| Adriamycin | 6.6 ± 0.3(24) | 7.0 ± 0.4(20) |
| Saline control | 7.0 ± 0.3(18) | 6.6 ± 0.3(16) |

There were no significant differences between the groups orbetween weeks 1 and 8 in either group.

Numbers in parenthesis represent the numbers of animals.

## 4.4 Pathology(tables 12 and 13)

The results in this section represent data from both instrumented and uninstrumented animals. At the end of the study the mean wet heart weights were significantly higher in the adriamycin group when compared to the saline control group, a difference of 49%. The liver weights were also increased in the adriamycin group by 35% when compared to controls. There was no significant difference in lung weights between the groups.

Total body weight fell slightly over a 10 week period in the adriamycin group and showed a non-significant increase in the saline control group, such that at week 10, the mean body weight was significantly higher in the saline control group.

Analysis of paired data from those animals which survived to the end of the study revealed a non-significant decrease in body weight in the adriamycin group from $3.3 \pm 0.06$Kg to $3.2 \pm 0.07$Kg, n=18. There was a non-significant increase in the mean body weight in the saline control group from $3.3 \pm 0.08$Kg to $3.5 \pm 0.1$Kg, n=16.

When the heart weights were expressed as a percentage of total body weight, heart weight was 57% higher in the adriamycin-treated animals than controls, $p<0.01$. The liver weight /total body weight ratio was 42% higher in

Table 12

Comparison of heart,liver and lung weights between the adriamycin and saline control groups.

| | Adriamycin | Saline Control | p |
|---|---|---|---|
| Heart weight(g) | 10.8±0.6(17) | 7.2±0.3(16) | 0.001 |
| Liver weight(g) | 87.8±6(18) | 64.9±4(15) | 0.01 |
| Lung weight(g) | 18.5±1.4(18) | 18±1.1(15) | NS |

Numbers in parenthesis represent numbers of animals.

NS = Not significant.

Table 13.

Comparison of body weight and heart, liver and lung to body weight ratios between the adriamycin and saline control groups.

| | Adriamycin | Saline control | p |
|---|---|---|---|
| Body weight(Kg): | | | |
| Week0 | 3.3±0.05(24) | 3.27±0.08(19) | NS |
| Week10 | 3.2±0.07(18) | 3.49±0.1(16) | 0.05 |
| HW/BW % | 0.33±0.02(18) | 0.21±0.02(15) | 0.01 |
| LW/BW % | 2.77±0.2(18) | 1.95±0.2(15) | 0.05 |
| LUW/BW% | 0.58±0.5(18) | 0.52±0.04(15) | NS |

Numbers in parenthesis represent numbers of animals.

HW = heart weight

LW = liver weight

LUW = lung weight

BW = body weight.

There were no significant differences in body weights between weeks 0 and 10 within either group.

the adriamycin-treated animals, $p<0.05$. There was a tendency for the lung/total body weight ratio to be higher in the adriamycin group when compared to the saline control group.

The wet/dry weight ratio of the ventricular samples were 5.8 $\pm$ 0.95 in the adriamycin group vs 4.47 $\pm$ 0.29 in controls. Although there was a tendency to higher tissue water content in the adriamycin-treated hearts, this change was not statistically significant and implies that the increase in heart weight in the adriamycin group was due to an increase in tissue dry matter and not simply due to oedema.

Histology

Light microscopy of the adriamycin-treated hearts showed widespread atrophy and lysis of cardiac myocytes associated with interstitial fibrosis. Extensive vacuolation and reduction in myocyte size was also seen. No histological abnormalities were observed in the control group.

## 4.5 Discussion

The principal finding in this chapter is of a progressive decrease in right ventricular effective refractory period and repolarisation time measured in vivo in a model of chronic cardiac failure. These changes are independent of sinus cycle length, since they were made at a constant heart rate. There were no significant changes in diastolic pacing threshold which might influence ERP. The elecrophysiological changes also appear to be unrelated to neurohormonal activation as no significant increase in plasma noradrenaline or renin concentrations were detected in the adriamycin-treated animals. The shortening in repolarisation and refractoriness are also independent of changes in plasma $K^{+}$ levels, and the decrease in plasma $Mg^{2+}$ could account for no more than 2% shortening in action potential duration if we extrapolate from previous in vitro studies (Watanabe, Y. & Dreifus, L.S.(1972)). The most likely explanation therefore is that the effects on ventricular repolarisation and refractoriness are related to an intrinsic myocardial phenomenon caused by the adriamycin cardiomyopathy, and this premise is further supported by the in vitro data presented in the next chapter.

Although no direct haemodynamic measurements were made in this study to support the premise that the animals treated

with adriamycin were in heart failure, the significant increases in heart and liver weights which were found in the animals treated with adriamycin would tend to support this hypothesis.

One possible explanation may be that adriamycin administration produces a toxic change in myocytes which is manifest as a shortening in action potential duration analagous to that seen during myocardial hypoxia or ischaemia (Janse, M.J. & Kleber, A.G.(1981); McDonald, T.F., Hunter, E.G. & MacLeod, D.P.(1971)).

Two principle mechanisms of adriamycin-induced cardiac damage have been proposed as discussed previously in chapter 1. An increased cytosolic calcium concentration has been suggested by some authors (Bers, D.M., Philipson, K.D. & Langer, G.A.(1981); Singal, P.K. & Pierce, G.N.(1986); Dhalla, N.S. et al(1982); Olson, H.M. et al(1974)), while others have proposed damage to cell and organellar membranes by the generation of oxygen-derived free radicals which produce an increase in the rate of endogeneous lipid peroxidation (Bachur, N.R. et al(1983); Kalyanaramun, B., Perez-Reyes, E. & Mason, R.P.(1980); Land, E.J. et al(1983); Doroshow, J.H.(1983); Myers, C.E. et al (1977)).

The former mechanism may well be more important in some ways to the shortening in ventricular repolarisation and refractoriness which has been demonstrated. As discussed

previously, it has been demonstrated that an increase in intracellular calcium concentration may shorten action potential duration (Carmeliet, E.(1978); McDonald, T.F. & MacLeod, D.P.(1973); Isenberg, G.(1975) and Reuter, H.(1974)).

The results suggest that the changes in repolarization and refractoriness are unrelated to any acute direct electrophysiological action of adriamycin however, as no changes were demonstrated in either the stimulus-T interval or ERP when these parameters were measured for 5 consecutive days during the first week of the study. There was also no reversal of the shortening in repolarisation and refractoriness after week 8 when the adriamycin injections were stopped. Further evidence that the electrophysiological changes are unrelated to any direct action of the drug is demonstrated in the next chapter when in vivo shortening in repolarization and refractoriness are seen to occur 2 weeks following the last injection of adriamycin.

The histological findings of widespread myocyte damage persisting 2 weeks after the last injection of adriamycin, suggests that the myopathic process itself may well be responsible for the electrophysiological changes.

Another possible mechanism for the in vivo shortening in the stimulus-T interval and the ERP may be an increase in afterload. It has been previously demonstrated that right

atrial pressure increases by 100% in animals treated with adriamycin according to the protocol used in this study, and that there was an increase in systemic arterial resistance (Wanless, R.B. et al(1987)). Although pulmonary arterial pressure was not measured in this or the previous study, it may well be that there was a chronic increase in pulmonary pressure and hence right ventricular afterload as a result of left ventricular dysfunction.

Several authors have investigated the effects of acute changes in afterload on ventricular repolarisation and refractoriness as discussed in chapter 1. Reiter, M.J. et al(1988) have demonstrated that in the isolated perfused rabbit heart, as left ventricular volume was increased by the dilatation of a balloon in the ventricular cavity, a progressive decrease in left ventricular effective refractory period was demonstrated. It was also noted that ventricular fibrillation and tachycardia were easily induced by the single extra-stimulus technique, after, but not before, balloon distention. The results in this chapter differ from those of Reiter, M.J. et al(1988) in that no arrhythmias were induced by the single extra-stimulus technique in any animal, although we did detect evidence of spontaneous ventricular arrhythmias in the adriamycin-treated animals, while no spontaneous arrhythmias were detected in the Reiter study. This

difference between the studies could be related to the obvious differences between an in vivo preparation and an isolated buffer-perfused heart. Reiter, M.J. et al(1988) also demonstrated an intra and inter ventricular heterogeneity of refractoriness which they related to the inducibility of arrhythmias. No comment can be made on the heterogeneity of refractoriness in the present study, since stimulation was only performed from a single fixed site.

Lermann, B.B. et al(1985) have also demonstrated that increasing the left ventricular end diastolic volume from 10ml to 30ml in the cross circulated, servo-controlled canine heart was associated with a 7% reduction in left ventricular ERP. The effects of mechanical stress on the electrophysiology of isolated muscle preparations has also been investigated by Lab, M.J.(1982), as previously discussed in chapter 1.5. Lab demonstrated that high muscle tension, in the form of isovolumetric contraction, is associated with a shortening in action potential duration in both isolated muscle preparations and the intact frog heart.

From a survey of existing literature, this is the first time that chronic changes in ventricular repolarisation time and refractoriness have been investigated in vivo in a model of cardiac failure. Thus comparison of this electrophysiological data with other in vivostudies is

not posssible. The paucity of pre-existing studies of chronic electrophysiological changes in heart failure is most likely related to the limitations bestowed by the available models of heart failure, as previously discussed in chapter 1.12.

Although haemodynamic measurements were not undertaken to confirm the presence of heart failure, such measurements were undertaken in an identical model (Arnolda, L. et al (1985) and Wanless, R.B. et al(1987)). Increases in both heart and liver weights consistent with cardiac failure were found in the present study. The increase in heart weight could not be explained by an increase in water content alone. One significant difference between the results in this study and the study by Arnolda is that I observed no activation of the renin-angiotensin system in the adriamycin-treated animals, in contrast to their observation of an increase in both plasma renin and noradrenaline after 4 and 8 weeks treatment with adriamycin respectively. Another difference between the studies was that the baseline levels of noradrenaline were considerably higher in the present study, 5.1+1.3nmol.l-1 vs 0.7+0.1nmol.l-1. This may imply that the animals were stressed possibly by the procedure of blood sampling from the marginal ear vein. The method of sampling in the study by Arnolda, L. et al(1985) is not specified but may have been from an indwelling catheter.

The absence of stimulation of the renin-angiotensin system in the adriamycin-treated animals in this study, does not disprove the hypothesis that the animals were in heart failure. It has been demonstrated previously that patients with untreated heart failure may in fact have normal levels of plasma renin, and that only when diuretics are introduced into the treatment regime does activation of the renin-angiotensin system occur (Brown, J.J. et al(1970) and Bayliss, J. et al(1987)). Plasma noradrenaline levels in the untreated patients with heart failure in the study by Bayliss showed a mean value of 4.2 nmol.l-1, compared to 5.4±0.8 nmol.l-1 by week 8 in the adriamycin-treated animals in this study. The similarity between these results and those of Bayliss lends further support to the argument that this model of experimental heart failure is compatible in some respects with the clinical syndrome of heart failure. However, the possibility of inter-species variability should also be borne in mind. This model is limited however by the fact that the incidence of sudden death in those animals treated with adriamycin was low, only 3 animals in the adriamycin group died suddenly and unexpectedly. There was also a paucity of complex ventricular arrhythmias as detected by continuous ambulatory monitoring. However, as with the clinical situation, defining which animals died suddenly, presumably from malignant ventricular

arrhythmias, is difficult. It is possible however that the incidence of sudden death may be greater in the prescence of intervention with diuretics.

Despite the limitations of the existing experimental model, the data suggests that shortening of ventricular repolarisation and refractoriness in heart failure may represent an intrinsic mechanism predisposing to sudden death in heart failure.

# CHAPTER 5

# IN VITRO ELECTROPHYSIOLOGICAL CHANGES IN ADRIAMYCIN-INDUCED CHRONIC HEART FAILURE IN THE RABBIT.

## 5.1 Introduction.

The previous chapter has outlined the chronic changes in ventricular repolarisation and refractoriness which occur in vivo in adriamycin-induced heart failure in the rabbit. The shortening in repolarization and refractoriness which was demonstrated appears to be unrelated to neurohormonal activation or plasma electrolyte depletion. The most likely explanation would therefore seem to be that either adriamycin produces a toxic change in myocytes which is manifest as a shortening in action potential duration, or that the electrophysiological changes are related to a chronic increase in pulmonary arterial pressure secondary to left ventricular dysfunction.

Electrophysiological experiments performed in vitro in the isolated papillary muscle preparation would provide further evidence as to the aetiology of the changes in repolarisation and refractoriness. This preparation by its very nature would be uninfluenced by the plasma concentrations of adrenaline, noradrenaline and

angiotensin II. The electrophysiological effects of changes in plasma electrolyte concentrations in heart failure are corrected by superfusion with a standardized physiological saline solution. Any electrophysiological changes which occur in such a preparation are therefore likely to be related to an intrinsic myocardial phenomenon or possibly to an adaptive change in the muscle which has occurred in response to a chronic increase in afterload.

## 5.2 Methods.

Two experimental groups of animals were used in this study, and the experimental protocol and electrophysiological techniques used are described in chapter 3. Animals used in this study did not undergo intracardiac electrode implantation, since previous studies in this laboratory have shown that prolonged implantation of a right ventricular electrode results in surface fibrosis of right ventricular papillary muscles, rendering intracellular microelectrode impalements extremely difficult.

## 5.3 Results.

### 5.3.1 Survival data.

A total of 11 animals entered the study in the adriamycin group and 9 animals in the control group. No deaths occured in either group over the 10 week period of the study.

### 5.3.2 Electrophysiology.(table 14).

The mean action potential duration at 90% repolarisation from the right ventricular papillary muscles in the adriamycin group of animals was 202±10ms (range:168-236ms) vs 264±14ms in controls (range :216-296ms), n = 6 and 5 respectively and $p<0.01$. The mean effective refractory period was 189±10ms (range :154-220ms) in the adriamycin-treated group, vs 290±9ms in controls (range :249-343ms), n = 6 and 5 respectively and $p<0.001$, table 14. These results represent a 23% shortening in $APD_{90}$ and a 35% reduction in effective refractory period in the adriamycin group.

Table 14.

In vitro changes in action potential duration and effective refractory period in the adriamycin and saline control groups.

| | Adriamycin | Control | p |
|---|---|---|---|
| $APD_{90}$(ms) | 202 ± 10(6) | 264 ± 14(5) | 0.01 |
| ERP(ms) | 189 ± 10(6) | 290 ± 9(5) | 0.001 |

$APD_{90}$ = Action potential duration at 90% repolarisation.

ERP = Effective refractory period.

Numbers in parenthesis represent the numbers of animals.

### 5.3.3 Biochemistry.

Neurohormonal data

The mean levels of plasma noradranaline, renin and atrial natriuretic peptide showed no significant differences between the instrumented animals discussed in chapter 4 and the un-instrumented animals in this study, when compared at week 0. For this reason, the results have been pooled and analysed in chapter 4, table 8.

Plasma electrolytes(tables 9, 10 and 11).

The pooled data for the instrumented and uninstrumented animals has been discussed in chapter 4.

5.3.4 Pathology(tables 12 and 13).

The pooled data for the instrumented and uninstrumented animals have been discussed in chapter 4.

5.4 Discussion.

The principal finding in this chapter is of a significant shortening in action potential duration and effective refractory period in right ventricular papillary muscles from the hearts of rabbits treated with adriamycin for 8 weeks, and then left for a further 2 week period, (Wanless, R.B. et al(1987)). These results are similar to the shortening in ventricular repolarisation and refractoriness which was demonstrated in vivo in the previous chapter, although the magnitude of the decrease was greater in the experiments in this study. Other workers have also described a significant reduction in action potential duration in right ventricular papillary muscles from rabbits treated with adriamycin, (Shenasa, H. et al 1990).

The fact that these changes occured at least 2 weeks after the last injection of adriamycin is further evidence that the electrophysiological changes are unrelated to any direct action of the drug itself. It was also demonstrated in the previous chapter that no acute direct

electrophysiological effect of the drug was seen in vivo. The nature of this isolated preparation also lends support to the premise that the shortening in action potential duration and refractoriness observed in vivo, are unrelated to either neurohormonal activation or plasma electrolyte depletion. The most likely explanation for the in vitro electrophysiological changes remains the toxic cytopathic effect of adriamycin(as previously discussed in chapter 1). One possible mechanism which has been proposed is the accumulation of intracellular calcium. The typical sarcolemmal changes which are associated with adriamycin toxicity are thought to be a consequence of intracellular calcium overload and subsequent loss of structural integrity of the cell. Sarcolemmal calcium bound at the low affinity sited on the membrane has been associated with cellular calcium influx and is correlated with the developed force, (Bers, D.M. etal(1981)). Adriamycin has been shown in-vitro to stimulate low-affinity calcium binding but depress contractile function,(Singal, P.K. & Pierce, G.N.(1986)). Other workers have shown that adriamycin stimulates calcium ATPase in vitro, and this enzyme activity has been associated with low affinity calcium binding and contractile force, (Dhalla, N.S. et al(1982)). These observations may imply a change in sarcolemmal permeability leading to an influx of calcium. Previous workers have demonstrated that an increase in

intracellular calcium concentration, independent of the aetiology, shortens the action potential duration, (Carmeliet, E.(1978); Mc Donald, T.F. & MacLeod, D.P.(1973); Isenberg, G.(1975) and Reuter, H.(1974)). The mechanism by which an increase in intracellular calcium concentration shortens action potential duration, may be mediated by inhibition of the slow inward calcium current, by an increase in potassium conductance, or both. Another possible explanation for the electrophysiological changes could be an adaptive response to a chronic increase in afterload, as discussed in the previous chapters. The majority of studies in which a major element of hypertrophy is present have demonstrated an increase in action potential duration.The shortening in action potential duration and refractoriness in this in vitro preparation from animals with heart failure, is in accordance with the in vivo results presented in the previous chapter. The results therefore support the hypothesis that the electrophysiological changes are unrelated to neurohormonal activation or plasma electrolyte depletion.

# CHAPTER 6

# THE EFFECT OF CAPTOPRIL ON THE ELECTROPHYSIOLOGICAL CHANGES ASSOCIATED WITH ADRIAMYCIN-INDUCED CHRONIC HEART FAILURE IN THE RABBIT.

## 6.1 Introduction.

Activation of the renin-angiotensin system is one of the neurohormonal changes which occur in chronic heart failure in an attempt to maintain circulatory homeostasis and preserve renal function. It has been proposed however that the increased activation of this system may predispose to sudden unexpected death, by increasing ventricular wall stress and myocardial oxygen consumption, (Massie, B. et al (1982)), and also by causing depletion of both potassium and magnesium. The evidence for the proarrhythmic effects of depletion of potassium and magnesium is discussed in chapters 1.6 and 1.7.
As discussed in chapter 1.8 however, the subgroup of patients with more severe heart failure and greater activation of the renin-angiotensin system, are at no higher risk of dying suddenly, (Burgraff, G.W. & Packer, M.(1975); Massie, B. et al(1981); Cohn, J.N. et al(1984) and Wilson, J.R. et al(1983)). As patients with more severe heart failure have greater activation of the renin-

angiotensin system, this would appear to indicate that activation of this mechanism was not of paramount importance in arrhythmogenesis in heart failure. Studies which have examined the effect of inhibition of the renin-angiotensin system in patients with chronic heart failure have shown that there is a reduction in the frequency and complexity of ventricular ectopic rhythms, (Cleland, J.G.F. et al(1984)), and an increase in exercise capacity and overall prognosis, (The Consensus trial study group,(1987); Magnani, B. & Magelli, C.(1986); Ader, R. et al.(1980); Dzau, V.J. et al(1980); Massie, B. et al(1982); The SOLVD Investigators,(1991)). As previously mentioned however, only one study has demonstrated a reduction in the incidence of sudden death in heart failure in patients treated with enalapril, (Cohn, J.N. et al(1991)).

The principal finding of the experiments conducted in chapters 4 and 5 is that of a progressive shortening in right ventricular repolarisation and refractoriness in adriamycin-induced chronic heart failure in the rabbit. These electrophysiological changes were unrelated to activation of the renin-angiotensin system, as there was no significant increase in plasma renin activity over an 8 week period in those animals treated with adriamycin. As previously discussed in chapter 1.9 however, captopril,

the angiotensin converting-enzyme inhibitor, has an alternative vasodepressor effect which is mediated through an increase in prostaglandin E2 synthesis. Although there is only limited clinical evidence that inhibition of the renin-angiotensin system reduces the incidence of sudden unexpected death, administration of the drug to patients with heart failure has been shown to reduce the frequency and complexity of ventricular arrhythmias. The experiments described in this chapter were designed to assess the effects of captopril on the shortening in repolarisation and refractoriness which occurred in the animals with heart failure in the previous chapters. An attempt was also made to assess the effects of captopril on spontaneous and induced ventricular arrhythmias and plasma electrolytes.

The possibility existed prior to these experiments that captopril could theoretically reduce afterload by virtue of its stimulation of the production of prostaglandin E2, even in the absence of activation of the renin-angiotensin system, and therefore by reducing ventricular wall stress and myocardial oxygen consumption, reduce the incidence of sudden death: Massie, B. et al(1982).

## 6.2 Methods.

Two experimental groups of animals were used in this study, and the surgical techniques and electrophysiological and other measurements are as described in chapter 2.

## 6.3 Experimental protocol.

Following the implantation of the permanent pacing electrodes and the subcutaneous ECG electrodes, a 3 week period was allowed during which all electrophysiological measurements were allowed to stabilise. The animals were then randomly allocated to receive either adriamycin injections $1mg.Kg^{-1}$ twice weekly intravenously for 8 weeks, or 0.9% saline in equivolumetric doses. Each animal was also treated with captopril in a dose of $2.14mg.Kg^{-1}.day^{-1}$, and the total daily dose was dissolved in 100ml of the animals drinking water. This dose corresponds to an equivalent dose of $150mg.day^{-1}$ in man, (Overturf, M.L., Sybers, H.D. & Smith, S.A.(1985)). The captopril was introduced after 6 completed weeks of treatment with either adriamycin or saline. This was to allow time for heart failure to develop in the animals, (Wanless, R.B. et al(1987)), and also to avoid the possibility that captopril might inhibit the cardiotoxic

effects of adriamycin by its role as an oxygen-derived, free radical scavenger, (Westlin, W. & Mullane, K. (1988)).

The electrophysiological measurements were made weekly for up to 10 weeks. The animals were venesected via the marginal ear vein at weeks 1 and 8 of the study for subsequent biochemical analysis, and in addition, plasma levels of angiotensin II were measured in the animals both prior to the introduction of captopril at the end of week 6, and after 2 weeks of treatment with captopril at the end of week 8, using methods previously described, (Morton, J.J. & Webb, D.J.(1885)). The blood samples which were taken for the subsequent analysis of angiotensin II were drawn into syringes which were pre-filled with an angiotensin converting-enzyme inhibitor consisting of (per 100ml) 4.64g of EDTA, neomycin 0.2g, orthopenanthroline 0.5g in 1ml of absolute ethanol. The amount of inhibitor used was 1ml per 20ml of venous blood. The samples thus taken were immediately centrifuged and the plasma stored at -50°C for subsequent assay.

When the animals died or when they were electively killed at the end of the study, the heart, liver, kidneys and lungs were removed, blotted dry and weighed. Samples were taken from each of these organs for histological examination and from the heart and liver for assessment of the wet/dry weight ratio.

## 6.4 Results.

Results are presented for the adriamycin/captopril and saline/captopril groups. In addition, comparison is made with the data from the adriamycin and the saline treated groups(chapter 4), where appropriate.

### 6.4.1 Survival data(table 15).

A total of 11 animals entered the study in the adriamycin/captopril group, and 9 animals in the saline control/captopril group. 2 animals in the adriamycin plus captopril group died with evidence of renal failure. One of those animals died suddenly and the other developed ventricular fibrillation during routine electrophysiological testing at a paced cycle length of 160ms. 3 other animals were electively killed as a result of anorexia and weight loss of over 10% of the total body weight. 6 animals survived to the end of the 10 week period. Only 1 animal died in the saline control/captopril group, as a result of a broken back during transfer between cages. 8 animals survived 10 weeks.

If we compare the incidence of sudden death in the adriamycin plus captopril group with the group of animals treated with adriamycin alone, we find that none of the 5

Table 15.

Survival data in the adriamycin/captopril and saline control/captopril groups.

| | Initial | OM | SD | SBE | Other |
|---|---|---|---|---|---|
| Adriamycin/Captopril | 11 | 5 | 0 | 0 | 5 |
| Saline control/Captopril | 9 | 1 | 0 | 0 | 1 |

Initial = Initial number of animals entering study.

OM = Overall mortality.

SD = Sudden death.

SBE = Subacute bacterial endocarditis.

deaths in the former group were sudden and unexpected. This is in comparison to the adriamycin treated group where 3 of the 6 deaths were classified as sudden. However, the numbers of sudden deaths are insufficient for statistical comparison.

## 6.4.2 Electrophysiology.

Diastolic pacing threshold.(table 16).

The mean diastolic pacing threshold did not change significantly in either the adriamycin/captopril or saline control/captopril groups over the 10 weeks of the study. The levels were 3.2±0.3V at week 0 vs 3.2±0.2V at week 10 in the adriamycin/captopril group, and 2.8±0.3V vs 2.9±0.5V in the saline control/captopril group. There were no significant differences between the groups.

Stim-T and ERP

The effects of 8 weeks treatment with adriamycin or saline, and 4 weeks treatment with captopril, on the stimulus-T interval and ERP are tabulated in table 17 and illustrated in figures 8 and 9. There was no significant difference in stim-T or ERP between the groups at week 0. Adriamycin induced a progressive shortening in both stim-T and ERP which was significant by weeks 3 and 4 respectively($p<0.05$ and $p< 0.01$). The shortening continued up to week 10, ie after 4 weeks treatment with captopril. Adriamycin caused an overall shortening of 14%

Table 16.

Changes in the diastolic pacing threshold in the adriamycin/captopril and saline control/captopril groups.

| Week | 0 | 10 |
|---|---|---|
| Adriamycin/Captopril:n | 11 | 5 |
| Threshold(Volts) | 3.2 ± 0.3 | 3.2 ± 0.2 |
| Saline control/Captopril:n | 9 | 6 |
| Threshold(Volts) | 2.8 ± 0.3 | 2.9 ± 0.5 |

There were no significant differences between the groups or within the 2 groups between weeks 0 and 10.

Table 17.

Changes in stim-T and ERP in the adriamycin/captopril and saline control/captopril groups.

| | Stim-T(ms) | | ERP(ms) | |
|---|---|---|---|---|
| | Week0 | Week10 | Week0 | Week10 |
| Adriamycin/ Captopril | 118±1(11) *** | 102±2(5) ** | 117±3(11) ** | 108±3(5) ** |
| Saline control/ Captopril | 119±1(9) | 119±2(6) | 118±0.6(9) | 121±1(6) |

*** = $p<0.001$.

** = $p<0.01$.

The numbers in parenthesis represent the numbers of animals.

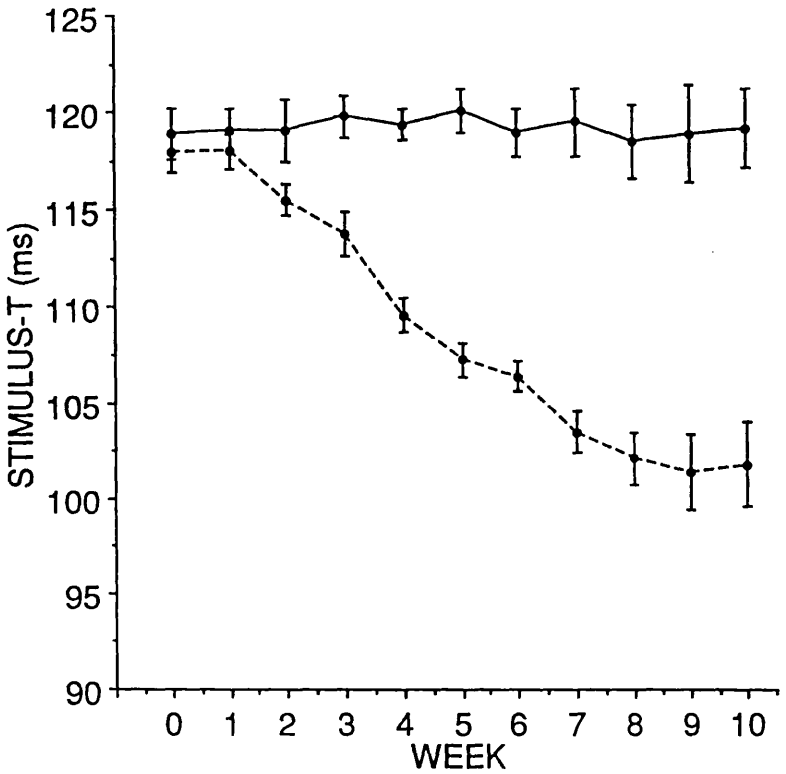


Figure 8.

Graph demonstrating the effect of captopril on ventricular repolarisation in heart failure. The adriamycin treated animals are represented by the broken line and the control animals by the continuous line. Captopril does not prevent the progressive shortening in Stim-T interval.

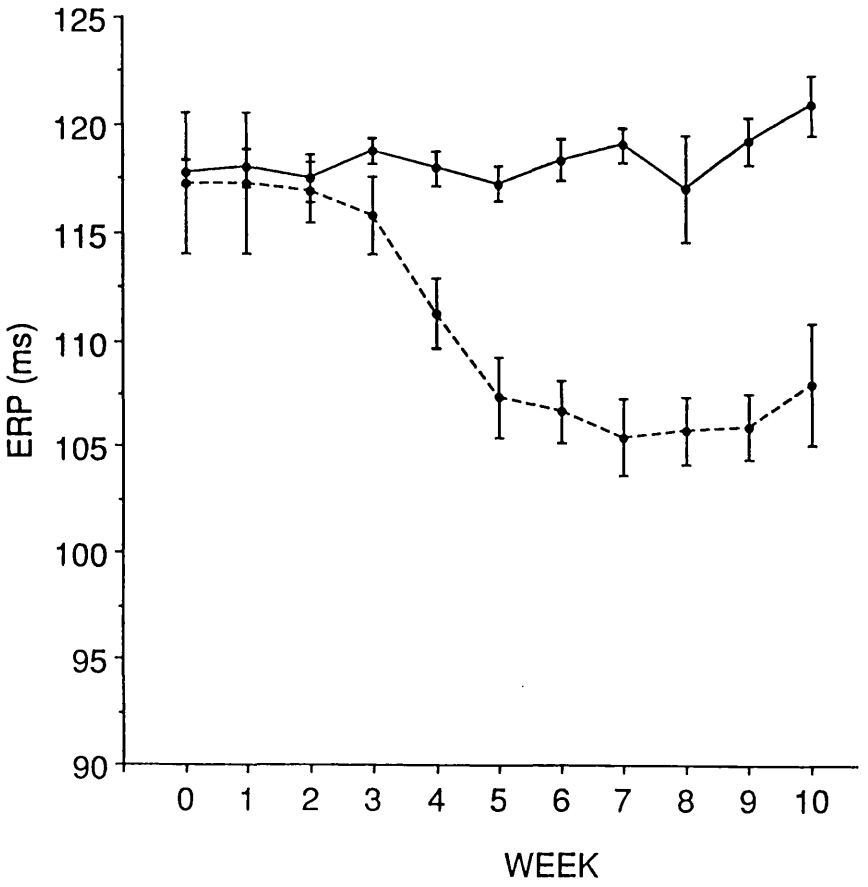


Figure 9.

Graph illustrating the effect of captopril on the right ventricular effective refractory period in heart failure. The adriamycin treated animals are represented by the broken line and the control animals by the continuous line. Captopril does not prevent the significant shortening in ERP.

in stim-T, and 8% in ERP compared with baseline($p < 0.001$ and $p<0.01$ respectively). No significant change was observed in the stim-T interval in the saline control/captopril group over the 10 weeks of the study. The ERP however was significantly prolonged by week 10($p<0.05$) in the saline control/captopril group.

Analysis of the stimulus-T interval in animals which survived to the end of the study revealed a significant decrease in the adriamycin/captopril group between weeks 0 and 10. The stim-T interval decreased from $118\pm2$ms to $102\pm2$ms, $p<0.01$ and $n=5$. There was a non-significant increase in the saline control/captopril group from $118\pm2$ms to $119\pm2$ms, $n=6$.

The ERP significantly decreased in the animals in the adriamycin/captopril group which survived to the end of the study, from $118\pm2$ms at week0 to $108\pm3$ms at week10, $p<0.01$ and $n=5$. There was a significant increase in the mean ERP in the saline control/captopril group from $117\pm1$ms to $121\pm1$ms, $p<0.05$ and $n=6$.

When the adriamycin/captopril group was compared with the group treated with adriamycin alone, (table 18), no significant differences were detected in stim-T at week 0 or week 10. Neither were there any significant differences at baseline or week 10 in the ERP. No significant differences were seen at baseline or week 10 in either stim-T or ERP, when the saline control/captopril

Table 18.

Comparison of stim-T and ERP in the adriamycin/captopril, saline control/captopril, adriamycin and saline control groups.

| | Stim-T(ms) | | ERP(ms) | |
|---|---|---|---|---|
| | Week0 | Week10 | Week0 | Week10 |
| Adriamycin/ captopril | 118±1(11) | 102±2(5) | 117±3(11) | 108±3(5) |
| Adriamycin | 116±1(16) | 102±2(10) | 115±2(16) | 98±3(10) |
| Saline control/ captopril | 119±1(9) | 119±2(6) | 118±0.6(9) | 121±1(6) |
| Saline control | 120±3(11) | 120±4(8) | 113±4(11) | 120±3(8) |

The numbers in parenthesis represent the numbers of animals.

There were no significant differences between the groups.

group was compared with the saline control group of animals.

Sinus cycle length significantly increased by week 10 when compared to baseline in the adriamycin/captopril group($p<0.01$). No significant change was detected in the saline control/captopril group over the 10 week period, and there was no significant difference between the 2 groups at weeks 0 or 10, (table 19).
Analysis of paired data also revealed a significant increase in sinus cycle length in the adriamycin/captopril group from $239\pm6$ms to $288\pm18$ms, $p<0.05$ and $n=5$. There was a non-significant increase in the saline control/captopril group from $250\pm4$ms to $256\pm7$ms, $n=6$.

There were no significant differences at week 0 between the adriamycin/captopril and the adriamycin groups of animals. At week 10 however, the sinus cycle length was significantly greater in the adriamycin/captopril group. A similar pattern was observed when the saline control/captopril and saline control groups were compared, (table 19).

Table 19.

Comparison of sinus cycle length in the adriamycin/captopril, saline control/captopril, adriamycin and saline control groups.

| | Sinus cycle length(ms) | | |
|---|---|---|---|
| | Week0 | | Week10 |
| Adriamycin/captopril | 242±7(11) | * | 288±18(5) |
| | | | ** |
| Adriamycin | 238±7(16) | | 221±8(10) |
| Saline control/ captopril | 250±3(9) | | 255±7(6) |
| | | | * |
| Saline control | 230±4(11) | | 238±3(11) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$

** = $p<0.01$

Ambulatory ECG recording

A total of 30 ambulatory ECG recordings were attempted although only 11 were successful, 6 in the adriamycin plus captopril group and 5 in the control plus captopril group. No evidence of arrhythmias was detected in either of the 2 groups.

### 6.4.3 Biochemistry.

Neurohormonal data

Plasma noradrenaline levels significantly increased between weeks 1 and 8 in the adriamycin/captopril group. There was no change in the saline control/captopril group over a similar period. There were no baseline differences between the groups of animals, and there were no differences between the groups at week 8, (table 20).

Analysis of the mean plasma noradrenaline concentration in those animals which survived to the end of the study demonstrated a non-significant increase in the adriamycin/captopril group from $4.1\pm0.5 nmol.l^{-1}$ to $9.3\pm2.4 nmol.l^{-1}$, n=7. There was a non-significant decrease in the saline control/captopril group from $10.4\pm5.3 nmol.l^{-1}$ to $3.5\pm0.6 nmol.l^{-1}$, n=3.

Table 20.

Comparison of plasma noradrenaline levels in adriamycin/captopril, saline control/captopril, adriamycin and saline control groups.

| | Plasma noradrenaline($n.mol.l^{-1}$) | | |
|---|---|---|---|
| | Week1 | | Week8 |
| Adriamycin/captopril | 5.0±0.76(10) | * | 11.3±2.9(8) |
| | | | * |
| Adriamycin | 5.1±1.3(19) | | 5.4±0.8(19) |
| Saline control/ captopril | 6.5±(7) | | 4.1±0.97(5) |
| Saline control | 3.5±0.5(18) | | 5.2±1.2(11) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.

Comparison of the mean levels of plasma noradrenaline between the adriamycin/ captopril and the group treated with adriamycin alone showed that there were no baseline differences between the 2 groups. At week 8 however, the mean noradrenaline level was significantly higher in the former group; 11.3 $\pm$ 2.9 $nmol.l^{-1}$ vs 5.4 $\pm$ 0.8 $nmol.l^{-1}$ ($p<0.05$). No significant differences were observed at baseline or week 8 when the saline control/captopril group was compared with the saline control group, (table 20).

Plasma renin concentration increased significantly between weeks 1 and 8 in both the adriamycin/captopril and the saline control/captopril groups, as a consequence of the treatment with captopril, (table 21). No paired analysis was performed because of the magnitude of the increase in plasma renin concentration.

When the adriamycin/captopril group was compared to the adriamycin group, plasma renin was significantly higher at baseline and at week 8 in the group treated with captopril. There were no significant differences at baseline between the saline control/captopril and the saline control groups, although plasma renin was significantly higher at week 8 in the saline control/captopril group, (table 22). No significant differences were detected at baseline or week 8 between

Table 21.

Changes in plasma renin concentration in the adriamycin/captopril and saline control/captopril groups.

| Week | 1 | | 8 |
|---|---|---|---|
| Adriamycin/captopril: n | 11 | | 8 |
| Renin($uU.ml^{-1}$) | 125.8±22.7 | *** | 4558.4±1131.7 |
| Saline control/captopril: n | 9 | | 6 |
| Renin($uU.ml^{-1}$) | 99.9±13.7 | *** | 1807±331.7 |

***= $p < 0.001$

There were no significant differences between the groups at weeks 1 or 8.

Table 22.

Comparison of plasma renin levels in the adriamycin/captopril, saline control/captopril, adriamycin and saline control groups.

| | Plasma renin concentration($uU.ml^{-1}$) | | |
|---|---|---|---|
| | Week 1 | | Week 8 |
| Adriamycin/captopril | 125.8±22.7(11) | *** | 4558.4±1131.7(8) |
| | * | | *** |
| Adriamycin | 74.2±6.8(11) | | 66.6±8.5(18) |
| Saline control/ captopril | 99.9±13.7(9) | *** | 1807±331.7(6) |
| | | | *** |
| Saline control | 72.6±7.2(12) | | 56.1±4.2(17) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.

*** = $p<0.001$.

the adriamycin/captopril and the saline control/captopril groups.

There were no significant changes in plasma ANP concentration between weeks 1 and 8 in the adriamycin/ captopril and the saline control/captopril groups. Neither were there any significant differences between the 2 groups in plasma ANP levels at baseline or week 8, (table 23). Analysis of the mean plasma ANP cocncentration in those animals which survived to the end of the study revealed a non-significant decrease in the adriamycin/captopril group from $176\pm57 pg.ml^{-1}$ to $145\pm34 pg.ml^{-1}$, n=7, and a non-significant increase in the saline control/captopril group from $126\pm28 pg.ml^{-1}$ to $135\pm38 pg.ml^{-1}$, n=6.

There were no significant differences between the adriamycin/captopril and the adriamycin groups of animals at either week, and there were no significant differences between the saline control/captopril and saline control groups, (table 23).

There were no significant differences in angiotensin II concentrations pre-captopril between the groups, and post-captopril the levels of angiotensin II were undetectable in every animal except for 1 in the saline

Table 23.

Comparison of plasma atrial natriuretic peptide(ANP) concentration between the adriamycin/captopril, saline control/captopril, adriamycin and saline control groups.

| | Plasma ANP($pg.ml^{-1}$) | |
|---|---|---|
| | Week 1 | Week 8 |
| Adriamycin/captopril | 181.2±49.1(10) | 144.9±34.3(8) |
| Adriamycin | 137.7±24.9(13) | 94.3±10.7(17) |
| Saline control captopril | 110.7±20(9) | 135.3±38.4(6) |
| Saline control | 118.7±12.5(18) | 118.8±14.4(14) |

The numbers in parenthesis represent the numbers of animals.

There were no significant differences between the adriamycin/captopril and adriamycin groups, nor between the saline control/captopril and saline control groups.

control/captopril group, (table 24). These changes are a result of the treatment with captopril

Plasma electrolytes

No significant change was detected in plasma $Mg^{2+}$ levels over an 8 week period in the adriamycin/captopril group. The mean level of plasma $Mg^{2+}$ significantly decreased however between weeks 1 and 8 in the saline control/captopril group, (table 25). Plasma $Mg^{2+}$ levels were significantly higher at baseline in the saline control/ captopril group when compared to the adriamycin/captopril group. When the data were normalised, there were no significant differences between the groups at week 8.

Analysis of the plasma magnesium levels in those animals which survived to the end of the study, revealed a non-significant increase in the adriamycin/captopril group between weeks 1 and 8 from $0.72\pm0.03 mmol.l^{-1}$ to $0.75 mmol.l^{-1}$, n=7. Plasma magnesium levels significantly decreased in the saline control/captopril group from $0.83\pm0.02 mmol.l^{-1}$ to $0.72\pm0.03 mmol.l^{-1}$, $p<0.05$ and n=7.

The mean level of plasma magnesium was significantly less at week 1 in the the adriamycin/captopril group when

Table 24.

The effect of captopril on the plasma concentration of angiotensin II in the adriamycin/captopril and saline control/captopril groups.

| | Angiotensin II($pg.ml^{-1}$) | |
|---|---|---|
| | Pre-captopril | Post-captopril |
| Adriamycin/captopril | 18±7.59(11) | Undetectable(6) |
| Saline control/ captopril | 13.2±3.3(9) | Undetectable(6) |
| Saline control/ captopril | 12(1) | 6.3(1) |

The numbers in parenthesis represent the numbers of animals.

There were no significant differences between the groups.

One animal in the saline control group had a level of 6.3 after captopril treatment, and this had fallen from 12 pre-captopril.

Table 25.

Changes in plasma magnesium and potassium concentrations in the adriamycin/captopril and saline control/captopril groups.

| | Adriamycin/captopril | | Saline control/captopril |
|---|---|---|---|
| $K^+$ week1 | 4.8 ± 0.13(8) | | 5.2 ± 0.3(7) |
| $K^+$ week8 | 5.5 ± 0.6(6) | | 5.0 ± 0.16(8) |
| $Mg^{2+}$ week1 | 0.72 ± 0.03(7) | * | 0.83 ± 0.02(7) |
| $Mg^{2+}$ week8 | 0.75 ± 0.08(8) | | 0.73 ** ± 0.02(8) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$

** = $p<0.01$

compared to the adriamycin group, (table 26). When the data was normalised however, the magnesium levels showed a significant decrease in the adriamycin group, (table 27). There was no baseline difference in plasma $Mg^{2+}$ levels between the saline control/captopril and the saline control groups. The mean level of plasma $Mg^{2+}$ was however significantly lower in the saline control/captopril at week 8, (table 26).

Plasma $K^{+}$ levels tended to increase over the 8 week period in the adriamycin/captopril group, although this increase was not statistically significant. There was no significant change in the saline control/captopril group, and there were no significant differences between the groups at baseline or week 8, (table 25). Analysis of the mean plasma potassium concentration in the animals which survived to the end of the study demonstrated a non-significant increase in the adriamycin/captopril group from $4.8\pm0.2 mmol.l^{-1}$ to $5.5\pm0.6 mmol.l^{-1}$, n=6, and a non-significant decrease in the saline control/captopril group from $5.2\pm0.3 mmol.l^{-1}$ to $5.1\pm0.1 mmol.l^{-1}$, n=7.

No significant difference in the mean plasma $K^{+}$ level was detected between the adriamycin/captopril and the adriamycin groups at baseline. The plasma $K^{+}$ concentration was however significantly higher at week 8

Table 26.

Comparison of plasma magnesium concentration in the adriamycin/captopril, saline control/captopril, adriamycin and saline control/groups.

| | Plasma $Mg^{2+}$ ($mmol.l^{-1}$) | |
|---|---|---|
| | Week1 | Week8 |
| Adriamycin/captopril | 0.72±0.03(7) | 0.75±0.08(8) |
| | * | |
| Adriamycin | 0.95±0.05(16) | 0.70±0.02(20) |
| Saline control/ captopril | 0.83±0.02(7) | 0.73±0.02(8) |
| | | ** |
| Saline control | 0.95±0.05(16) | 0.85±0.03(16) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.

** = $p<0.01$.

Table 27.

Comparison of the normalised plasma magnesium concentration between the adriamycin/captopril and adriamycin groups.

| | % change in plasma magnesium |
|---|---|
| Adriamycin | -24.5±4.5(16) |
| | * |
| Adriamycin/captopril | 6.4±13.9(7) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.

in the adriamycin/captopril group. There were no significant differences at baseline or week 8 between the saline control and the saline control/captopril groups, (table 28).

The mean value of plasma $Na^+$ tended to increase in both the adriamycin/captopril and the saline control/captopril groups over an 8 week period although this increase was not statistically significant. There were no statistically significant differences between these 2 groups at baseline or week 8, (table 29).

Analysis of the plasma sodium concentration in those animals which survived to the end of the study revealed a non-significant increase in both the adriamycin/captopril and saline control/captopril groups. The mean plasma sodium concentration increased from $143\pm1 mmol.l^{-1}$ to $146\pm3 mmol.l^{-1}$ in the adriamycin/captopril group, n=6. The levels in the saline control/captopril group increased from $143\pm2 mmol.l^{-1}$ to $145\pm1 mmol.l^{-1}$, n=7.

No change was seen at baseline or week 8 between the adriamycin/captopril group and the adriamycin group, and there were no differences between the saline control and the saline control/captopril groups.

Table 28.

Comparison of plasma potassium concentration in the adriamycin/captopril, saline control/captopril, adriamycin and saline control groups.

| | Plasma $K^+$ ($mmol.l^{-1}$) | |
|---|---|---|
| | Week1 | Week8 |
| Adriamycin/captopril | 4.8±0.1(8) | 5.5±0.6(6) * |
| Adriamycin | 4.9±0.1(23) | 4.6±0.1(18) |
| Saline control/ Captopril | 5.2±0.3(7) | 5.0±0.2(8) |
| Saline control | 4.8±0.1(17) | 4.8±0.1(16) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.

Table 29.

Changes in plasma urea and sodium in the adriamycin/captopril and saline control/captopril groups.

| | Adriamycin/ Captopril | Saline control/ Captopril | p |
|---|---|---|---|
| Urea week1 | 6.4±0.3(8) | 7.4±0.1(7) | 0.05 |
| | ** | | |
| Urea week8 | 35.1±20.4(6) | 7.0±0.3(8) | 0.05 |
| Sodium week1 | 142.1±0.9(8) | 142.6±1.5(7) | NS |
| Sodium week8 | 146.3±3.4(6) | 146.7±2.1(8) | NS |

Urea = plasma urea concentration in $mmol.l^{-1}$

Sodium = plasma sodium concentration in $mmol.l^{-1}$

NS = not significant.

** = $p<0.01$ between weeks 1 and 8 in the adriamycin/captopril group.

The numbers in parenthesis represent the numbers of animals.

Plasma urea levels increased significantly over the 8 week period in the adriamycin/captopril group($p<0.01$), There was no significant change in the saline control/captopril group over a similar period, (table 29). When the groups were compared, the mean plasma urea level was significantly higher in the saline control/captopril group when compared to the adriamycin/captopril group at baseline ($p<0.05$). When the data for plasma urea was normalised however, the mean level of plasma urea showed a significant increase in the adriamycin/captopril group, (table 30).

No baseline differences were seen between the adriamycin/ captopril group and the adriamycin group. However at week 8 the mean level of plasma urea was significantly higher in the former group($p<0.05$). There were no differences at baseline or at week 8 between the saline control/captopril group and the saline control group, (table 31).

The significant increase in the plasma urea levels in the adriamycin/captopril group probably reflects a combination of the nephrotoxicity of adriamycin and the potential for captopril to cause a deterioration in renal function by reducing glomerular filtration rate.

Table 30.

Comparison of the normalised plasma urea levels between the adriamycin/captopril and saline control/captopril groups.

| | % change in plasma urea |
|---|---|
| Adriamycin/captopril | 137±59(6) |
| | * |
| Saline control/captopril | -3±4.9(7) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.

Table 31.

Comparison of plasma urea concentration in the adriamycin/captopril, saline control/captopril, adriamycin and saline control groups.

| | Plasma urea($mmol.l^{-1}$) | |
|---|---|---|
| | Week1 | Week8 |
| Adriamycin/captopril | 6.4±0.3(8) | 35.1±20.4(6) * |
| Adriamycin | 6.6±0.3(24) | 7.0±0.3(20) |
| Saline control/ captopril | 7.4±0.1(7) | 7.0±0.3(8) |
| Saline control | 7.0±0.3(18) | 6.6±0.3(16) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.

6.4.4 Pathology.(tables 32-35).

The mean wet heart weight in the adriamycin/captopril group tended to be higher than in the saline control/captopril group, a difference of 10% . There were no significant differences in the heart weights when the above groups were compared to the adriamycin and saline control groups.

There were no differences in the mean liver weights when the adriamycin/captopril group was compared with the saline control/captopril group, and there were no significant differences when comparisons were made with the adriamycin and saline control groups.

There were no significant changes in the lung weights between the adriamycin/captopril and saline control/captopril groups, and neither were there any significant differences between these groups and the adriamycin and saline control groups.

Total body weight increased significantly in both the groups treated with captopril between weeks 0 and 10($p<0.05$). There were no differences between the groups at weeks 0 or 10.

In those animals which survived to the end of the study the mean body weight showed a non-significant increase in the adriamycin/captopril group from $3.06\pm0.03$Kg to $3.09\pm0.1$Kg, n=6. The mean body weight in the saline

control/captopril group increased from 3.05±0.05Kg to 3.63±0.1Kg, n=8 and $p<0.01$.

The mean total body weight was significantly less at baseline in the adriamycin/captopril group when compared to the adriamycin group, although there were no significant differences between the saline control and saline control/captopril groups. When the data for the body weights was normalised however, there were no significant differences between the adriamycin/captopril and adriamycin groups at week 10.

Both the heart/body weight, liver/body weight and lung/body weight ratios were higher in the adriamycin/captopril group when compared to the saline control/captopril group, although these changes were not statistically significant. There was no difference with respect to both of these parameters when the groups treated with captopril were compared to the saline control and adriamycin groups.

The wet/dry weight ratios of the heart and liver tended to be higher in the animals in the adriamycin/captopril group than in the saline control/captopril group, although the difference was not statistically significant. There were no significant differences between the wet/dry weight ratios of the hearts in the animals treated with captopril and those in the adriamycin and saline control groups.

Table 32.

Comparison of the heart, liver and lung weights in the adriamycin/captopril, saline control/captopril, adriamycin and saline control groups.

| | Heart(g) | liver(g) | lung(g) |
|---|---|---|---|
| Adriamycin/captopril | 9.8±0.8(7) | 77.1±3.1(7) | 19.6±2.7(6) |
| Adriamycin | 10.8±0.6(17) | 87.8±5.8(18) | 18.5±1.4(18) |
| Saline control/ captopril | 8.9±1.4(7) | 73.5±6.4(7) | 19.5±1.3(5) |
| Saline control | 7.2±0.3(16) | 64.9±3.9(15) | 18±1.1(15) |

The numbers in parenthesis represent the numbers of animals.

There were no significant differences between the adriamycin/captopril and adriamycin groups, nor between the saline control/captopril and saline control groups.

Table 33.

Comparison of body weights between the adriamycin/captopril, saline control/captopril, adriamycin and saline control groups.

| | Body weight(Kg) Week0 | Body weight(Kg) Week10 |
|---|---|---|
| Adriamycin/captopril | 3.04±0.03(12) * | 3.3±0.15(7) |
| Adriamycin | 3.3±0.05(24) | 3.2±0.07(18) |
| Saline control/ captopril | 3.06±0.04(8) | 3.68±0.17(7) |
| Saline control | 3.27±0.08(19) | 3.49±0.1(16) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.01$

Table 34.

Comparison of the heart, liver and lung to body weight ratios in the adriamycin/captopril, saline control/captopril, adriamycin and saline control groups.

| | H/BW | L/BW | LU/BW |
|---|---|---|---|
| Adriamycin/captopril | 0.3±0.03(7) | 2.36±0.14(7) | 0.59±0.09(6) |
| Adriamycin | 0.33±0.02(18) | 2.77±0.2(18) | 0.58±0.05(18) |
| Saline control/ Captopril | 0.23±0.02(7) | 1.98±0.1(7) | 0.51±0.05(5) |
| Saline control. | 0.21±0.02(15) | 1.95±0.2(15) | 0.52±0.04(15) |

H/BW=Heart to body weight ratio.
L/BW=Liver to body weight ratio.
Lu/BW=Lung to body weight ratio.

The numbers in parenthesis represent the numbers of animals.

There were no significant differences between the adriamycin/captopril and adriamycin groups, nor between the saline control/captopril and saline control groups.

Table 35.

Comparison of the wet/dry weight ratio of the heart in the adriamycin/captopril, saline control/captopril, adriamycin and saline control groups, and the liver wet/dry weight ratio between the adriamycin/captopril and saline control/captopril groups.

| | Heart Wet/dry | Liver Wet/dry |
|---|---|---|
| Adriamycin/captopril | 5.6±0.9(4) | 3.6±0.2(3) |
| Adriamycin | 5.5±0.7(4) | |
| Saline control/ captopril | 4.6±0.1(7) | 3.5±0.1(4) |
| Saline control | 4.5±0.3(4) | |

The numbers in parenthesis represent the numbers of animals.

There were no significant differences between the adriamycin/captopril and adriamycin groups, nor between the saline control/captopril and saline control groups.

## 6.5 Discussion.

The principal finding in the experiments described in this chapter is that the introduction of captopril following 6 weeks of treatment with adriamycin did not reverse the shortening in stim-T and ERP which had developed by this time. Neither was there any statistically significant difference in stim-T or ERP at the end of the study between the animals in the adriamycin/captopril group and those in the adriamycin group described in chapter 4. The possible aetiological mechanisms proposed for the shortening in stim-T and ERP in the experiments described in chapter 4 were either the direct cytoxic effects of adriamycin, or an increase in pulmonary arterial pressure and consequently right ventricular afterload. The results from the experiments in this chapter and from those described in chapter 4, would suggest that the former explanation is the more likely. The angiotensin converting enzyme inhibitor captopril was introduced specifically after 6 weeks treatment with adriamycin, as at this time it had been demonstrated that in the animals treated with adriamycin in the experiments described in chapter 4, the stim-T interval and ERP were significantly shortened. Another reason for specifically introducing captopril at this time was to avoid the drug preventing

the development of heart failure in this model. One of the possible mechanisms proposed for the myopathic effect of adriamycin is by an increased production of oxygen-derived free radicals, (Bachur, N.R. et al(1977); Doroshow, J.H. (1983); Kalyanaraman, B. et al(1980)). Captopril is a potent free radical scavenger, (Westlin, W. & Mullane, K.(1988)), and because of this particular property, the introduction of the drug was delayed until week 7 of the study.

A progressive and parallel reduction in repolarisation and refractoriness was observed in the adriamycin group which was treated with captopril. The reduction of 14% and 8% respectively was similar in magnitude to the 12% and 14% reduction which occured in these parameters in the adriamycin group. The lack of effect of captopril on repolarisation and refractoriness does not support the hypothesis that the shortening in stim-T and ERP in adriamycin cardiomyopathy is caused by an increase in right ventricular afterload.

Although haemodynamic measurements were not made in these experiments it would be expected that captopril would reduce pulmonary arterial pressure, (Sharpe, D.N.(1980); Ader, R. et al(1980); Topic, N.(1980); Levine, T.B. & Cohn, J.N.(1982)).

There is some indirect evidence from the data in these experiments that captopril had a beneficial effect on

afterload and also improved cardiac failure. The mean wet heart weight of the animals in the adriamycin/captopril group was only 10% higher than in the corresponding saline control/adriamycin group, a non significant difference. The mean wet heart weight of the animals in the initial group treated with adriamycin was however 58% greater than the saline control group. A reduction in heart weight would be consistent with a reduction in ventricular afterload and an improvement in the heart failure state. Similarly, the mean wet liver weight was only increased by 5% in the adriamycin/captopril group when compared to the saline control/captopril group, whereas in the initial group treated with adriamycin, the liver weight was increased by 35%. Again the reduction in hepatic congestion would be consistent with an improvement in heart failure. Sinus cycle length also progressively lengthened over the 10 weeks of the study in the adriamycin/captopril group, and was significantly longer when compared to the adriamycin group. This reduction in heart rate may also imply an improvement in cardiac performance. Angiotensin converting-enzyme inhibition has been shown clinically to lower heart rates significantly in patients with congestive heart failure, (The consensus trial study group,(1987)).

The failure of captopril to reverse the shortening in stim-T and ERP which had been proposed as a possible

mechanism for sudden arrhythmic death in heart failure, would suggest that the addition of the drug would have no effect on the incidence of sudden death in the study. Examination of the mortality data however reveals that there were no sudden unexplained deaths in the adriamycin group of animals treated with captopril (although 1 animal did die suddenly with coexisting renal failure). However, the small size of the study groups and the low incidence of sudden death in the adriamycin treated animals makes meaningful comparisons difficult. It is only possible to say that there was a tendency for captopril to reduce the incidence of sudden death. If this were so, this would be in disagreement with the majority of existing clinical evidence which so far has failed to demonstrate any effect of inhibition of the renin-angiotensin system on the incidence of sudden arrhythmic death, (The concensus trial study group,(1987); Furberg, C. & Yusuf, S.(1985); Creager, M.A. et al(1982); Magnani, B. & Magelli, C.(1986); The SOLVD Investigators,(1991)).

It is interesting to note however that the incidence of ventricular arrhythmias as detected by 24 hour continuous ambulatory monitoring was qualitatively reduced in the animals treated with adriamycin and captopril. Indeed there was no evidence of ventricular ectopic activity in any animals in this group. This is in contrast to the animals in the adriamycin group with every animal in the

group demonstrating evidence of ventricular ectopy. The experimental finding of a reduction in ventricular ectopic activity is in accord with the majority of clinical studies using angiotensin converting-enzyme inhibitors which have addressed the same problem, (Cleland, J.G.F. et al(1984); Dargie, H.J. et al(1987)). One possible explanation for this reduction may be that by preventing the decrease in plasma $Mg^{2+}$ which was observed in the adriamycin group over an 8 week period, captopril eliminated one axis of a multifactorial system, and thus reduced the arrhythmogenic tendency. It is also interesting to note from the experimental data presented here that captopril did not prevent the decrease in plasma $M^{2+}$ over an 8 week period in the saline control animals. The adriamycin group of animals treated with captopril also tended to an increase in plasma $K^{+}$ at week 8 when compared to baseline, while those animals in the adriamycin group demonstrated a tendency for the mean plasma $K^{+}$ to fall over the 8 week period. This effect could also be potentially anti-arrhythmic. Data from clinical studies have also demonstrated that in patients with heart failure, captopril causes an increase in plasma $K^{+}$ and a reduction in the frequency of ventricular ectopic activity, (Dargie, H.J. et al(1987); Cleland, J.G.F. et al(1984)).

The effect of captopril on the renin-angiotensin system in this experimental model was as expected on theoretical grounds. After 2 weeks of therapy with captopril, ie at week 8 of the study, the mean plasma renin activity was significantly higher in both the groups of animals which were treated with captopril. At week 8 the levels of renin were also higher in the adriamycin/captopril group when compared to the saline control/captopril group, although the difference was not statistically significant. These results would suggest that captopril has a greater effect on inhibition of the renin-angiotensin system in heart failure than in controls. The data from the animals treated with adriamycin in the experiments described in chapter 4 demonstrated no activation of the renin-angiotensin system by week 8 however. In parallel with the increase in plasma renin was as expected a decrease in the levels of angiotensin II. There were no significant baseline differences in angiotensin II between the 2 groups of animals treated with captopril and after commencement of the drug the levels of angiotensin II were undetectable in every animal except for one in the control group whose levels of aangiotensin II were halved.
The other limb of the neurohormonal axis, the sympathetic nervous system showed some surprising changes in this part of the study. The mean level of plasma noradrenaline in the adriamycin group of animals treated with captopril was

significantly increased at week 8 of the study when compared to baseline. It was also higher when compared to the animals treated with adriamycin alone. This result would appear unusual as the heart rates in the adriamycin group of animals treated with captopril were significantly reduced by the end of the study when compared to baseline. It would be expected that the increase in sympathetic stimulation would have caused an increase in heart rates. This result is also unusual in another respect. The probable improvement in heart failure which was caused by captopril and manifest by a lessening in the ventricular hypertrophy and hepatic congestion, would if anything again be associated with a decrease in noradrenaline levels. The clinical study by Cleland, J.G.F. et al(1984) demonstrated that in patients with heart failure treated with captopril the plasma noradrenaline levels were significantly decreased.

One possible explanation for the increase in plasma noradrenaline levels in the adriamycin/captopril group is that the animals could have had a high sympathetic drive as renal function deteriorated. The nephrotoxic effect of captopril was manifest as a significant increase in plasma urea levels. Another possible explanation is that the dose of captopril used in the study was too high and resulted in a hypotensive response in the adriamycin/captopril group. The fact that there was no

biochemical deterioration in renal function in the animals in the saline control/captopril group would tend to indicate that the dose of captopril used was not enough to be directly nephrotoxic.

The fact that renal function did deteriorate in the group of animals with heart failure which were treated with adriamycin is not entirely unexpected. Previous clinical studies have reported similar findings, (Cleland, J.G.F. et al(1984)). On theoretical grounds, some authorities believe that in low-output states the primary purpose of activation of the renin-angiotensin system is the preservation of renal function. Interference with the formation of angiotensin II would predictably result in a reduction in glomerular filtration rate. This expectation has been confirmed experimentally in the dog, (Kastner, P.G., Hall, J.E., & Guyton, A.C.(1982); Hall, J.E. et al(1977); Lohmeier, T.E. et al(1977)).

In conclusion therefore, the addition of captopril to the adriamycin treated animals with heart failure did not reverse the changes in repolarisation and refractoriness which had developed prior to the administration of the drug. This would tend to support the theory that the electrophysiological changes which occured in this study were secondary to the toxic myopathic effect of adriamycin and unrelated to activation of the renin-angiotensin system. The changes in heart and liver weight in the

study were consistent with the known effect of captopril in improving cardiac output in heart failure. The improvement in ventricular ectopic activity in the animals treated with adriamycin is also similar to that detected clinically and may be related to the repletion of plasma electrolytes, in particular $K^+$ and $Mg^{2+}$. Although there was a tendency for captopril to reduce the incidence of sudden unexplained death in the adriamycin treated animals, the failure of the drug to reverse the changes in repolarisation and refractoriness may be one possible explanation for the failure of inhibition of the renin-angiotensin system clinically to reduce the incidence of sudden death in congestive cardiac failure.

# CHAPTER 7

## THE EFFECT OF FRUSEMIDE ON THE ELECTROPHYSIOLOGICAL CHANGES IN ADRIAMYCIN-INDUCED CHRONIC HEART FAILURE IN THE RABBIT.

### 7.1 Introduction.

Diuretic therapy has historically been the mainstay of first-line management in patients with congestive heart failure. The most widely used agent has been the loop diuretic frusemide which acts by inhibiting the specific enzymes concerned with the pumping of chloride across the lining cell of the ascending limb of the loop of Henle. The result is that chloride, sodium, potassium and hydrogen ions all remain within the renal tubule with the diuresis of sodium. Frusemide has also been shown to inhibit 15-hydroxyprostaglandin dehydrogenase, the first-step enzyme involved in the degradation of prostaglandins, (Stone, K.J. & Hart, M.(1976)). The consequence of this is that prostaglandins, especially prostaglandin E2, accumulate in the renal medulla and directly suppress anti-diuretic hormone dependent water permeability of the collecting tubules, (Grantham, J.J. & Orloff,J.(1968)).

Prostaglandins may further prevent the reabsorption of sodium chloride from the thick ascending loop of Henle, (Stokes,J.B.(1979)). Among the most important side-effects of treatment with frusemide are hypokalaemia and hypomagnesaemia. Current evidence would suggest however that the use of standard doses of frusemide such as 40mg or 80mg daily in the management of chronic heart failure, causes less hypokalaemia than thiazide diuretics, (Davidov, M.E., M$^{c}$Knight, J.E. & Osborne, J.L.(1979); Morgan, D.B. & Davidson, C.(1980)). The theoretical risk exists however that electrolyte depletion may occur in patients treated with frusemide, who have congestive cardiac failure. As previously mentioned in chapter 1.6 and 1.7, a number of reports have demonstrated that depletion of $K^{+}$ and $Mg^{2+}$ may be associated with arrhythmias, and that repletion of these electrolytes may improve the arrhythmias without the use of convential anti-arrhythmic therapy, (Bertuso, J.R. et al (1984); Chadda, K. et al(1984)). The presence of hypokalaemia has also been shown to be associated with the severity of ventricular ectopic activity in patients with heart failure, (Dargie, H.J. et al(1987)), and the presence of arrhythmias in patients with hypertension, (Medical Research Council working party on mild to moderate hypertension,(1983); Holland, O.B. et al(1981); Papademetriou, V. et al(1983)).

In addition to the possible arrhythmogenic effect of frusemide in causing electrolyte depletion, the drug has important interactions with the renin-angiotensin and sympathetic nervous systems. Acute administration of the drug is associated with an increase in the release of renin, an effect which is thought to be prostaglandin dependent, (Johnston, G.D. et al(1983)). The diuresis and fall in circulating volume produced by frusemide, also result in stimulation of the renin-angiotensin and sympathetic nervous systems, (MacKay, I.G., Muir, A.L. & Watson, M.L.(1984); Cannella, G. et al(1983)). The increased activity of the renin-angiotensin system and the stimulation of the sympathetic nervous system are potentially arrhythmogenic in patients with heart failure. The purpose of the experiments described in this chapter was to assess the experimental effect of frusemide on the renin-angiotensin system in animals with heart failure, and also the interaction between this system and the changes in repolarisation and refractoriness which have been described previously. The results from the experiments in the previous chapters would suggest that the shortening in repolarisation and refractoriness in heart failure was unrelated to to an increase in plasma renin activity. There are theoretical grounds at least which would indicate however that stimulation of the renin-angiotensin system by virtue of the increase in

afterload, may cause a reduction in action potential duration: Lab, M.J.(1982); Reiter, M.J. et al (1988). This effect may be potentially arrhythmogenic and cause an increase in the incidence of sudden death.

## 7.2 Methods.

Two experimental groups of animals were used in this study. The surgical techniques and the electrophysiological and other measurements are as described in chapter 2.

## 7.3 Experimental protocol.

As in the other experiments, a 3 week period was allowed following implantation of the permanent pacing electrodes during which time the electrophysiological measurements were allowed to stabilise. The animals were then randomized to receive either adriamycin or saline according to the protocol described in chapter 2.
In addition to the injections with adriamycin or saline, each animal was treated with frusemide in a dose of 5mg.$Kg^{-1}$ intravenously twice daily. The dose of frusemide was chosen on the basis of information obtained in the veterinary formularly and also from a small pilot study

which assessed the effect of differing doses of the drug on body weight, urine output, fluid intake and plasma electrolytes.

A total number of 7 animals were assessed in the pilot study which was conducted over a 7 day period. One animal was treated with 0.5 $mg.Kg^{-1}$ twice daily of intravenous frusemide. Another animal with 1 $mg.Kg^{-1}$ twice daily, 2 animals with 2.5 $mg.Kg^{-1}$ and 2 animals with 5 $mg.Kg^{-1}$. A further animal was given 1ml of 0.9% saline intravenously twice daily as a control. One of the animals treated with 5$mg.Kg^{-1}$ of frusemide observed a fall in body weight of 3% over a week and the other had no change. Fluid intake increased by 240% and 150% respectively in the 2 animals. The average urine output was 60mls and 65mls in 24 hours. Plasma $K^+$ decreased and plasma urea increased in both the animals at the end of the 7 days. With respect to the 2 animals treated with 2.5$mg.Kg^{-1}$, both had a fall in body weight, a substantial increase in fluid intake and average urine outputs of 42ml and 27ml. Plasma $K^+$ decreased in only one of the animals and plasma urea decreased slightly in both animals. The animal treated with 1$mg.Kg^{-1}$ had no change in body weight, a 20% increase in fluid intake and an average urine output of 2,5ml. Plasma $K^+$ decreased and urea increased after 7 days. The animal treated with 0.5$mg.Kg^{-1}$ had a 4% increase in body weight, a 27%

increase in fluid intake and an average urine output of 56ml. Both plasma $K^+$ and urea decreased over the 7 days. The dose of frusemide eventually chosen as mentioned above was $5mg.Kg^{-1}$. From the information obtained in the pilot study this was the dose which produced the greatest response in terms of urine output, and also resulted in a decrease in plasma potassium concentration.

## 7.4 Results

### 7.4.1 Survival data.(table 36).

A total number of 8 animals entered the study in the adriamycin group and 7 animals in the control group. 2 animals died in the adriamycin group, both during week 8 of the study. Both of the animals had been unwell during the 24 hours prior to death and no cause was found at post-mortem examination. 6 animals survived to the end of the study and were then electively killed. There were no deaths in the saline control/frusemide group during the 10 weeks of the study.

Table 36.

Survival data in the adriamycin/frusemide and saline control/frusemide groups.

| | Initial Number | Overall Mortality | SD | SBE | Others |
|---|---|---|---|---|---|
| Adriamycin/frusemide | 8 | 2 | 0 | 0 | 2 |
| Saline control/ frusemide | 7 | 0 | 0 | 0 | 0 |

SD = Sudden death.

SBE = Subacute bacterial endocarditis.

Initial number = Initial number of animals in the study.

### 7.4.2 Electrophysiology.

Diastolic pacing threshold

The diastolic pacing threshold tended to increase between weeks 0 and 10 in both the adriamycin/frusemide and saline control/frusemide groups, although these changes were not statistically significant. There were no differences between the groups at either week(table 37).
Analysis of the pacing threshold in those animals which survived to the end of the study demonstrated a non-significant increase in the adriamycin/frusemide group from 2.2±0.3V to 3.0±0.3V, n=6. The diastolic pacing threshold was significantly higher in the adriamycin group from chapter 4, when compared to the adriamycin/frusemide group at week 0, (table 38). The data were thus normalised and there was no significant difference between the normalised means at week 10.
There were no differences in the pacing threshold between the saline control and saline control/frusemide groups at weeks 0 or 10.

Table 37

Changes in the diastolic pacing threshold in the adriamycin/frusemide and saline control/frusemide groups.

| Week | 0 | 5 | 10 |
|---|---|---|---|
| Adriamycin/frusemide:n | 8 | 8 | 6 |
| Threshold(volts) | 2.3±0.4 | 2.7±0.4 | 3.0±0.3 |
| Saline control/:n frusemide | 7 | 7 | 7 |
| Threshold(volts) | 2.9±0.6 | 3.1±0.6 | 3.5±0.5 |

There were no significant differences between groups or within the groups between weeks 0 and 10.

Table 38.

Comparison of the diastolic pacing threshold in the adriamycin/frusemide, saline control/frusemide, adriamycin an saline control groups.

| | Diastolic pacing threshold(volts) | |
|---|---|---|
| | Wk0 | Wk10 |
| Adriamycin/frusemide | 2.3±0.4(8) * | 3±0.3(6) |
| Adriamycin | 4.4±0.5(16) | 4±0.6(10) |
| Saline control/ frusemide | 2.9±0.6(7) | 3.5±0.5(7) |
| Saline control | 3.8+0.6(11) | 3.1+0.2(8) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.01$.

Stimulus-T interval

The stimulus-T interval in the group of animals treated with adriamycin and frusemide decreased significantly by 7% over the 10 weeks of the study ($p<0.05$). There was no significant diference however when the adriamycin/frusemide group of animals was compared to saline control/frusemide group at weeks 0 or 10, (table 39 and Fig 10). There was no significant change in stim-T in the saline control/frusemide group of animals over the 10 weeks of the study.

The stim-T interval in those animals which survived to the end of the study in the adriamycin/frusemide group also demonstrated a significant decrease between weeks 0 and 10. The mean value of stim-T was $106\pm1$ms at week 0, and $99\pm3$ms at week 10, $n=6$ and $p<0.05$.

The baseline stimulus-T interval was significantly shorter in the adriamycin/frusemide group when compared to the animals treated with adriamycin alone, (table 40). The baseline values were therefore normalised. Although the stim-T interval tended to decrease more in the adriamycin group, the difference between the groups was not statistically significant, (table 41). The stimulus-T interval was also significantly greater at week 0 in the saline control group when compared to the saline control/frusemide group, (table 40). This data was also

Table 39.

Changes in stim-T and ERP in the adriamycin/frusemide and saline control/frusemide groups.

| Week | 0 | 5 | 10 |
|---|---|---|---|
| Adriamycin:n | 8 | 8 | 6 |
| Stim-T(ms) | 105.8±1.3 | 106.1±2.6 | 98.8±2.8 |
| ERP(ms) | 102.5±2.5 | 95.5±2.7 | 86±2.5 |
| Control:n | 7 | 7 | 7 |
| Stim-T(ms) | 104.7±2.3 | 106.1±2.1 | 105.8±3.3 |
| ERP(ms) | 102.6±1.9 | 106.3±1.2 | 104±1.1 |

Stim-T significantly decreased between weeks 0 and 10 in the adriamycin/frusemide group, $p<0.05$. No change occured in the saline control/frusemide group between weeks 0 and 10. There were no significant differences between the groups.

The ERP significantly decreased by week 6 in the adriamycin/frusemide group, $p<0.01$, and this difference continued to week 10, $p<0.001$. No change occured in the control group. The ERP was significantly shorter in the adriamycin/frusemide group at week 10, $p<0.001$, when compared to the saline control/frusemide group.

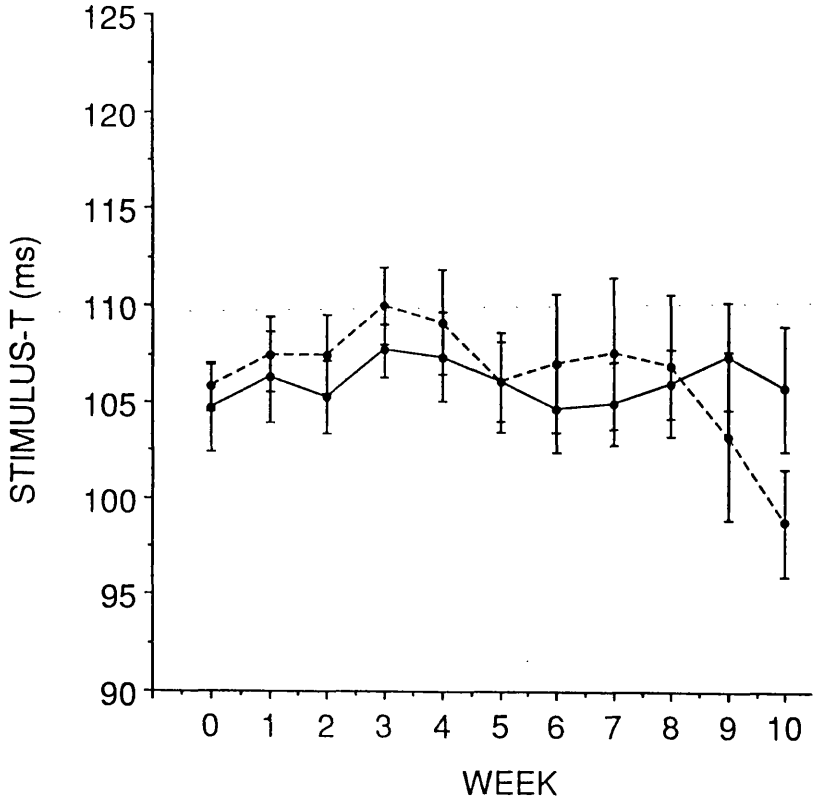


Figure 10.

Graph illustrating the effect of frusemide on ventricular repolarisation in heart failure. The adriamycin treated animals are represented by the broken line and the control animals by the continuous line. Although there was no progressive shortening, stim-T was significantly less at week 10 in the adriamycin group.

Table 40.

Comparison of stim-T and ERP in the adriamycin/frusemide, saline control/frusemide, adriamycin and saline control groups.

| | Stim-T(ms) | | ERP(ms) | |
|---|---|---|---|---|
| | Wk0 | Wk10 | Wk0 | Wk10 |
| Adriamycin/ frusemide | 106±1(8) * | 99±3(6) | 102±2(8) *** | 86±2(6) |
| | ** | | ** | |
| Adriamycin | 116±2(16)*** | 102±2(10) | 115±2(16)*** | 98±3(10) |
| Saline control/ frusemide | 105±2(7) | 106±3(7) | 103±2(7) | 104±1(7) |
| | ** | | | ** |
| Saline/ control | 120±3(11) | 120±4(8) | 113±4(11) | 120±3(8) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$
** = $p<0.01$.
*** = $p<0.001$

Table 41.

Comparison of the normalised stim-T and ERP data between the adriamycin/frusemide and adriamycin groups, and between the saline control/frusemide and saline control groups.

| | % change in stim-T. | % change in ERP. |
|---|---|---|
| Adriamycin/frusemide | -7±2.2(6) | -14.7±2.2(6) |
| Adriamycin | -11±1.7(8) | -13.5±1.3(9) |
| Saline control/frusemide | 1.3±1.8(7) | |
| Saline control | -2±1.6(8) | |

The numbers in parenthesis represent the numbers of animals.

There were no significant differences between the adriamycin/frusemide and adriamycin groups, nor between the saline control/frusemide and saline control groups.

normalised and there was no significant difference between the means at week 10, (table 41).

Effective refractory period

The ERP shortened by 16% in the adriamycin/frusemide group of animals by week 10, ($p<0.001$). The ERP was significantly shortened within this group by week 6, ($p<0.01$). When the adriamycin/frusemide group was compared to the saline control/frusemide group of animals, the ERP was significantly shorter in the former at week 3($p< 0.05$), (table 39) and Fig.11. Analysis of the ERP in those animals which survived to the end of the study in the adriamycin/frusemide group also demonstrated a significant decrease from 101$\pm$3ms to 86$\pm$2ms at week 10, $n=6$ and $p<0.01$.

As with the stim-T results, the baseline ERP was significantly shorter in the adriamycin/frusemide group when compared to the group of animals treated with adriamycin alone, (table 40), thus the ERP data was normalised. There were no significant differences between the normalised means at week 10, (table 41).
Although there was no difference at week 0, the ERP was significantly greater at week 10 in the saline control group when compared to the saline control/frusemide group, (table 40).

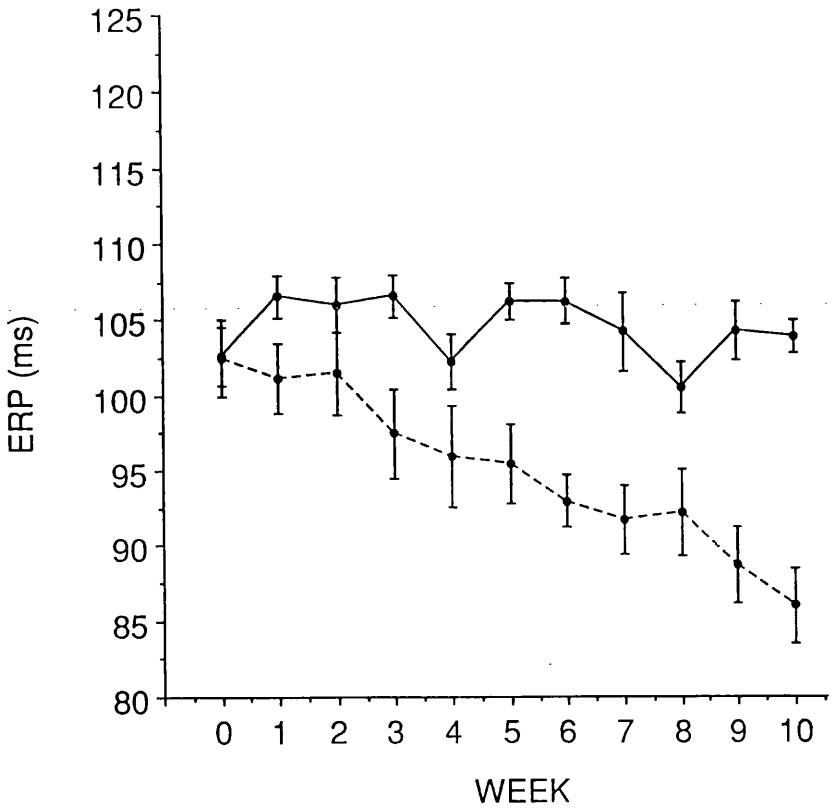


**Figure 11.**

Graph illustrating the effect of frusemide on the right ventricular effective refractory period in heart failure. The adriamycin treated animals are represented by the broken line and the controls by the continuous line. ERP progressively shortens in the adriamycin group.

Sinus cycle length

Sinus cycle length showed a non-significant decrease in both the adriamycin/frusemide and saline control/frusemide groups between weeks 0 and 10. There were no differences between the groups at week 0 or week 10, (table 42). There was also a non-significant decrease in the sinus cycle length in the animals which survived to the end of the study in the adriamycin/frusemide group, from 266±9ms to 260±14ms, n=6.

The baseline sinus cycle length was significantly greater in the adriamycin/frusemide group when compared to the group treated with adriamycin alone, (table 43), the data were thus normalised for comparison. At week 10 the normalised means were not significantly different. There were no significant differences between the saline control group and the saline control/frusemide group, (table 43).

Ambulatory ECG recording

One complete ambulatory 24 hour ECG recording was made in each of the animals in the study both prior to the introduction of frudemide and afterwards. There was no evidence of ventricular ectopic activity at any time in either the adriamycin or control groups.

Table 42.

Changes in sinus cycle length in the adriamycin/frusemide and saline control/frusemide groups.

| Week | 0 | 5 | 10 |
|---|---|---|---|
| Adriamycin/frusemide:n | 8 | 8 | 6 |
| Sinus cycle length(ms) | 262±7.7 | 267±8.5 | 260±13.7 |
| Saline control/frusemide:n | 7 | 7 | 7 |
| Sinus cycle length(ms) | 281±21.4 | 269±13.1 | 257±6.2 |

There were no significant differences between the groups, or within the groups between weeks 0 and 10.

Table 43

Comparison of sinus cycle length in the adriamycin/frusemide, saline control/frusemide, adriamycin and saline control groups.

| | Sinus cycle length(ms) | |
|---|---|---|
| | Wk0 | Wk10 |
| Adriamycin/frusemide | 262±7.7(8) * | 260±13.7(6) |
| Adriamycin | 238±6.7(16) | 221+7.9(10) |
| Saline control/ frusemide | 281±21.4(7) | 257±6.2(7) |
| Saline control | 230±3.8(11) | 238±2.8(8) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.

7.4.3 Biochemistry.

Neurohormonal data

Plasma noradrenaline increased significantly in the adriamycin group of animals treated with frusemide from 3.9 $\pm$ 0.2 $nmol.l^{-1}$ at week 1 to 7.9 $\pm$ 1.7 $nmol.l^{-1}$ at week 8, $p<0.05$. There was no significant difference when this group was compared to the corresponding saline control group treated with frusemide at weeks 1 or 8, (table 44). There was no significant difference between weeks 1 and 8 in the saline control/frusemide group. The mean plasma noradrenaline level demonstrated a non-significant increase in the animals in the adriamycin/frusemide group which survived to the end of the study. The mean concentration at week1 was 3.8$\pm$0.2$nmol.l^{-1}$ and at week8 8.2$\pm$2.2$nmol.l^{-1}$, n=6. There were no significant differences between the adriamycin/frusemide group and the adriamycin group at weeks 1 or 8, neither were there any significant differences between the saline control/frusemide group and the saline control group, (table 44).

There was no significant difference in plasma ANP either between the adriamycin/frusemide and saline control/frusemide groups or within the groups over an 8 week period, (table 45).

Table 44.

Comparison of plasma noradrenaline concentration between the adriamycin/frusemide and adriamycin groups, and between the saline control/frusemide and saline control groups.

| | Plasma noradrenaline concentration($nmol.l^{-1}$) | | |
|---|---|---|---|
| | Wk1 | Wk8 | p |
| Adriamycin/frusemide | 3.9±0.2(8) | 7.9±1.7(8) | 0.05 |
| Adriamycin | 5.1±1.3(19) | 5.4±0.8(19) | NS |
| Saline control/ frusemide | 5.8±1.9(6) | 4.8±1.1(6) | NS |
| Saline control | 3.5±0.5(18) | 5.2±1.2(11) | NS |

The numbers in parenthesis represent the numbers of animals.

There were no significant differences between the groups other than indicated.

Table 45.

Changes in plasma atrial natriuretic peptide concentration in the adriamycin/frusemide and saline control frusemide groups.

| | Week1 | Week8 | p |
|---|---|---|---|
| Adriamycin/frusemide:n | 7 | 8 | |
| ANP($pg.ml^{-1}$) | 110.4±29.5 | 152.9±27.3 | NS |
| Saline control/:n frusemide | 6 | 7 | |
| ANP($pg.ml^{-1}$) | 77.2±10.3 | 96.6±15.4 | NS |

ANP = atrial natriuretic peptide.

NS = not significant.

There was no significant difference between the groups at week 1 or week 8.

The mean plasma ANP concentration did however increase significantly from 86±24pg.ml$^{-1}$ to 138±25pg.ml$^{-1}$ in the animals which survived to the end of the study in the adriamycin/frusemide group, n=5 and $p<0.05$.
Although there was no significant difference between the adriamycin and the adriamycin/frusemide groups at week 1, the ANP level was significantly higher in the adriamycin/frusemide group at week 8, (table 46). There were no significant differences between the saline control/frusemide group and the saline control group, (table 46).

The mean plasma renin concentration did not change significantly between the adriamycin/frusemide and saline control/frusemide groups, nor between weeks 1 and 8 within the groups, (table 47). There was however a significant decrease in plasma renin from 139±24uU.l$^{-1}$ to 86±21uU.l$^{-1}$ in those animals which survived to the end of the study in the adriamycin/frusemide group, n=6 and $p<0.05$. The mean plasma renin level was significantly higher at week 1 in the adriamycin/frusemide group when compared to the adriamycin group, (table 48), but there was no significant difference between the means at week 8 when the data was normalised, (table 49). Similar results were found when the saline control/frusemide and the saline control groups were compared, (tables 48 and 49).

Table 46.

Comparison of plasma atrial natriuretic peptide concentration between the adriamycin/frusemide and adriamycin groups, and between the saline control/frusemide, and saline control groups.

| | Plasma ANP concentration($pg.ml^{-1}$) | |
|---|---|---|
| | Wk1 | Wk8 |
| Adriamycin/frusemide | 110.4±29.5(7) | 152.9±27.3(8) * |
| Adriamycin | 137.7±24.9(13) | 94.3±10.7(17) |
| Saline control frusemide | 77.2±10.3(6) | 96.6±15.4(7) |
| Saline control | 118.7±12.5(18) | 118.8±14.4(14) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.

Table 47.

Changes in plasma renin concentration in the adriamycin/frusemide and saline control/frusemide groups.

| | Week1 | Week8 | p |
|---|---|---|---|
| Adriamycin/frusemide:n | 8 | 7 | |
| Renin($uU.l^{-1}$) | 128.2±20.2 | 79.3±18.9 | NS |
| Saline control/:n frusemide | 7 | 6 | |
| Renin($uU.l^{-1}$) | 144.3±24.4 | 79.3±19 | NS |

NS = not significant

There were no significant differences between the groups at week 1 or 8, nor between the groups.

Table 48.

Comparison of plasma renin concentration between the adriamycin/frusemide and adriamycin groups, and between the saline control/frusemide and saline control groups.

| | Plasma renin concentration($uU.ml^{-1}$) | |
|---|---|---|
| | Wk1 | Wk8 |
| Adriamycin/frusemide | 128.2±20.2(8) | 79.3±18.9(7) |
| | * | |
| Adriamycin | 74.2±6.8(11) | 66.6±8.5(18) |
| Saline control/ frusemide | 144.3±24.4(7) | 79.3±19(6) |
| | ** | |
| Saline control | 72.6±7.2(12) | 56.1±4.2(17) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.

** = $p<0.01$.

Table 49

Normalised renin data for comparison between the adriamycin/frusemide and adriamycin groups, and between the saline control/frusemide and saline control groups.

| | % change in plasma renin concentration |
|---|---|
| Adriamycin/frusemide | -36±12.4(7) |
| Adriamycin | 21.7±9.9(11) |
| Saline control frusemide | -39.2±11.4(6) |
| Saline control | -8.9±13.1(12) |

The numbers in parenthesis represent the numbers of animals.

There were no significant differences between the groups.

Plasma electrolytes

The mean plasma $K^+$ levels were significantly higher at week 1 in the saline control/frusemide group when compared to the adriamycin/frusemide group, (table 50). There was no difference when the normalised data was compared at week 8 however. There was no significant difference between weeks 1 and 8 in either group. The mean plasma potassium concentration demonstrated a non-significant increase in those animals which survived to the end of the study in the adriamycin/frusemide group, from $4.3 \pm 0.05 mmol.l^{-1}$ to $4.4 \pm 0.07 mmol.l^{-1}$, n=6.

Comparison of the week 1 data revealed that plasma $K^+$ was significantly higher in the adriamycin group when compared to the adriamycin/frusemide group, (table 51). When the data were normalised however, the potassium levels demonstrated a significant decrease in the adriamycin group $p<0.05$, (table 52). There were no significant differences between the saline control/frusemide and the saline control groups at weeks 1 or 8.

Plasma magnesium levels showed a non-significant decrease between weeks 1 and 8 in both of the groups which were treated with frusemide and there were no significant differences between the 2 groups, (table 50).

Table 50.

Changes in plasma potassium and magnesium concentration in the adriamycin/frusemide and saline control/frusemide groups.

| | Adriamycin/frusemide | Saline control/frusemide | p |
|---|---|---|---|
| $K^+$Week1 | 4.2±0.04(8) | 4.7±0.13(7) | 0.01 |
| $K^+$Week8 | 4.6±0.25(7) | 4.6±0.09(6) | NS |
| $Mg^{2+}$Week1 | 0.77±0.02(8) | 0.83±0.04(7) | NS |
| $Mg^{2+}$Week8 | 0.72±0.05(7) | 0.76±0.02(6) | NS |

$K^+$ = plasma potassium in $mmol.l^{-1}$

$Mg^{2+}$ = plasma magnesium in $mmol.l^{-1}$

NS = not significant.

There was no difference in the adriamycin/frusemide or saline control/frusemide groups between weeks 1 and 8.

The numbers in parenthesis represent the numbers of animals.

Table 51.

Comparison of plasma potassium concentration between the adriamycin/frusemide and adriamycin groups, and between the saline control/frusemide, and saline control groups.

| | Plasma potassium concentration($mmol.l^{-1}$) | |
|---|---|---|
| | Wk1 | Wk8 |
| Adriamycin/frusemide | 4.2±0.04(8) | 4.6±0.25(7) |
| | ** | |
| Adriamycin | 4.9±0.12(23) | 4.6±0.08(18) |
| Saline control/ frusemide | 4.7±0.13(7) | 4.6±0.09(6) |
| Saline control | 4.8±0.11(17) | 4.8±0.12(16) |

The numbers in parenthesis represent the numbers of animals.

** = $p<0.01$.

Table 52.

Comparison of the normalised plasma potassium concentration between the adriamycin/frusemide and adriamycin groups.

| | % change in plasma potassium concentration |
|---|---|
| Adriamycin/frusemide | 9.7±5(7) * |
| Adriamycin | -6±3.6(16) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.

The mean plasma $Mg^{2+}$ concentration also showed a non-significant decrease from $0.78\pm0.03 mmol.l^{-1}$ to $0.73\pm0.04 mmol.l^{-1}$ in those animals in the adriamycin/frusemide group which survived to the end of the study, n=6.

The mean plasma magnesium level was significantly higher at week 1 in the adriamycin group when compared to the adriamycin/frusemide group, (table 53). When the data were normalised however, there was a significant decrease in the adriamycin group when compared to the adriamycin/frusemide group, (table 54). There was no significant difference between the saline control/frusemide group and the saline control group, (table 53).

There were no significant differences in plasma sodium levels between the adriamycin/frusemide and the saline control/frusemide groups. Neither were there any differences within the groups between weeks 1 and 8, (table 55).

There was a non-significant increase in the mean plasma sodium concentration from $140\pm0.6 mmol.l^{-1}$ to $141\pm0.4 mmol.l^{-1}$ in those animals which survived to the end of the study in the adriamycin/frusemide group, n=6.

The mean plasma sodium concentration was significantly higher at week 1 in the adriamycin group when compared to the adriamycin/frusemide group, (table 56). There was no

Table 53.

Comparison of plasma magnesium comcentration between the adriamycin/frusemide and adriamycin groups, and between the saline control/frusemide, and saline control groups.

| | Plasma magnesium concentration($mmol.l^{-1}$) | |
|---|---|---|
| | Wk1 | Wk8 |
| Adriamycin/frusemide | 0.77±0.02(8) * | 0.72±0.05(7) |
| Adriamycin | 0.95±0.05(16) | 0.7±0.02(20) |
| Saline control/ frusemide | 0.83±0.04(7) | 0.76±0.02(6) |
| Saline control | 0.95±0.05(16) | 0.85±0.03(16) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.

Table 54.

Comparison of the normalised plasma magnesium concentration between the adriamycin/frusemide and adriamycin groups.

| | % change in plasma magnesium comcentration |
|---|---|
| Adriamycin/frusemide | -6.8±4.8(7) |
| | * |
| Adriamycin | -24.5±4.5(16) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.

Table 55.

Changes in plasma urea and sodium concentration in the adriamycin/frusemide and saline control/frusemide groups.

| | Adriamycin/frusemide | Saline control/frusemide | p |
|---|---|---|---|
| Urea week1 | 7.3±0.3(8) | 7.3±0.4(8) | NS |
| | * | | |
| Urea week8 | 9.1±0.6(7) | 8.2±0.4(6) | NS |
| $Na^+$ week1 | 140.1±0.64(8) | 141.1±0.88(7) | NS |
| $Na^+$ week8 | 140.8±0.46(7) | 141.0±0.52(6) | NS |

Urea = plasma urea concentration in $mmol.l^{-1}$.

$Na^+$ = plasma sodium concentration im $mmol.l^{-1}$

NS = not significant.

There was no significant difference between weeks 1 and 8 in the saline control/frusemide group.

* = $p<0.05$.

The numbers in parenthesis represent the numbers of animals.

Table 56.

Comparison of plasma sodium concentration between the adriamycin/frusemide and adriamycin groups, and between the saline control/frusemide and saline control groups.

| | Plasma sodium concentration($mmol.l^{-1}$) | |
|---|---|---|
| | Wk1 | Wk8 |
| Adriamycin/frusemide | 140±0.6(8) | 141±0.5(7) |
| | * | |
| Adriamycin | 143±0.7(24) | 143±0.5(20) |
| Saline control/ Frusemide | 141±0.9(7) | 141±0.5(6) |
| | * | |
| Saline control | 143±0.5(18) | 142±0.4(16) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.

significant difference at week 8 when the data were normalised, (table 57). Similar results were found when the saline control/frusemide group was compared to the saline control group, (tables 56 and 57).

Plasma urea increased significantly in the adriamycin/frusemide group from 7.3±0.3 $mmol.l^{-1}$ at week 1 to 9.1±0.6 at week 8, $p<0.05$. There were no significant differences between this group and the saline control/frusemide group, (table 55). There was no significant change in plasma urea between weeks 1 and 8 in the saline control/frusemide group. The mean plasma urea concentration also increased significantly in the animals which survived to the end of the study in the adriamycin/frusemide group from 7.0±0.3$mmol.l^{-1}$ to 8.6±0.4$mmol.l^{-1}$, $n=6$ and $p<0.01$.

No significant differences were observed at week 1 between the adriamycin/frusemide and the adriamycin group. Plasma urea was however significantly higher at week 8 in the adriamycin/frusemide group. Similar results were found when the saline control/frusemide group was compared to the saline control group. (table 58).

Table 57.

Normalised plasma sodium concentration for comparison between the adriamycin/frusemide and adriamycin groups, and between the saline control/frusemide and saline control groups.

| | % change in plasma sodium concentration |
|---|---|
| Adriamycin/frusemide | 0.34±0.58(7) |
| Adriamycin | 0.41±0.57(19) |
| Saline control/ frusemide | 0.45±0.38(6) |
| Saline control | -0.8±0.38(16) |

The numbers in parenthesis represent the numbers of animals.

There were no significant differences between the groups.

Table 58.

Comparison of plasma urea concentration between the adriamycin/frusemide and adriamycin groups, and between the saline control/frusemide and saline control groups.

| | Plasma urea concentration(mmol.l-1) | |
|---|---|---|
| | Wk1 | Wk8 |
| Adriamycin/frusemide | 7.3±0.3(8) | 9.1±0.6(7) |
| | | ** |
| Adriamycin | 6.6±0.3(24) | 7.0±0.4(20) |
| Saline control/frusemide frusemide | 7.3±0.4(8) | 8.2±0.4(6) |
| | | * |
| Saline control | 7.0±0.3(18) | 6.6±0.3(16) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.
** = $p<0.01$)

7.4.4 Pathology.(tables 59-63).

The mean wet heart weight was 12% higher in the adriamycin/frusemide group when compared to the saline control/frusemide group,although this difference was not statistically significant. There was no significant difference when the mean wet heart weights were compared between the adriamycin/frusemide and the adriamycin groups, although the mean was greater in the adriamycin group. The heart weights were significantly higher in the saline control/frusemide group when compared to the saline control group.

The mean wet liver weight was 10% higher in the adriamycin/frusemide group when compared to the saline control/frusemide group, a non-significant difference. There was no significant difference when the liver weights were compared between the adriamycin and adriamycin/frusemide groups, and the saline control/frusemide and saline control groups.

There was a tendency for the mean wet lung weights to be higher in the adriamycin/frusemide group when compared to the saline control/frusemide group, a non-significant increase. There were no significant differences between the adriamycin/frusemide and adriamycin groups, and no

difference between the saline control/frusemide and saline control groups.

When the heart and liver weights were expressed as a percentage of the total body weight, they were 8% and 7% respectively higher in the adriamycin/frusemide group when compared to the saline control/frusemide group, a non-significant increase. The heart/body weight ratio was significantly higher in the adriamycin group when compared to the adriamycin/frusemide group, but there was no significant difference between the saline control/frusemide and the saline control groups. There was no significant difference in the liver/body weight ratios between the adriamycin/frusemide and adriamycin groups, nor between the saline control/frusemide and the saline control groups. There were no significant differences in the lung/body weight ratios between the 4 groups, although the mean value was greatest in the adriamycin group.

Total body weight increased by 8% in the adriamycin/frusemide group and 9% in the saline control/ frusemide group over the 10 weeks of the study, although the increases were not statistically significant. There were no significant difference between the 2 groups.
In those animals which survived to the end of the study in the adriamycin/frusemide group, the mean total body weight increased significantly from 3.56$\pm$0.1Kg to 3.75$\pm$0.1, n=6

and $p<0.05$. Paired analysis of the animals in the saline control/captopril group also revealed a significant increase between weeks 0 and 10, $p<0.05$.

There were no baseline differences between the adriamycin/frusemide and the adriamycin groups, but the mean body weights were significantly higher at week 10 in the adriamycin/frusemide group $p<0.05$. There was no significant difference between the saline control/frusemide and the saline control groups.

The wet/dry weight ratio of the hearts was similar in the adriamycin/frusemide and the saline control/frusemide groups, $4.8 \pm 0.3$ in the adriamycin/frusemide animals and $4.8 \pm 0.1$ in the saline controls/frusemide group. There were no significant differences when these groups were compared to the adriamycin and saline control groups which did not receive frusemide, although the greatest value was seen in the adriamycin group.

There were no significant differences in the liver wet/dry weight ratios between the adriamycin/frusemide and saline control/frusemide groups, $4.2 \pm 0.3$ in the adriamycin/frusemide group vs $3.9 \pm 0.4$ in the saline control/frusemide group.

Table 59.

Comparison of heart, liver and lung weight between the adriamycin/frusemide and adriamycin groups, and between the saline control/frusemide and saline control groups.

| | Heart(g) | Liver(g) | Lung(g) |
|---|---|---|---|
| Adriamycin/frusemide | 9.4±0.4(8) | 79.1±6(8) | 20.3±3.3(6) |
| Adriamycin | 10.8±0.6(17) | 87.8±5.8(18) | 18.5±1.4(18) |
| Saline control/ frusemide | 8.4±0.3(7) * | 71.7±4.1(7) | 17.6±1.8(7) |
| Saline control | 7.2±0.3(16) | 64.9±3.9(15) | 18±1.1(15) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.

Table 60.

Comparison of body weight and heart, liver and lung to body weight ratios between the adriamycin/frusemide and saline control/frusemide groups.

| | Adriamycin/ frusemide | Saline control/ frusemide | p |
|---|---|---|---|
| Body weight (Kg): | | | |
| Week 0 | 3.46±0.1(8) | 3.2±0.08(7) | NS |
| Week 10 | 3.75±0.1(6) | 3.5±0.1(7) | NS |
| HW/BW | 0.26±0.01(8) | 0.24±0.02(7) | NS |
| LW/BW | 2.17±0.12(8) | 2.02±0.05(7) | NS |
| LU/BW | 0.54±0.09(6) | 0.5±0.05(7) | NS |

The numbers in parenthesis represent the numbers of animals.

HW = heart weight.

LW = liver weight.

BW = body weight.

LU = lung weight.

NS = not significant.

Table 61.

Comparison of the heart, liver and lung to body weight ratios between the adriamycin/frusemide and adriamycin groups, and between the saline control/frusemide and saline control groups.

| | H/BW | L/BW | LU/BW |
|---|---|---|---|
| Adriamycin/ frusemide | 0.26±0.01(8) * | 2.17±0.12(8) | 0.54±0.09(6) |
| Adriamycin | 0.33±0.02(18) | 2.77±0.2(18) | 0.58±0.05(18) |
| Saline control/ frusemide | 0.24±0.02(7) | 2.02±0.05(7) | 0.5±0.05(7) |
| Saline control | 0.21±0.02(15) | 1.95±0.2(15) | 0.52±0.04(15) |

The numbers in parenthesis represent the numbers of animals.

* = $p<0.05$.

H/BW = Heart to body weight ratio.

L/BW = Liver to body weight ratio.

LU/BW = Lung to body weight ratio.

Table 62.

Comparison of body weights between the adriamycin/frusemide and adriamycin groups, and between the saline control/frusemide and saline control groups.

| | Body weight(Kg) | |
|---|---|---|
| | Wk0 | Wk10 |
| Adriamycin/frusemide | 3.46±0.1(8) | 3.75±0.1(6) ** |
| Adriamycin | 3.3±0.05(24) | 3.2±0.07(18) |
| Saline control/ frusemide | 3.2±0.08(7) | 3.5±0.1(7) |
| Saline control | 3.27±0.08(19) | 3.49±0.1(16) |

The numbers in parenthesis represent the numbers of animals.

** = $p<0.01$.

Table 63.

Comparison of the wet/dry weight ratio of the heart and liver between the adriamycin/frusemide and adriamycin groups, and between the saline control/frusemide and saline control, groups.

| | Heart wet/dry | Liver wet/dry |
|---|---|---|
| Adriamycin/frusemide | 4.8±0.3(8) | 4.2±0.3(8) |
| Adriamycin | 5.5±0.72(4) | |
| Saline control/ frusemide | 4.8±0.1(7) | 3.9±0.4(7) |
| Saline control | 4.5±0.29(4) | |

The numbers in parenthesis represent the numbers of animals.

There were no significant differences between the groups.

## 7.5 Discussion.

The electrophysiological results from the experiments in this chapter are somewhat surprising. Firstly, the stimulus-T interval only decreased by 7% over the 10 weeks of the study in the animals treated with adriamycin and frusemide. This is in comparison to the 12% shortening in stim-T which was observed in the initial group of animals treated with adriamycin. The shortening in repolarisation was only significant at week 10 and did not show the progressive decrease which occured in the adriamycin group of animals. Neither was there any significant difference between the 2 groups treated with frusemide at any week. In contrast to the stim-T interval, the ERP did decrease progressively and significantly by week 10. This result is also surprising as repolarisation and refractoriness normally change in concert. Although an explanation for the discrepancy between the changes in refractoriness and the lack of significant changes in repolarisation is not readily available, there is some evidence which may explain the latter observation. If the basis of the electrophysiological changes which were seen in the animals treated with adriamycin in the initial series of experiments was, as presumed, related to the development of heart failure and the myopathic effect of adriamycin, then if the animals in the group treated with adriamycin

and frusemide did not have the same degree of heart failure or a similar degree of histological evidence of cardiac damage then the electrophysiological changes might not occur. Although there were no haemodynamic measurements made in this study or in the previous studies there is some indirect evidence that the animals in the adriamycin plus frusemide group did not have the same degree of heart failure. The mean wet heart weight in the adriamycin plus frusemide group was only 12% higher than the corresponding control group, and the liver weights were only 10% higher. This is in comparison to increases of 49% and 35% in heart and liver weights in the adriamycin group. Likewise, the heart and liver to body weight ratios were only 8% and 7% higher when compared to the saline control/frusemide group. This is in comparison to increases of 57% and 42% in these parameters in the initial adriamycin group of animals. The absence of significant increases in heart and liver weights would be consistent with the absence of a significant degree of heart failure. The cardiac ventricular muscle samples which were prepared for histological examination were unfortunately not assessed at the time of publication and no comment can therefore be made about the presence or absence of histological changes in the animals treated with adriamycin and frusemide.

Another measurement which is important in assessing the toxic effects of adriamycin is the body weights of the animals. Although the cytopathic effect of the drug on the heart muscle was not examined histologically, one method of looking at the systemic effects of adriamycin is by measuring the change in body weight, which by virtue of adriamycin's inhibition of protein synthesis, (Arena, B. et al(1974)), and its anorectic effect could result in a decrease in body weight or at least a lack of increase over a 10 week period. This hypothesis is confirmed in so much as the body weights in the adriamycin/frusemide group demonstrated a non-significant increase over 10 weeks, and in the adriamycin group a non-significant decrease over the same period of time such that at week 10, they were significantly different with no difference at baseline. This is despite the fact that frusemide would be expected to reduce body weight. Another general observation which was made was that the animals in the adriamycin plus frusemide group appeared well throughout the duration of the study in comparison to the animals in the adriamycin group which in general were at times unwell and anorectic. One possible explanation for the supposed lack of toxic effect of adriamycin in the animals discussed in this chapter may be a biological variation in response to any given dose of the drug. Previous studies have demonstrated considerable variation both clinically and

experimentally in the extent of myocardial damage, as assessed by endomyocardial biopsy, in response to a standard dose of adriamycin, (Bristow, M.R. et al(1978); Bristow, M.R. et al(1981)). The rabbits were all obtained from the same supplier throughout the study, but an unrecognised variation in susceptibility to adriamycin cannot be excluded.

Another important factor which may explain the probable decrease in cardiotoxicity induced by adriamycin in the animals treated with frusemide may be variations in the dietary content of elements which reduce the activity of free radicals. Previous reports have emphasised the importance of selenium and alpha-tocopherol in determining the extent of cardiac damage induced by adriamycin. One such study demonstrated that the regular administration of an alpha-tocopherol-selenium mixture to rabbits lessened the degree of adriamycin induced cardiomyopathy, (Van Fleet, J.F., Greenwood, L. & Ferrans, V.J.(1978)). Although the dietary source remained constant throughout this study, it is possible that the raw materials incorporated into the diet may have come from different suppliers with undetected variations in the concentrations of antioxidants.

The role of selenium in preventing the cardiotoxic effects of adriamycin is attractive on theoretical grounds. As mentioned previously the increased production

of oxygen derived free radicals has been proposed as one possible mechanism for the cytopathic action of adriamycin. This mechanism of action of adriamycin could potentially be reversed by selenium which increases the tissue concentration of of the free radical protection enzyme, glutathione peroxidase, (Chow, C.K. & Tappel, A.L.(1974)). A higher concentration of selenium in the diet of the animals treated with adriamycin and frusemide could be associated with a decrease in the level of heart failure which occurred. Previous work has in fact shown that the selenium content of commercial animal feed may vary from deficient to toxic, (Diplock, A.T.(1976)). Other studies have also demonstrated that the cardiotoxicity induced by adriamycin may be attenuated by alpha-tocopherol, (Myers, C.E., Mc Guire, W. & Young, R.(1976); Milei, J. et al(1986)).

Clinical studies have demonstrated that the treatment of patients with heart failure with diuretics may result in activation of the renin-angiotensin system, (Bayliss, J. et al(1987); Brown, J.J. et al(1970)). It was expected therefore that the animals in this study which were treated with adriamycin and frusemide would have an increase in plasma renin concentration. Surprisingly however there was no significant increase in plasma renin between weeks 1 and 8 of the study in the adriamycin group

treated with frusemide nor between the 2 frusemide treated groups. The reason for the lack of activation of the renin-angiotensin system in the animals may have been that the dose of frusemide was inadequate to cause salt and water depletion or that the animals were not in heart failure(as indicated by the absence of significant increases in heart and liver weights). Another possibility is that the blood samples could have been taken too early after the onset of frusemide treatment.

Another interesting observation in this study is that the mean plasma noradrenaline concentration significantly increased between weeks 1 and 8 in the adriamycin plus frusemide group. There was no significant difference however between the 2 groups or between the adriamycin/frusemide and the group treated with adriamycin alone. One possible explanation for an increase in sympathetic nervous activity could be that the animals treated with frusemide were volume depleted and thus had a reflex increase in noradrenaline. This would seem unlikely however as there was no concomitant activation of the renin-angiotensin system. Previous workers have suggested however that the neuroendocrine response to heart failure is sequential with the sympathetic nervous system being activated before the renin-angiotensin system, (Bayliss, J. et al(1987)).

Other biochemical changes which are commonly associated with diuretics are a decrease in plasma $K^+$ and $Na^+$ concentrations. There were no significant changes in either of these parameters between weeks 1 and 8 or between groups. It has also been postulated that the decrease in plasma magnesium concentration which occurs in heart failure and is potentiated during teatment with diuretics may be potentially arrhythmogenic. Although there was a significant decrease in plasma magnesium concentration between weeks 1 and 8 in the initial group of animals treated with adriamycin, and this decrease was associated with ventricular ectopic activity as detected by ambulatory ECG monitoring, there was surprisingly no significant change in plasma magnesium in the adriamycin group of animals treated with frusemide. It would seem however that the diuretic therapy did have some effect as the mean plasma urea concentration was significantly increased between weeks 1 and 8 in the adriamycin plus frusemide group. This could imply that the animals were becoming dehydrated by an overvigorous diuresis.

In conclusion therefore, the reproducibility of the electrophysiological results from the first 2 experimental series have been called into question by the results in this chapter. For some reason it seems that the cardiomyopathic effect of adriamycin was significantly less or even absent in the animals treated with adriamycin

and then subsequently with frusemide. This is supported by the lack of a significant increase in heart and liver weights in the animals, and also by the increase in body weight which would suggest that the drug had less systemic toxic effects. The possible reasons for the variability in the effects of adriamycin have been outlined above, but these results do indicate that the conclusions and possible extrapolations which were drawn from the results in chapters 4 and 6 should be treated with caution.

# CHAPTER 8

## ASSESSMENT OF THE RELATIONSHIP BETWEEN CERTAIN INDICES OF ADRIAMYCIN TOXICITY AND CHANGES IN VENTRICULAR REPOLARISATION AND REFRACTORINESS.

### 8.1 Introduction.

As discussed in the previous chapter, the absence of a progressive decrease in right ventricular repolarisation and refractoriness in the animals in the adriamycin/frusemide group could have been related to the absence of the cardiotoxic effects of adriamycin in this group.

If this theory were true, the possibility exists that there may be a relationship between certain indices of the toxic effects of adriamycin, both cardiac and systemic, and the changes in repolarisation and refractoriness which were seen in the animals in the adriamycin group. It might follow therefore that the animals with the greater degree of adriamycin toxicity, as manifest by a greater heart weight and lower body weight, would have the greater change in repolarisation and refractoriness.

This chapter therefore examines the relationship between these parameters as assessed by simple regression analysis.

## 8.2 Statistical analysis.

The data in this chapter have been compared using simple regression analysis to generate the correlation coefficient and its significance level.

## 8.3 Results.

Comparison was made between the electrophysiological variables; ERP, change in ERP, Stim-T, change in stim-T and the parameters which may have reflected evidence of adriamycin toxicity; body weight, change in body weight, heart weight, liver weight and the heart and liver weights as expressed as a ratio of body weight.
The analysis of the data was disappointing. There was no significant correlation between any of the electrophysiological variables and the indices of adriamycin toxicity, when the data from all of the animals treated with adriamycin in chapters 4, 6 and 7 were analysed. Although the animals in the adriamycin/frusemide group did not have significant increases in heart and liver weights, and the mean body weights were significantly lower in the adriamycin group when compared to the adriamycin/frusemide group, there is

no statistical relationship between the electrophysiological changes and these variables which would support the proposal that the animals in the adriamycin/frusemide group did not develop a cardiomyopathy.

As previously discussed however, there is evidence that the systemic toxicity of adriamycin can be assessed by changes in body weight(Arena, B. et al(1974)). There is also evidence that there is considerable variation in the cytopathic effect of a given dose of adriamycin(Bristow, M.R. et al(1978)).

The results in this chapter do not therefore explain why the electrophysiological data in the adriamycin/frusemide group was not reproducible.

# CHAPTER 9

## GENERAL DISCUSSION ON THE OVERALL FINDINGS IN THE EXPERIMENTS DESCRIBED IN THIS THESIS, AND ASSESSMENT OF THE STRENGTHS AND WEAKNESSES OF THE STUDY.

The initial series of experiments described in chapter 4 of this thesis produced some interesting results. The most important observation which was made was the progressive and significant shortening in ventricular repolarisation and refractoriness which occured in the animals treated with adriamycin. These electrophysiological changes were unrelated to neurohormonal activation and were not associated with changes in the diastolic pacing threshold. The animals in the adriamycin group also had significant increases in heart and liver weights when compared to the saline control group. Although no haemodynamic measurements were made in this study, the increases in these parameters were proposed as indirect evidence that the animals in the adriamycin group were in heart failure.

A significant decrease was also observed in plasma magnesium concentration in the adriamycin group, although the magnitude of the decrease in magnesium could not explain the the 12% shortening in repolarisation if we

extrapolate from previous in vitro studies, (Watanabe, Y. & Dreifus, L.S.(1972)).

Although there was no evidence of arrhythmias in the saline control group, each of the animals in the adriamycin group had evidence of infrequent ventricular ectopic activity, which may have reflected electrolyte depletion.

One of the most important issues which this study was designed to investigate was the relationship between the electrophysiological changes and sudden death. Three animals died suddenly and unexpectedly in the adriamycin group, and no animal died suddenly in the saline control group.

The principal finding in the in vitro experiments which were described in chapter 5, was a significant decrease in action potential duration and effective refractory period in animals treated with adriamycin when compared to controls.

These findings were similar but of a greater magnitude to the in vivo shortening in repolarisation and refractoriness which were described in chapter 4. The nature of the in vitro preparation also meant that these changes were unrelated to neurohormonal mechanisms and electrolyte depletion.

The combination of the electrophysiological changes fron chapters 4 and 5 seemed to indicate that these changes

were most likely the result of the direct cytopathic effect of adriamycin. In chapter 6, the effect of captopril was assessed on the electrophysiological changes associated with adriamycin cardiomyopathy.

The introduction of captopril did not result in the reversal of the shortening in repolarisation and refractoriness which had resulted from the treatment with adriamycin. By the end of the study, the stim-T interval and ERP were reduced by 14% and 8% respectively in the adriamycin/captopril group. These results are similar in magnitude to the changes seen in the adriamycin group.

If the direct cytopathic effect of adriamycin and not an increase in afterload is the cause of the electrophysiological changes in this thesis, then captopril would not have been expected to reverse these changes.

Captopril was associated with an improvement in certain indices which would indicate, at least indirectly, an improvement in cardiac function. The heart weight in the adriamycin/captopril group was only 10% greater than in the saline control/captopril group. This compares with a 49% increase in the adriamycin group when compared to the saline control group. Neither was there a significant increase in liver weight in the adriamycin/captopril group.

Captopril treatment also prevented the significant decrease in plasma magnesium which had occured in the initial adriamycin group, and caused a non-significant increase in plasma potassium at week 8 of the study, which resulted in the mean level of plasma potassium being significantly greater in the adriamycin/captopril group when compared to the adriamycin group

No evidence of arrhythmias was observed in any of the animals in the adriamycin/captopril group, which is in contrast to the adriamycin group, and may have been related to the absence of electrolyte depletion.

The mean level of plasma renin significantly increased in both the groups treated with captopril, as expected. One surprising finding in the animals in the adriamycin/captopril group was the significant increase in plasma noradrenaline by week 8 of the study. If captopril was associated with an improvement in heart failure then it would have been expected that sympathetic nervous activity would have decreased.

Although captopril did not reverse the shortening in stim-T and ERP,, no animals died suddenly in the adriamycin/captopril group.

The results from the final series of experiments described in chapter 7 were disappointing. Unlike the results in chapters 4 and 6, there was no progressive and parallel shortening in stim-T and ERP over the 10 weeks of the

study. Although the ERP significantly decreased by 16% in the adriamycin/frusemide group, there was no significant decrease in stim-T when compared to the saline control/frusemide group. Both of these parameters should change in concert, and the results from these experiments therefore cast some doubt on the electrophysiological changes previously described in chapters 4 and 6. It was suggested that an explanation for this anomaly could be a variation in the response of the animals to adriamycin, such that the animals in the adriamycin/frusemide group were not in heart failure.

This explanation is supported by the lack of a significant increase in heart and liver weights in the adriamycin/frusemide group, and a non-significant increase in body weight implying the absence of significant systemic toxicity of adriamycin.

No significant increase was observed in plasma renin in either of the groups treated with frusemide. This is unusual as diuretics are known to activate the renin-angiotensin system in heart failure. A possible explanation for this is that the blood samples for plasma renin assay were taken before the drug had taken full effect.

Plasma noradrenaline levels significantly increased in the adriamycin/frusemide group. This could possibly have been secondary to diuretic-induced dehydration, as manifest by

the significant increase in plasma urea which occured in this group of animals.
Diuretics are known to produce depletion of plasma potassium and magnesium although no change was seen in either of these indices.
In chapter 8, there was no significant correlation between certain indices which reflect the toxic effects of adriamycin, and the electrophysiological changes. These results do not support the hypothesis that the absence of a progressive shortening in ventricular repolarisation in the adriamycin/frusemide group, was related to a lack of adriamycin cytotoxicity.

The importance of the experiments presented in this thesis is that for the first time, chronic changes in ventricular repolarisation and refractoriness have been measured in an experimental model of heart failure.
The significant shortening in both of these parameters in the adriamycin and adriamycin/captopril groups, is proposed as a possible mechanism for sudden death in heart failure. The shortening in action potential duration and effective refractory period in the in vitro experiments lends further support to the hypothesis that the electrophysiological changes in the in vivo experiments are a consistent feature of this model of heart failure.

Although these electrophsiological changes were present, the incidence of sudden death was low in the adriamycin group and subsequent comparison of the mortality rates with other groups is therefore difficult.
The absence of similar electrophysiological changes in the adriamycin/frusemide group questions the validity of the results in chapters 4 and 6. The variability in response of the rabbits to adriamycin is however a significant drawback with this model of heart failure.

Another weakness with this series of experiments is that no haemodynamic measurements were made to confirm the presence of heart failure, but the significant increase in heart and liver weight in the adriamycin group is indirect evidence that adriamycin did cause heart failure. As discussed in chapter 1.14, previous studies have shown that an identical injection protocol with adriamycin, induces heart failure in the rabbit as assessed by haemodynamic abnormalities.
Another further weakness with the study is the absence of activation of the renin-angiotensin and sympathetic nervous systems, both of which are known to occur in heart failure clinically. It has been previously described however, that untreated heart failure may be associated with normal levels of plasma renin, (Bayliss, J. et al(1987); Brown, J.J. et al(1970)).

Although there were significant electrophysiological changes in the animals treated with adriamycin as described in chapters 4 and 6, the incidence of sudden death was low. As this study was designed to attempt to elucidate the possible mechanisms predisposing to sudden death, further experiments would be required in which the incidence of sudden death was increased. One possibility may be that the use of digoxin or a higher dose of diuretics for a longer period of time may achieve this. It is possible that the activation of neurohormonal mechanisms by a higher dose of diuretics may increase the incidence of sudden death.

It would also be interesting to apply the electrophysiological techniques employed in this study to other experimental models of heart failure, both in the rabbit and other mammals. If similar electrophysiological changes were found in other models of heart failure, this would give more weight to the premise that the shortening in stim-T and ERP could be a potential mechanism for sudden death.

# CHAPTER 10

# CONCLUSIONS

1. Adriamycin induced heart failure in the rabbit is associated with significant shortening in ventricular repolarisation and refractoriness which could provide a mechanism for sudden death.
2. The electrophysiological changes in this study are independent of changes in plasma electrolytes and neurohormones.
3. Variation in response to adriamycin toxicity may occur in the rabbit, and the electrophysiological changes could be related to the severity of heart failure.
4. Captopril does not reverse the shortening in repolarisation and refractoriness induced by adriamycin, but reduces the frequency of ventricular arrhythmias possibly by preventing plasma electrolyte depletion. The animals treated with adriamycin and captopril did not show significant increases in heart and liver weights, which implies an improvement in heart failure.
5. The duration and dose of frusemide used to treat the animals in this study was not sufficient to cause activation of the renin-angiotensin system.

6. The absence of a progressive and parallel shortening in stim-T and ERP in the animals treated with adriamycin and frusemide, may reflect the absence of heart failure in these animals as a result of variability in the response to adriamycin.

# REFERENCES

Ader, R. et al(1980), Immediate and sustained haemodynamics and clinical improvement in chronic heart failure by an oral angiotensin converting-enzyme inhibitor, Circulation, 61, 931-937.

Alexander, M. Hinshaw, L.B. & Drury, D.R.(1957), Mechanism of congestive heart failure following aortic constriction in rabbits, Circulation Research, 5, 375-381.

Appelbaum, F.R. et al(1976), Acute lethal carditis caused by high-dose combination chemotherapy. A unique clinical and pathological entity, Lancet, 1, 58.

Appelfield, M.M. & Egorin, M.J.(1984), The anthracycline antibiotics depress left ventricular contractility: a clinical fact or laboratory fancy? International Journal of Cardiology, 6, 351-353.

Arena, E. et al(1974), DNA, RNA and protein synthesis in heart, liver and brain of mice treated with daunorubicin or adriamycin, International Research Communications, Systemic Medical Science, 2, 1053-1061.

Arnolda, L. et al(1985), Adriamycin cardiomyopathy in the rabbit: an animal model of low output cardiac failure with activation of vasoconstrictor mechanisms, Cardiovascular Research, 19, 378-382.

Aronson, R.S.(1980), Characteristics of action potentials of hypertrophied myocardium from rats with renal hypertension, Circulation Research, 47, 443-454.

Bachur, N.R., Gordon, S.L. & Gee, M.V.(1977), Anthracycline antibiotic augmentation of microsomal electron transport and free radical formation, Molecular Pharmacology, 13, 901-910.

Bajusze, E. et al(1969), Spontaneous hereditary myocardial degeneration and congestive heart failure in a strain of Syrian hamsters, Annals of the New York Academy of Sciences, 156, 105-129.

Basset, A.L. & Gelband, H.(1973), Chronic partial occlusion of pulmonary artery in cats, Circulation Research, 32, 15-26.

Bayliss, J. et al(1987), Untreated heart failure: clinical and neuroendocrine effects of introducing diuretics, British Heart Journal, 57, 17-22.

Bazett, H.C.(1920), An analysis of the time relations of the electrocardiogram, Heart, 7, 353-370.

Bers, D.M., Phillipson, K.D. & Langer, G.A.(1981), Cardiac contractility and sarcolemmal calcium binding in several cardiac muscle preparations, American Journal of Physiology, 240, H576-H585.

Bertuso, J.R. et al(1984), Do patients with cardiac arrest and hypokalaemia require antiarrhythmic drug therapy? Circulation, 70(Suppl II), II-443.

Bigger, J.T.Jr.(1973), Electrical properties of cardiac muscle and and possible causes of cardiac arrhythmias, In Cardiac Arrhythmias, eds. Dreifus, L.S. & Likoff, W. Ch. 13, New York: Grune and Stratton.

Bigger, J.T.Jr. et al(1985), Effects of digitalis treatment on survival after myocardial infarction, American Journal of Cardiology, 55(6), 623-630.

Billingham, M.E.(1979), Some recent advances in cardiac pathology, Human Pathology, 10, 367-386.

Blum, R.H. & Carter, R.H.(1974), Adriamycin. A new anti-cancer drug with significant clinical activity, Annals of Internal Medicine, 80, 249-259.

Bristow, M.R. et al(1978), Doxorubicin cardiomyopathy: Evaluation by phonocardiography, endomyocardial biopsy and cardiac catherisation, Annals of Internal Medicine, 88, 175-197.

Bristow, M.R. et al(1981), Dose-effect and structure-function relationships in doxorubicin cardiomyopathy, American Heart Journal, 102, 709-718.

Brooks, H. Holland, R, & Al-Sadir, J.(1977), Right ventricular performance during ischaemia: an anatomic and haemodynamic analysis, American Journal of Physiology, 233, 500-513.

Brown, J.J. et al(1970), Renin relationships in congestive heart failure, treated and untreated, American Heart Journal, 80, 329-342.

Brown, M.J., Brown, D.C. & Murphy, M.B.(1983), Hypokalaemia from beta2-receptor stimulation by

circulating epinephrine, New England Journal of Medicine, 309, 1414-1419.

Browne, K.F. et al(1982), Influence of the autonomic nervous system on the QT interval in man, American Journal of Cardiology, 50, 1099-1103.

Buja, L.M. et al(1973), Cardiac ultrastructural changes induced by daunorubicin therapy, Cancer, 32, 771-778.

Burgraff, G.W. & Parker, J.O.(1975), Prognosis in coronary artery disease: Angiographic, haemodynamic and clinical factors, Circulation, 51, 146-156.

Butrous, G.S.(1986), The QT interval: Its clinical implications, Current Opinion Cardiology, 1, 29-34.

Calcroft, S.C.W., Gavin, J.B. & Herdon, P.B.(1973), Fine structure changes in rat myocardium induced by daunorubicin, Pathology, 5, 99-105.

Calkins, H. et al(1989), Effect of acute volume load on refractoriness and arrhythmia development on isolated, chronically infarcted canine hearts, Circulation, 79, 687-697.

Cannella, G. et al(1983), Sequential changes in plasma renin activity and plasma catecholamines in mildly hypertensive patients during acute furosemide-induced body-fluid loss, European Journal of Clinical Pharmacology, 25, 299-302.

Carabello, B.A. et al(1981), Contractile function in chronic gradually developing subcoronary aortic stenosis, American Journal of Physiology, 240, 80-86.

Carmeliet, E.(1978), Cardiac transmembrane potentials and metabolism, Circulation Research, 42, 577-587.

Chadda, K., Ballas, M. & Bodenheimer, M.M.(1984), Efficacy of magnesium replacement in patients with hypomagnesaemia and cardiac arrhythmias, Circulation, 70(Suppl III), 444.

Chakko, C.S. & Gheorghiade, M.(1985), Ventricular arrhythmias in severe heart failure: incidence, significance and effectiveness of anti-arrhythmic therapy, American Heart Journal, 109, 497-504.

Chow, C.K. & Tappel, A.L.(1974), Response of glutathione peroxidase to dietary selenium in rats, Journal of Nutrition, 104, 444-451.

Cleland, J.G.F., Dargie, H.J. & Ford, I.(1987), Mortality in heart failure: clinical variables of prognostic value, British Heart Journal, 58, 572-582.

Cleland, J.G.F. et al(1984), Captopril in heart failure: A double blind controlled trial, British Heart Journal, 52, 530-535.

Cobbe, S.M. et al(1985), Day to day variations in morphology and and duration of fragmented ventricular potentials during the late post-myocardial infarction phase in conscious dogs, European Heart Journal, 6, 672-680.

Cohn, J.N., Archibald, D.G. & Ziesche, S.(1986), Effect of vasodilator therapy on mortality in chronic congestive heart failure. Results of a veterans

administration cooperative study, New England Journal of Medicine, 314, 1547-1552.

Cohn, J.N. et al(1984), Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heat failure, New England Journal of Medicine, 311, 819-823.

Cohn, J.N. et al.(1991), A comparison of enalapril with hydralazine-isosorbide dinitrate in the treatment of chronic congestive heart failure, New England Journal of Medicine, 325, 303-310.

Cohn, K.E., Kleiger, R.E. & Harrison, D.C.(1967), Influence of potassium depletion on myocardial concentration of tritiated digoxin, Circulation Research, 20, 473-476.

Cohn, M., Packer, M. & Gorlin, R.(1983), Indications for ventricular aneurysmectomy, Circulation, 67, 717-722.

Coltart, D.J. & Meldrum, S.J.(1970), Hypertrophic cardiomyopathy: an electrophysiological study, British Medical Journal, 4, 217-218.

Creager, M.A., Faxon, D.P. & Halperin, J.L.(1982), Determinants of clinical response and survival in patients with congestive heart failure treated with captopril, American Heart Journal, 104, 1147-1154.

Curtiss, C. et al(1978), Role of the renin-angiotensin system in the systemic vasoconstriction of chronic congestive heart failure, Circulation, 58, 763-770.

DaPrada, M. & Zurcher, G.(1976), Simultaneous radioenzymatic determination of plasma and tissue adrenaline, noradrenaline and dopamine within the femtomole range, Life Science, 191, 1161-1173.

Dargie, H.J. et al(1987), Relation of arrhythmias and electrolyte abnormalities to survival in patients with severe chronic heart failure, Circulation, 75(Suppl IV), IV98-107.

Davidoff, M.E., McKnight, J.E. & Osborne, J.L.(1979), A cross-over comparison between chlorthalidone and furosemide in essential hypertension, Current Therapeutic Research, 25, 1-9.

DeBruyn, J.H.B. et al(1980), Captopril affects blood pressure equally in renovascular and essential hypertension and in the fluid depleted anephric state, Clinical Science, 59, 835-865.

DeCarli, C., Sprouse, G. & La Rosa, J.C.(1986), Serum magnesium levels in symptomatic atrial fibrillation and their relation to rhythm control by intravenous digoxin, American Journal of Cardiology, 57, 956-959.

DeLean, A. et al(1984), Specific receptor-mediated inhibition by synthetic atrial natriuretic factor of hormone mediated steroidogenesis in cultured adrenal cells, Endocrinology, 115, 1636-1638.

Dhalla, N.S. et al(1982), Calcium movements in relation to heart function, Basic Research in Cardiology, 77, 117-139.

Dhalla, N.S. et al(1982), Calcium movements in relation to heart function, Basic Research in Cardiology, 77, 117-139.

Diplock, A.T.(1976), Metabolic aspects of selenium action and toxicity, CRC Critical Reviews in Toxicology, 5, 271-329.

Donaldson, R.M. et al(1983), Study of the electrophysiological effects of early or subendocardial ischaemia with intracavity electrodes in the dog, Clinical Science, 65, 579-588.

Doroshow, J.H.(1983), Effect of anthracycline antibiotics on oxygen radical formation in rat heart, Cancer Research, 43, 460-472.

Dzau, V.J. et al(1980), Sustained effectiveness of converting enzyme inhibition in patients with severe congestive heart failure, New England Journal of Medicine, 302, 1373-1379.

Einzig, S., Jankus, E.F. & Moller, J.H.(1972), Round Heart disease in turkeys: a haemodynamic study, American Journal of Veterinary Research, 30, 557-561.

Einzig, S. et al(1980), Regional myocardial blood flow and cardiac function in a naturally occuring congestive cardiomyopathy of turkeys, Cardiovascular Research, 14, 396-407.

Einzig, S. et al(1981), Cellular electrophysiological changes in "Round heart disease" of turkeys: a potential basis for dysrrhythmias in

myopathic ventricles, Cardiovascular Research, 15, 643-651.

El-Sherif, N. et al(1983), Reentrant ventricular arrhythmias in the late myocardial infarction period. Interpretation of reentrant circuits by cryothermal techniques, Circulation, 68, 644-656.

El-Sherif, N., Scherlag, B.J. & Lazzara, R.(1975), Electrode catheter recordings during malignant ventricular arrhythmias following experimental acute myocardial ischaemia: Evidence for re-entry due to conduction delay and block in ischaemic myocardium, Circulation, 5, 1003-1014.

Ferrans, V.J.(1978), An overview of cardiac pathology in relation to anthracycline cardiotoxicity, Cancer Treatment Reports, 62, 955-961.

Flaim, S.F. et al(1979), Chronic arteriovenous shunt: evaluation of a model for heart failure in the rat, American Journal of Physiology, 236, 698-704.

Ford, E.L. & Campbell, N.P.(1980), Effects of myocardial shortening velocity on duration of electrical and mechanical systole, British Heart Journal, 44, 179-183.

Forester, J.S. et al(1972), Early increase in left ventricular compliance after myocardial infarction, Journal of Clinical Investigation, 51, 598-603.

Francis, G.S. et al(1985), Neurohormonal mechanisms involved in congestive heart failure, American Journal of Cardiology, 55, 15A-21A.

Francis, G.S. et al(1984), The neurohormonal axis in congestive heart failure, Annals of Internal Medicine, 101, 370-377.

Friers, G.G. et al(1985), Effects of first dose doxorubicin on cardiac rhythm as evaluated by continuous 24 hour monitoring, Cancer, 56(12), 2762-2764.

Furberg, C.D., Morton-Hawkins, C.M. & Lichstein, E. for the Beta-Blocker Heart Attack Trial Study Group(1984), Effect of propranolol in post-infarction patients with mechanical or electrical complications, Circulation, 69, 761-765.

Furberg, C.D. & Yusuf, S.(1985), Effect of vasodilators on survival in chronic congestive heart failure, American Journal of Cardiology, 55, 1110-1113.

Gelband, H. & Basset, A.L.(1973), Depressed transmembrane potentials during experimentally indiced ventricular failure in cats, Circulation Research, 32, 625-634.

Gettes, L. & Surawicz, B.(1968), Effects of low and high concentrations of potassium on the simultaneously recorded Purkinje and ventricular action potentials of the perfused pig moderator band, Circulation Research, 23, 717-729.

Gomes, J.A.L. et al(1984), Programmed electrical stimulation in patients with high grade ventricular

ectopy: electrophysiological findings and prognosis for survival, Circulation, 70, 43-51.

Gottlieb, J.A. et al(1973), Fatal adriamycin cardiomyopathy: prevention by dose limitation, Proceedings of the American Association of Cancer Research, 14, 88.

Grantham, J.J. & Orloff, J.(1968), Effect of prostaglandin E2 on the permeability response of the isolated collecting tubule to vasopressin, adenosine 3'5' monophosphate and theophylline, Journal of Clinical Investigation, 47, 1154-1161.

Gulch, R.W., Baumann, R. & Jacob, R.(1979), Analysis of myocardial action potential in left ventricular hypertrophy of Golblatt rats, Basic Research in Cardiology, 774, 69-82.

Hall, J.E. et al(1977), Control of glomerular filtration rate by renin-angiotensin system, American Journal of Physiology, 233, 366-372.

Holland, O.B., Nixon, J.V. & Koohnert, L.(1981), Diuretic-induced ventricular ectopic avtivity, American Journal of Medicine, 70, 762-768.

Hollifield, J.W.(1984), Potassium and magnesium abnormalities: diuretics and arrhythmias in hypertension, American Journal of Medicine, 77, 28-32.

Hollifield, J.W.(1986), Thiazide treatment of hypertension: Effects of thiazide diuretics on serum potassium, magnesium and ventricular arrhythmias, American Journal of Medicine, 80(Suppl 4A), 8-12.

Holmes, J.(1985), Arrhythmias in ischaemic and non-ischaemic dilated cardiomyopathy: prediction of mortality by ambulatory electrocardiography, American Journal of Cardiology, 55,146-151.

Huang, S.K., Messer, J.V. & Denes, P.(1983), Significance of ventricular tachycardia in idiopathic dilated cardiomyopathy: observations in 35 patients, American Journal of Cardiology, 51, 507-512.

Inoue, H. et al.(1985), Effects of bretylium tosylate on inhomogeneity of refractoriness and ventricular fibrillation threshold in canine hearts with quinidine-induced long QT interval, Cardiovascular Research, 19(10), 623-630.

Isenberg, G.(1975), Is potassium conductance of cardiac purkinje fibres controlled by (Ca2+)i ?, Nature, 253, 273-274.

Janke, R.A.(1974), An anthracycline antibiotic-induced cardiomyopathy in rabbits, Laboratory Investigation, 30, 292-303.

Janse, M.J. & Kleber, A.G.(1981), Electrophysiological changes and ventricular arrhythmias in the early phase of regional myocardial ischaemia, Circulation Research, 49, 1069-1081.

Johnstone, G.D. et al.(1983), Factors modifying the early non-diuretic vascular effects of furosemide in man, Circulation Research, 53, 630-635.

Kahn, D.S., Rona, G. & Chappell, C.I.(1969), Ispprotenerol-induced cardiac necrosis, Annals of the New York Academy of Science, 156, 286-293.

Kalyanaraman, B., Perez-Reyes, E. & Mason, R.P.(1980), Spin-trapping and direct electron spin resonance investigations of the redox metabolism of quinone anticancer drugs, Biochim Biophys Acta, 630, 119-130.

Kastner, P.G., Hall, J.E. & Guyton, A.C.(1982), Renal haemodynamic response to increased renal venous pressure: role of angiotensin II, American Journal of Physiology, 243, F260-264.

Kaul, U. et al.(1987), Prognostic implications of complex ventricular ectopy in patients with and without structural heart disease: A study based on programmed electrical stimulation, International Journal of Cardiology, 14, 79-89.

Kirk, E.S. et al(1978), Mechanism of beneficial effects of vasodilators and inotropic stimulation in the experimental failing ischaemic heart, American Journal of Medicine, 65, 189-196.

Kleinert, H.D. et al(1984), Atrial natriuretic factor inhibits angiotensin, norepinephrine and potassium-induced vascular contractility, Hypertension, 6(Suppl I), 143-147.

Lab, M.J.(1982), Contraction-excitation feedback in myocardium: Physiological basis and clinical relevance, Circulation Research, 50, 757-766.

Laks, M.M., Morady, B.A. & Swann, H.J.C.(1973), Myocardial hypertrophy produced by subhypertensive doses of norepinephrine in the dog, Chest, 64, 75-78.

Lambertenghi-Delliers, G., Zanon, P.L. & Pozzoli, E.F.(1976), Myocardial injury induced by a single dose of adriamycin: an electrom microscopic study, Tumori, 62, 517-528.

Land, E.J. et al.(1983), One electron reduction of adriamycin: Properties of the semi-quinone, Archives of Biochemistry and Biophysics, 225, 116-121.

Lee, W. & Packer, M.(1984), Prognostic value of serum sodium concentration in severe heart failure and its modification by converting enzyme inhibition, Circulation, 70(Suppl II), 113.

Lefrak, E.A. et al.(1973), A clinicopathologic analysis of adriamycin cardiotoxicity, Cancer, 32, 302-314.

Lermann, B.E. et al.(1985), Mechano-electrical feedback: independent role of preload and contractility in modulation of canine ventricular excitability, Journal of Clinical Investigation, 76, 1843-1850.

Levine, J.N. et al.(1988), Changes in myocardial repolarisation changes in patients undergoing balloon valvuloplasty for congenital pulmonary stenosis: evidence for contraction-excitation feedback in humans, Circulation, 77, 70-77.

Levine, T.B. & Cohn, J.N.(1982), Determimants of acute and long term response to converting enzyme

inhibitors in congestive heart failure, American heart Journal, 104, 1159-1164.

Lluch, S. et al.(1969), A reproducible model of cardiogenic shock in the dog, Circulation, 39, 205-217.

Lohmeier, T.E. et al.(1977), Effects of endogeneous angiotensin II on renal sodium excretion and renal haemodynamics, American Journal of Physiology, 233, F388-395.

Lossnitzer, K. et al.(1975), Disturbed myocardial calcium metabolism: a possible pathologic factor in the hereditary cardiomyopathy of the Syrian hamster, Recent advances in studies on cardiac structure and metabolism, 6, 207-217.

Mackay, I.G., Muir, A.L. & Watson, M.L.(1984), Contribution of prostaglandins to the systemic and renal vascular response to frusemide in normal men, British Journal of Clinical Pharmacology, 17, 513-519.

Magnani, B. & Magelli, C,(1986), For the multicentre research group on mild heart failure, Captopril in mild heart failure: preliminary observations of a long-term, double-blind, placebo-controlled multicentre trial, Postgraduate Medical Journal, 62(Suppl I), 153-158.

Manley, B.S. et al.(1989), An animal model for the chronic study of ventricular repolarisation and refractory period, Cardiovascular Research, 23, 16-20.

Marks, E.S., Bing, R.F. & Thurston, H.(1980), Vasodepressor property of the converting enzyme inhibitor captopril(SQ 14,225): The role of factors

other than renin-angiotensin blockade in the rat, Clinical Science, 58, 1-6.

Massie, B et al.(1981), Long term vasodilator therapy for heart failure: clinical response and its relationship to haemodynamic measurements, Circulation, 63, 269-278.

Massie, B. et al(1982), Haemodynamic and radonuclide effects of acute captopril therapy for heart failure: changes in left and right ventricular volumes and function at rest and during exercise, Circulation, 65, 1374-1381.

McDonald, T.F., Hunter, E.G. & Macleod, D.P.(1971), Adenosine triphosphatase partition in cardiac muscle with respect to transmembrane electrical activity, Pflugers Archives, 322, 95-108.

McDonald, T.F. & Macleod, D.P.(1972), Metabolism and the electrical activity of anoxic ventricular muscle, Journal of Physiology, 229, 559-582.

Medical Research Council working party on mild to moderate hypertension, Ventricular extra-systoles during thiazide treatment: substudy of MRC mild hypertension trial, British Medical Journal. 287, 1249-1253.

Meinertz, T. et al.(1984), Significance of ventricular arrhythmias in idiopathic dilated cardiomyopathy, American Journal of Cardiology, 53, 902-907.

Mercadier, J.J. et al.(1981), Myosin isoenzyme changes in several models of rat cardiac hypertrophy, Circulation Research, 49, 525-532.

Milei, J. et al.(1986), Amelioration of adriamycin-induced cardiotoxicity in rabbits by prenylamine and vitamins A and E, American Heart Journal, 111, 95-102.

Millar, J.A. et al.(1980), A microassay for active and total renin concentration in human plasma based on antibody trapping, Clin Chim Acta, 101, 5-15.

Miller, R.R., Awan, N.A. & Mason, D.T.(1978), Nitroprusside therapy in acute and chronic coronary heart disease, American Journal of Medicine, 65, 167-172.

Moore, T.J. et al.(1981), Contribution of prostaglandins to the antihypertensive action of captopril in essential hypertension, Hypertension, 3, 168-173.

Morgan, D.B. & Davidson, C.(1980), Hypokalaemia and diuretics: an analysis of publications, British Medical Journal, 280, 905-908.

Morgan, J.M., Cunningham, A.D. & Rowland, E.(1990), Relationship of the effective refractory period and monophasic action potential duration after a step increase in pacing frequency, PACE, 13, 1002-1008.

Morton, J.J. & Webb, D.J.(1985), Measurement of plasma angiotensin II, Clinical Science, 68, 483-484.

Myers, C.E. et al.(1977), Adriamycin: the role of lipid peroxidation in cardiac toxicity and tumour response, Science, 19, 165-167.

Myers, C.E., McGuire, W. & Young, R.(1976), Adriamycin: Amelioration of toxicity by alpha-tocopherol, Cancer Treatment Reports, 60(7), 961-962.

Naccarelli, G.U. et al.(1983), Role of electrophysiologic testing in managing patients who have ventricular tachycardia unrelated to coronary artery disease, American Journal of Cardiology, 50, 165-171.

Natarajan, G. et al.(1979), A new technique for production of chronic atrial septal defect in cat without thoracotomy, Proceeds of the Society of Experimental Biology Medicine, 161, 515-518.

Neri, T. et al.(1987), Ventricular arrhythmias in dilated cardiomyopathy: Efficacy of amiodarone, American heart Journal, 113, 707-715.

Olson, H.M. et al.(1974), Electrolyte and morphologic alterations of myocardium in adriamycin-treated rabbits, American Journal of Pathology, 77, 439-454.

Overturf, M.L., Sybers, H.D. & Smith, S.A.(1985), Captopril-induced hyperreninaemia in cholesterol-fed rabbits, Research Communications in Chemical Pathology and Pharmacology, 47(2), 229-253.

Packer, M.(1985), Sudden unexpected death in patients with congestive heart failure. A second frontier, Circulation, 72, 681-685.

Packer, M.(1987), Why do the kidneys release renin in patients with congestive heart failure? A nephrocentric view of converting enzyme inhibition, American Journal of Cardiology, 60, 179-184.

Packer, M., Lee, N.H. & Kessler, P.D.(1986), Preservation of glomerular filtration rate in human heart failure by activation of the renin-angiotensin system, Circulation, 74, 766.

Packer, M., Medina, N. & Yushak, M.(1984), Haemodynamic and clinical limitations of long-term inotropic therapy with amrinone in patients with severe chronic heart failure, Circulation, 70, 1038-1047.

Papademetriou, V. et al.(1983), Diuretic-induced hypokalaemia in uncomplicated systemic hypertension: effect of plasma potassium correction on cardiac arrhythmias, American Journal of Cardiology, 52, 1017-1022.

Parmley, W.W. & Chatterjee, K.(1986), Congestive heart failure and arrhythmias: an overview, American Journal of Cardiology, 57, 34-37.

Pfeffer, J.M., Pfeffer, M.A. & Braunwald, E.(1987), Haemodynamic benefits and prolonged survival with long term captopril therapy in rats with myocardial infarction and heart failure, Circulation, 75(Suppl I), 149-155.

Poll, D.S. et al.(1984), Sustained ventricular tachycardia in patients with idiopathic dilated cardiomyopathy: Electrophysiologic testing and lack of

response to antiarrhythmic drug therapy, Circulation, 70, 451-456.

Promise Study Research Research Group.(1991), Effect of oral milrinone on mortality in severe chronic heart failure, New England Journal of Medicine, 325, 1468-1475.

Ramee, S.T. et al.(1985), Torsades de pointes and magnesium deficiency, American Heart Journal, 109, 164-167.

Reichenbach, D.D. & Benditt, E.P.(1970), Catecholamines and cardiomyopathy. The pathogenesis and potential importance of myofibrillar degeneration, Human pathology, 1, 125.

Reiter, M.J., Synhurst, D.P. & Mann, D.E.(1988), Electrophysiological effect of acute ventricular dilatation in the isolated rabbit heart, Circulation Research, 62, 554-562.

Reuter, H.(1974), Exchange of calcium ions in the mammalian myocardium, Circulation Research, 34, 599-605.

Revis, N. & Marusic, N.(1979), Effects of doxorubicin and its agyclone metabolite on calcium sequestration by rabbit heart, liver and kidney mitochondria, Life Science, 25, 1055-1064.

Richards, A.M. et al.(1987), Radioimmunoassay for plasma alpha human atrial natriuretic peptide: A comparison of direct and pre-extracted methods, Journal of Hypertension, 5, 227-236.

Roden, D.M. et al.(1986), Clinical features and basic mechanisms of quinidine-induced arrhythmias, Journal of the American College of Cardiology, 8, 73A-78A.

Roden, D.M. & Hoffman, B.F.(1985), Action potential prolongation and induction of abnormal automaticity of low quinidine concentrations in canine purkinje fibres: Relationship to potassium and cycle length, Circulation Research, 56, 857-867.

Roden, D.M. & Iansmith, D.H.S.(1987), Effects of low potassium or magnesium concentrations on isolated cardiac tissue, American Journal of Medicine, 82(Suppl IIIA), 18-23.

Roden, D.M., Woosley, R.L. & Primm, R.K.(1986), Incidence and clinical features of the quinidine-induced long QT interval: implications for patient care, American Heart Journal, 111, 1088-1093.

Rossner, K.L. & Sachs, H.G.(1978), Electrophysiological study of Syrian hamster cardiomyopathy, Cardiovascular Research, 12, 436-443.

Sharpe, D.N.(1980), Low dose captopril in chronic heart failure: Acute haemodynamic effects and long term treatment, Lancet, 2, 1154-1157.

Shenasa, H. et al.(1990), Chronic doxorubicin induced cardiomyopathy in rabbits: mechanical, intracellular action potential, and beta adrenergic characteristics of the failing myocardium, Cardiovascular Research, 24(7), 591-604.

Silberbauer, K., Stanek, B. & Templ, H.(1982), Acute hypotensive effect of captopril in man modified by prostaglandin synthesis inhibition, British Journal of Clinical Pharmacology, 14, 87-93.

Singal, P.K. et al.(1985), Changes in lysosomal morphology and enzyme activities during the development of adriamycin-induced cardiomyopathy, Canadian Journal of Cardiology, 1, 139-147.

Singal, P.K., Deally, C.M.R. & Weinberg, C.E.(1987), Subcellular effects of adriamycin in the heart: a concise review, Journal of Molecular and Cellular Biology, 19(8), 817-828.

Singal, P.K. & Pierce, G.N.(1986), Adriamycin stimulates low-affinity Ca2+ binding and lipid peroxidation but depresses myocardial function, American Journal of Physiology, 250, H419-H425.

Smith, H.J. et al(1976), Cardiomyopathy associated with amphetamine administration, American Heart Journal, 91(6), 792-797.

Smith, H.J. & Nuttall, A.(1985), Experimental models of heart failure, Cardiovascular Research, 19, 181-186.

Sordahl, H.J., Wood, W,G. & Schwarz, A.(1970), Production of cardiac hypertrophy and failure in rabbits with ameroid clips, Journal of Molecular and Cellular Cardiology, 1, 341-344.

Spear, J.F., Michelson, E.L. & Moore, E.N.(1983), Cellular electrophysiologic characteristics of chronically infarcted myocardium in dogs susceptible to

sustained ventricular tachyarrhythmias, Journal of the American College of Cardiology, 14, 1099-1110.

Stokes, J.B.(1979), Effect of prostaglandin E2 on chloride transport across the rabbit thick ascending limb of Henle: selective inhibition of the medullary portion, Journal of Clinical Investigation, 64, 485-502.

Stone, K.J. & Hart, M.(1976), Inhibition of PGE2-9-ketoreductase by diuretics, Prostaglandins, 12, 197-207.

Surawicz, B.(1967), Relation between electrocardiogram and electrolytes, American heart Journal, 73, 814-834.

Surawicz, B. et al.(1959), Effect of potassium and calcium deficiency on the monophasic action potential, electrocardiogram and contractility of isolated rabbit hearts, American Journal of Physiology, 196, 1302-1307.

Swedberg, K. et al.(1979), Prolongation of survival in congestive cardiomyopathy by beta-receptor blockade, Lancet, 1, 1374-1376.

Taggart, P. et al.(1988), Monophasic action potentials at discontinuation of cardiopulmonary by-pass: Evidence for contraction-excitation feedback in man, Circulation, 77, 70-77.

The Consensus Trial Study Group.(1987), Effects of enalapril on mortality in severe congestive heart failure, New England Journal of Medicine, 23, 1429-1435.

The SOLVD Investigators.(1991), Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure, New England Journal of Medicine, 325, 293-302.

Topic, N.(1982), Acute and long term effects of captopril on exercise cardiac performance and exercise capacity in congestive heart failure, American Heart Journal, 104, 1172-1179.

Tritthart, H. et al.(1975), Right ventricular hypertrophy in the cat: an electrophysiological and anatomical study, Journal of Molecular and Cellular Cardiology, 7, 163-174.

Uhley, H.N.(1961), Study of the transmembrane action potential, electrocardiogram and vectorcardiogram of rats with left ventricular hypertrophy, American Journal of Cardiology, 7, 211-217.

Van Fleet, J.F. et al.(1978), Effect of Selenium-vitamin E on adriamycin-induced cardiomyopathy in rabbits, American Journal of Veterinary Research, 39, 997-1010.

Vassalle, M.(1965), Cardiac pacemaker potentials at a different extra and intracellular K+ concentration, American Journal of Physiology, 208, 770.

Von Hoff, D.D. et al.(1979), Risk factors for doxorubicin-induced congestive heart failure, Annals of Internal Medicine, 91, 710-717.

Von Olshausen K. et al.(1984), Ventricular arrhythmias in idiopathic dilated cardiomyopathy, British Heart Journal, 51, 195-201.

Walton, C., Economides, A.P. & Gergeley, S.(1985), A new approach to the unipolar paced evoked response, In Perez Gomes F, ed. Cardiac pacing, electrophysiology, tachyarrhythmias. Madrid; Editorial Grouz, 857-864.

Wanless, R.B. et al.(1987), An experimental model of chronic cardiac failure using adriamycin in the rabbit: central haemodynamics and regional blood flow, Cardiovascular Research, 21, 7-13.

Watanebe, Y. & Dreifus, L.S.(1972), Electrophysiological effects of magnesium and its interaction with potassium, Cardiovascular Research, 6, 79-88.

Welham, K.C., Silove, E.D. & Wyse, R.K.H.(1978), Experimental right ventricular hypertrophy and failure in swine, Cardiovascular Research, 12, 61-65.

Wellens, H.J.J., Duren, D.R. & Lie, K.I.(1976), Observations on mechanisms of ventricular tachycardia in man, Circulation, 54, 237-244.

Westlin, W. & Mullane, K.(1988), Does captopril attenuate reperfusion-induced myocardial dysfunction by scavenging free radicals? Circulation, 77(Suppl I), I-30 - I-39.

Wilson, J.R. et al.(1983), Prognosos in severe heart failure: Relation to haemodynamic measurements and

ventricular ectopic activity, Journal of the American College of Cardiology, 2, 403-410.

Woods, W.T. et al.(1979), Electrophysiological effects of magnesium on cells in the canine sinus node and false tendon, Circulation Research, 44, 182-188.

Wortmann, J.E. et al.(1979), Sudden death during doxorubicin administration, Cancer, 44, 1588-1591.

Zipes, D.P., Besch, H.R. Jr. & Watanabe, A.M.(1975), Role of the slow current in cardiac electrophysiology, Circulation, 51, 761-766.

# PUBLICATIONS

## Peer Review Journals.

Doherty, J.D. & Cobbe, S.M.(1990), Electrophysiological changes in animal model of chronic cardiac failure, Cardiovascular Research, 4, 309-316.

Doherty, J.D. & Cobbe, S.M., The effect of Captopril on electrophysiological changes in adriamycin cardiomyopathy in the rabbit, Cardiovascular Research,(In Press)

## Published Abstracts.

Doherty,J.D., Manley,B.S. & Cobbe, S.M.,(1988) Electrophysiological changes in an animal model of congestive cardiac failure, Clinical Science (Suppl 18), 30p.

Doherty, J.D.(1989), Electrophysiological changes in an animal model of experimental chronic cardiac failure, British Heart Journal 61, 104-105.

The experiments described in chapters 4 and 5 of this thesis were presented as a finalist in the British Cardiac Society Young Investigators Prize 1989.