ANALYSIS OF THE EFFECTS OF BRAIN DEATH ON BIVENTRICULAR FUNCTION AND PROLONGED MYOCARDIAL PRESERVATION, AND THE EFFECT OF COMPLETE ATRIOVENTRICULAR TRANSPLANTATION ON CARDIAC FUNCTION IN THE RECIPIENT.

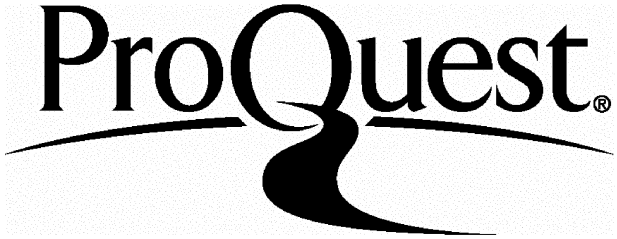
by

Simon William Henry Kendall

M.B. B.S., B.Sc. Hons (Lond), F.R.C.S. (Ed.), F.R.C.S. (C / Th.).

Thesis submitted for the
degree of
Master of Surgery
of the
University of London.

Faculty of Surgery
Michaelmas Term 1995.



ABSTRACT

Brain stem death is confirmed prior to donation in clinical cardiac transplantation. This condition is associated with haemodynamic instability and eventual circulatory collapse. The effect of brain death on right ventricular function is unknown, as is its subsequent interaction with prolonged myocardial preservation. The technique for orthotopic cardiac transplantation, usually ventricular transplantation with atrioplasty, might be improved by complete atrioventricular implantation.

The canine model was used to validate brain death (n = 13) whilst the optimal surgical method for implantation was assessed (n=40). Two-way variate analysis compared four experimental transplant groups for the main effects of brain death and four hour preservation, and the interaction between them (n=68). Rght ventricular function was also analysed post brain death when challenged with an increased pulmonary vascular impedance, as may occur in clinical transplantation (n=9).

Systolic function was assessed for left and right ventricles using load independent analyses of pressure volume relationships; pre load independent recruitable stroke work (PRSW). Fourier analysis of pulmonary artery flow and pressure was utilised to calculate pulmonary vascular impedance and right ventricular hydraulic power. In addition atrial systole was analysed to compare surgical techniques of implantation.

Complete transplantation conferred significant advantages in conserving sinus rhythm, right ventricular PRSW, and atrial systolic function. Intracranial balloon inflation was validated in causing brain stem death and these techniques were applied in remaining studies. Brain death significantly impaired PRSW by 21.2% and 29.4% for left and right ventricles respectively. Moreover an acute increase of pulmonary vascular impedance revealed that brain death significantly abolishes the right ventricle's reserve of hydraulic power. Four hours of preservation superimposes further injury to the right ventricle significantly reducing PRSW to 52% of baseline. This is reflected by the significant inotrope requirements to wean from cardiopulmonary bypass.

Right ventricular function is significantly impaired by brain death. Prolonged preservation, for four hours, adds to this injury, suggesting future studies in prolonged myocardial preservation should use brain dead donors. Complete atrioventricular transplantation is superior to the standard technique although the clinical role for this method requires evaluation.

To Sue, Thomas and Emma,
for their love, support and endurance.

TABLE OF CONTENTS

CHAPTER 3

CHAPTER 4

PRELIMINARY STUDIES

CHAPTER 4 CONT.

CHAPTER 5

VALIDATION OF BRAIN DEATH

CHAPTER 6

COMPLETE ATRIOVENTRICULAR TRANSPLANTATION

CHAPTER 6 CONT.

CHAPTER 7

MYOCARDIAL FUNCTION FOLLOWING TRANSPLANTATION FROM A BRAIN DEAD DONOR.

CHAPTER 8

CARDIAC FUNCTION FOLLOWING TRANSPLANTATION USING A BRAIN DEAD DONOR AND PROLONGED PRESERVATION.

CHAPTER 9

HAEMODYNAMIC FUNCTION IN THE BRAIN DEAD MODEL FOLLOWING AN ACUTE INCREASE IN PULMONARY VASCULAR RESISTANCE.

CHAPTER 10

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

The studies described in this thesis were performed during a year spent as a Cardiovascular Surgical Fellow to Dr. Peter VanTrigt at Duke University Medical Center, Durham, North Carolina. Dr. VanTrigt encouraged my interests in this area of cardiovascular research. I am grateful to him for his help and instruction in experimental design and preparation for each phase of the project, and for his continued support and guidance throughout the year.

I am grateful to Professor David C. Sabiston, Jr., M.D., Chairman and James B. Duke Professor of Surgery, Department of General and Cardiothoracic Surgery, Duke University Medical Center, for providing a funded research fellowship within a premier research establishment, where he has created a very stimulating academic environment.

In particular Mr. Kurt Campbell was fundamental to the experiments; the preparation of the animals, the administration of anaesthesia, the control of cardiopulmonary bypass and general maintenance of the laboratory was attributable to his hard work. I am also grateful to Mr. George Quick for his expert guidance in the animal laboratory. Both these gentlemen gave their tireless help and cheerful endurance throughout all of my studies.

Dr. Hartmuth Bittner M.D., and Dr. David Peterseim M.D., were extremely helpful in the organisation and running of the experiments; Dave Peterseim worked in the laboratory prior to my arrival and had laid the foundations for a laboratory capable of cardiac transplantation and adequate data acquisition; 'Hacky' Bittner was a fine colleague throughout the year and an outstanding asset in the successful outcome of the experiments.

I would like to acknowledge the help of Damian Craig, MS, who was instrumental in setting up the sophisticated computer programs for the analysis of the haemodynamic data, and his patient tuition in the use of the equipment for the collection, and subsequent organisation, of the data.

William White BS, MHS, collaborated in the statistical analysis and I greatly appreciated his guidance in ensuring appropriate appraisal of the data.

Thomas Montine, MD PhD, is a neuropathologist who very generously gave his time to evaluate the specimens from studies in chapter 5.

I am grateful to Professor K.M. Taylor, M.D., FRCS, FRCSE, FSA, for agreeing to act as my supervisor, and for his guidance and support in the preparation of the manuscript.

Ronald 'Butch' Usery in medical photography and Jerry Schoendorf in medical art, both part of the department of Audio-visual education at Duke Medical Center, were extremely helpful in preparing the illustrations used in this thesis.

During the year I have gratefully received financial support from the following sources;

Wellcome research travel grant from the Wellcome trust.

Travel grant from the Society of Cardiothoracic Surgeons of Great Britain and Ireland.

Research grants from;

Baxter UK.

Datascope Ltd.

Oxford Cardiac Surgical Research Fund.

Sarns Medical.

Sherwood Medical Ltd.

Sorin Biomedica.

St. Jude Medical.

STATEMENT OF ORIGINALITY

The work embodied in this thesis represents original ideas of the author who carried out all of the experimental work, including both design and data collection analysis. The work represents an attempt to validate brain death in an animal model and to understand the interactions between brain death and myocardial function, with particular regard to preservation prior to transplantation. The project also attempts to study the effect of complete atrioventricular transplantation on the physiology of cardiac function in the recipient with a study of diastolic function.

The responsibility to coordinate and supervise the experiments was the author's, as was the instrumentation of all the animals and the collection of data. Dr. Hartmuth Bittner was responsible in chapter 5 for the collection of blood and myocardial samples, and the subsequent organisation to analyse these specimens. The vast majority of the surgery was performed by the author, the remainder of which he supervised Dr. Bittner.

The expansion of heart transplantation as a successful therapeutic modality in the treatment of end-stage heart failure has revealed the importance of optimising donor organ preservation, as primary donor organ dysfunction is still a significant cause of morbidity and mortality in transplant programmes. Furthermore a thorough study into the physiological effect of complete atrioventricular transplantation may reveal a significant benefit from this modification of the surgical procedure and improve exercise tolerance in heart transplant patients.

Frontispiece.

Duke University Medical Center, North Carolina.

The author carried out the work embodied in this thesis while on a cardiothoracic surgical research fellowship at this institute.


Duke University Today

CHAPTER 1

BACKGROUND

Introduction

Over the last decade solid organ transplantation has rapidly increased in frequency, and has become an accepted treatment in end-stage cardiac, hepatic, pancreatic, pulmonary and renal disease. This expansion is attributable to the discovery by Borel of the immunosuppression agent, cyclosporin A, a lipophilic cyclic polypeptide from the fungus *Tolypocladium inflatum* (Borel et al, 1976). This drug has dramatically curtailed the rejection process by impairing the action of interleukin-2 on T-cells in cell mediated immunity.

As transplant programmes have developed worldwide, the limitations of this treatment modality have become apparent. Primarily, solid organ transplantation currently relies on organs from a limited number of brain dead 'heart-beating' cadavers. The supply of donors was initially increased in the 1980's with substantial investment in the education of the public and the medical profession. However, this donor pool is no longer expanding, unlike the number of potential recipients, and transplant activity has plateaued in the established programmes. Research in this field now encompasses the pathophysiology and medical management of the donor to increase the number of available organs for orthotopic transplantation, as well as extensively investigating the potential of xenotransplantation which might eventually satisfy the demand for organs.

Although there is a high financial cost with this treatment modality, it may become cost effective over time. The most frequent solid organ transplant is the kidney, for patients with end-stage renal failure supported by chronic peritoneal or haemo- dialysis. In this situation transplantation significantly reduces health-care costs while vastly improving the quality and duration of the patients' lives. As experience with transplantation of the other organs increases, and results improve, the financial implications of orthotopic transplantation will become more evident.

Unlike the other major organs, orthotopic cardiac and pulmonary transplantation depends on **immediate** and adequate function to support the recipient. Whereas the recipient of a kidney can have a further two weeks of dialysis, awaiting the resolution of acute tubular necrosis in the donor

kidney, the recipient of a heart / lung will not reliably survive a duration of mechanical support awaiting improved function of the donor organ. Therefore the cardiothoracic surgeon has the paramount interest in the condition of the donor organs.

Brain death prior to organ donation has an established deleterious effect on the cardiovascular system. Despite this, the vast majority of research groups investigating preservation in intra-thoracic organs have studied organs from anaesthetised animals, often obtaining results not reproducible in the clinical setting. The aim of this thesis is to study the effects of brain death on orthotopic cardiac transplantation. Firstly a model of brain death will be thoroughly validated in the canine model, while quantifying **biventricular** function. The assessment of the cardiovascular system is performed using sonomicrometry, micromanometry and flow-probes with ensuing complex data analysis to obtain accurate information regarding the pre-load recruitable stroke work and impedance spectra. Then, having quantified the effects of brain death on left and right ventricular function, the effect of brain death on prolonged myocardial preservation may be investigated. Subsequently interventional studies will be performed at the three stages of transplantation; prior to organ retrieval, during preservation and after implantation.

This study will be coupled to an investigation of the surgical technique for implantation of the donor heart. With the sophisticated methods available to measure cardiac function, outlined above, the influence of complete atrioventricular transplantation will be assessed and compared to the standard method of implantation; ventricular transplantation with atrioplasty.

Before describing the experiments, and the resultant data, it is important to review the background of this topic. Firstly there follows a historical perspective of heart transplantation, and then reviews of the research into the pathophysiology of brain death and of the research into prolonged myocardial preservation. The subsequent chapter outlines in detail the methods of measurement employed in this thesis.

History of Heart Transplantation

To the present day there have been approximately 18,000 human orthotopic cardiac transplants performed with an expected 5 year survival of approximately 75% of all recipients. Not only is longevity increased but there is a huge improvement in the quality of life. However, in the decade prior to the clinical use of cyclosporin, pioneered by Sir Roy Calne (Calne et al, 1978), there were under 400 cardiac transplants performed, one hundred of which were performed in 1968 alone. It is therefore interesting to reflect on the early days of heart transplantation and the problems that were encountered.

The first human cardiac transplant was performed in December 1967 by Barnard. This stimulated an intense worldwide enthusiasm for clinical transplantation followed by a precipitous decline in activity due to the low survival rates (Wandall, 1970). Indeed clinical cardiac transplantation had proceeded without adequate scientific background, even though at this time there had been decades of laboratory research. This had tried to address four fundamental problems;

1) Design of a simplified and routinely successful surgical technique;
2) Development of techniques for organ preservation;
3) Documentation of satisfactory early and late post-operative function of the denervated heart and
4) Early diagnosis and treatment of rejection

Carrel and Guthrie reported the first cardiac transplant in 1905 (Carrel and Guthrie, 1905). Canine hearts were transplanted heterotopically into the cervical region of the host by anastomoses of the recipient's jugular vein and carotid artery to the great vessels, vena cava and one of the pulmonary veins of the donor heart. There were sporadic reports following this paper, with investigators attempting to simplify the surgical technique of heterotopic transplantation (Mann et al, 1933). Further advances were achieved by Demikhov in the 1950's who performed heterotopic transplants in the intrathoracic position. In most of his experiments the heart served as an auxiliary pump but in several instances he was able to exclude the recipient's own heart and transfer the entire

circulatory load to the donor organ and achieve short term survival (Demikhov, 1960). It is remarkable that he succeeded with this work in the absence of cardiopulmonary bypass and no established methods for effective myocardial preservation.

The first reported attempts of orthotopic transplantation of the canine heart were reported by Goldberg in 1958. They met with limited success and a maximum survival time of two hours (Hairston, 1965).

The first fully successful orthotopic cardiac transplantations were described by Lower and Shumway in 1960 (Lower and Shumway, 1960). The principal technical procedures culminating in this achievement included removal of both the recipients' and donor hearts at the mid-atrial level (a maneuver that permitted rapid implantation), myocardial preservation by simple hypothermia during interruption of the coronary circulation and reliable methods for cardiopulmonary bypass. Long term survivors became routine using these methods (Lower et al, 1965). Indeed the efficacy of simple hypothermia for myocardial preservation was subsequently investigated not only for use in transplantation but also for routine cardiac surgical procedures (Greenberg and Edmonds, 1961). As will be seen below in the section 'Prolonged Myocardial Preservation', there was abundant documentation regarding the efficacy of topical cooling with cold saline for preservation of cardiac grafts. In 1977 this method was combined with aortic root perfusion of hypothermic potassium rich solution (Reitz et al, 1974) to allow the procurement of hearts from distant donors, and so replace the unsatisfactory practice of moving the donor to the transplant centre (Watson et al, 1979).

Even the earliest experiences in experimental heart transplantation demonstrated immune responses from the host, as had been encountered in other solid organ grafts. In cardiac transplants though rejection of the graft usually resulted in death. Therefore considerable attention was directed towards the diagnosis and treatment of cardiac rejection during the early stages of laboratory experience. In 1962 it was shown that rejection of the canine heart was manifested by decreases in the generated QRS voltage, as measured in standard electrocardiograms (Lower et

al, 1966). It was also found that atrial and ventricular dysrhythmias were associated with rejection episodes. Reemsta was the first investigator to achieve prolonged survival in recipients of cardiac allografts by using methotrexate and then azathioprine (Reemsta et al, 1962), results that were reproduced by Blumenstock, with dogs surviving up to 42 days using a similar immunosuppression regime (Blumenstock, 1963). Before cyclosporin the primary drugs used to prevent rejection in the clinical setting were corticosteroids and azathioprine, supplemented with anti-thymocyte sera raised from the rabbit or the horse. In the Stanford series of 136 patients undergoing cardiac transplantation prior to 1978, which was the largest active centre at that time, the survival at 5 years was 31% using the methods described above (Baumgartner et al, 1978).

The expression of acute rejection in orthotopically transplanted canine hearts using histopathological specimens was studied in detail (Kosek et al, 1968). The defining of the histopathological features associated with cardiac graft rejection became the foundation of transvenous endomyocardial biopsies used in the clinical setting for the early detection of rejection (Caves et al, 1973).

Therefore three major obstacles to cardiac transplantation had been overcome; simple surgical technique; reliable organ preservation and early detection of rejection. The fourth obstacle of suppressing rejection was removed with the introduction of cyclosporin. This has allowed the development of multiple transplant centres around the world who now obtain similar results with their cardiac transplant programmes.

Cardiac transplant activity is now limited by the number of donors; the 'heart-beating' cadaver is haemodynamically unstable and many organs are wasted before organ procurement. Subsequently research into the pathophysiology of brain death has become increasingly important.

Pathophysiology of Brain Death

The surgical procedure to harvest organs for transplantation will not usually begin until the donor has been brain dead for at least 24 hours. This is a result of the complex processes required from both the donor hospital and the teams sent from the transplant units. As described below it is a challenge, both from a medical and an administrative standpoint.

Firstly brain death must be established according to legal requirements laid down by that hospital's government. The primary physician diagnosing brain death must be entirely satisfied that the patient is free from sedative and paralysing agents before brain stem tests can be performed. With a positive diagnosis of brain death the next of kin may be approached regarding the possibility of organ donation. At this time the national transplant centre may be informed to start co-ordination of the recipient teams. A second set of brain stem tests are performed by a different physician after a statutory delay from the first set. If positive, and with the consent of the next of kin, arrangements may be made for organ retrieval by the renal, liver and thoracic transplant teams who are usually from distant centres. Therefore the commencement of physically harvesting the organs will be at least 24 hours from onset of brain death.

An intensive care unit will primarily attend to the patients who may survive. The care of the brain dead patient is not standardised, and the majority of the attending medical and nursing staff may not be familiar with the optimisation of brain dead patients prior to organ procurement. Brain death is well known to be associated with haemodynamic deterioration (Darby et al, 1989)(Bodenham and Park, 1989) and it is estimated many potential transplants have been lost due to the physiological demise of the donor (Smart et al, 1991)(Jenkins, 1976)(Cooper et al, 1982). In the latter report 20% of potential donor hearts became untransplantable due to haemodynamic deterioration, unresponsive to medical intervention. Even in the early years of clinical heart transplantation there was concern as to the suitability of donor hearts from patients with head-injuries, with regard to myocardial injury (DePasquale and Burch, 1969). These observations have prompted great interest in the pathophysiology of brain death.

Myocardial injury in association with cerebral pathology has been recognised for many years. Initial reports were from observations of recognisable changes in the electrocardiograms of patients following cerebrovascular accidents (Burch et al, 1954) (Shuster, 1960) and subsequently correlated with sub-endocardial haemmorrhage of the left ventricle (Koskelo et al, 1964). Greenshoot observed that the morphological changes of the myocardium in patients with subarachnoid haemmorrhage strongly resembled changes induced by high dose catecholamines (Greenshoot and Reichenbach, 1969)(Chappel et al, 1959). Although not specific for catecholamine injury alone the histological appearance is of contraction bands within the myocardium. These lesions represent scattered focal areas of myocardial cell necrosis and are surrounded by mononuclear cells (Rose et al, 1988)(Karch and Billingham, 1986). Indeed this cardiac injury could be reproduced experimentally by stimulation of the stellate ganglion (Kaye et al, 1961). The 'Cushing Response' of severe systemic hypertension due to raised intracranial pressure is well recognised (Cushing, 1901) and enormous levels of adrenaline (1100% increase) and noradrenaline (1300% increase) have been recorded in the baboon model, 5 minutes after the induction of intracranial hypertension (Novitzky et al, 1984). This work promoted further studies demonstrating that cardiac sympathectomy prior to induction of intracranial hypertension abolished the myocardial injury (Novitzky et al, 1986).

The management of the brain dead donor has more recently focused on hormonal derangement with regard to the hypothalamic-pituitary axis. Diabetes insipidus has long been associated with severe head injury (Hiatt and Lowis, 1957) and 80% of organ donors are polyuric resulting in intravascular hypovolaemia and hypernatraemia (Gramm et al, 1992). Central venous pressure may not be routinely monitored in brain dead donors and the resultant systemic hypotension, due to hypovolaemia, is often treated erroneously by the donor hospital with inotropic agents. The administration of anti-diuretic hormone (ADH) to treat the diabetes insipidus in brain dead donors is controversial. Cooper first advocated the use ADH in organ donors with low systemic pressure and already on inotropic support (Cooper et al, 1982). The doses used were high (10-20 mU/min iv)

where the effect primarily is mediated by the powerful vasoconstrictive action of ADH, and secondarily by decreasing polyuria and electrolyte imbalances. However, these high doses have been shown to significantly reduce coronary, renal and hepatic flow (Cowley, 1982)(Gaskill et al, 1983) and low dose ADH (2-10 μU/kg/min) is adequate to reverse diabetes insipidus in the porcine brain-dead model (Blaine et al, 1984).

Novitzky et al in Cape Town, South Africa, has been a prolific researcher into brain death and its relevance to cardiac transplantation. His initial primate work used the chacma baboon for a brain dead model; ten animals were studied using inflation of a 20 ml intracranial balloon to cause brain death, although herniation of the midbrain through the foramen magnum was not validated. Initial hypertension, dysrhythmias and a sudden large rise of serum catecholamines was recorded in the first five minutes following brain death. This was followed by a fall of catecholamine levels to sub-control levels along with a fall in thyroid hormones (T_3 and T_4), cortisol and insulin levels (Novitzky et al, 1984). Myocardial energy stores were also evaluated showing a significant depletion of adenosine triphosphate and creatine phosphate. This primate work was then followed by experimental and clinical studies investigating the effects of hormonal manipulation after brain death. In the porcine model, brain death was induced by ligation of the major vessels at their origin from the aorta, Novitzky investigated hormonal intervention in the donor, also studying the harvested isolated heart preparation following 24 hours of hypothermic perfused preservation. The results showed a significant benefit from hormonal therapy with regard to myocardial function. Thyroxine, cortisol and insulin were administered after brain death and appeared to replenish myocardial energy stores with resultant better function following preservation, although at the expense of increased anaerobic metabolism not reversed by hypothermic perfusion (Novitzky et al, 1987b). The results of this study resulted in a clinical trial. Twenty one consecutive donors were treated with thyroxine (T_3 2μg/iv/hr), cortisol (100mg/iv/hr) and insulin (20units/iv/hr). This intervention resulted in a reduction in the inotropic and ADH requirements, a reversal of the metabolic acidosis, and excellent graft function. All donors from this group were used, as opposed to 20% of 26 donors who were unusable in the non-treatment group, due to haemodynamic

deterioration (Novitzky et al, 1987a). These results have been reproduced by other investigators using this hormonal mixture in the clinical setting (Wheeldon et al, 1992). Novitzky correlates the thyroid status in the brain dead animal with the 'euthyroid sick syndrome' where there is a reduction of free triiodothyronine (fT_3), a low or normal thyroxine (T4) and elevated reverse triiodothyronine (rT_3), whereas thyroid-stimulating hormone remains unchanged (Novitzky, 1992).

However, several investigators have found differing results. In the canine model Finkelstein et al found a significant increase in T_3, cortisol and catecholamines, which persisted after brain death. They instigated brain death by ligation of the head and neck vessels but this was only verified using electroencephalography (Finkelstein et al, 1987). In a clinical study of twenty potential organ donors Masson et al found thyroid function to resemble the 'euthyroid sick syndrome' but also found this to be present before brain death. They found no correlation between thyroid status, haemodynamic status and immediate allograft function (Masson et al, 1990). These findings were echoed by a prospective clinical trial of 25 organ donors; Randell and Hockerstedt compared a triiodothyronine treated group to a non treated group. Their conclusions were that triiodothyronine therapy for organ donors had no effect on haemodynamic status nor did it improve metabolic acidosis, which in fact worsened in this study (Randell and Hockerstedt, 1992). Sub-normal levels of fT_3 and cortisol were found in a study of 32 potential organ donors by Gramm et al but these were comparable to levels in patients with severe head injuries (Gramm et al, 1992). In this study the TSH was markedly elevated but no improvement was made to the status of the donor with T_3 supplementation. Their conclusions were similar to those of Randell and Masson; that brain death does not necessarily lead to endocrine failure and there is little rationale in the treatment of organ donors with T_3 and cortisol. Studies using the porcine model also concur with these clinical reports. In the University of Cape Town, South Africa, Professor Hickman's group found no indication for T_3 treatment in potential organ donors based on the results of liver transplantation in the pig model (Schwartz et al, 1993). Meyers et al studied myocardial blood flow and load-independent left ventricular function following brain death in pigs, and discovered that T_3 had no effect on myocardial function and significantly reduced coronary blood flow (Meyers et al, 1993).

The changes in serum catecholamines following brain death in the baboon model already described above (Novitzky et al, 1984) are not so clear cut in clinical practice. In a small study, by Powner et al, of 15 patients with severe head injury, of whom nine became brain dead, there were wide variations in catecholamine levels (Powner et al, 1992). The small numbers involved, and the wide variations in results, meant that no statistical conclusions could be made, although the author stated that catecholamine levels were probably not a useful predictive measure of outcome as previously described (Hamill et al, 1987)(Woolf et al, 1987). Clinical studies are difficult due to the majority of donors requiring inotropic support to maintain adequate perfusion pressure. Brain death is also associated with a decrease in systemic vascular resistance (Novitzky et al, 1984) so the most effective inotropic therapy for these patients is dopamine and/or noradrenaline with their vasoconstrictive effects at higher doses (Nishimura and Sugi, 1984). This benefit may be outweighed by inadequate perfusion to the viscera, and compromise donor organ function.

The need for inotropic support after brain death has provoked interest in myocardial ß-adrenergic receptor performance. This has been studied in the canine model comparing a control brain dead group to an adrenaline treated brain dead group. ß-adrenergic receptor affinity was similar in each group but ß-adrenergic receptor density decreased in the adrenaline group (Sakagoshi et al, 1992). In a clinical study post-transplant ß-adrenergic receptor density was normal despite myocardial denervation but these findings have not been correlated to pre-harvest status (Caturla et al, 1992).

The pathophysiology of brain death is difficult to define as seen from this summary of the published literature. The establishment of a reliable animal model has not been achieved nor has brain death been verified in the majority of these experiments. When verification has been attempted then unreliable indirect methods are employed such as testing brain stem reflexes in the pig soon after anaesthesia (Lanza et al, 1984). In the clinical setting even more variation is encountered making interventional studies difficult to interpret. The aim of this thesis is to establish a model of brain death in the dog and verify brain stem death, so that the model may represent the changes that

occur in the potential human organ donor. Using this model the effects on myocardial preservation in terms of post-transplant load-independent performance may be investigated and therein the effects of intervention in the donor, and in the technique of preservation.

Myocardial Preservation for Heart Transplantation

Since the introduction of oxygenated extra-corporeal circulation, research has been directed towards the prolongation of myocardial preservation; not only to allow the surgeon more time for cardiac operations but to also utilise donor organs from distant centres for cardiac transplantation. Therefore research in myocardial preservation tends either to be directed towards preservation of the diseased heart in cardiac surgery or towards long-term preservation of the 'normal' heart for transplantation.

Prolonged ischaemic cardiac preservation of 24 hours in the canine model was reported in 1969 (Feemster and Lillehei, 1969) and up to 72 hours in 1971 (Proctor et al, 1971). Both these methods employed hypothermia with continuous perfusion using a balanced salt solution. These studies were followed by attempts at survival studies such as Copeland et al with 48 hour survival after 24 hours of ischaemia (Copeland et al, 1973). These results were obtained by other investigators (Watson, 1977). This latter study used a remarkably different perfusate based on an intracellular mixture with high phosphate (11gm/litre), high glucose (20gm/litre) and a resultant high osmotic pressure of 460 mOsm. Watson, and also Lillehei (Toledo-Pereyra et al, 1979) investigated intermittent versus continuous perfusion with no significant improvement, though they agreed on the importance of both hypothermia, between 3^{o}C to 5^{o}C, and the hyperosmolarity of the perfusate. Swanson concurred that for preservation beyond 12 hours, hyperosmolarity was vital (Swanson et al, 1979). The benefits of preservation with perfusion have not only been found with functional studies but also morphological studies (Spray et al, 1977). The marked variety in the contents of these successful preservation solutions is displayed in table 1.

A completely alternative approach has been investigated using normothermic oxygenated blood on the beating heart for preservation up to 12 hours (Solis et al, 1985). Despite the good results this method has not gained popularity probably due to the complexity and fragility of the apparatus if it were applied to a clinical setting.

Research has also studied the optimal pressures and temperatures for initial arrest of the heart and subsequent perfusion (Johnson et al, 1982). Initial arrest with 4°c solution at 100 mmHg pressure is satisfactory with subsequent perfusion at 20-25 mmHg. However prolonged hypothermic preservation inevitably leads to significant oedema of the heart (Bethencourt and Laks, 1981). This oedema leads to decreased relative coronary artery flow to the left ventricle and increased relative coronary artery flow to the right ventricle with an overall increase in coronary vascular resistance (Wieshammer et al, 1979).

Subsequent to these studies other investigators have had success in 24 hour preservation with modifications of the perfusate, the use of simple immersion and agonal arrest (Guerraty et al, 1981)(Morishita et al, 1985)(Burt and Copeland, 1986a)(Shirakura et al, 1989)(Qayami et al, 1991).

More recently attention has been focused on preservation using University of Wisconsin solution (UW). In renal and hepatic transplantation this solution has significantly increased the safe ischaemic times in clinical practise to 72 and 12 hours respectively. Initial work by Swanson with 12 hour preservation in UW solution using dog hearts in a Langendorff preparation were encouraging (Swanson et al, 1988) and this was reinforced by Wechsler studying UW preservation for the rat heart (Yeh et al, 1990). Swanson's work was repeated by Gott who also found improved preservation using UW solution after 6 hours ischaemia in the isolated dog heart model (Gott et al, 1990). Okouchi and colleagues have employed a heterotopic rat model (Okouchi et al, 1990) which also found superior preservation using UW solution. Measurement of myocardial function in canine orthotopic transplantation was performed after 14 hour preservation by Elbeery et al and found UW solution to preserve function equal to immediate reimplantation (Elbeery et al, 1991). Despite the

Table 1.

Main ionic and osmolar constituents of eleven cardioplegic solutions used in studies of prolonged myocardial preservation, and the solution used in chapter 8 of this thesis for four hour preservation.

These solutions have all been tested for hypothermic cardiac preservation, with adequate restoration of cardiac function in experimental animal models. There is notable variation in contents and concentrations between solutions, and their resultant pH and osmolarity.

1. St. Thomas' solution; Yeh et al, 1990; Tian et al, 1991; Choong and Gavin, 1991.
2. University of Wisconsin solution; Swanson et al, 1988; Belzer and Southard, 1988; Weng et al, 1992; Tian et al, 1991; Sunamori et al, 1993.
3. Stanford solution; Billingham et al, 1980; Swanson et al, 1988; Weng et al, 1992.
4. Modified Collins solution; Swanson et al, 1979.
5. Krebs-Henseleit solution; Tian et al, 1991.
6. Modified Krebs-Henseleit solution; Bethencourt andLaks, 1981.
7. Plegisol; Weng et al, 1992.
8. Watson et al, 1979.
9. Swanson et al, 1979.
10. Wicomb et al, 1982.
11. Cooper et al, 1983.
12. Solution used in this thesis, chapter 8.

		Sodium *mEq/ml*	Potassium *mEq/ml*	Phosphate *mEq/ml*	Bicarbonate *mEq/ml*	Magnesium *mEq/ml*	Calcium *mEq/ml*	Hetastarch *%*	Glucose *g/l*	Mannitol *g/l*	pH	Osmolarity *mOsm*
1	St THOMAS'	100	26	1.2	10	16	1.2	0	0	0	7.5	320
2	U. WISCONSIN	30	125	25	0	4.8	0.1	5	0	0	7.4	327
3	STANFORD	30	27	0	20	0	0	0	50	12.5	7.7	380
4	Mod. COLLINS	45	20	57.5	10	8	0	0	7	20	7.3	460
5	KREBS	118	4.7	1.2	25	1.2	1.75	0	11	0	7.4	295
6	Mod. KREBS	140	20	4.1	10	3	4.1	0	2	0	7.4	330
7	PLEGISOL	65	8	0	10	16	1.2	0	0	0	7.3	150
8	WATSON	16	17	100	20	60	0	0	20	20	7.2	460
9	SWANSON	80	40	1.2	25	0	0	0	0	20	7.3	380
10	WICOMB	111	11	0	25	1.2	1.1	0	2	0	7.4	300
11	COOPER	102	10	0	4	14	1.1	0	50	0	7.4	320
12	CHAPTER 8	138	25	0	0	7.65	0.7	6	15	20	7.5	420

MAIN IONIC AND OSMOLAR CONSTITUENTS OF TWELVE HYPOTHERMIC MYOCARDIAL PRESERVATION SOLUTIONS USED IN ANIMAL MODELS

majority of evidence in favour of UW solution Choong found St. Thomas' cardioplegia to be superior after 5 hours storage in the isolated rat heart model (Choong and Gavin, 1991). Jeevanandam in New York has published his results using UW in primates and clinical transplantation including the results of a prospective randomised blinded trial (Jeevanandam et al, 1992a)(Jeevanandam et al, 1992b). These studies show significant improvements with UW solution.

Research in myocardial preservation for elective and emergency cardiac surgery has revealed the importance of controlled reperfusion. This work has been applied to prolonged cardiac preservation for transplantation. Reperfusion with warm blood cardioplegia and glutamate was assessed on the isolated canine heart after 24 hours storage (Milliken et al, 1989). This showed improvement of diastolic function only and supported the earlier work of Swanson using the same model after 2 hours of ischaemia (Swanson and Myerowitz, 1983). More recently Fukushima et al found excellent improvement in preservation after 24 hours in canine orthotopic transplantation using leukocyte-depleted blood reperfusion (Fukushima et al, 1992).

Primate research is restricted but excellent results have been achieved in baboons. Successful survival studies up to 27 months after 24 to 48 hours ischaemia have been achieved (Wicomb et al, 1986b) using hypothermic continuous perfusion. The perfusate used in these experiments was based on an extracellular solution.

Brain death has significant effects on the cardiovascular system as described in the section 'Pathophysiology of Brain Death'. In **all** of the animal studies above, investigating myocardial preservation for transplantation, the donor heart was removed from an anaesthetised animal and no attempt has been made to recreate the clinical scenario of brain death prior to explantation. Wicomb studied the effect of 24 hour storage using hypothermic perfusion on the isolated pig heart following brain death. He caused brain death by ligation of the head and neck vessels at aortic level, a method that had not been carefully evaluated in a previous study (Lanza et al, 1984). 72 pigs were studied in 9 treatment groups whose population groups varied from 6 to 10. The

treatment groups were divided in as follows;

STUDY GROUP	HAEMODYNAMIC SUPPORT	STORAGE
A Anaesthetised (n=10)		None
B Anaesthetised plus cerebral ischaemia(n=6)		None
C Anaesthetised plus brain death(n=6)		None
D Anaesthetised plus brain death(n=8)	IV fluids + dobutamine	None
E Anaesthetised (n=10)		24 hours
F Anaesthetised plus cerebral ischaemia(n=6)		24 hours
G Anaesthetised plus brain death(n=7)		24 hours
H Anaesthetised plus brain death(n=9)	IV fluids + dobutamine	24 hours
I Anaesthetised plus brain death (n=10)	IV fluids + vasopressin	24 hours

The hearts were studied on an isolated preparation varying pre-load and afterload. The findings were that brain death appeared to depress cardiac function and this function was further insulted by hypothermic perfusion. Furthermore the dobutamine treatment group had the worst function of all the treatment groups (Wicomb et al, 1986b).

Galinanes and Hearse have studied the rat model, causing brain death with an intracranial balloon and verifying this maneuvre with electroencephalography and fixed dilated pupils. Their findings, using an isolated heart preparation, were that myocardial contractility decreased after 60 minutes of brain death. However, in contradiction to Wicomb's results this decrease in function was reversed by reperfusion after immediate transplantation and also reversed after 6 hours of hypothermic preservation (Galinanes and Hearse, 1992).

One other study has investigated the effect of brain death on myocardial preservation. Shivalkar et al compared two canine models of brain death; one model with rapid inflation of an intracranial balloon and the other with slow inflation of an intracranial balloon (Shivalkar et al, 1993). Cardiac impairment was most marked in the rapid inflation group with no 'heart-beating' survivors beyond two hours (n=6). When the hearts from this model were used after one hour of brain death, for transplantation, only 2 from 4 hearts could be weaned from bypass. The slow inflation group survived in the 'heart-beating' state for longer and could be more reliably weaned from cardiopulmonary bypass following transplantation. This was attributed to lower peak levels of catecholamines in the latter model during the Cushing response. This paper further shows the difficulty in the validation of an animal brain dead model due to the wide variation in response, as already alluded to in the clinical situation. Their study concentrated on left ventricular function using load dependent analysis of function and preservation, and brain stem death was not validated nor was the presence of diabetes insipidus mentioned. A model of brain death requires validation using studies of the brain stem, and hormonal changes measured directly or indirectly such as the presence of diabetes insipidus. Reliance on electroencephalography or brain stem reflexes in an animal under general anaesthesia may not be sufficient.

In summary the effect of brain death on myocardial preservation has not been fully investigated nor has myocardial function been studied in these preparations using a load-independent system. In particular there is a need to investigate right ventricular function/ preservation in future studies as it is a leading cause of morbidity and mortality. A canine model of brain death must first be developed with verification of brain stem injury and subsequent studies performed to asses cardiac function in the transplanted heart.

Surgical Method of Implantation; Complete Atrio-Ventricular Transplantation

More than 18,000 orthotopic human transplants have been performed worldwide of which the vast majority have been implanted in a standard fashion that was first described by Lower and Shumway (Lower and Shumway, 1960). This involved 4 anastomoses, between donor and recipient, of left atrium, right atrium, pulmonary artery and aorta. These anastomoses are relatively straightforward to perform and hence this technique gained popularity even if the final result is **biventricular transplantation with atrioplasty**. However there are criticisms of the anatomical result. Transthoracic, and in particular trans oesophageal, ultrasonic echocardiography reveals asynchronous contracture of the recipient and donor atria and distortion of the atrioventricular valves (Angerman et al, 1990). This results in a significant incidence of mitral and tricuspid regurgitation an observation already reported by Stevenson et al (Stevenson et al, 1987).

This 'standard' technique of cardiac implantation was a simplified form of an earlier method used in dogs by Cass and Brock and also by Webb. This involved multiple anastomoses of left and right pulmonary veins, superior vena cava, inferior vena cava, pulmonary artery and aorta (Cass and Brock, 1959) (Webb et al, 1959). Due to technical difficulty this was abandoned but has recently been reported in the clinical setting (Yacoub and Banner, 1989) (Dreyfus et al, 1991). At Papworth Hospital, England, 30 patients have been transplanted with 6 anastomoses; left pulmonary veins, right pulmonary veins, IVC, SVC, pulmonary artery and aorta. This results in **complete atrioventricular transplantation**. These patients were in a prospective randomised clinical trial of 60 consecutive transplants and there were no differences between the groups for total cross clamp time, duration of cardiopulmonary bypass and perioperative complications (Kendall et al, 1993). Clinical evaluation six months post transplant with right heart catheter studies, contrast ultrasonic echocardiography and exercise tolerance testing showed no advantage to the more complex method.

These clinical assessments of graft function are relatively insensitive, particularly in a heterogeneous population. The theoretical advantage of complete atrioventricular transplantation is that normal atrial function is maintained and hence gives improved atrioventricular transportation of blood with improved atrioventricular valve function. To study diastolic function accurately a combination of flow, pressure and geometric measurements are required, and simultaneously atrial systolic function may be quantified.

Therefore a further aim of this thesis is to compare **ventricular transplantation with atrioplasty** to **complete atrioventricular transplantation** in the canine model, using accurate methods to assess diastolic and systolic function.

Figure 1. (New)

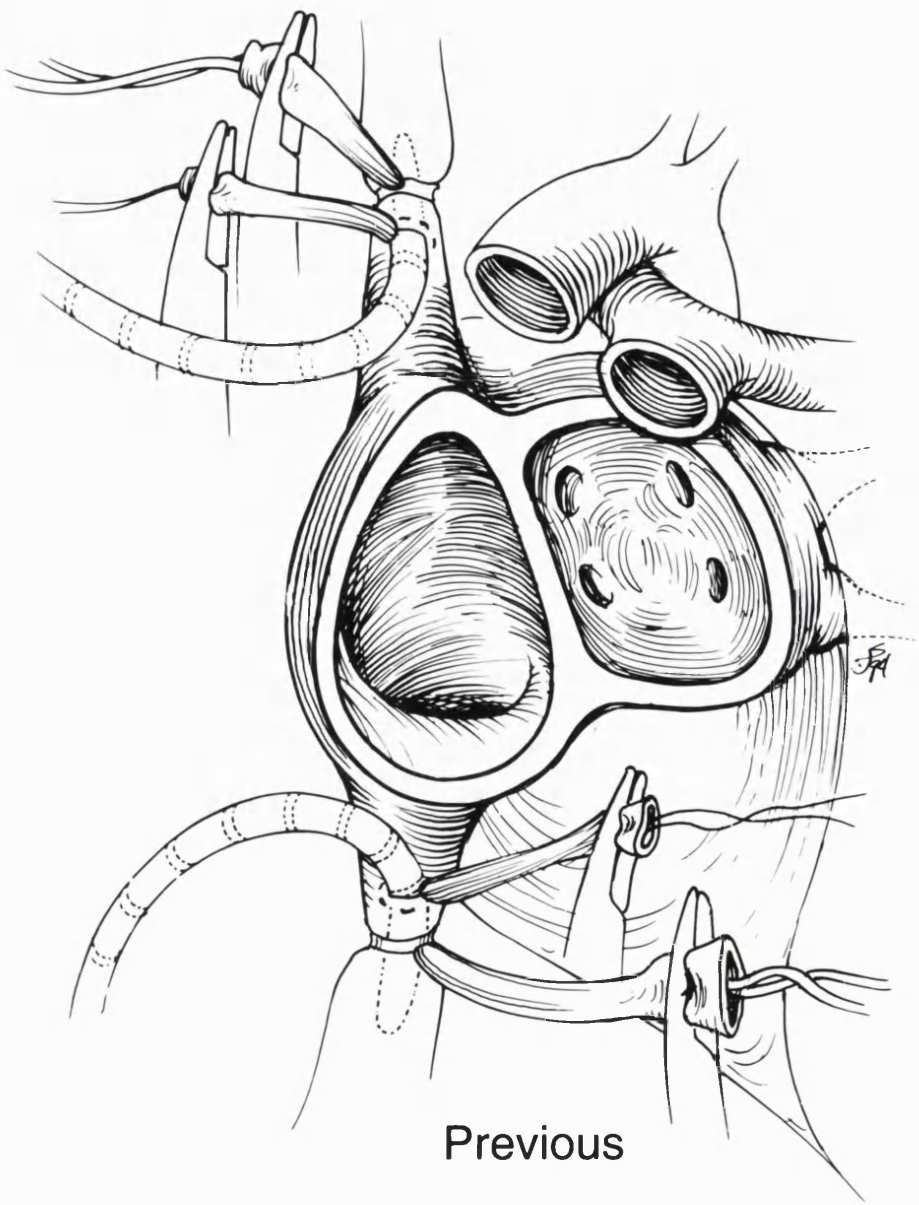
Complete atrioventricular transplantation

The mediastinum prepared for complete atrioventricular transplantation;
Aorta, pulmonary artery, inferior vena cava, superior vena cava and the left and right pulmonary veins on Carrel patches prepared for anastomosis to the donor heart.

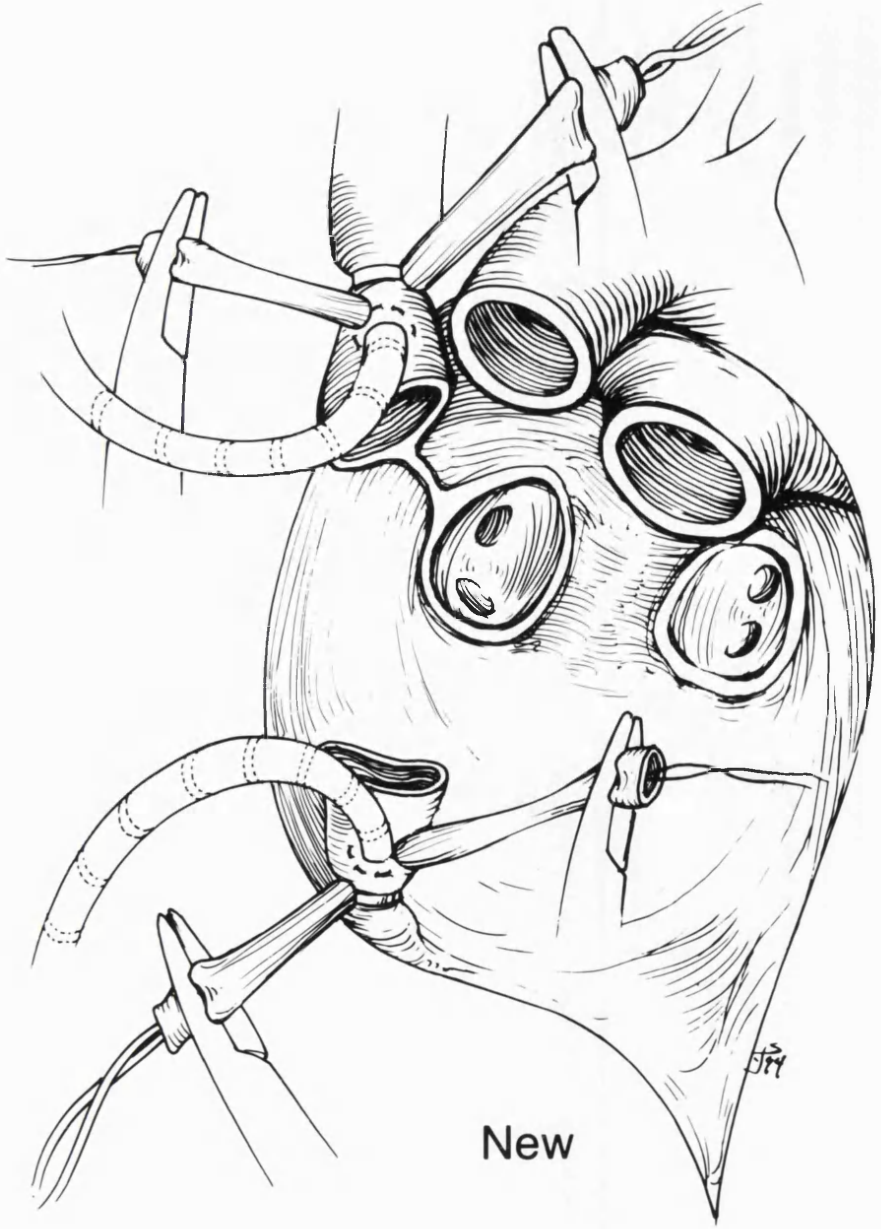
Figure 2. (Previous)

Ventricular transplantation with atrioplasty; 'Standard technique'.

The mediastinum prepared for ventricular transplantation with atrioplasty;
Aorta, pulmonary artery, right atrial and left atrial cuffs prepared
for anastomosis to the donor heart.



Previous



New

CHAPTER 2

ASSESSMENT OF DONOR / RECIPIENT CARDIAC FUNCTION

Haemodynamic Assessment

Cardiovascular dynamics is one of the oldest lines of medical research. Speculation about circulatory function began in the dawn of history and existing theories were catalogued by Galen in the second century A.D. (Cournand, 1964). During the middle ages most of Galen's work was lost in the west but his concepts of cardiac function were maintained in the Islamic world through the writings of Avicenna. In the fifteenth and sixteenth centuries Padua, Italy, became the focus of cardiovascular research and it was here that Servetus and Columbus described the passage of blood through the lungs. It was also here that William Harvey studied Columbus' theories, learned of the directionality of blood flow and was influenced by the scientific method of Galileo (Hamilton and Richards, 1964). On his return to London Harvey made a series of direct experimental observations of circulatory dynamics in animal and human cadavers which he published in '*De Motu Cordis*' (Harvey, 1628). This was a full description of the circulation and was probably the single most revolutionary and important advance in the history of medical science. The majority of Harvey's principles are still relevant today, 365 years later.

Experimental cardiac physiology became established with the development of the Kymograph pressure recording device by Carl Ludwig in 1849 (Williams, 1948). This rudimentary technology evolved over the next 50 years into highly accurate optical recording systems. Using these techniques Ludwig and his students Adolph Fick, Julius Cohnheim, Henry Bowditch, and Otto Frank, established most of the important principles of contemporary cardiac physiology including the length dependency of myocardial function.

Ernest Starling began his experiments at University College, London in the early 1900's (Chapman and Mitchell, 1965). He worked with isolated mammalian heart lung preparations and discovered that the heart adapts to increasing arterial pressure by a compensatory rise in filling pressure, which tends to maintain cardiac output. From this he stated: " The law of the heart is thus the same as the law of muscular tissue generally, that the energy of contraction, however measured, is a function of the length of the muscle fibre ..... Many lines of argument converge similarly in the direction of the

view that the chemical changes, occurring in a muscle as a result of excitation, affect surfaces arranged longitudinally in the muscle. Now the effect of lengthening the muscle will be to increase the extent of active surface, so that we may restate our general proposition in the following way. Any increase in the extent of active surface increases the energy of change." This statement preceded by decades the theory of myocardial contraction due to sliding actin/myosin cross bridges.

In the 1950's and 1960's Sarnoff and his co-investigators made major advances in regard to cardiac function working at the National Institute for Health, Maryland. He showed that the external work of the heart (manifested as cardiac output and arterial blood pressure) rose steeply as filling pressure increased, and then at a certain level of filling the work output was maximised and plateaued (Sarnoff and Mitchell, 1961). With this and further work Sarnoff suggested that the relationship between stroke work and end-diastolic volume might be linear, although technical limitations precluded direct volume measurements at that time. However the experimental work of this group together with later studies of afterload reduction and pulmonary artery balloon catheters is the basis of clinical cardiac diagnosis and management to the present day.

The development of technology permitting direct measurement of ventricular geometry has allowed further advances in cardiac physiology.

Ventricular Geometry

A comprehensive analysis of myocardial performance with regard to pre-load recruitable stroke work requires an understanding of the geometric characteristics of the heart under physiological conditions. The ventricles eject blood into the aorta and pulmonary artery during systole, having filled with blood during diastole. Diastolic filling occurs in three phases: early rapid filling, during which the velocity and extent of filling are greatest; diastasis in mid-diastole; and atrial systole in late diastole. Rankin et al showed that ninety percent of the change in left ventricular volume with filling is produced by expansion of the minor axis circumference, and that ten percent relates to

major axis elongation (Rankin et al, 1976). The consequent change in chamber geometry produces a more spherical ventricular shape at end-diastole (Olsen et al, 1981). During systole ventricular geometry appears to be different, in that dimensional changes during ejection occur in a more concentric fashion. It is hypothesised that ejection phase geometry is related almost solely to average fibre orientation which is more evenly distributed in all directions. In contrast, during diastole the filling and consequent change of geometry is influenced by many other factors such as myocardial collagen distribution and papillary-mitral continuity. However there is a further hypothesis to describe ventricular geometric filling characteristics by Arts that is based on a 'basket-weave' fibre angle distribution (Arts et al, 1982). As a consequence of differing systolic and diastolic geometry, the ventricle undergoes significant shape changes during the transitional periods of isovolumic contraction and relaxation and these become more prominent at lower ventricular volumes (Rankin et al, 1976).

Another important geometric consideration is diastolic ventricular interaction due to the common septum shared by both ventricles (Olsen et al, 1983). During the cardiac cycle complex transmural forces exist across the interventricular septum and its position depends on the transseptal pressure. For example, pulmonary artery occlusion increases right ventricular pressure relative to left and is accompanied by shifting of the septum towards the left ventricular free wall with little change in the other diameters. The physiologic effect of septal shifting is related primarily to altered diastolic compliance. However this change in left ventricular shape has little effect on its systolic function. When pulmonary artery occlusion produces the maximum of tolerated right ventricular hypertension there is a slight augmentation of left ventricular systolic function via transseptal energy transmission (Feneley et al, 1989). In the reverse situation, maximal left ventricular decompression by a left atrial-aortic assist device causes minimum change to right ventricular function. Therefore the geometric position of the septum has little influence on right ventricular function. This statement is not meant to undermine the very significant contribution of the septum to right ventricular systolic function, which has been measured to be approximately 65% (Damiano et al, 1991).

These geometric studies rely on the validity of using orthogonal ventricular diameter measurements to assess changes in chamber volume. In normal conditions the minor axis circumference of the left ventricle fills and ejects fairly concentrically. Measurements of the anterio-posterior minor axis and base-apex major axis diameters, coupled with a prolate ellipsoid model, allow an accurate and highly reproducible estimate of changes in absolute ventricular volume (Olsen, et al, 1983). The limitations of this method include significant under-estimation of absolute volume change and slightly different linear relationships between calculated and absolute cavitary volume for systole and diastole (Rankin et al, 1976).

Another potential problem in the assessment of ventricular geometry is the need to measure ventricular wall volume in order to subtract it from the epicardial shell volume in the calculation of cavitary volume. In early studies at Duke Medical Center an additional pair of ultrasonic transducers were used to measure dynamic wall thickness (Rankin et al, 1976), but these results were variable and subject to error (Rankin et al, 1977). More recently however two techniques have been developed to yield more accurate measurements. The first technique to directly measure free wall volumes by saline displacement at the termination of the study (Glower et al, 1985), and the second technique is to use echocardiography (Feneley et al, 1988). This method is also simple and accurate and in chronic studies it allows serial assessment of changing wall volume, for example during the development of ventricular hypertrophy.

Addition of a right ventricular free wall transducer that allows measurement of right ventricular free wall to septal diameter gives accurate simultaneous assessment of dynamic cavitary volume in both ventricles using a shell subtraction model (Feneley et al, 1987).

The assessment of intracavitary ventricular volumes is made by considering the left ventricle and the biventricular shell as a composite of a prolate ellipsoid and prolate cylindrical model. The right ventricular volume is then calculated by subtracting the left ventricular volume and right ventricular free wall volume from the biventricular volume. The biventricular epicardial shell volume (BVV epi)

is the sum of the left ventricular epicardial shell volume (LVV epi), the endocardial right ventricular shell volume (RVV endo), and the volume of the right ventricular free wall (RFWV).

This is represented by the equation;

$$\text{BVV epi} = \text{LVV epi} + \text{RVV endo} + \text{RFWV}$$

It therefore follows that;

$$\text{RVV endo} = \text{BVV epi} - \text{LVV epi} - \text{RFWV}$$

The left ventricular epicardial volume and biventricular volume for the prolate ellipsoidal model are;

$$\text{LVV epi} = \pi/6\, a \times b \times c$$

$$\text{BVV epi} = \pi/6\, a \times b \times (c + d)$$

$$\text{RVV endo} = \pi/6\, a \times b \times (c + d) - \pi/6\, a \times b \times c - \text{RFWV}$$

$$= \pi/6\, (a \times b \times d) - \text{RFWV}$$

where a = long axis; b = minor axis; c = left free wall to septal distance; d = right free wall to septal distance

The position of the piezo-electric crystals and diagrammatic representation of the measured axes of the heart are depicted in figures 3 and 4.

The cylindrical ellipsoidal model considers the LVV epi and BVV epi to be the combination of a prolate hemi-ellipsoid and prolate cylinder of length $a/2$. The prolate hemi-ellipsoid LV has a volume of:

$$1/2 \times \pi/6 \times a \times b \times c = \pi/12 \times a \times b \times c$$

the prolate cylinder has a volume of:

$$a/2 \times \pi \times b/2 \times c/2 = \pi/8 \times a \times b \times c$$

Summing the two volumes yields:

$$5\pi/24\, a \times b \times c$$

The biventricular ellipsoid shell is similarly calculated with (c + d) replacing c in the above equations. The cylindrical ellipse model thus yields:

$$\text{RVV endo} = 5\pi/24 \times a \times b \times d - \text{RFWV}$$

The ability to obtain the fundamental measurements of ventricular geometry (a, b, c and d) throughout the cardiac cycle is made possible with ultrasonic transducers. These are coupled to a sonomicrometer, a system pioneered and developed by Rushmer et al in the 1950's. This method relies on the speed of sound to be 1585 m/s across the heart, and allows real-time measurement to a resolution of 0.08mm (Rushmer et al, 1956). Due to the development of microprocessors these measurements may be instantaneously converted to ventricular volumes. With this ability to study physiological and pathological changes in the geometry of the left and the right ventricles, further studies are possible to study systolic and diastolic mechanics.

Figure 3. (A)

Diagram showing the measured geometric axes of the heart.

Also shows positions of the piezo - electric crystals and micromanometers.

B_a = Base crystal

A_p = Apical crystal

A = Anterior crystal

P = Posterior crystal

R = Right free wall crystal

F = Left free wall crystal

S = Septal crystal

Geometric Axes;

(a) B_a to A_p = Long axis

(b) A to P = Minor axis

(d) R to S = Right free wall to septal distance

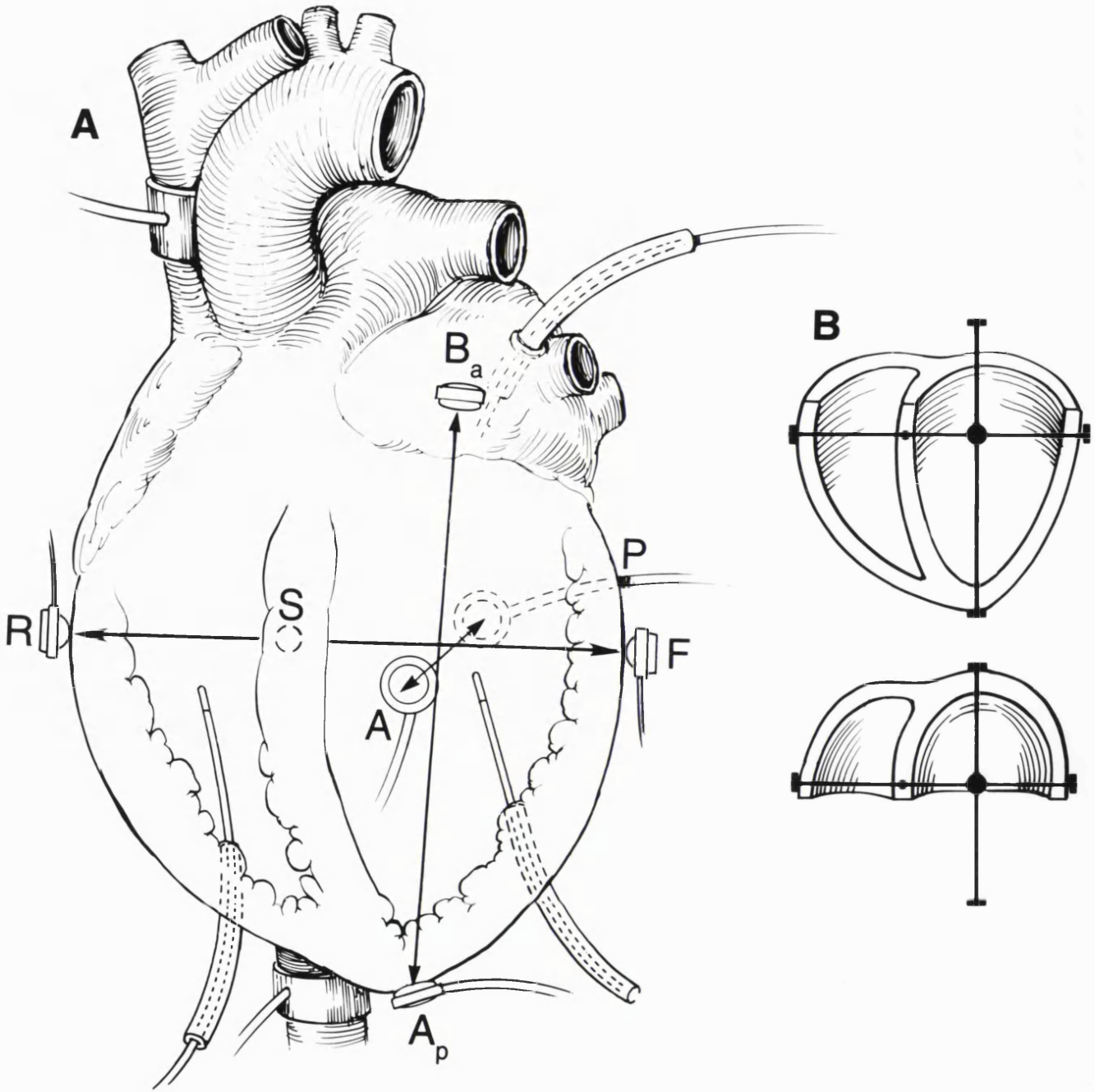
(c) F to S = Left free wall to septal distance

Micromanometers shown in the left ventricle, left atrium and right ventricle.

Caval occluders are drawn on the inferior and superior vena cava.

Figure 4. (B) (Inset)

Diagrammatic representation of coronal and sagittal sections to demonstrate the measured geometric axes of the heart.


A
B
B_a
P
S
R
F
A
A_p

Systolic Function; Pre-Load Recruitable Stroke Work

The fundamental unit of muscular contraction is the myosin cross-bridge (Squire, 1981). With electrical depolarisation of the myocardial cell membrane, ionised calcium fluxes into the cytoplasm causing the myosin molecule to hydrolyse adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and inorganic phosphate. When ATP is split a considerable amount of chemical energy is released from the ATP molecule and transferred into a conformational change in the myosin cross bridge via calcium release from the sarcoplasmic reticulum. This chemo-mechanical alteration in the cross-bridge produces sliding of the myosin filaments relative to actin and hence shortens the sarcomere. Over the physiologic range of sarcomere lengths (1.6 - 2.2 μm) the surface area of available cross-bridge interactions, and therefore the metabolic energy transferred into mechanical energy during sarcomere contraction, is linearly proportional to end-diastolic sarcomere length. The length-dependency of cross-bridge interaction at the sarcomere level constitutes the histochemical basis of the Frank-Starling relationship. Following contraction the cytoplasmic reticulum actively transports calcium out of the cell and high energy phosphate bonds are regenerated by the mitochondria by aerobic metabolism of oxygen and substrates. This chemical energy is transmitted back to the cross-bridge area via the creatine phosphate shuttle.

The work-dimension relationship proposed by Sarnoff was, and is, investigated using dimensional analysis. Initially there was controversy regarding fundamental aspects of the pre-load recruitable stroke work (PRSW) of the ventricle. This arose from differences in the methodology of the early studies. Laboratories that used the less accurate and reliable mercury-in-silastic gauges to measure cardiac dimension demonstrated non-linearity of the PRSW (Warbasse et al, 1963). Ultrasonic measurement using sonomicrometry had been validated for geometric models to calculate ventricular volumes (Rushmer et al, 1956), and laboratories using this method demonstrated linearity of the PRSW (Suga and Sagawa, 1974). The earlier investigators also had differing methods to alter preload and afterload. Preload has been varied using volume infusion (Mahler et al, 1975), decreased venous return (Rankin et al, 1976) and repeated bolus' of a vasopressor agent (Ross and Braunwald, 1964). This latter method was questionable due to the profound effect on

afterload, myocardial blood flow and autonomic reflexes (Rosenfeldt et al, 1979). Afterload has been manipulated using diverse techniques such as arteriovenous fistulas (Wilcken et al, 1964), rapid aortic occlusion (Imperial et al, 1961) or infusions of vasoconstrictor agents (Sonnenblick and Downing, 1963). These differences in techniques probably render these early studies incomparable because of ß-adrenergic effects in the absence of autonomic blockade or because of differences in cardiac responses to transient versus steady-state loading alterations. At present vena-caval occlusion is the most satisfactory and reproducible method to vary pre-load. In the intact circulation a steady-state infusion of phenylephrine in the presence of autonomic blockade is considered the standard reference for varying afterload (Tajimi et al, 1984).

In early studies there was a reliance placed on aortic flow / cardiac output to calculate the external potential work of the heart. This is a useful entity to measure but does not fully describe the net mechanical work done by the heart per cardiac cycle, since diastolic work performed on the ventricle was assessed inaccurately. Calculation of net mechanical work was not possible until the advent of methods for measuring continuous ventricular volume. Stroke work should be an appropriate measure of systolic performance in the heart and several investigators supported the finding that there was minimum afterload sensitivity (Rosenfeldt et al, 1979)(Imperial et al, 1961). However other investigators showed evidence of that stroke work fell either with increasing or decreasing ventricular afterload (Weber et al, 1974) (Sagawa, 1967). These differences may well relate to a more prominent afterload dependence of isolated or open-chest anaesthetised preparations. These ventricles have depressed myocardial function as compared with the ventricles of conscious dogs, and afterload sensitivity may be greater because of a more prominent impedance matching phenomenon (Ross and Braunwald, 1964). Even in the conscious dog there is the obvious certainty that external stroke work must be zero at zero and infinite afterload, which implies a plateau in the work-afterload relationship. This explains the apparent small effect of arterial load on stroke work over the physiologic range of arterial pressures. Despite the differences between studies of conscious dogs and isolated or open-chest anaesthetised preparations, this model maintains linearity for determining stroke work, and negates the need for modification of data

analysis to account for afterload dependence (Sagawa, 1967).

Conceptual understanding of global ventricular performance is enhanced by considering the heart as a pump that imparts energy to the circulation. Basic variables for the analysis include left ventricular pressure and volume, and each cardiac cycle may be characterised by a distinct pressure-volume loop (fig. 5). The area of each loop represents net external mechanical energy, or stroke work, expended for that cycle, while end-diastolic volume reflects the end-diastolic fibre length, or pre-load. The relationship between stroke work and end-diastolic volume, as described above, has been shown to be quite linear under all conditions thus far tested, and has been termed the pre-load recruitable stroke work relationship. This is a clear representation of ventricular inotropism and provides a useful framework for analysis and understanding of ventricular performance.

The calculation for stroke work from each pressure - volume loop is;

$$SW = \int P \cdot dV$$

During a vena caval occlusion the end-diastolic volume and intracavitary pressure declines with each beat, as does the stroke work. This linear relationship between stroke work and end - diastolic volume is represented by the equation;

$$SW = M_W (EDV - V_0)$$

where M_W is the linear slope constant and V_0 is the X intercept. It is this linear slope constant that represents the recruitable stroke work available per unit volume of myocardium (erg / $cm^3 \cdot 10^3$) and, as described above, is relatively independent of changes in preload and afterload. The linearity of the slope is represented by the R^2 coefficient, and the closer this value is to 1 the greater the

Figures 5 and 6

Pressure Volume Loops plotted for consecutive cardiac cycles during occlusion of both vena cava.

Top picture; Left ventricle (figure5)

Bottom Picture; Right ventricle (figure 6)

Left graph;

X axis = intracavitary ventricular volume (ml)

Y axis = intracavitary pressure (mmHg)

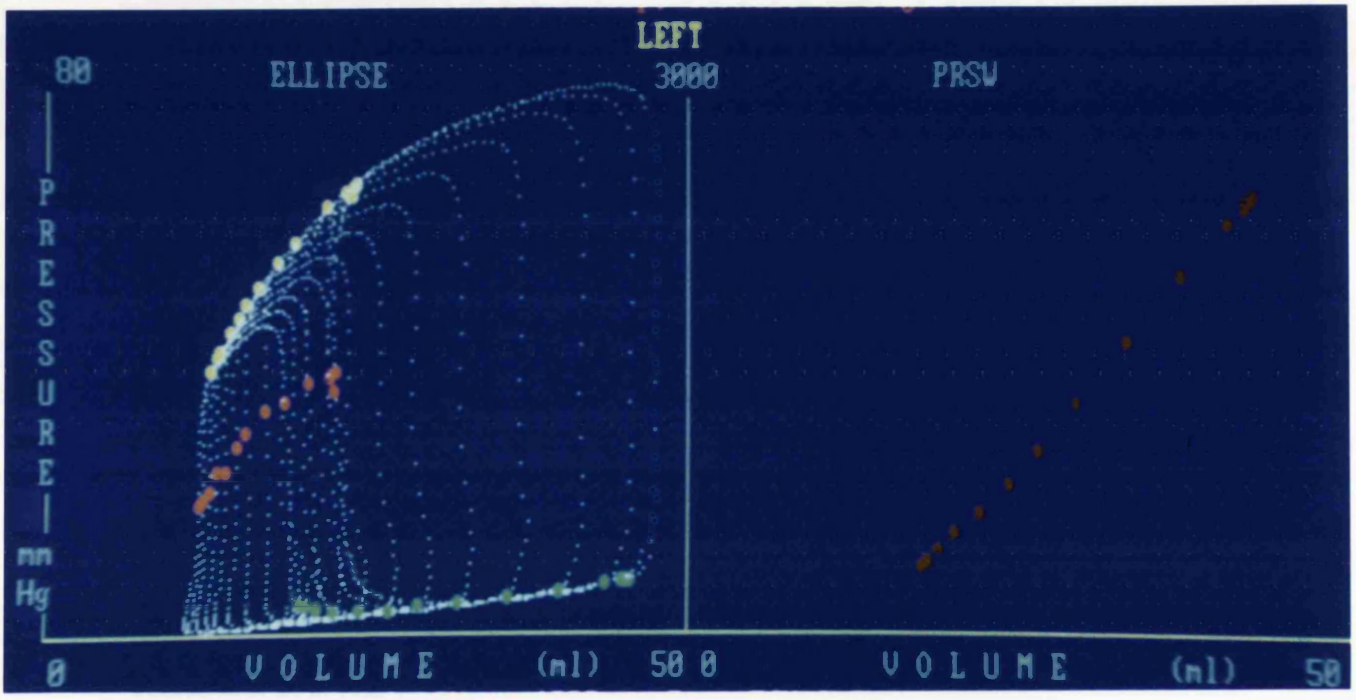
Green marker indicator for end-diastole

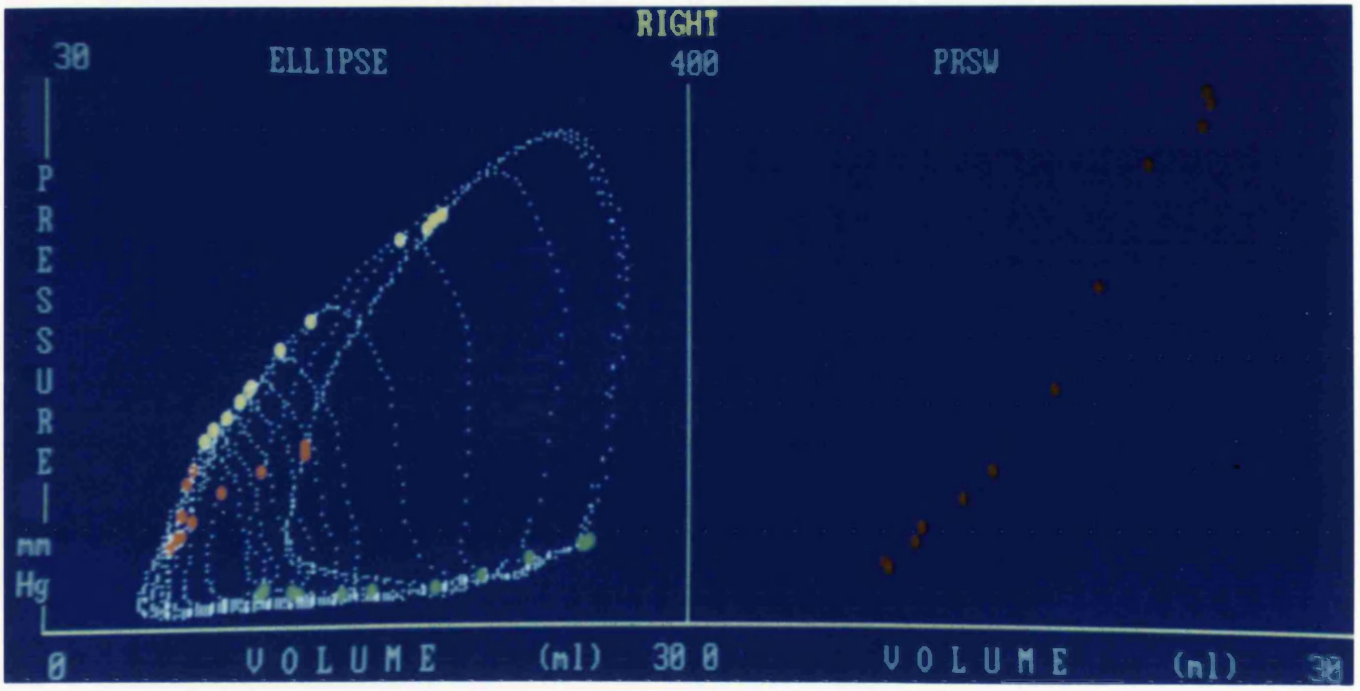
Yellow marker for peak flow and

Red marker for end-systole.

Right graph;

Stroke work (area of loop) plotted for each cardiac cycle on same axis.


LEFT
80
ELLIPSE
3000
PRSW
PRESSURE
mm Hg
0
VOLUME (ml) 500
VOLUME (ml) 50


RIGHT
30
ELLIPSE
400
PRSW
PRESSURE
mm Hg
0
VOLUME (ml) 300
VOLUME (ml) 30

linearity of the slope. V_0 is the value in millilitres representing the theoretical end-diastolic volume where zero stroke work is performed. This point is rarely reached as there is always some venous return to the heart (ie coronary sinus). Mean values for the slope, x intercept, and correlation coefficient were calculated for each study condition by averaging values for two to five vena caval occlusions.

Data analysis is accomplished using interactive software developed in the laboratory on a PC computer (Zenith data systems Z-386/20, Glenview Illinois). Right and left ventricular dP/dt cardiac values are determined by a third order polynomial approximation. Individual beats are defined by software analysis of the waveforms with interactive, semi-automated editing. Simultaneous right and left ventricular cardiac cycles are each defined by ventricular dP/dt criteria and determination of epicardial dimensional events based on global chamber events. For the left ventricle, end-diastolic length was determined as the dimension 20 msec before the point of peak positive left ventricular dP/dt (250mmhg/sec) (figure 7). Review of analogue tracings confirm this to be a readily identifiable point marking the early portion of the near isometric phase before initiation of left ventricular ejection. Since a truly isometric phase of systole is not apparent in all dogs, this point is taken as a consistent approximation of end-diastole. Left ventricular end-systolic length is determined as the dimension 10 msec before peak negative left ventricular dP/dt as previously described (Abel, 1981). For the right ventricle, end-diastolic length (EDL) is determined as the dimension 20 msec before peak positive right ventricular dP/dt (150mmhg/sec) and end-systolic length (ESL) as the dimension at peak negative dP/dt. These determinations are also confirmed by review of analogue tracings and have been accurately shown to mark the initiation and end of right ventricular ejection, respectively (Morris et al, 1986).

Right ventricular function

Estimation of right ventricular function has only become possible in recent years. Prior to this time it was the left ventricle that has been extensively researched, due to its role in physiological and pathological conditions such as ischaemic heart disease; this has been aided by the greater ease with which its performance may be assessed and its more definable geometry . In comparison the right ventricle has a minor role in propelling blood through the pulmonary circulation and is more commonly only affected secondary to lung pathology or left sided cardiac failure. However in cardiac transplantation the right ventricle is a major cause of morbidity and mortality as it is believed that the recipients have an elevation of their pulmonary vascular resistance secondary to left ventricular failure (Kirklin et al, 1988).

The more complex geometry of the right ventricle has prevented a practical algorithm deriving the continuous measurements of right ventricular volumes. In the past investigators have avoided this problem by using measurements of regional right ventricular dimensions as indexes of changes in right ventricular volume (Hamm et al, 1984) (Morris et al, 1986). This method does have its limitations as it has been well demonstrated that left ventricular volume changes effect right ventricular shape (Olsen et al, 1983)(Feneley et al, 1989).

However the recent work of Feneley et al has discovered a reliable method to calculate right ventricular volumes based on a shell subtraction model, which involved the addition of three ultrasonic transducers to the right ventricular freewall, the ventricular septum and the left free wall (Feneley et al, 1990). (The equations for this algorithm are described above). This study showed the weakness of models that ignored left ventricular volumes; it revealed that an increase in left ventricular volume reduced the diameter between the septum and the right ventricular free wall, which is accommodated by an increase in the dimensions of the right ventricular free wall. Their work also showed the ellipsoidal shell subtraction model to be insensitive to changes in left ventricular volume. The variability between animals, of slope and intercept in the actual versus predicted right ventricular relation, was attributed to alignment of the ultrasonic dimension

transducers and / or variation in cardiac geometry, and is not dissimilar to the variation found in studies on the left ventricle (Glower et al, 1985). When this model was applied in vivo the dimensional estimates of right ventricular stroke volume were highly linear related to measured stroke volume. They demonstrated the potential of this model by generating right ventricular pressure - volume loops during vena caval occlusions. Right ventricular stroke work / end - diastolic volume / end - systolic pressure - volume relations were qualitatively similar to those obtained for the left ventricle, and provided quantitative linear indices of right ventricular systolic function. (fig 6)

Two other methods have been described for the continuous recording of right ventricular volumes, which have a reasonably high sampling rate (Santamore et al, 1981)(Schwiep et al, 1988). These rely on biplane cineradiography of surgically implanted radiopaque markers and in Schwiep's method this entails 18 markers around the right ventricular wall. Although this method does not rely on any geometric assumptions it is limited to a sampling rate of 60 Hz i.e., the cineradiographic frame rate. In contrast the sonomicrometer has a sample rate of 200Hz.

It was noted from this study that there was a discrepancy between the data from the isolated hearts and the in vivo studies. In the isolated heart studies the slope of the relation between calculated and measured volumes remained less than 1.0. Consequently if the this relation remained constant throughout ejection it would be predicted that the shell subtraction model would underestimate the right ventricular stroke volume. In vivo, however, they found the reverse in that the slope of the relation between calculated and measured volumes slightly exceeded 1.0, suggesting the model tended to overestimate stroke volume. This discrepancy has been attributed to a change in the shape of the right ventricle during ejection with a consequent underestimation of the volume at end - ejection relative to the volume estimated at the onset of ejection. The consequence of this flaw is that the shell subtraction model provides a more accurate estimate of stroke volume than absolute volume and is best regarded as a volume index. Despite this, the ellipsoidal shell subtraction model remains the best method available to assess right ventricular volumes and is the method employed in this thesis.

Figure 7

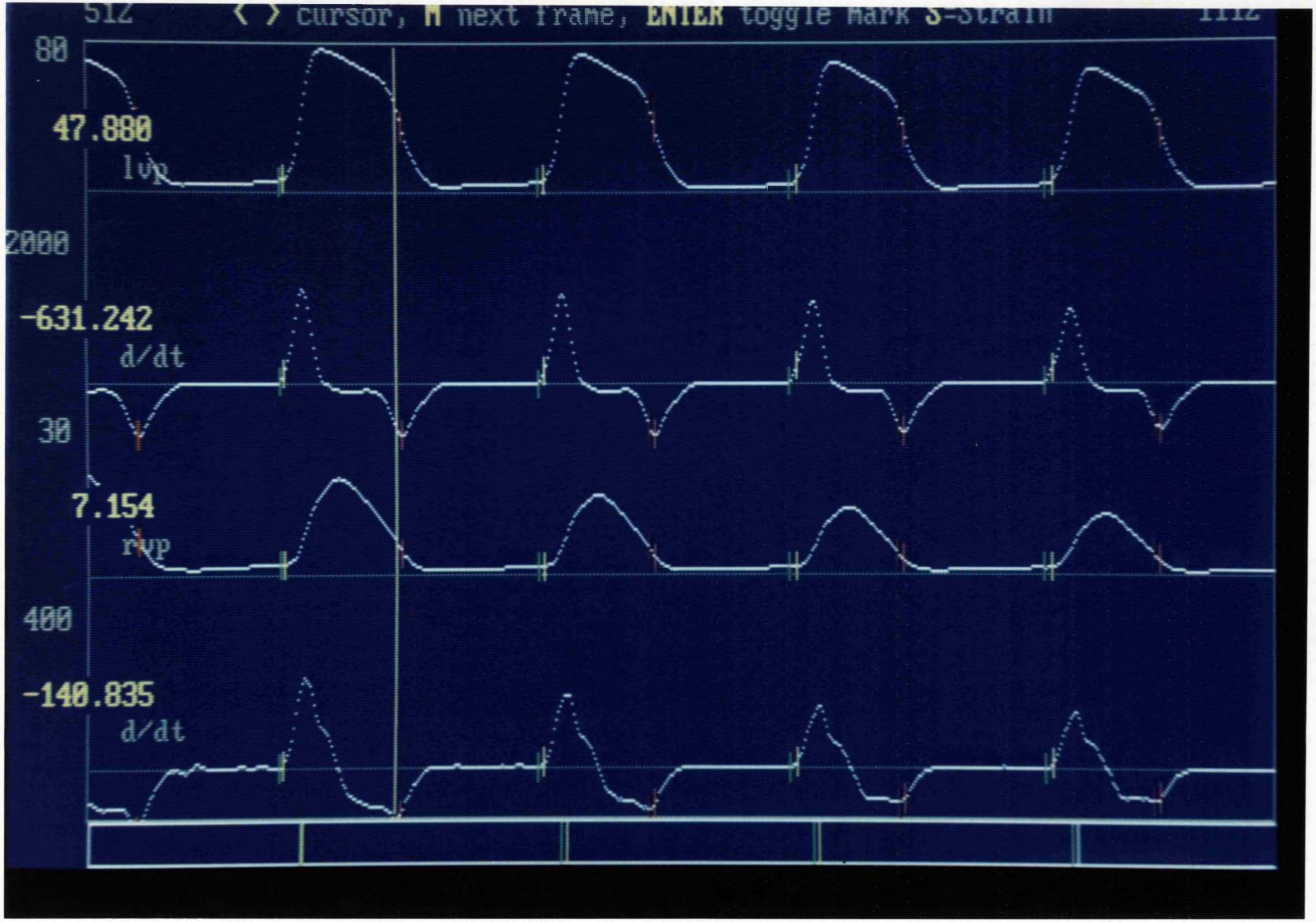
Sequencing of the Cardiac Cycles (500Hz file)

Two channel display of left and right intracavitary ventricular pressures

with their respective derivative channels (dP/dt) plotted below.

Green	Left ventricular end-diastole	20 msec prior to maximum positive dP/dt
Yellow	Right ventricular end-diastole	20 msec prior to maximum positive dP/dt
Red	Left ventricular end-systole	Peak negative dP/dt
Brown	Right ventricular end-systole	Peak negative dP/dt
		(Morris, JI et al, 1986)

Yellow / Green markers below channels indicate maximum positive dP/dt


512
〈 〉 cursor, M next frame, ENTER toggle mark S-Strain
80
47.880
lvp
2000
-631.242
d/dt
30
7.154
rvp
400
-140.835
d/dt

To date right ventricular function following transplantation has not been assessed using the above model, or any other model as a means to calculate pre-load independent recruitable stroke work. It is therefore a specific aim of this thesis to assess this method of biventricular analysis when applied to cardiac transplantation.

Diastolic Function

The study of cardiac performance excluding the systolic phase of the cardiac cycle has become a controversial area. As already described above systolic function of the left ventricle, and more recently the right ventricle, has been the primary area of research. Diastolic function has been applied to a multitude of mathematical models made necessary by the three distinct phases of diastole: early rapid filling during which the velocity and extent of filling are greatest; diastasis in mid-diastole; and atrial systole in late diastole. These phases are not isolated in that ventricular filling and atrial emptying are mutually interacting, coupled processes. For instance if there is impaired global relaxation of the left ventricle then the trans-mitral pressure gradient is diminished. This will impede early passive filling but augment the role of active atrial contraction in late filling.

For the purpose of this thesis diastole will be defined as the portion of the cardiac cycle in which all influences of systolic contraction are absent. Therefore the isovolumic relaxation phase of systole will not be included. This is the period of systole where the myocardium makes a transition from systolic energy generation to a passive resting diastolic state. Relaxation is not fully complete until the first diastolic minimum of ventricular pressure, when half of rapid filling is already accomplished (Rankin et al, 1976). There is some evidence that active restorative forces associated with relaxation assist rapid filling during this period (Yellin et al, 1986), but this theory is disputed.

Thus diastolic mechanics relate to the passive mechanical characteristics of the fully relaxed myocardium and the atrial contraction in late filling. These properties have been shown to be relatively independent of acute pharmacologic alterations in systolic function (VanTrigt et al, 1980).

The relationship between diastolic ventricular pressure (P) and volume (V) is exponential, as with most biological tissues, and also has a finite *x* - intercept. An exponential model predicting zero pressure at a finite volume must be used to quantify this relationship, and a modification of the Glantz equation is ideal for this purpose (Rankin et al, 1977). This uses a non-linear least-squares regression analysis utilising the measurements from several diastoles during a vena caval occlusion:

$$P = \alpha \, (e^{\beta \varepsilon} - 1)$$

where α and β are nonlinear elastic coefficients (Rankin et al, 1980).

$$\varepsilon = (V - V_0) / V_0$$

ε is the strain according to the Lagrangian strain definition, with V being the instantaneous diastolic volumes and V_0 is the *x* - intercept or the unstressed diastolic volume. During rapid filling in diastole the measured ventricular pressure is always higher than would be predicted by a simple exponentially elastic relationship, probably reflecting viscous forces. To reduce these effects the dynamic data are excluded by only sampling values where the dV/dt is less than 5% per second ie, only diastatic measurements. With this technique, directional changes or differences in diastolic mechanical properties can be quantified using standard covariant analysis of: 1) the unstressed volumes, and 2) the nonlinear elastic coefficients α and β.

Atrial Systole

The implantation of the intact heart, using complete atrioventricular transplantation, maintains the integrity of the left and right atria. To explore any benefit of this technique the examination of atrial function was vital, and in particular the comparatson of function during atrial systole.

The benefits of good atrial function during atrial systole are apparent to the majority of cardiac

surgeons; the advantages are evident using atrial pacing or atrio-ventricular sequential pacing to assist weaning a weak ventricle from cardiopulmonary bypass. Therefore any demonstration of conserved atrial systolic function would be strong evidence to support this new technique of orthotopic cardiac transplantation.

The pressure and dimensional data from the experimental preparation afforded an opportunity to specifically scrutinise events during atrial systole. With this aim, a unique and specific computer program was designed.; the analysis focused on the following parameters.

1. 500Hz files were used. The cardiac cycles were defined to allow ensembling of all defined beats, using the ten raw channels of data and the calculated channels of pressure derivative, ventricular volumes and the volume derivatives.

2. The period of atrial systole was defined by a semi-automated process; the left atrial pressure and its derivative channel was displayed. The start of atrial systole was defined as 0 mmHg/sec dP/dt prior to the rise of pressure in atrial systole. The end of atrial systole was defined as end diastole, 20 ms before maximum left ventricular dP/dt.

3. With atrial systole defined, the data during this period was selected for all eighteen channels.

4. Duration of atrial systole was calculated and also as a percentage of the cardiac cycle; this percentage prevented the use of data from animals in first degree heart block.

5. Left atrial pressure was analysed for; value at baseline; maximum value; increase of pressure; time to achieve maximum; time to achieve maximum as a percentage of atrial systole. The derivative of left atrial pressure was analysed for the same parameters including the minimum dP/dt during atrial systole.

This analysis was also applied to right ventricular pressure.

6. Likewise left and right ventricular volumes were analysed for; value at baseline; maximum value; increase of volume; time to achieve maximum; time to achieve maximum as a percentage of atrial systole. The derivatives of ventricular volumes were analysed for the same parameters, and the maximum volumes attained by the left and right ventricles during the cardiac cycle were recorded.

The programme concentrated on parameters that may be influenced by atrial systolic function; pressure change and rate of pressure change in the left and right sides of the heart, and increase plus rate of increase of intraventricular volumes. Right atrial pressure analysis was not included as the pressure signals from the micromanometers in the right atrium are poor quality and not suitable for analysis.

Overall this analysis of atrial systole was designed to identify function by pressure and volume changes during this period of the cardiac cycle for left and right sides of the heart.

PULMONARY IMPEDANCE

Introduction

As techniques in cardiac surgery have improved, with particular regard to myocardial preservation of the left ventricle, it has become apparent that right ventricular dysfunction is a significant source of morbidity and mortality. This is most evident in the field of orthotopic cardiac transplantation where most recipients, due to their left ventricular dysfunction, have an element of increased pulmonary vascular resistance. This may be so marked that the donor heart, despite being matched to the recipient for size and weight, may be unable to function adequately in the short term due primarily to right ventricular failure. Pulmonary vascular hypertension in these patients does resolve in a short time period (Bhatia et al, 1987) and during this time they may be helped by pulmonary vasodilators. However current clinical practice tends to implant 'oversize' donor hearts to recipients,

who have mild elevation of pulmonary vascular resistance, so that the right ventricle can cope with elevated pulmonary pressures acutely.

The interactions of the right ventricle and its afterload, the pulmonary vasculature and pressure in the left atrium, may be assessed by measurement of the pulmonary vascular energetics and their oscillatory nature. This assessment is made by using an ultrasonic flow probe for pulmonary artery flow and a micromanometer for pulmonary artery pressure. These high-fidelity phasic measurements allows Fourier analysis of the waveforms to define pulmonary vascular input impedance and determine the hydraulic work.

Historical Background

Historically assessment of cardiovascular performance is usually performed by measurements of flow and pressure averaged over several cardiac cycles, as is the cardiac output which is made by the average of ventricular output over a period of time. These studies are governed by the principles of steady flow haemodynamics and were limited due to the inability to measure instantaneous flow in vivo and the complex calculations to analyse the results. Now the development of electromagnetic and ultrasonic flow probes, and the increasing ability of digital computers has overcome these limitations and allows further study of pulsatile flow, along with the superimposed pulsations.

The Windkessel theory for arterial wave form originated from Hales in the eighteenth century (Hales, 1733). This postulated that the volume of blood ejected from the ventricle in each systole distends the proximal aorta and its branches, and the elastic energy thus stored in the walls of these vessels is converted to the energy of flow, as the walls recoil during diastole. This explains the gradual decline of arterial pressure during diastole, contrasting with the abrupt drop that would be found in a rigid system of tubes, and is a 'buffering' action. This theory was developed further by Lamb who produced complete equations of wall motion in terms of the elastic modulus of the wall (Lamb, 1898) and Witzig developed a complete analysis of wave propagation, including the effects

of viscosity, that could be adapted to physiological studies (Witzig, 1914). Womersley and McDonald adopted Lamb's formulation of the relations between pressure and radial motion of the vessel wall using Fourier analysis of pressure and flow waves (Womersley, 1955a)(Womersley, 1955b). This analysis was also applied by them to the pulmonary vasculature so that pulmonary artery flow and pressure could be translated into mean and harmonic pulsatile terms (McDonald, 1955).

The pulmonary circulation is a highly compliant, low resistance conduit subject to nervous, hormonal and intrinsic control. As with the systemic circulation, flow pulsations in the pulmonary arterial tree diminish as they move distally (Milnor, 1972a)(Milnor, 1972b). Traditional evaluation of right ventricular function includes the measurement of mean pulmonary blood flow (cardiac output), right ventricular pressure, pulmonary artery pressure (PAP) and either left atrial pressure (LAP) or pulmonary capillary wedge pressure. The external work performed by the right ventricle is related to these terms and is either expressed directly or assessed in relation to the mean driving pressure (PAP - LAP), divided by the cardiac output, which yields the pulmonary vascular resistance. Through Fourier analysis of phasic pulmonary artery flow and pressure waveforms the harmonic components of flow and pressure are generated (fig.9).

Each harmonic is a multiple of the fundamental frequency (heart rate) and represents an oscillatory component of the basic waveform at that frequency. When all harmonics are summed with the mean harmonic the flow and pressure waveforms are exactly reconstituted. The division of modulus (or value) of pressure $|P|$ by the modulus of flow $|Q|$ at each harmonic frequency yields the oscillatory counterpart of resistance which is the impedance $|Z|$.

$$|Z| = |P| \div |Q|$$

This technique allows a complete and quantitative description of pulmonary vascular events in the form of vascular impedance and is theorized to give information regarding the physical state of the

pulmonary vasculature (Caro and McDonald, 1961).

The ratios of pressure to flow harmonics constitute the impedance spectrum and their products give the power spectrum (fig.10). The greater the number of harmonics that are calculated from a single pulse the more precise will be the sum of the Fourier terms in matching the original data. Cardiovascular pulsations of pressure and flow can be approximated quite closely by the sum of 10 harmonics. The hydraulic power represented by the first 10 harmonics is more than 99.5% of that in the original pulsations (Milnor et al, 1966), and beyond the tenth harmonic signals fall below the 'noise' level in most experimental situations (i.e., the harmonic moduli usually decrease as the harmonic number rises).

A phase angle is generated for each harmonic of flow and pressure. The difference in the phase angles of flow and pressure is the impedance phase angle (ZpH), which reflects the degree to which flow leads pressure or vica versa at each frequency.

$$ZpH = P(phase) - Q(phase)$$

The significance of this analysis is apparent when the power produced by the right ventricle is considered in mean and oscillatory terms (fig.10). In normal circumstances 30% of right ventricular work is used to create pulsations in the pulmonary bed (Milnor et al, 1966) but when the characteristic impedance is elevated the amount of this work is proportionately increased (Hammon et al, 1977).

The largest moduli of impedance are found at frequencies below1 Hz and then decline with frequency to a minimum between 2 and 4 Hz, rising subsequently to a maximum at 6 to 8 Hz. Beyond 10 Hz the impedance moduli remain almost constant as the frequency increases. Impedance phase is negative (i.e., flow leads pressure) at low frequencies becoming less negative as frequency rises (figure 8). The phase changes from negative to positive at about the same

frequency as the minimum modulus. A positive maximum of phase usually appears between 6 and 8 Hz followed by a return toward zero at higher frequencies. The range of values for impedance phase recorded experimentally between 1 and 12 Hz is ordinarily ± 1.0 radian.

The value usually plotted for the impedance modulus at a frequency of zero is that of the pulmonary vascular resistance (Milnor 1972a). However this is calculated from pressure-drop across the bed and input resistance (Rin) may be calculated using the mean pulmonary artery pressure along the bed. This is more applicable as other impedance moduli are calculated from the pulmonary artery pressure and flows without regard for the pulsations at the terminus of the bed, and so in this thesis Rin is used to denote the zero-frequency impedance.

The average of moduli between 7 and 11 Hz is a useful value in describing impedance spectra and is an estimate of *characteristic impedance* |Zo| (Hammon et al, 1981). The minimum and maximum moduli can then be expressed as proportions of this average impedance. The extent to which |Zx| rises and falls with frequency depends on the size of waves reflected from the periphery. Peripheral vasoconstriction increases reflections and dilation reduces them, producing similar changes in the fluctuation of |Zx| with frequency. Reflections determine the ratio Zx/Zo, and a useful measure of oscillations in the spectrum is the difference between the amplitudes of Zx at the first minimum and the first maximum, divided by Zo.

$$(Zx(max) - Zx(min)) \div Zo$$

Therefore, changes in the amplitude of the minimum and maximum impedance moduli may be attributable to changes in the distal part of the bed, while alterations of the frequency of the *phase zero crossing* suggest alterations in the elasticity of the larger arteries.

Therefore the impedance spectrum summarizes the effects of the properties of the vascular bed on pressure-flow relationships at the input. However these relationships will apply to any input signal only if the system is *linear*.

Figure 8

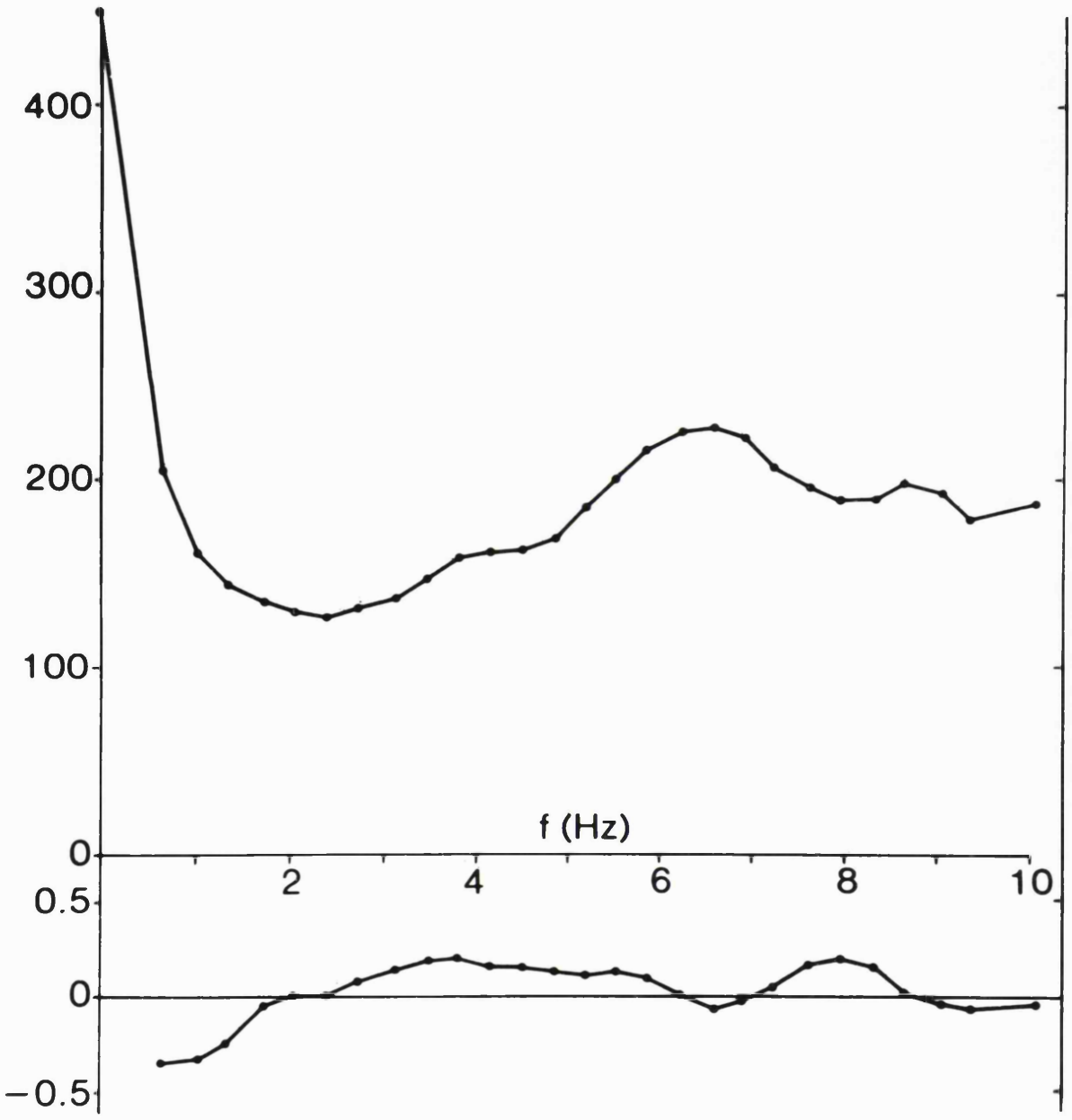
Input Impedance.

Input impedance in the main pulmonary artery of an anaesthetised dog.

Y axis top graph; Impedance modulus, dyn/sec/cm^{-5}.

Y-axis lower graph; Impedance phase, radians. Positive phase indicates that pressure leads flow.

(Milnor, 1989)


400
300
200
100
0
f (Hz)
2
4
6
8
10
0.5
0
−0.5

Linearity of a Haemodynamic System

The value of the concept of impedance relies on the assumption that the arterial system is linear with respect to pressure and flow. This assumption presupposes that a periodic signal is equivalent to the sum of a series of sinusoidal waves, a proposition that may be demonstrated mathematically. Linearity may have a variety of definitions but in this context it means two things:

(1) Pressure waves at a given frequency produce flow waves at that frequency and no other; and

(2) that the pressure / flow ratio at any frequency is independent of the size and shape of the pressure pulse.

In a non - linear system an input of sinusoidal pressure waves at one frequency may produce flow waves at other frequencies, and the magnitude of flow waves may increase non - linearly as pressure increases.

If a haemodynamic system is linear and its properties remain constant, the amplitude and phase of any flow harmonic will be linear functions of the amplitude and phase of the corresponding pressure harmonic, regardless of the size of that pressure harmonic or of other frequency components that are present at the same time (McDonald, 1974). Linearity therefore guarantees that impedance is an indication of the physical state of the blood vessel, not of the particular pulsations that exist at that moment. Perfect linearity is not to be expected and rather than treat the arterial system as linear it is better to allow for errors introduced by treating it as if it were linear (Milnor, 1972a).

One standard technique is to measure the output of a system in response to several different inputs, applied singly or in combination. Sinusoidal pressure waves are taken as inputs in the present context and flow waves as the output. Whilst sinusoidal generators may be used to produce inputs, the natural pulsations in vivo provide an equally good source since they already contain an

assortment of different frequencies and amplitudes. Thus a variety of input signals may be provided by changes in rate and if all other factors are controlled so that the physical state of the artery can be assumed to be constant, then the derived impedances should remain the same if the system is linear. Bergel and Milnor have shown this to be the case with the pulmonary artery behaving in an approximately linear fashion (Bergel and Milnor, 1965). Milnor has also demonstrated that linearity is not a prerequisite for Fourier analysis. The sum of a Fourier series can be made to approximate any waveform more closely by increasing the number of Fourier terms, whether or not the original pulsation was recoded from a linear system (Milnor, 1989).

Fourier Analysis

J.B.J. Fourier (1768 - 1830) was a French savant who accompanied Napoleon to Egypt. He was the first to show that repetitive pulsations of any shape are equivalent to the sum of a series of sinusoidal waves. Therefore the most effective manner to express the numerical properties of haemodynamic waveforms is to break them down into their component frequencies. He originally applied these problems to the transfer of heat from one body to another and is now valuable in fields ranging from astronomy to molecular biology (Judson, 1979). The great value of this technique arises from the subsequent mathematical techniques that have been developed for sinusoidal functions. Once the response of a linear system to sinusoidal signals of various frequencies is known then its response to any periodic waveform can be predicted.

The number of **sample intervals** required is determined by two principles:

1) The frequencies present in the record; and

2) The number of harmonics that are needed.

In the latter principle the rule is simply N / 2 to calculate the number of harmonics to be determined where N is the number of intervals taken in one cycle. Any harmonics that are higher are indeterminable (McDonald, 1974). Thus, if ten points are taken, five harmonics would be valid. Where the sampling rate is set per second, the same rule applies to the maximum frequencies that are analysable, i.e., 100 samples/sec allows up to 50 Hz to be analysed. The number of harmonics

this represents depends on the heart rate, e.g. if this is 2.0 Hz it is twenty-four harmonics, whereas with a rate of 3.0 Hz it is only sixteen harmonics. If there are frequencies present beyond the limit set by the sampling rate (the 'folding' frequency) , then they will introduce errors into the components analysed. This phenomenon is known as 'aliasing' (Blackman and Tukey, 1958).

In this study the use of analogue-to-digital conversion places an upper frequency limit on Fourier analysis by limiting the number of discrete observations made of the contiguous analogue data. The sampling rate is 500 Hz allowing 500 observations/cycle at a heart rate of 60 beats/minute or 1 Hz. This will enable calculations of harmonics to be performed with frequencies as high as 250 Hz (no. of observations/2 harmonics) (Attinger et al, 1966).

Pulmonary artery flow data will be subjected to Fourier analysis such that

$$Q(t) = Q_0 + \sum_{n=1}^{10} Q_n \sin (n\omega t + \Phi_n)$$

where Q_0 is mean flow and Q_n is the amplitude of the nth harmonic.

Pressure waveforms are similarly expressed,

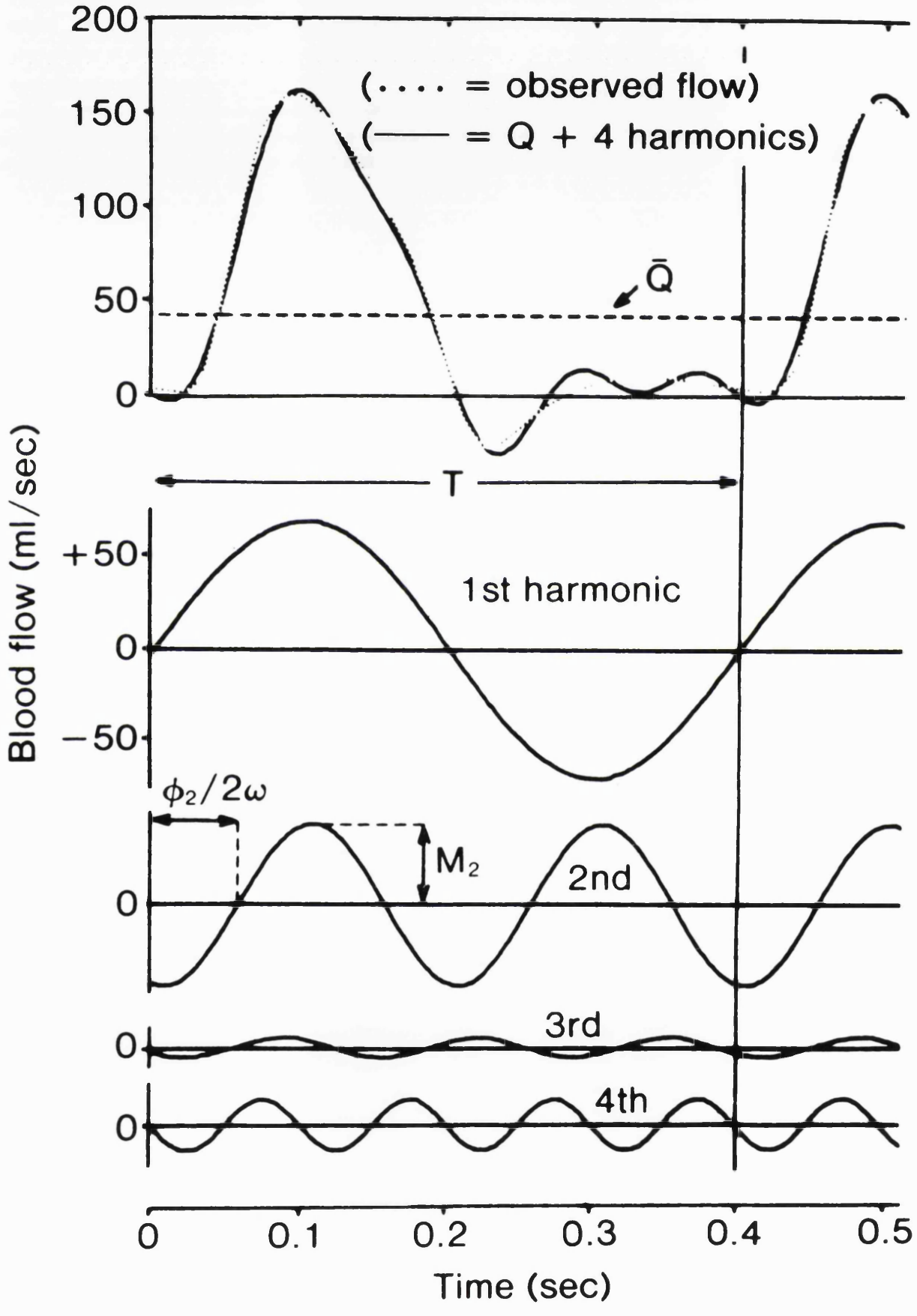
$$P(t) = P_0 + \sum_{n=1}^{10} P_n \sin (n\omega t + \beta_n)$$

where P_0 is mean pressure, P_n is the amplitude of the nth harmonic and β_n is the phase angle at the nth harmonic.

Figure 9.

Fourier Analysis

Diagrammatic representation of Fourier series derived from an experimentally recorded flow pulsation. The uppermost graph is a record of pulmonary artery flow in a dog which was converted to digital form and subjected to harmonic analysis. The resulting Fourier series consists of the mean flow (Q) and a series of sinusoidal waves, or harmonics. The first harmonic is at the frequency of the heart beat, the second is twice that frequency etc. The sum of all terms in the Fourier series approaches the original wave more and more closely as additional harmonics are computed. In this example the sum of the four harmonics is plotted as a continuous line on the top graph (original flow graph is dotted line). The amplitude of each harmonic is termed its modulus (labeled *M* in the second harmonic) and the timing of each sinusoidal wave in relation to others is called its phase angle (Φ). (Milnor, 1989).


200
150
100
50
0
(···· = observed flow)
(—— = Q + 4 harmonics)
$\bar{Q}$
T
Blood flow (ml/sec)
+50
0
−50
1st harmonic
$\phi_2/2\omega$
M_2
2nd
0
3rd
0
4th
0
0
0.1
0.2
0.3
0.4
0.5
Time (sec)

Division of each of the sinusoidal terms (P_n / Q_n) gives the input impedance (Z_n for the nth harmonic. The corresponding impedance phase angle Θ_n is derived from the subtraction of the flow phase angle from the pressure phase angle (β_n - Φ_n). The first harmonic term has the frequency of the resting heart rate, or fundamental frequency.

Analysis of Hydraulic Power.

The computations of impedance and hydraulic power are based on harmonic analysis where the observed pressure and flow waves are expressed as Fourier series. Flow can be represented as a function of time, Q(t), by: (1)

$$Q(t) = Q_0 + \sum_{n=1}^{10} Q_n \sin (n\omega t + \Phi_n)$$

where Q_0= mean flow; the subscript n indicates the harmonic number; N = the total number of harmonics included in the series; Q_n is the amplitude of the nth harmonic and Φ_n its phase angle; and ω the fundamental frequency of pulsation in radians/second. Pressure waves can be expressed in the same form, and if P_0 = mean pressure, P_n = amplitudes of the pressure harmonics and β_n their phases, then the impedance modulus, Z_n, at a frequency of $n\omega$ is: (2)

$$Z_n = P_n / Q_n$$

and impedance phase at that frequency, Θ_n, is: (3)

$$\Theta_n = \beta_n - \Phi_n$$

The hydraulic energy of the blood at any site in the circulation is of three different kinds:

(a) Pressure energy, W, is a kind of potential energy. The product of intravascular pressure and the volume rate of blood flow at any instant equals potential power expressed as potential energy per unit time.

(b) Kinetic energy, K, which is equal to one half of the blood mass, m, multiplied by the square of its velocity ($m = \rho v$, where ρ = blood density, and v = the volume of blood considered).

(c) Energy of position which is energy conferred by virtue of the vertical height of the blood above the level of reference. In pulmonary impedance studies for example the inlet and outlet of the pulmonary bed are at almost the exact same level and therefore assumed to be negligible.

Accurate determination of the hydraulic energies involved in blood flow requires continuous measurements of instantaneous blood flow and pressure, and integration of the appropriate quantities throughout the cardiac cycle. Hydraulic pressure energy and kinetic energy can each be divided into a portion associated with mean pressure and flow, which are the energies that would exist if there were no oscillations around the mean, and a fraction of energy involved in pulsations.

The average energy per unit time, or average steady power, was calculated for pressure energy associated with mean terms by multiplying mean pressure and mean flow: (4)

$$W_m = P_0 \; Q_0$$

The oscillatory fraction of pressure energy, W_0, can be calculated by multiplying the oscillatory terms of the Fourier expressions for pressure and flow. This may be put in a convenient form for this calculation by substituting the impedance-flow relationships for pressure in equations 2 and 3 giving the following equation: (5)

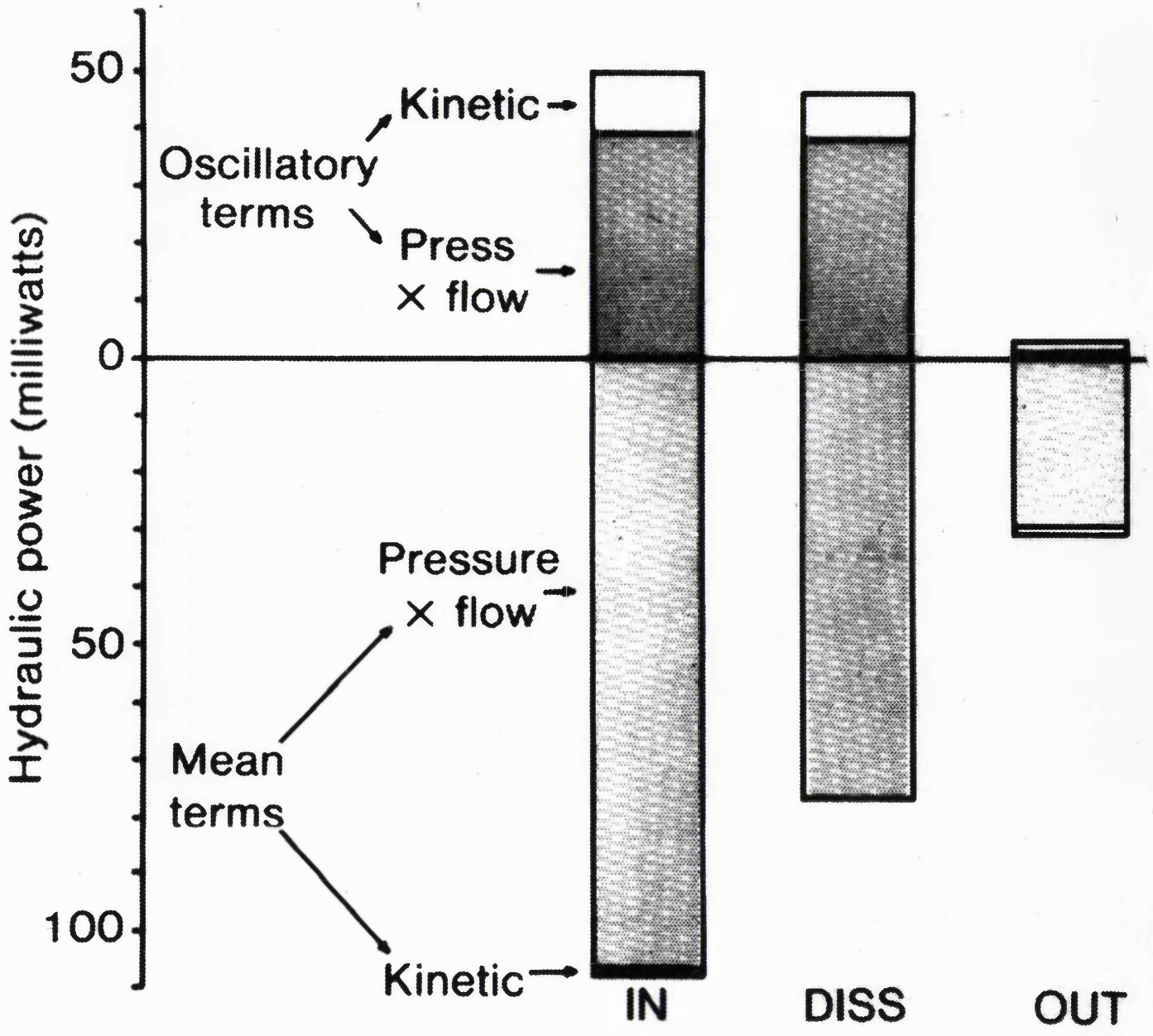
Figure 10.

Profile of Hydraulic Power from the Right Ventricle.

Average hydraulic power supplied by the right ventricle (IN), dissipated in the pulmonary vessels (DISS), and remaining at the terminus of the pulmonary veins (OUT) in an anaesthetised, open chested dog.

Each bar is divided into kinetic and potential (pressure X flow) power components, subdivided into oscillatory and steady (mean) parts.

(Milnor et al, 1966)


50
0
50
100
Hydraulic power (milliwatts)
Oscillatory terms
Kinetic
Press × flow
Pressure × flow
Mean terms
Kinetic
IN
DISS
OUT

$$W_O = 1/2 \sum_{n=1}^{10} (Q_n)^2 Z_n \cos \Theta_n$$

The total hydraulic power as pressure energy (W_T) is the sum of these two components: (6)

$$W_T = W_M + W_O$$

Vascular Input Impedance.

Vascular input impedance completely describes the resistance to pulsatile blood flow in a vascular bed. The mean terms are the direct ratio of mean pressure to mean flow while calculation of the oscillatory terms requires Fourier analysis of the pulsatile pressure and flow waveforms. Fourier analysis is computationally intensive as it requires multiple calculations of trigonometric functions and is only practically possible on computers.

In this thesis the pulmonary blood flow is measured using an ultrasonic flow probe (Transonic Systems Inc., Ithaca, New York) positioned around the main pulmonary artery just distal to the pulmonary valve, while pressure was simultaneously measured at the same location using a micromanometer - tipped catheter (Millar Inc., Houston, Texas) coupled to a pressure amplifier (Hewlett - Packard Inc., Palo Alto, California) (figures 18 & 19). All analogue flow and pressure data were digitised at 500 Hz with 12 bit resolution via a 10 channel, differential input, analogue-to-digital converter (Scientific Solutions Inc.,WSolon, Ohio). Phase delay between data channels was 25.0 x 10^{-6} sec. Digital data were analysed using a personal computer (Zenith Data Systems Z-386/20).

The mechanics of respiration has significant effects on right ventricular filling, pulmonary blood flow, pulmonary impedance, left ventricular filling and cardiac output (Milnor, 1989). Therefore to negate any effects from mechanical respiration the ventilator was always disconnected prior to any haemodynamic data collection.

Software responsible for data acquisition was developed in this laboratory and was written in assembly language in order to minimise phase delay between data channels. The soft programmes responsible for mathematical calculations and graphical waveform display were written in C to take advantage of the graphics capabilities and input/output functions of a high-level language.

Cardiac cycle endpoints of end-systole and end-diastole were defined graphically using dp/dt trace of right ventricular pressure. This was done with a semi-automated process using user-defined trigger levels for the entire six seconds of cardiac cycles. (figure 7).

Therefore pressure and flow data were acquired at 500Hz for six seconds and the waveforms of each cardiac cycle was analysed by software implementation of the discreet Fourier transform. No attempt was made to normalise data sets to an arbitrary number of points. The sampling interval was 0.005 seconds with the number of samples per cardiac cycle was inversely proportional to heart rate.

The vascular input impedance was determined by division of the modulus of pressure by the modulus of flow at each harmonic frequency, while the corresponding phase angles were determined by subtraction of the flow phase angles from the pressure phase angles. Characteristic impedance was calculated as the average modulus of impedance from 7 to 11 Hz. Post-acquisition analysis of each cardiac cycle was displayed graphically in Fourier transformations and subsequently the impedance moduli and phase angles were processed to produce complete impedance spectra.

Conventionally 6 seconds of continuous data were stored on a hard drive in binary sequential format to comprise one data set that was individually labelled. Without further processing these files were available for further analysis and processing. This analysis would take five minutes for each data set from which the relevant data could be transferred to a spreadsheet for assessment. As little

as five years ago the calculation of impedance was a laborious process involving the storage of analogue data on magnetic tape for later digitisation and then computational analysis, a lengthy procedure taking approximately 40 minutes for each data set.

Therefore computational speed is essential for Fourier analysis due to the iterative calculation of trigonometric functions. Speed is greatly enhanced by math co-processors in microcomputers as they include many high level functions, such as tangent function, which can be performed on an 80-bit floating point number in less than 500 clock cycles, or approximately 42 microseconds at 12 MHz.

Further increases in the speed of calculation of Fourier transforms are possible when appropriate assumptions are made with respect to the input data. Algorithms that use these assumptions are collectively termed Fast Fourier Transform routines. It has been shown that 99.5% of the information present in the original analogue signal is contained in the first ten harmonics of heart rate and little significant information is gained above 12Hz. Therefore to increase speed only the first ten harmonics are calculated. From this statement it follows that fewer harmonics are required as heart rate increases, but to ensure maximum retention of information from the original analogue waveform the Fourier analysis is always applied to the first ten harmonics.

ß-Receptor Function

The effect of brain death on ß-receptor function has not been clearly evaluated. The catecholamine storm combined with cardiac denervation may have superimposed or conflicting effects. Initial research into the influence of denervation on ß-receptor function was controversial. Fortin described denervation did not effect B-receptors in the rat kidney, results reinforced by Melvin who studied receptors in the rat parotid gland (Fortin and Sundaresan, 1989)(Melvin et al, 1988). Excess circulating catecholamines have been found to down-regulate the ß-adrenergic receptor system (Limas and Limas, 1984), whereas reduced catecholamines and chemical denervation has been shown to up-regulate the system(Fowler et al, 1984)(Glaubiger et al, 1978). This up-regulation has

also been reported with myocardial ischaemia and corticosteroid therapy (Mukherjee et al, 1982)(Davis and Lefkowitz, 1984). Sakagoshi et al studied ß-receptor function post brain death in the canine model, using inflation of an intracranial balloon to instigate brain death. They showed no change in ß-receptor function after six hours of brain death despite a recorded decrease in circulating adrenaline. Moreover when they administered catecholamines there was a down-regulation of the receptors (Sakagoshi et al, 1992). Work by the same author showed hypothermic preservation to increase ß-receptors, but if down regulation due to ischaemia had occurred prior to preservation, this increase of ß-receptors post preservation was abolished (Sakagoshi et al, in press).

The conclusions to be drawn from this work relate to management of the organ donor, in that inotropic support using ß-agonists prior to harvesting of the heart may be detrimental to cardiac function post implant. Moreover, if catecholamines are avoided in the donor there may be an up-regulation of the ß-receptor system, that could be beneficial to the outcome of the recipient.

The model of brain death used in this thesis provides an opportunity to further assess ß-receptor function post brain death, and provide insight to improve the protocols for management of the brain dead donor.

CHAPTER 3

PROJECT AIMS

The aims of the project will be to test the following hypotheses:

(A) That right ventricular function, along with left ventricular function, may be reliably measured in hearts that have been transplanted, in terms of pre-load recruitable stroke work derived from pressure volume relationships.

(B) Complete atrioventricular transplantation leads to improvement of post-transplant cardiac function compared to ventricular transplantation with atrioplasty, when assessed for systolic and diastolic function with particular regard for atrial systole.

(C) That a reliable animal model of brain death may be validated, with reproducible effects on haemodynamic, hormonal and metabolic parameters.

(D) Brain death causes damage to the myocardium as measured by its effect on haemodynamic function and performance, and by receptor and histological assessment.

(E) Prolonged hypothermic preservation is adversely effected by brain death with regard to haemodynamic function post transplantation.

(F) Following brain death the ability of the right ventricle to work against acutely raised pulmonary vascular resistance is impaired.

A to B, and D to F will be tested sequentially in parallel studies, with each hypothesis dependent on the validation of the preceding hypothesis.

Specific Aims

In testing the above hypotheses the studies will be planned to reach specific aims as follows;

1. To characterise the effect of acute increase of intracranial pressure on:

   direct cardiac effects as measured by preload recruitable stroke work, receptor status and histological changes:

   systemic effects assessed by haemodynamic, hormonal and urine output:

   the pulmonary vasculature with regard to pulmonary impedance.

   Combining these measurements with pathological assessment of the cerebrum and brain stem at the termination of the experiment, this animal model of brain death may be validated.

2. To perform complete atrioventricular transplantation in the canine model, not previously completed successfully, and to compare it to ventricular transplantation with atrioplasty, the "standard' technique. This will be accomplished using analysis of load independent systolic function as well as diastolic functional analysis, with particular attention addressed to atrial systole.

3. Having elucidated the effect of brain death on cardiac function, orthotopic transplantation of such hearts will be performed after two different periods of preservation. With comparison to control groups, the effect of brain death on the preservation characteristics of the heart can be measured with regard to function, post implantation.

4. The ability of the right ventricle to pump against an acutely raised pulmonary vascular resistance, as in the clinical situation of heart transplantation, will be assessed in the brain dead model.

Animal Model

The dog was chosen as the experimental animal due to its suitability as a large animal for orthotopic cardiac transplantation. Their hearts withstand the manipulation required for instrumentation without recurrent problems of tachyarrhythmias that are so often encountered in the

swine model . The anatomy of the canine heart is very similar to the human, lending itself to comparisons of surgical technique in the clinical situation (McLaughlin et al, 1961).

It has been extensively used in past studies not only for transplantation but also for the haemodynamic models that are used in this thesis (Milnor, 1972a). Therefore there is more information in the literature related to this work on canine physiology, and pathophysiology, allowing useful comparisons to other studies. Dogs are compliant animals to work with and relatively more available than the bovine or primate models.

The choice was therefore between the swine and canine model. The advantage of the pig is its availability but to perform these experiments on swine would have required very large numbers due to a high failure rate. Moreover the slight difference in anatomy, with regard to complete atrioventricular transplantation, would make any extrapolation from the pig to the human model very difficult. The dog, barring humane aspects, was by far the best animal to use, so as to obtain the most relevant data with the least possible sacrifice of animals. Results may also be compared to numerous studies, previously performed using the canine model.

Statistical Analysis.

Statistical analysis was performed on a Zenith personal computer using the SAS statistical software package (Cary, NC). All data were tested for normality prior to further analysis. Regular consults were made with Mr. William White, statistician for the Department of Surgery, regarding the application and validity of the analyses.

For animal studies in chapter 5, the validation of brain death, haemodynamic data were analysed using paired Student's t-tests. In all studies to compare data from a single animal this test was applied, such as comparing post to pre transplant values for pulmonary impedance.

The remaining statistical comparisons use derivations of analysis using Anova. This is applicable to

biological systems that assumes a normal distribution of variance. To compare animals in different experimental groups ie standard versus complete transplantation, an unpaired Student's t-test was used. This was also used to compare animals in chapter 9 to groups B, D and the validation group for haemodynamic changes post brain death.

In chapters 7 and 8 the experiments were designed to allow two-way multivariate analysis to assess the **main effects** of the **factors** brain death and four hour preservation, and also the **interaction** between them. In studies based on observational data, multifactorial analysis permits a ready evaluation of interaction effects and economizes on the number of cases required for analysis. However these advantages do not infer that the more factors should be included. Experiments involving many factors become complex. It is often better research strategy to begin with few factors, investigate the effect of these, and extend the investigation in accordance with the results obtained to date (Neter et al, 1985).

Finally in chapter 8 the Mantel-Haenszel test was used. This was to compare the need for inotropic agents post transplant in the 4 groups involved in the two way analysis. Due to three groups having zero requirements for inotropes, two way Anova analysis was not satisfactory, and the Mantel-Haenszel test used. In essence this test introduces inotrope as a third factor and performs two way analysis on eight cells rather than four.

Research-Ethical Aspects.

The research involves animal subjects. All animals will receive humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences (National Institutes of Health Publication no. 86-23, revised 1985). A minimal number of animals will be used (consistent with reaching sound statistical conclusions) and all invasive procedures to be performed under general anaesthesia.

CHAPTER 4

PRELIMINARY STUDIES

Introduction

The study of previously published literature revealed the need for further investigation into brain death and its effect on cardiac function. In particular load independent analysis of ventricular function can be applied to this model, also obtaining information regarding effects on right ventricular function. The aim of this thesis was to apply this knowledge to orthotopic cardiac transplantation and examine the effect of brain death on prolonged myocardial preservation.

The laboratory and associated facilities were equipped to an excellent standard with experienced personnel. However the complex techniques required to operate on the animals, so as to reliably instrument the heart and acquire data in a reproducible method, necessitated pilot experiments. These experiments were divided into two groups. The first group used the pig model to acquire practical experience in the application of piezo-electric crystals, micromanometers and flow probes. Subsequent to instrumentation, the aim of the first group of experiments was to also obtain data and analyse the results. The second group used the dog model to further practice the practical skills learnt from the pig model, acquire data, and to instigate brain death. This was in preparation for subsequent planned studies to validate brain death and to measure systemic and cardiac functions.

Therefore the overall aim of the pilot studies was to establish the feasibility of the model. They allowed experience in the induction and maintenance of anaesthesia and give familiarity with the technical equipment. These studies were essential to learn the methodology for instrumentation of the heart, recording data, the instigation of brain death and the techniques for tissue biopsies.

Materials and Methods; Swine Model

The pig (Sus scrofa) was used for the preliminary surgery. This animal was chosen as it is a large animal with similar anatomy to the dog and human appropriate for preliminary studies where technical difficulties may occur.

Figure 11.

The anaesthetised dog model and operative set-up within the laboratory.

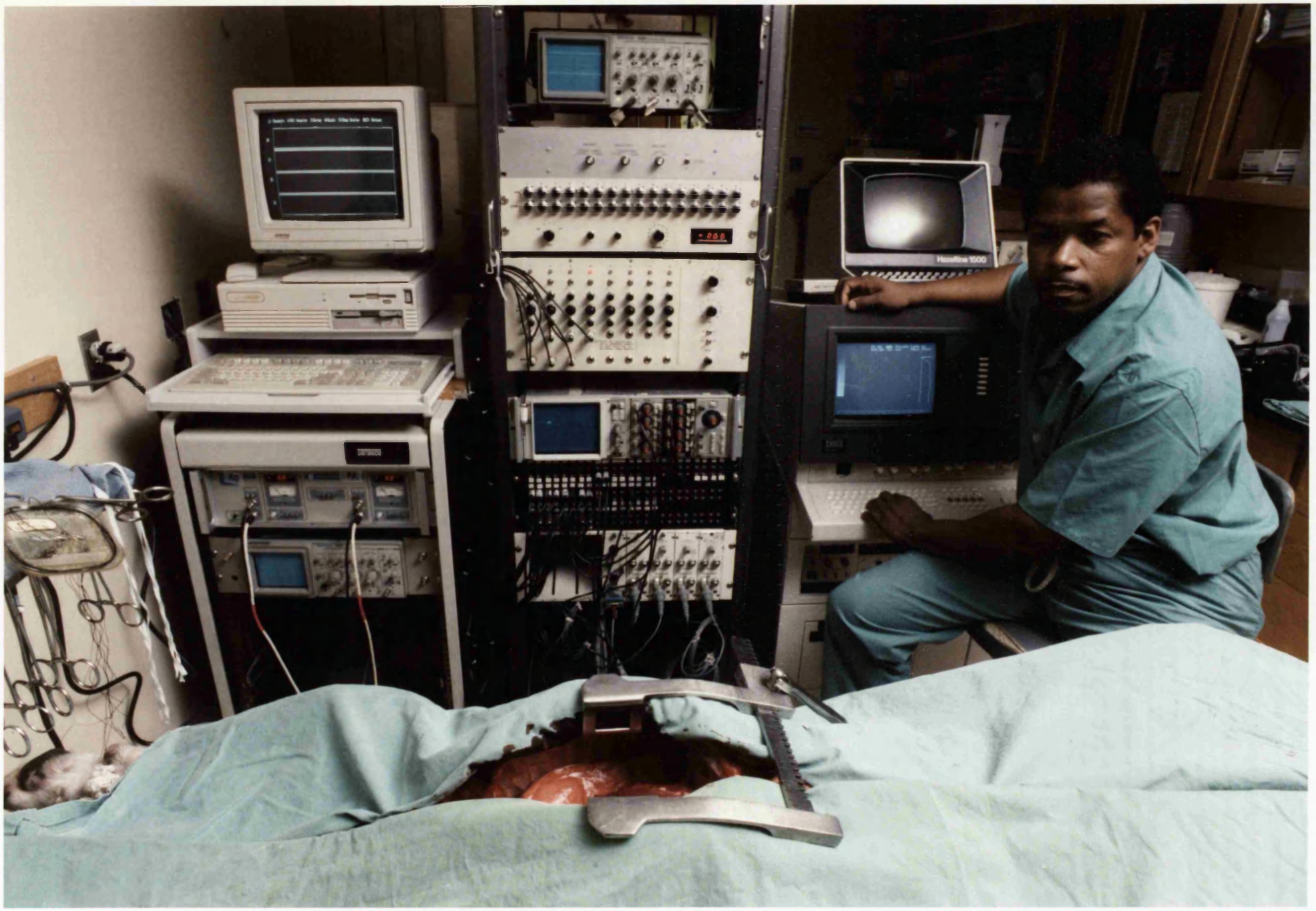
Foreground; Anaesthetised animal prior to cardiac instrumentation.

Right rack; PC computer for data assessment and collection. Situated below is the flowmeter.

Middle rack; Sonomicrometer with oscilloscope at top. Analogue to digital converter in the middle

and micromanometer amplifiers with oscilloscope on the bottom.

Mr. Kurt Campbell was fundamental to the successful outcome of the experiments; he worked hard in maintaining and running the laboratory to a very high standard.


Headline 1500

Three female pigs were used (28.5kg to 38.2kg) and prepared in the same way for surgery, while pressure and flow measurements were taken and analyzed using equipment to be described under "Haemodynamic data acquisition". Data acquired from these recordings were analyzed as described in the Chapter 2, page 46.

Anaesthetic Technique

Each animal was pre-medicated with an intramuscular injection of ketamine (22mg/kg) in combination with acetylpromazine (1.1 mg/kg). This was injected into the gluteal region and within 5 minutes the animal would become drowsy. A 16 French gauge intravenous catheter was then inserted into a marginal ear vein and 15 mg/kg of pentothal was injected intravenously. Prophylactic antibiotics of 1 vial of Combiotic and 2mg/kg of gentamicin were administered intramuscularly.

The animal was intubated with an 7.0 mm Murphy endotracheal tube and ventilated with a minute volume of 6 to 8 litres using a Bear Adult Volume Ventilator (the tidal volume was derived from the formula, 15 mls/kg of body weight), with a respiratory rate of 14-18 breaths / minute. Animals were maintained on a fractional inspired oxygen concentration (FiO_2) of 60% and serial arterial blood gas analysis was performed to assess stability and adequacy of ventilation. The arterial blood gas analysis included arterial pH, partial oxygen pressure, partial carbon dioxide pressure, oxygen saturation, haematocrit, sodium, potassium, calcium, base deficit and both corrected and uncorrected bicarbonate (Gem-Stat, Mallinckrodt Sensor Systems, Ann Arbor, MI). These were measured at hourly intervals and fifteen minutes after changes to ventilator settings or medications administered.

The animal was paralyzed using intravenous (IV) Pavulon, and analgesia was administered using fentanyl 0.03 mg/kg IV initially, and then 0.02 mg/kg IV thereafter as a bolus injection as required when heart rate and blood pressure increased. Intravascular volume was supplemented with intravenous Ringer's Lactate solution to maintain central venous pressure and arterial pressure. Wherever possible, drugs with the least effect upon cardiopulmonary function were administered.

Haemodynamic Monitoring

Using open exposure of the right femoral artery a 14 French gauge polyethylene cannula was inserted to the artery and connected via a pressure transducer to a Mennen - Medical (model 450) pressure amplifier.

Procedure

With the pig anaesthetised, paralysed and ventilated, electrocardiograph (ECG) electrodes were appropriately placed and connected to the ECG monitor (Mennen - Medical (model 450)). A diathermy plate was placed under the animal which was shaved over the anterior chest wall. Median sternotomy was performed with an oscillating saw and the thymus gland excised. The azygos vein was doubly ligated, and divided, and tapes passed around the superior and inferior vena cavae (SVC and IVC). The pericardium was opened in the midline and tapes passed around the ascending aorta and pulmonary artery.

Ventricular Geometric Measurement

Six piezo-electric ultrasound crystals (1.5 mm external diameter, No.1-1015-5A, Vernitron, Bedford, Ohio) for sonomicrometry were then stitched to the heart and one further crystal inserted into the septum (figures 12 & 14). Placement of the crystal at the base of the left ventricle is facilitated by rotating the entire heart 90^0 to lie in the right chest while retracting the pulmonary artery cranially and the left atrium caudally (fig 13). The crystal could then be accurately sutured posterior to the circumflex coronary artery between the pulmonary artery and base of the left atrium. The opposite crystal to measure the major axis was sutured to the apex of the heart. One pair of crystals was situated astride the minor axis of the left ventricle; anteriorly the crystal is placed between the left anterior descending coronary artery and the origin of the first diagonal branch from this vessel; posteriorly the crystal is placed near to the atrioventricular groove between the distal circumflex and posterior descending coronary arteries. A pair of crystals was sutured astride the transverse diameter of the left and right ventricles; the left ventricular crystal placed on the epicardium between the first and second obtuse marginal coronary arteries, and the right ventricular crystal placed

cranial to the marginal branch of the right coronary artery. A septal crystal (Fig. 14) was positioned 2cm deep into the interventricular septum, inserted to the right of the left anterior descending artery. Insertion of the septal crystal is achieved in the following manner; a 16 gauge needle is used to ascertain the correct direction into the septum before the crystal is inserted using a rigid introducer (fig 15). This introducer is removed from the heart over a Seldinger wire that keeps the crystal at the correct depth. The desired placement of this latter crystal was directly below the endocardium of the right ventricular septum on an imaginary line between the latter pair of crystals. To prevent ventricular dysrhythmias during insertion, a prophylactic bolus of 50 mg 1% lignocaine was administered intravenously.

Calibration of crystals

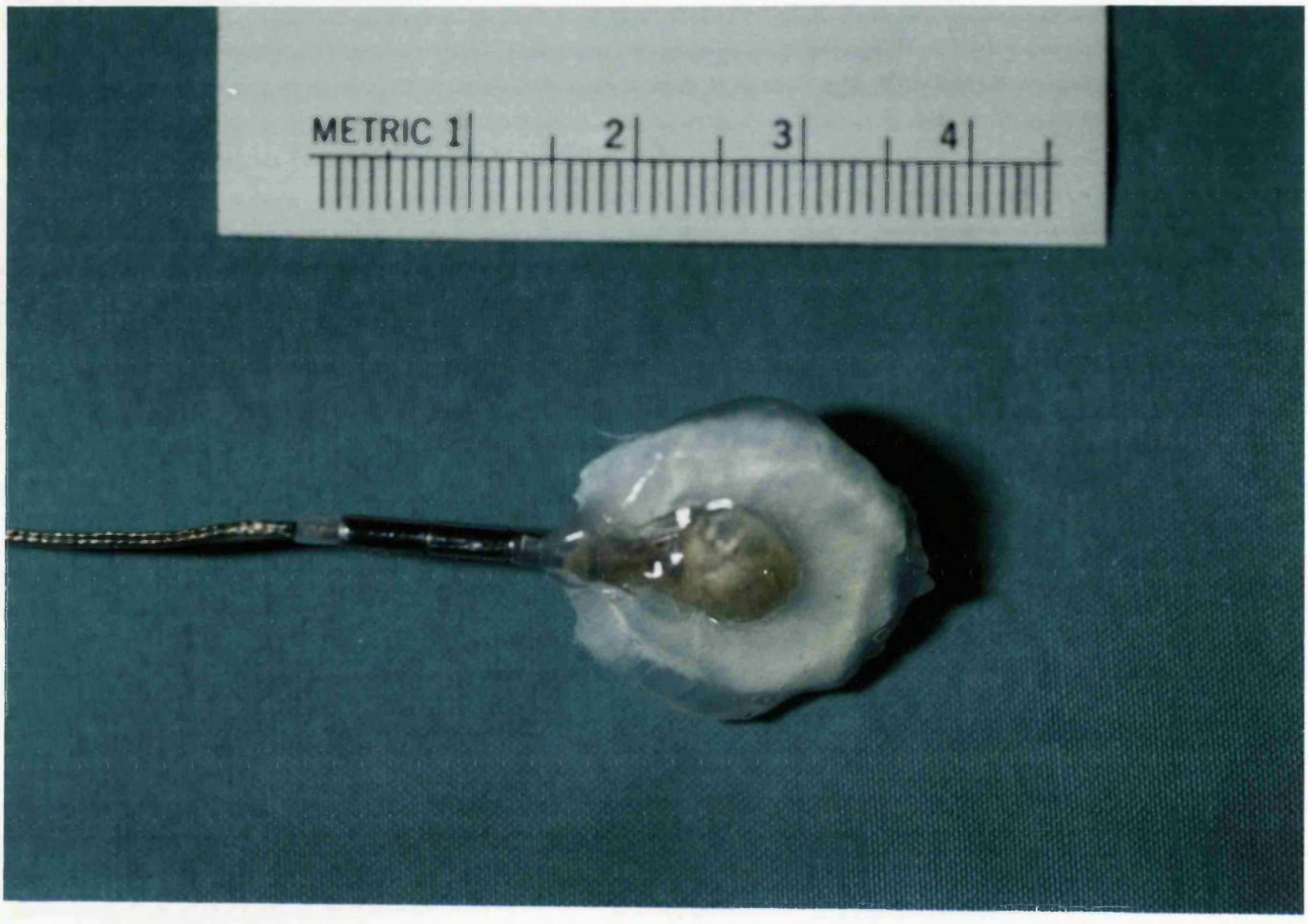
Pulse transit sonomicrometry measures the time delay from transmission of a burst of ultrasound from one piezo electric transducer to the reception of the sonic wave by an identical transducer. Since the velocity of sound in body tissues and blood is approximately constant the measured temporal delay is directly proportional to the distance between the transducers. Calibrations therefore are obtained by substituting an electronically generated time delay into the circuit. The sonomicrometer in this thesis (built by James W. Davis Consultant, Inc., Durham, NC) has a practical frequency response of 0-50 Hz, a minimal resolution of 0.08 mm, and a maximal electronic drift of 0.02 mm/hr. By using animals of similar weights, the dimensions for the four pairs of ultrasound crystals remained constant. This allowed calibration of each channel prior to each experiment, specifying a range of distance for an electronically generated time delay of -0.4mv to +0.4mv. Occasionally a dimension would fall outside the calibration range necessitating a shift in range and recalibration. Following application of the piezo-electric crystals each channel was studied on an oscilloscope display (fig. 16). The level of frequency gain, sensitivity of the trigger, distance of inhibition and focusing of the signal required adjustment throughout each experiment to ensure clean signals (fig. 17). An excellent dimension tracing could invariably be obtained with manipulation of these parameters to alter the signal and trigger. Only occasionally would a crystal need to be moved and resutured to the heart or changed for a new component.

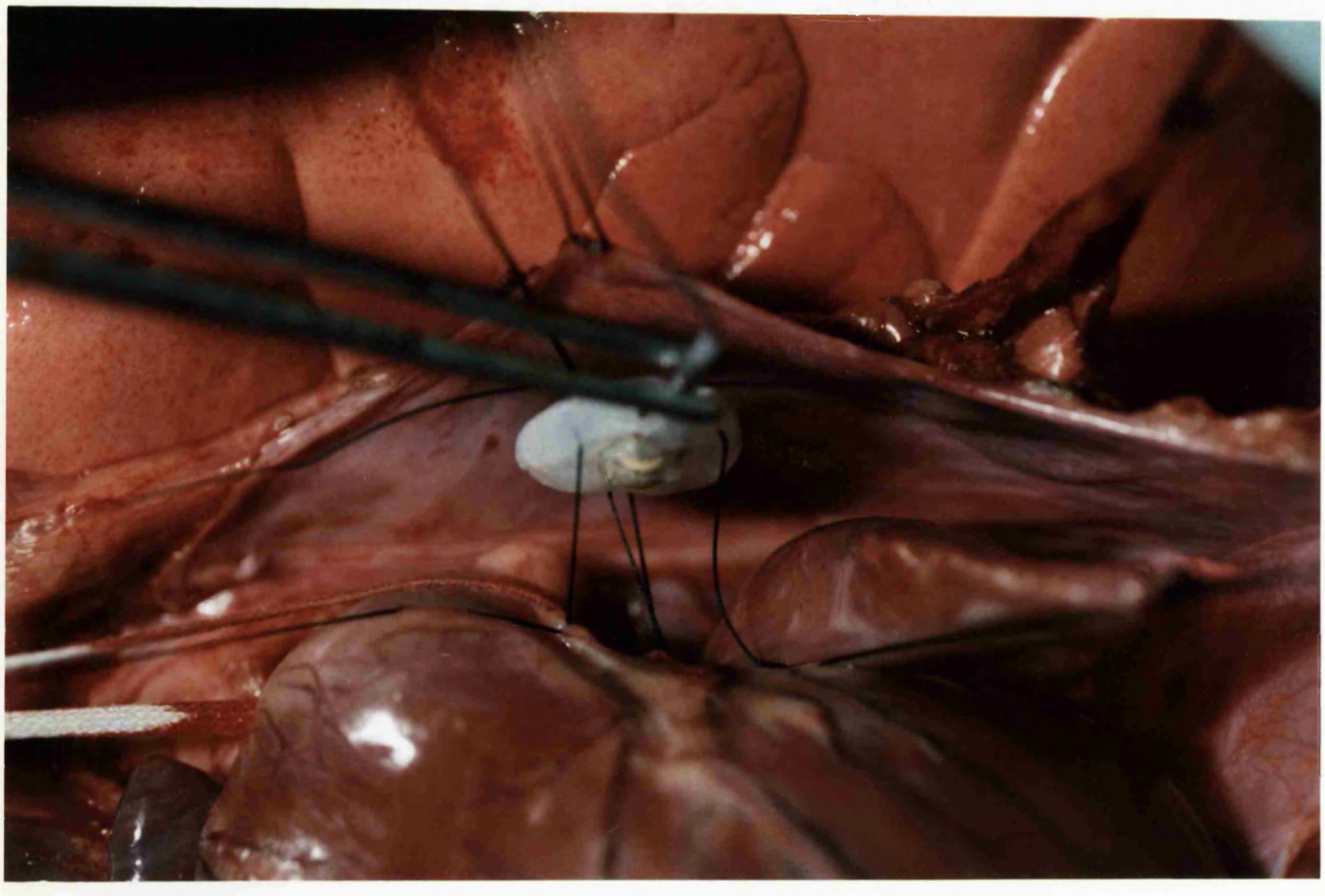
Figure 12 (Above)

Piezo - electric ultrasound crystal; it is mounted on felt and resin to assist suturing to the heart.

Figure 13 (Below)

Sutures in place on the base crystal, prior to tying of knots. Cranial aspect to left. This is placed between the pulmonary artery (on left with sling around it) and the base of the left atrial appendage (right).


METRIC 1
2
3
4



Intracavitary Manometry

Four 5 French gauge micromanometers (Model PC-350, Millar Instruments Inc, Houston) (fig. 18) were inserted through purse strings into the :

1. left atrium via the tip of the left atrial appendage,
2. left ventricle via the apex of the ventricle,
3. right ventricle via its anterior wall ,
4. pulmonary artery, via the infundibulum of the right ventricular outflow tract through the pulmonary valve.

The micromanometers were pre-warmed in an organ bath at 38°c under constant electrical excitement by a pressure amplifier (Model 8805-C, Hewlett-Packard, Waltham, Maryland). They were simultaneously balanced, zeroed in the warm water and calibrated against a mercury manometer before and after each experiment. Resultant drift was less than 0.5 mmHg per hour and the useful frequency response exceeded 10 kHz. In transplantation experiments the catheters were placed in the organ bath at 38°c during explantation, preservation and transplantation.

Blood Flow Measurement

Phasic pulmonary and aortic artery flow was determined with an ultrasonic flowmeter (Hartman et al, 1985) and flow probe (Transonic Systems Inc, Model T101/T201, Ithaca, New York).

The probes, 16-20 mm in diameter were placed around the aorta and pulmonary artery (Fig. 19). The size of the flow probe is carefully matched to that of the vessel, assisted with ultrasonic jelly to ensure good ultrasonic conduction. The probes were calibrated using an internal calibration before and after each study. The specification for this instrument reads a maximum error in zero baseline ± 50 mls/min and a maximum error and absolute volume flow ± 10% of the reading plus zero baseline error. The linearity (relative accuracy) is ± 1% of the reading.

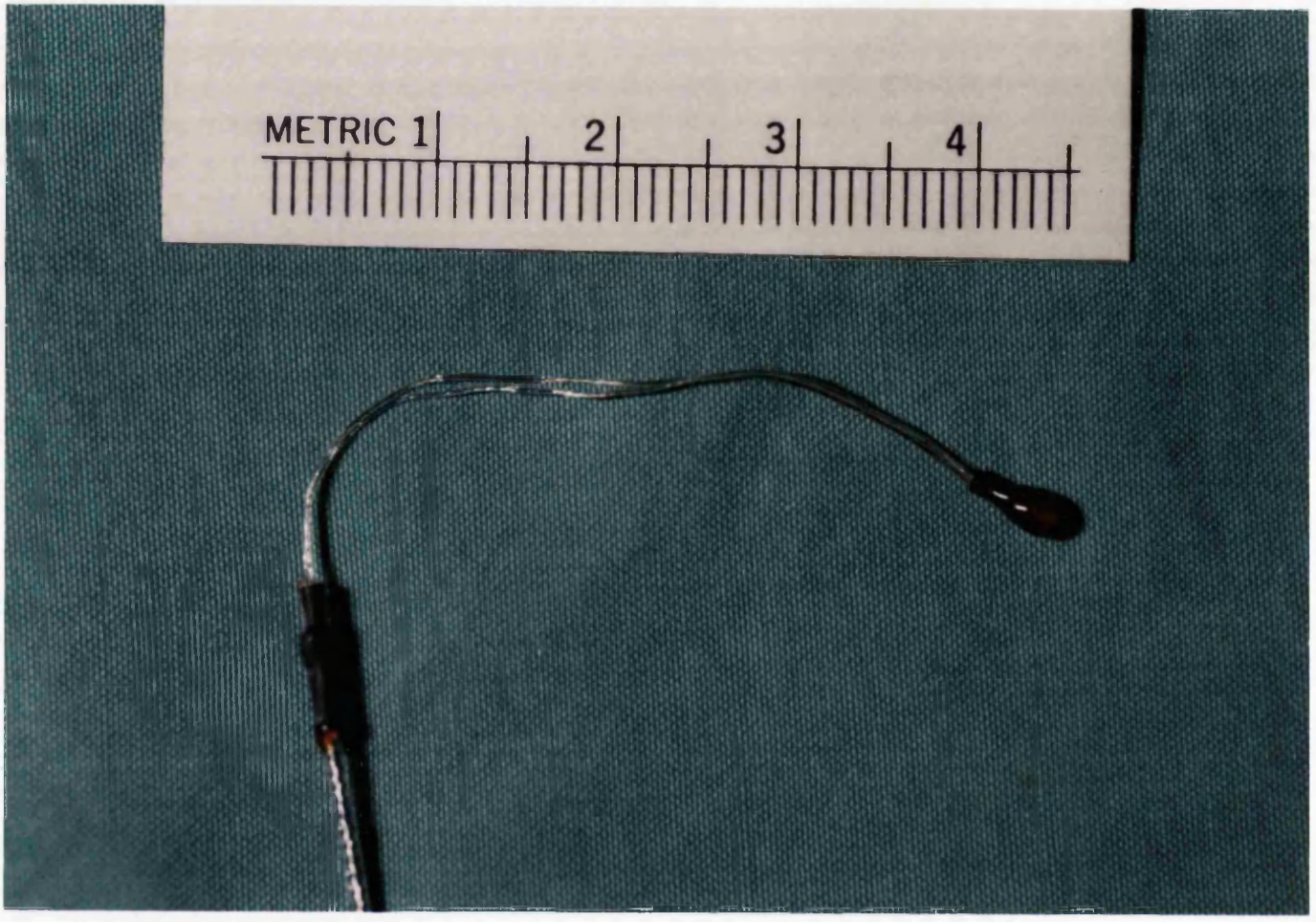
Figure 14 (Top)

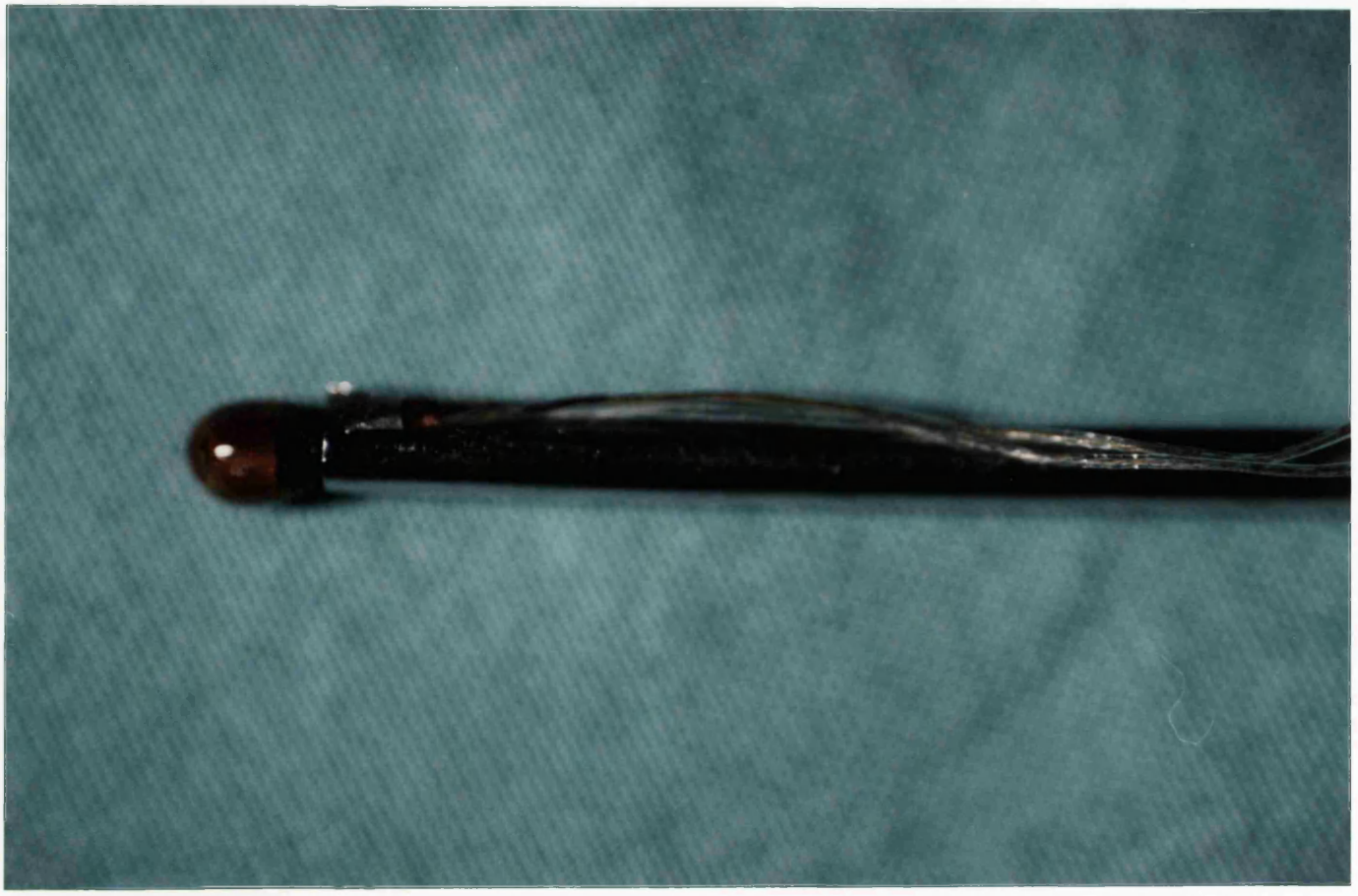
Septal piezo - electric crystal (ruler indicates 1 - 4 cm).

Figure 15 (Bottom)

Septal crystal mounted in introducer.

Following insertion of the purse string a 16 gauge needle is inserted to the septum to ascertain the correct direction for the septal crystal. The crystal is then inserted to the correct depth and the introducer is removed over a Seldinger wire, that is held firm, to prevent accidental withdrawal of the crystal. The purse string is then tied to secure the crystal.


METRIC 1
2
3
4



Haemodynamic Data Acquisition

All data were filtered by a 50 Hz low pass analogue filter and digitized in real time at a ten channel sweep speed of 200 Hz for 16 seconds by an analogue-to-digital converter built in this laboratory (James W. Davis, Consultant, Inc., Durham, NC). The conversion time per channel was 30 microseconds, imposing a phase delay between channels of less than 4.5 degrees. Data required for calculations of pulmonary impedance and diastolic function was acquired at 500Hz for 6 seconds. The digital data was stored on a personal computer (Compaq Prolinea 3/25 S). In early experiments this data was copied to microdisk and transferred for subsequent analysis. Each experiment would compile 2 to 4 megabytes of information and the process was enhanced and simplified by networking the system to allow direct data storage to the hard drive for data analysis. This allowed immediate study of files during experiments to ensure the 10 channels were being recorded properly, and dispensed with blind studies where experiments might be wasted.

Experiment 1.

After preparing and calibrating the technical equipment the surgical techniques were demonstrated by the chief technician, Mr. George Quick. The induction of anaesthesia and the surgical preparation of the 38.2kg animal was uneventful. Application of piezo-crystals and ultrasonic flow probes was straight forward, but on insertion of the fourth micromanometer through the apex of the left ventricle the heart went into ventricular fibrillation. This was unresponsive to repeated intrapericardial transventricular DC shocks (5 - 40 Joules) and intravenous lignocaine (100mg bolus' IV) and the experiment was aborted.

Experiment 2.

The author performed this experiment on a 35.9kg animal. The anaesthetic management and surgical instrumentation were uneventful and Mr. Damian Craig gave instruction regarding tuning of the signals from the crystals, and acquisition of data files.

Six steady state 500Hz files and six venal occlusion studies at 200 Hz files were collected and studied before termination of the experiment.

Figure 16. (Above)

Oscilloscope showing signal from one pair of piezo-electric crystals.

The signal is the lower line and is 'transmitted' from the right of the screen.

This signal is 'received' where the line suddenly increases.

The upper line is the sensor detecting the movement of the lower line at the point of 'reception'.

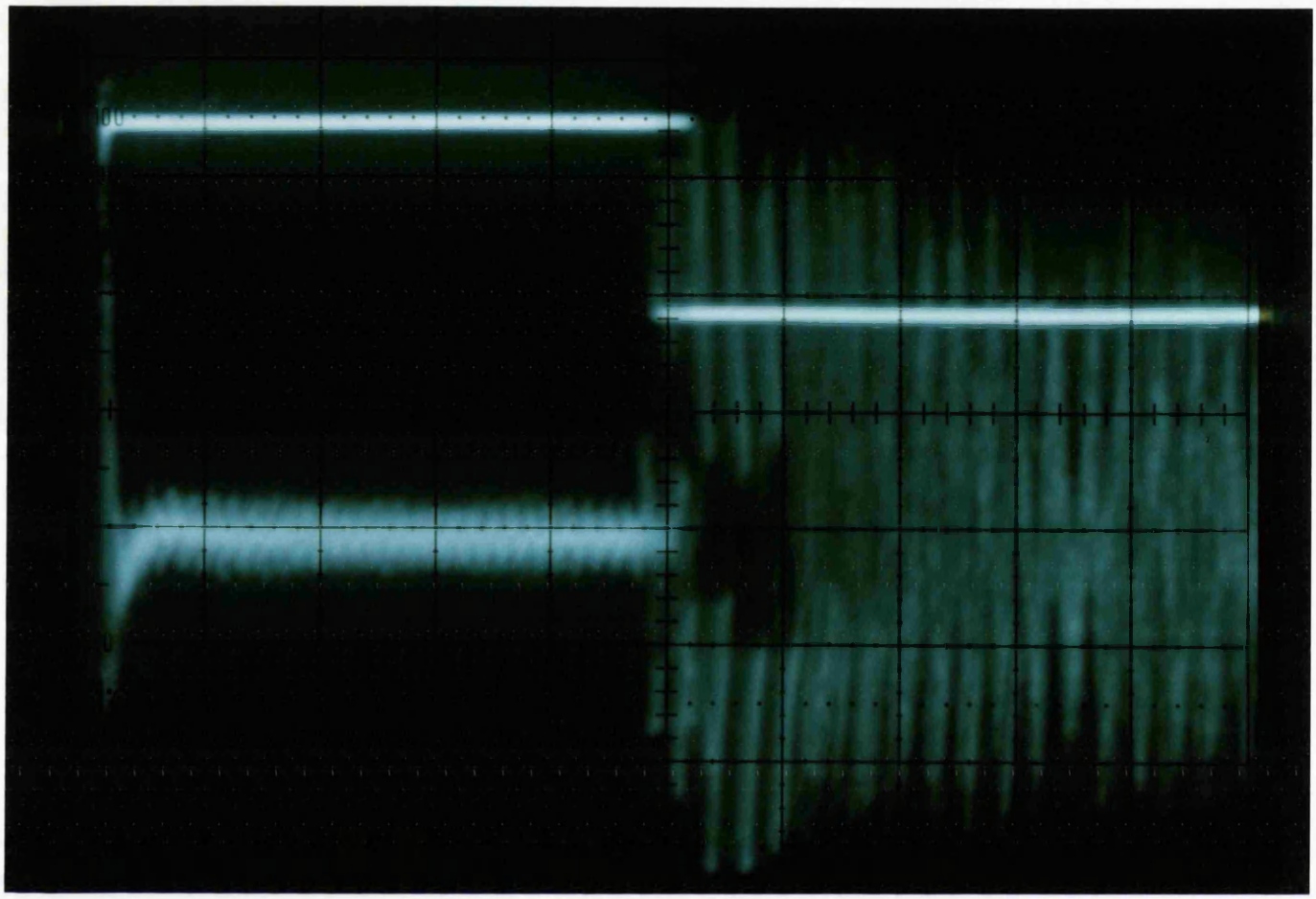
As the heart moves the point of reception correspondingly changes and is interpreted to distance by the micromanometer, based upon its calibration.

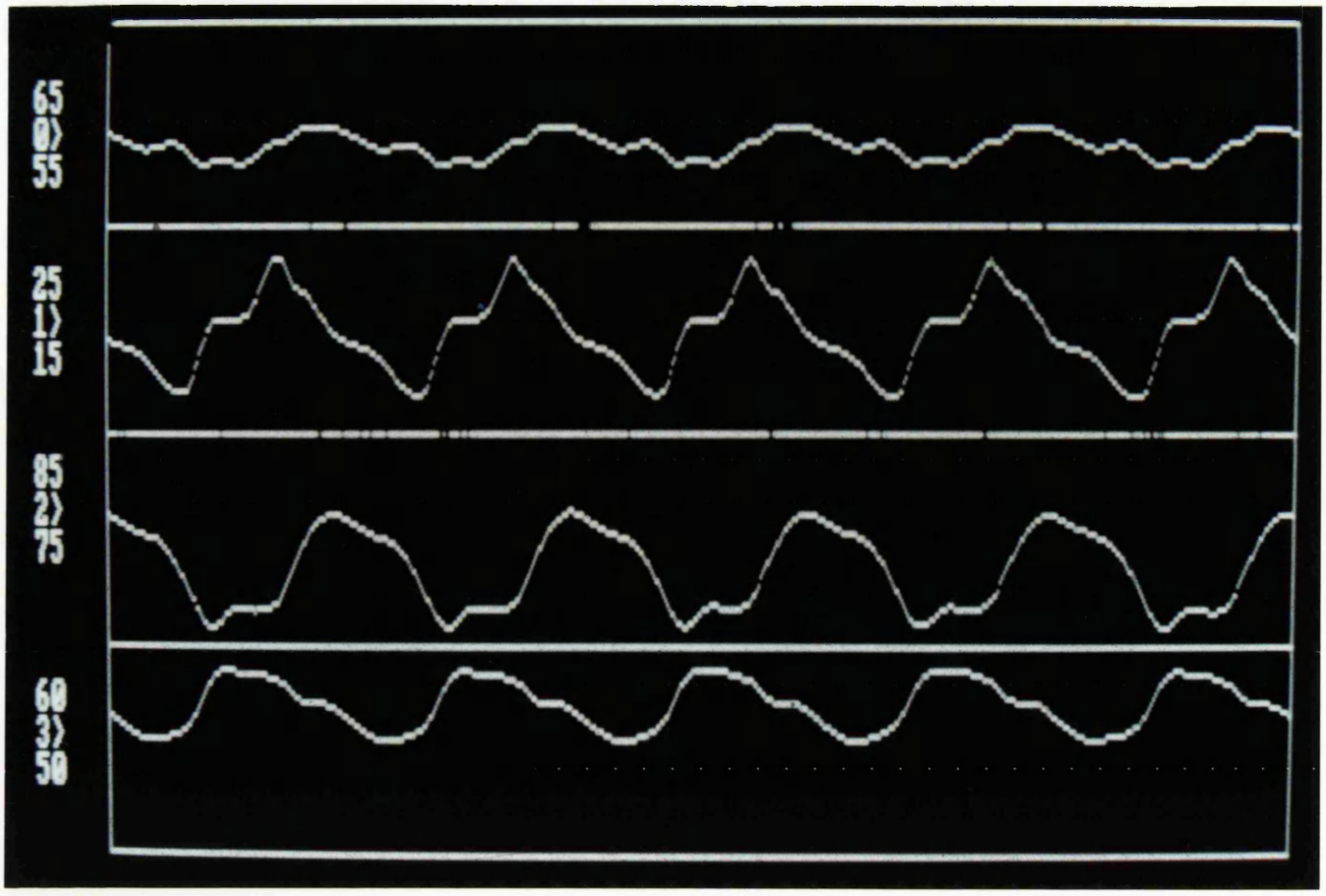
Figure 17. (Below)

Computer screen display of the four geometric axes during 4 cardiac cycles.

Top; minor axis. 2nd; septal to right free wall. 3rd; major axis. Bottom; septal to left free wall.

These changes of ventricular dimension are used to calculate the change of left and right ventricular volume through the cardiac cycle




65
55
25
15
85
75
60
50

Experiment 3.

As for the previous study the anaesthesia and surgery were uneventful on a 28.5kg animal. Two crystals were repositioned due to poor signal/reception. Data collection was successful.

Materials and Methods; Canine Model

The second study group consisted of three mongrel dogs whose weights ranged from 24.3 kg to 28.6 kg. These animals had been used in medical experiments in Duke University and were scheduled for euthanasia. In this regard they were ideal animals for a pilot group although they were not 100% fit. This pilot group was used to repeat the methods learnt in the pig group and to induce brain death, so as to prepare for the validation studies. All three animals were surgically prepared in exactly the same way, while pressure and flow measurements were taken and analyzed using equipment to be described under "Haemodynamic Data Acquisition". Data acquired from these recordings were analyzed as described in chapter 2.

Anaesthetic Technique

A 16 French gauge polyethylene cannula was inserted to a left foreleg vein. The dogs were premedicated with0.5mg/kg of morphine intramuscularly and 2mg/kg IM of diazepam. Anaesthesia was induced with intravenous etomidate (2mg/kg) and intramuscular ketamine (20mg/kg).

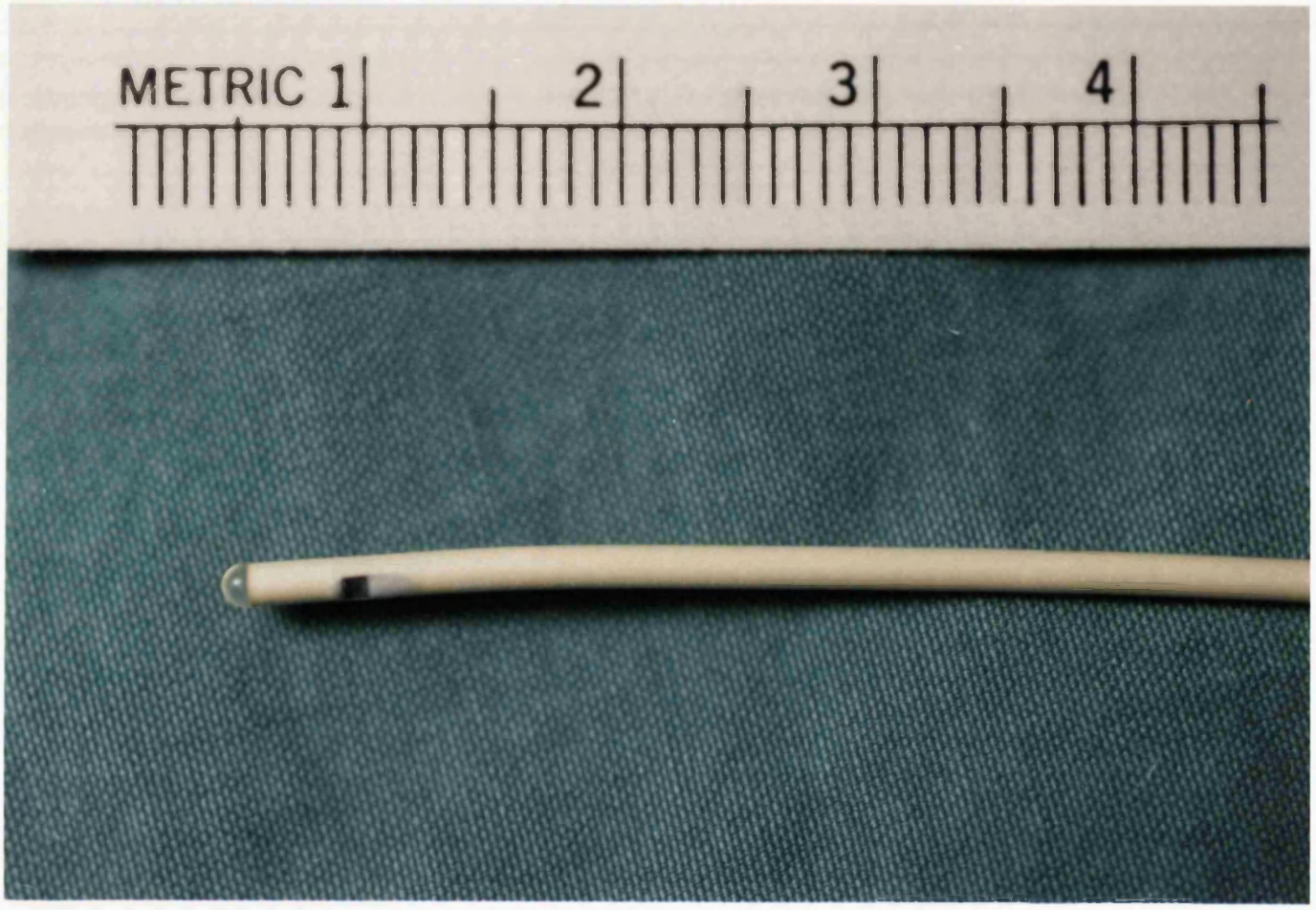
The animal was intubated with an 9.0 mm Murphy endotracheal tube and ventilated with a minute volume of 6 to 8 litres using a Bear Adult Volume Ventilator (the tidal volume was derived from the formula, 15 mls/kg of body weight), with a respiratory rate of 10-14 breaths / minute. Animals were maintained on a fractional inspired oxygen concentration (FiO_2) of 80-100% and serial blood gas analysis performed to assess stability and adequacy of ventilation.

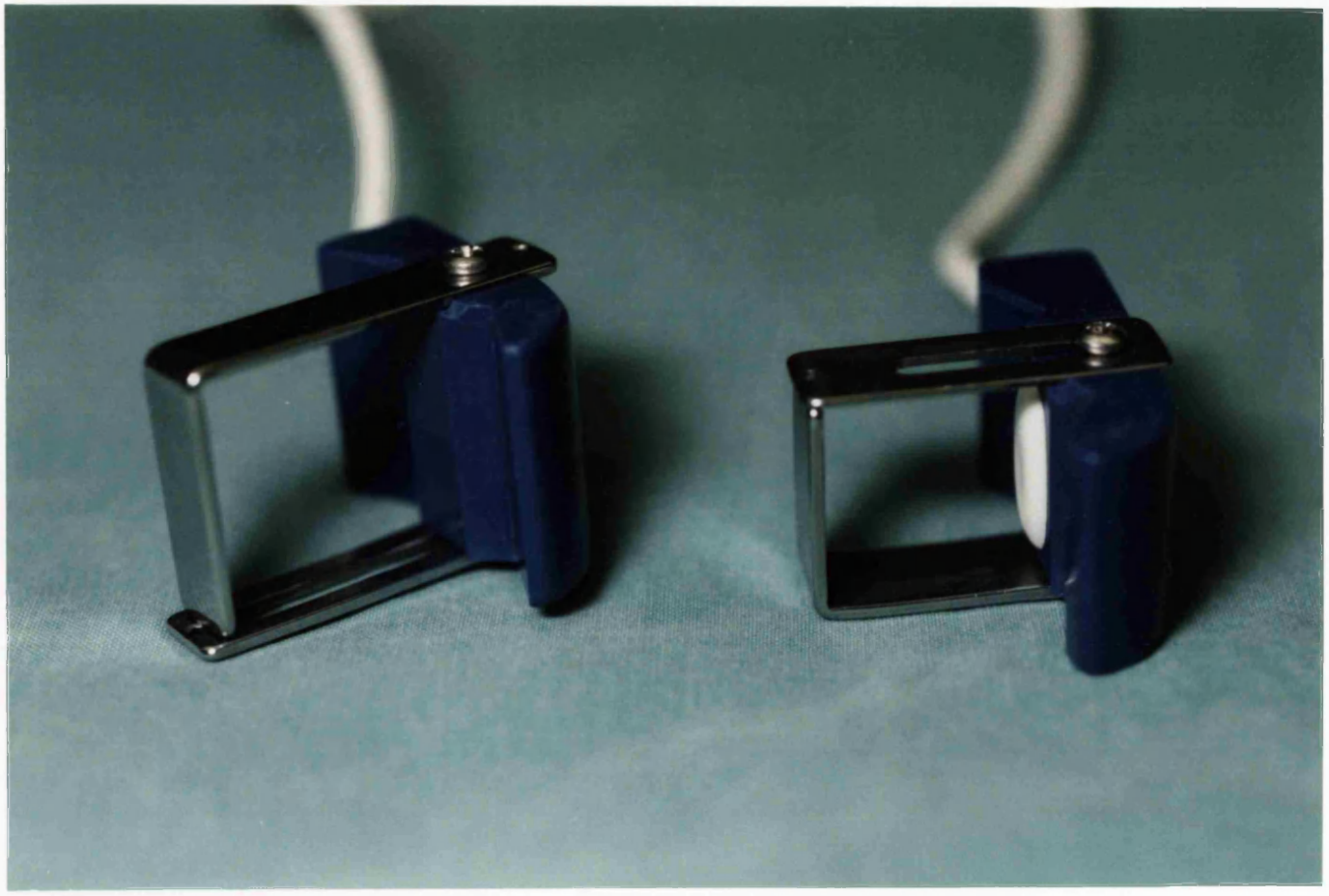
Figure 18 (Above)

Micromanometer, 5 French gauge (Model PC-350, Millar Instruments Inc, Houston).

Figure 19 (Below)

20mm aortic flow probe on left and 16mm pulmonary artery flow probe on left.


METRIC 1
2
3
4



The animal was paralyzed using intravenous (IV) succinylcholine (0.08mg/kg), and analgesia was administered using fentanyl 0.02 mg/kg IV initially, and then 0.01 mg/kg IV thereafter as a bolus injection, as required. Intravascular volume was supplemented with intravenous Ringer's Lactate solution to maintain central venous pressure greater than 2 mmHg, and arterial pressure greater than mean pressure of 60 mmHg. Wherever possible, drugs with the minimum effect upon cardiopulmonary function were administered.

Haemodynamic Monitoring

The method of haemodynamic monitoring was identical to the pig model.

Procedure

With the dog anaesthetised, paralysed and ventilated, electrocardiograph (ECG) electrodes were appropriately placed and connected to the ECG monitor (Mennen - Medical (model 450)). An oesophageal temperature probe was placed using a laryngoscope and forceps, and connected to the thermometer (Tele-thermometers, Yellow Springs Instrument Co, Inc, Ohio). A diathermy plate was placed under the animal which was then shaved over the cranium and the anterior chest wall. Electroencephalogram was recorded via bilateral needle electrodes to the parieto-occipital skull area, with a reference electrode in the right cheek (Bioelectric amplifier #8811A, Hewlett Packard, Mountain View, CA). Median sternotomy was performed with an oscillating saw. The azygos vein was doubly ligated, and divided, and tapes passed around the SVC and IVC. The pericardium was opened in the midline and tapes passed around the aorta and pulmonary artery.

Ventricular Geometric Measurement

The anatomy of the left and right ventricles of the dog are very similar to the pig. Therefore identical techniques are used for the application of the piezo-electric ultrasound crystals.

Intracavitary Manometry

Four Millar micromanometers were inserted in similar fashion to the pig model.

Figure 20

The heart fully instrumented as viewed from the right side of the animal (head to left)

White cables leading to the flow probes around the great vessels.

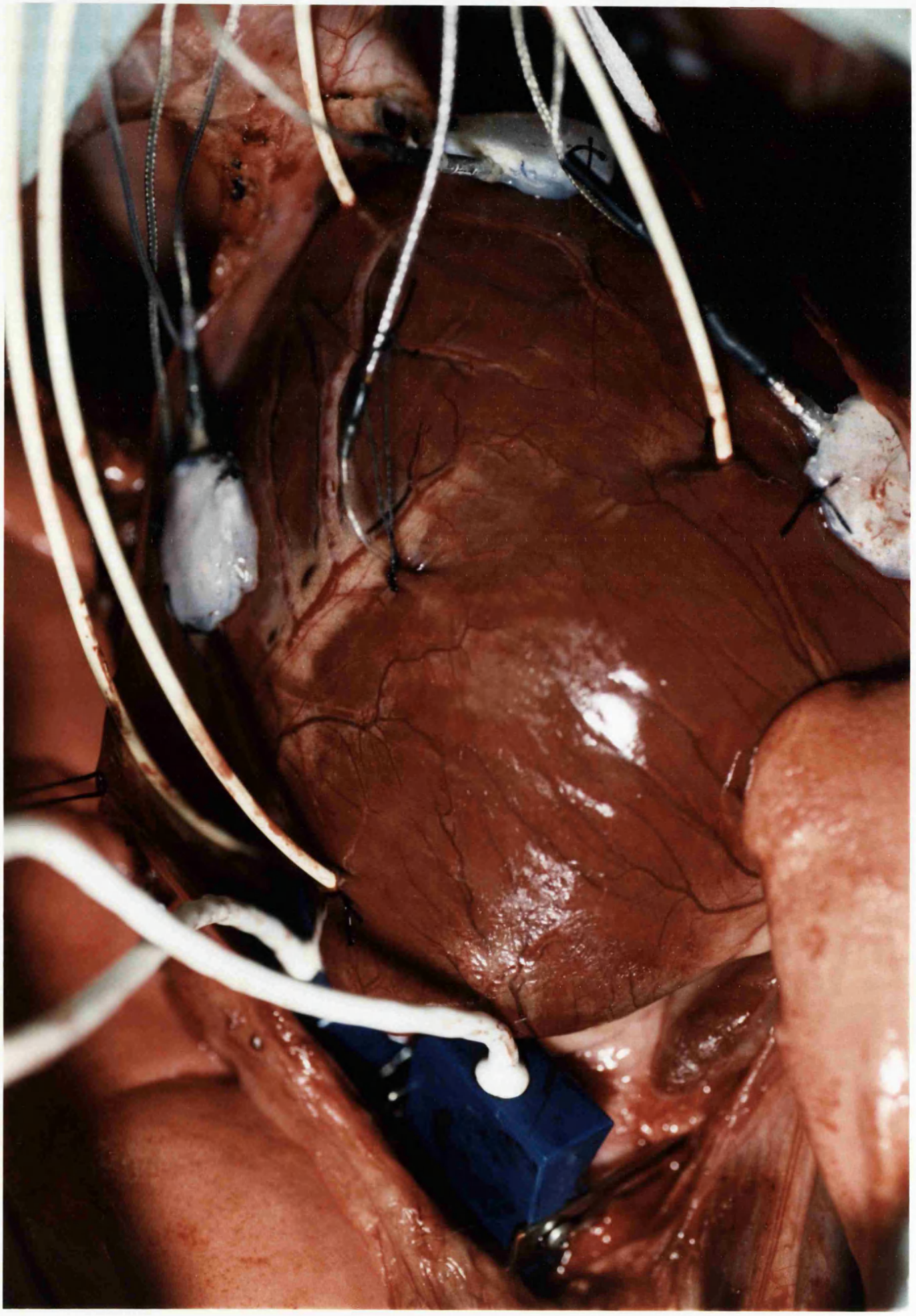
Yellow micromanometers seen inserted to right ventricular outflow tract (to pulmonary artery), left atrium (top), right ventricle (bottom) and left ventricle.

In the centre of the picture is the thin wire from the septal crystal.

The three crystals that are seen on the heart are; top, anterior (minor axis)

right, apical (major axis)

bottom, right free wall.



Blood Flow Measurement

The ultrasonic flow probes are awkward instruments to place around the major vessels due to their rigidity, rectangular shape and the blunt 0.5cm arm that must be passed posteriorly. This is not a major problem with the aorta as it is an inherently strong structure with a thick wall, and is positioned anteriorly. In comparison the main pulmonary artery is thin walled, more posteriorly placed and short due to its proximal bifurcation into left and right pulmonary arteries. These properties of the probe and pulmonary artery make this maneuver the most difficult of all the procedures. To prevent this potential iatrogenic insult, careful sharp dissection was performed between the major vessels before blunt dilatation of the tissues posterior to the pulmonary artery. Fortunately only one study in this thesis was terminated due to injury to the pulmonary artery at this stage.

Haemodynamic Data Acquisition

The methodology of data acquisition was identical to the pig model. The timing of acquisition was baseline, 15, 45, 90, 120 minutes post brain death and on every subsequent hour.

Hormonal Assay

The review of previous published literature on brain death indicated the timing of hormonal measurements. The catecholamine surge in the Cushing response is well documented and therefore no samples were to be taken in the immediate period following brain death. Sampling times were baseline, 15, 45, 90, 240, 360 and 420 minutes post brain death. At these times blood was withdrawn from the flushed femoral artery line. 18 ml was required at each sample time and divided into four prepared specimen bottles:

1st bottle; Heparin coated for subsequent assays of adrenaline, noradrenaline, dopamine, triiodothyronine, tetraiodothyronine and cortisol.

2nd bottle; Ethyl diamino tetra-acetic acid (EDTA) coated for assays of vasopressin, adrenocorticotrophic hormone (ACTH) and insulin.

3rd bottle; Untreated for assays of lactic dehydrogenase and also glucagon.

All specimens were placed on ice and immediately centrifuged for 10 minutes at 3000 rpm at 4°C and then stored at -80°C. The assays for each hormone were as follows:

Catecholamines; Dopamine, norepinephrine and epinephrine were analysed by on-line trace enrichment high performance liquid chromatography (HPLC-EC) with electrochemical detection (Kilts, 1983). Plasma catecholamines are extracted off-line using aluminium oxide and then enriched on-line on a cation exchange HPLC mini column. The enriched sample is then back-eluted using a potassium enriched mobile phase for further separation by ion-pair, reverse phase HPLC. This method offers two main advantages over other HPLC-EC assays: it is more **specific** as an interfering compound would need to be alumina-extractable, retained on cation exchange and reverse phase HPLC column, and electrically detected at +600mv: it is more **sensitive** as all the eluate from the off-line alumina extraction is injected onto the enrichment column leaving a low detector baseline noise with a high signal to noise ratio due to consistent solvent composition and the delivery of an enriched sample with low volume. The lower limit of detection is 10pg/ml plasma for norepinephrine, epinephrine and dopamine. Calibration curves are produced using stripped normal human plasma to which standards are added, thus avoiding possible matrix effects. Normal values are norepinephrine 100 -1700 pg/ml, epinephrine <10 - 140 pg/ml and dopamine <30pg/ml.

Thyroid hormones. Triiodothyronine (T3) and tetraiodothyronine(T4) are measured using fluorescence polarisation immunoassay technology on a clinical automated analyser (Abbott TDx, IL). These are standard clinical assays and are run without clinical modifications according to the manufacturers specifications. The assays are highly specific and free from interference due to abnormal levels of free fatty acids, lipids and haemoglobin (Gharib and Horn, 1972). The assay is accurate to 5% with normal canine values being T3, 0.4 - 1.0 ng/ml and T4, 1.5 - 6 μg/ml.

Cortisol. This is also measured by a fluorescent polarisation immunoassay on the Abbott TDx analyser. The assay is highly specific and shows only limited cross reactivity with corticosterone

and 11-deoxycortisol. The assay is calibrated against the European GC/MS reference method. All samples are run in duplicate and six levels of quality control are processed in each assay run. Accuracy is 11% and 4% at concentrations of 4.0 and 15.0 μg/dl respectively. Normal values for canine plasma are 6.25 to 22.5 μg/dl in the morning which is the time the experiments took place.

Vasopressin. This radioimmunoassay utilises an antiserum investigated and supplied by Dr. Greidanus (Dogterom et al, 1978) and a standard synthetic hormone, arg-8-vasopressin, from Bachem Inc, Torrance, CA. Radioactive vasopressin is prepared using radioactive iodination and the chloramine T technique. This is then purified on HPLC using a TFA methanol gradient. Standard curves are prepared in acidified vasopressin stripped plasma. Prior to assay the standards and samples are extracted on premade cartridges (Waters Associates, Milford, MA) and then concentrated by lyophilisation and reconstituted in assay buffer. The standards and samples are incubated with the antiserum for 24 hours at 4°C, and then the tracer is added for a further 24 hours incubation. The second antibody is added, goat anti-rabbit gamma globulin (Arnel products, New York, NY) for 3 hours incubation. The bound component is separated from the free by centrifugation at 6000 rpm for 30 minutes. The samples and standards are then analysed in the gamma counter (Packard Instruments, Downers Grove, IL). The sensitivity of the assay is 0.3 pg/ml in plasma. The antisera shows limited cross reactivity with lysine vasopressin and virtually no cross reactivity with oxytocin. The accuracy is 5% at a concentration of 10pg/ml, with a normal value of 35 - 60 pg/ml.

Insulin. This is measured using the Coat-A-Count radioimmunoassay available from Diagnostic Products Corp, VA. In summary ^{125}I - labelled insulin competes with insulin in the samples for binding sites on the insulin specific antibody that is immobilised on the wall of a polypropylene tube. After incubation the separation of bound from free is achieved by decanting, and the bound ^{125}I - insulin is measured in a gamma counter. The procedure detects down to 1.2 μIU/ml of insulin. The assay is standardised versus the WHO's First International Preparation of Insulin Immunoassay (#66/304). Accuracy is 10% across the range of normal values which are 3-35 μIU/ml.

Glucagon. This hormone is measured using the double - antibody radioimmune assay from Diagnostic Products Corp, VA. Labelled ^{125}I glucagon competes with glucagon in the sample for sites on a glucagon specific antibody. After incubation the bound is separated from the free by the accelerated double antibody method, followed by centrifugation and measurement by a gamma counter. The procedure can detect as little as 13pg/ml and is standardised against WHO's First International Preparation of Glucagon for Immunoassay (#69/194). There is no cross reactivity with preproglucagon and accuracy is within 5% in the normal range 40 to 130 pg/ml.

Adrenocorticotrophic hormone (ACTH). Plasma ACTH is measured using a two site immunoradiometric assay developed by the Nichols Institute (San Juan Capistrano, CA)(Raff and Findling, 1989). This assay has a working sensitivity of 1 pg/ml. Being a 2 site assay it is highly specific and exhibits no cross reactivity with related physiologic peptides. Normal morning ranges are 14 - 55 pg/ml and accuracy is within 6%.

ß - Adrenergic Receptor Analysis

For receptor and myocardial metabolite analysis at least 80 mg of myocardial tissue is required that is immediately frozen by immersion in liquid nitrogen. In the pilot studies this was attempted with multiple biopsies using a Tru-cut needle. Following storage at -80°C the first step in this analysis is the separation of the cellular membranes from the rest of the cardiac myocyte. These were prepared in the following manner: The sample is **immediately** placed in liquid nitrogen following extraction and then stored at -80°c. The sample is homogenised in 5ml of ice-cold lysis buffer (5mM Tris Cl, pH7.4 / 5mM EDTA / leupeptin 10 µg/ml / aprotinin 20 µg/ml). Nuclei and cellular debris were spun down at 500 x g for 15 minutes and then the supernatant was passed through a double layer of cheese cloth. Membranes were then pelletised by centrifugation at 40,000 x g for 15 minutes. Membranes were then washed with 5mls of binding buffer (75mM Tris Cl pH7.4 / 12.5mM $MgCl_2$ / 2mM EDTA) and resuspended in fresh binding buffer at approximately 1mg membrane protein / ml.

Following this preparation the membranes were analysed for adenyl cyclase activity. The freshly prepared membranes (5 - 20 μg protein) were incubated in 50 μl of assay mixture (30mM Tris Cl / 5mM $MgCl_2$ / 0.8mM EDTA / 2.7mM phosphoenolpyruvate / 0.05 mM GTP / 0.1mM cAMP / 0.12mM ATP / 1IU myokinase reaction / 3uCiα{P_{32}}-ATP). Adenyl cyclase activity is determined under basal conditions in the presence of progressively higher concentrations of isoproterenol (1 X 10^{-9} to 1 x 10^{-4}M) or in the presence of 10mM sodium fluoride. Incubation was for 10 minutes at 37°c and the reactions then terminated by the addition of 1ml ice cold 0,4mM ATP, 0.3mMcAMP, and {H3}cAMP (50,000cpms/ml). α{P_{32}}-ATP was isolated and quantitated as previously described (Salomon et al, 1974). Basal and isoproterenol stimulated cyclase activities were normalised as a per cent of the activity achieved with sodium fluoride and forskolin. Therefore the ß-receptor and its coupling to its signal transduction system (ie stimulation of adenylyl cyclase) is tested at **three levels**; the first level is the receptor tested by the response to isoproterenol, the second level is the stimulatory Gs protein tested by the response to sodium fluoride and the third level is the adenylyl cyclase moiety itself tested with forskolin.

Histological Assessment

Left ventricular biopsies were obtained from the apex of the left ventricle using a Trucut disposable biopsy needle (Travenol Laboratories Inc, Deerfield, Il). Five cores of tissue were obtained at baseline and at the termination of the experiment. The specimens were immediately placed in formaldehyde solution for histological preparation.

Instigation of Brain Death.

After collection of baseline samples and data, with the animal still supine, the neck was fully flexed to allow access to the cranium. A $1cm^2$ cutaneous flap was excised at the midline of the skull and the periosteum exposed at the fusion of the fronto-parietal plates. A 3mm drill was used to create a burr hole, 1cm to the left of the midline on the posterior aspect of the parietal bone. A Millar micromanometer, already calibrated as previously described, was inserted into the cranium via this Burr hole. A second burr hole was fashioned in the midline on the vertex of the cranium using a

6.25mm drill. Through this a size 16 Foley catheter was inserted subdurally into the cranium. To cause brain death the catheter balloon was inflated with 15 to 18 ml of normal saline. This method of causing brain death was chosen in preference to ligation of the head and neck vessels. The dog has many arterial collaterals to the brain and to ensure brain death venous and arterial ligation is necessary. This causes ischaemia to a large area of the head and neck, resulting in metabolic acidosis and hyperkalaemia. Therefore the intracranial method was preferred in attempting to mimic the clinical scenario of blunt head injury / intracranial bleeding that results in raised intracranial pressure, and avoid the systemic effects of ischaemic tissue.

Termination of Experiment

At the completion of the study with no further haemodynamic data, blood samples or tissue samples required, the heart was fibrillated and the ventilator switched off. The Millar micromanometers, the flow probes and the epicardial sonomicrometers were removed.

The heart was then excised and the position of the septal sonomicrometer was ascertained with sharp dissection to measure 'septal shift' in millimeters from the endocardial surface of the right ventricle. The right ventricular free wall was excised and its volume measured using a technique of volume displacement in normal saline. The volume of the left ventricle was then measured in the same fashion having excised the papillary muscles and all non muscular tissue including the valvular apparatus.

A circumferential craniotomy was then performed with an oscillating saw. The clavarium was opened to allow removal of the cerebrum in continuity with cerebellum, brain stem and a small portion of the cervical spinal cord *in toto*. The specimen was placed in five times of its own volume of 20% buffered formaldehyde and preserved for 7 to14 days. Immediately after the extraction / immersion in formaldehyde a macroscopic-neuropathologic examination was performed, although the evaluation is best made after fixation using commonly known macroscopic pathological criteria (Leestma et al, 1984). After the preservation period significant regions of the brain underwent

sections and tissue sampling for slide processing and histologic evaluation. The brains were first dissected by removing the brain stem and cerebellum via an axial cut at the level of the midbrain. The cerebrum was then sectioned coronally while the brain stem and cerebellum were sectioned axially. Histologic sections were stained with the haemotoxylin and eosin / Luxol fast blue method.

Experiment 1

A 28.6kg female mongrel dog was anaesthetised, paralysed, and ventilated as described in the previous Materials and Methods section. Urinary catheterisation was attempted but not possible, placement of piezo-electric crystals, insertion of Millar micromanometers and positioning of ultrasonic flow probes was uneventful and satisfactory signals were obtained from all instruments. Calibration was performed before and after the experiment as previously described.

Baseline data for haemodynamic parameters were recorded and samples taken of blood and cardiac tissue. Brain death was instigated accompanied by a fixed dilatation of the pupils, hypertension of 230 mmHg systolic pressure and a sinus tachycardia of 220 BPM. There was significant bleeding from the left atrial appendage at the point of insertion of the micromanometer which required application of a snare to the purse string. Subsequent data and samples were collected at 15, 45, 90 and 120 minutes following brain death before the experiment was terminated. The brain stem was obtained for histology and the heart dissected revealing 2mm of septal shift of the septal crystal, a right ventricular free wall volume of 40 mls and a left ventricular volume of 120 mls.

Experiment 2

A 24.3kg male mongrel dog was prepared as described in the previous Materials and Methods section. Urinary catheterisation was successful and much easier than in the female dog in experiment 1. Placement of piezo-electric crystals, insertion of Millar micromanometers and positioning of ultrasonic flow probes was uneventful. However the right ventricular crystal needed

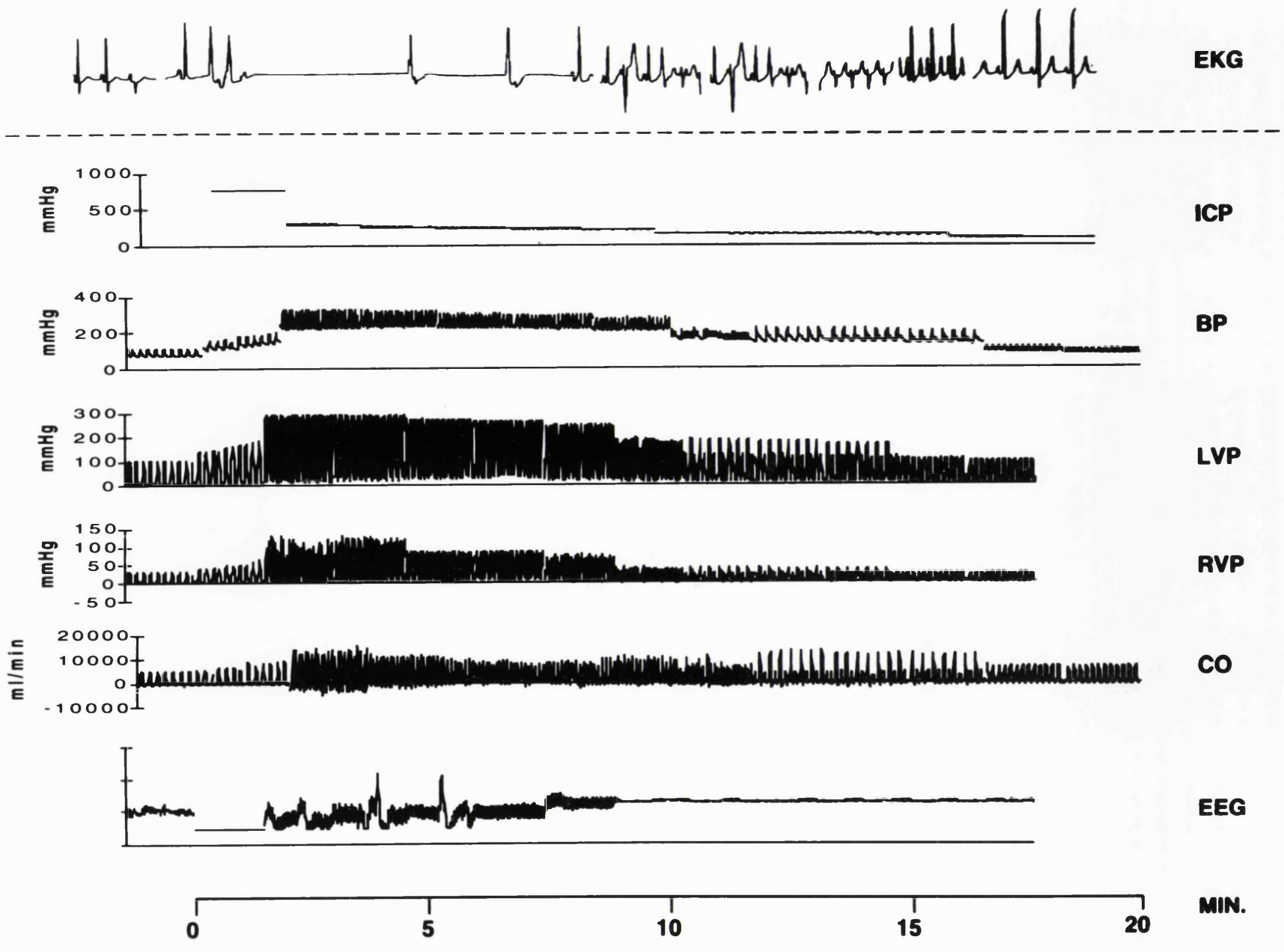
Figure 21

The Cushing response in one animal following inflation of the intracranial balloon and subsequent rise of intracranial pressure.

Top trace; this shows seven representative sections of the **electrocardiogram** (EKG) over the course of the Cushing response. First is baseline sinus rhythm followed by the profound bradycardia immediately following intracranial balloon inflation. The third and fourth sections are examples of severe tachycardia with multifocal ventricular ectopics. The fifth section is sinus tachycardia, the sixth is bigemini and the seventh is sinus rhythm following the Cushing response with residual ST depression.

The lower six traces are plotted against the X-axis (time) and are as follows; ICP, intracranial pressure; BP, peripheral arterial pressure; LVP, left ventricular pressure; RVP, right ventricular pressure, CO, cardiac output; EEG, electroencephalogram.

These traces show the severity of the Cushing response in achieving supra-normal pressures and flows. The intracranial balloon achieves supra-systolic pressures causing global brain ischaemia and this results in the flat line trace on the EEG. The bradycardia is so brief it does not show on pressure/flow traces, although the periods of multifocal ectopics and bigemini are recognisable on these traces between 8 and 15 minutes.


EKG
1000
500
0
mmHg
ICP
400
200
0
mmHg
BP
300
200
100
0
mmHg
LVP
150
100
50
0
-50
mmHg
RVP
20000
10000
0
-10000
ml/min
CO
EEG
0
5
10
15
20
MIN.

repositioning due to poor signals to the sonomicrometer. Calibration was performed before and after the experiment as previously described.

Baseline data for haemodynamic parameters were recorded along with collection of blood samples and cardiac tissue. Brain death was instigated accompanied by a fixed dilatation of the pupils, hypertension of 310 mmHg systolic pressure and a sinus tachycardia of 210 BPM with multiple multifocal ventricular ectopics. Duration of this hypertensive response was 4.5 minutes. Subsequent data and samples were collected at 15, 45, 90 and 120 minutes following brain death before the experiment was terminated. The brain stem was obtained for histology and the heart dissected revealing 4mm septal shift of the septal crystal, a right ventricular free wall volume of 35 mls and a left ventricular volume of 90 mls.

Experiment 3

A 27.2kg male mongrel dog was prepared in identical fashion to the prior animals and urinary catheterisation was successful with 200 mls immediately drained. It was noted that this animal and the previous animal were hypertensive (170/110mmhg) following induction of anaesthesia. This provoked a major review of the anaesthetic protocol and is discussed at the end of this chapter. Placement of piezo-electric crystals, insertion of Millar micromanometers and positioning of ultrasonic flow probes was uneventful. Calibration was performed before and after the experiment as previously described. Electroencephalography (EEG) was also achieved in this animal and the EEG tracing significantly changed to a flat line ten minutes after instigation of brain death.

Baseline data for haemodynamic parameters was recorded along with samples of blood and cardiac tissue. Brain death was instigated, again accompanied by fixed dilatation of the pupils, hypertension of 250 mmHg systolic pressure and a sinus tachycardia of 190 BPM with multiple multifocal ventricular ectopics. Duration of this hypertension was 6 minutes. Subsequent data and samples were collected at 15, 45 and 90 minutes following brain death. At 110 minutes the animal became hypotensive and went into ventricular fibrillation. The cause for these events was

unapparent as no intervention had occurred and arterial blood gas analysis had been stable. The experiment was terminated; the brain stem was obtained for histopathology and the heart dissected revealing 3mm septal shift of the septal crystal, a right ventricular free wall volume of 45 mls and a left ventricular volume of 115 mls.

RESULTS

Haemodynamic results

A summary of the analysed haemodynamic data is shown in tables 2 and 3. Baseline data was complete except in the second animal for impedance analysis. This was the result of a poor signal from the pulmonary artery flow probe preventing useful analysis and was not rectified until data collection at 45 minutes post brain death. The mean heart rate was 119 bpm with a mean cardiac output of 1428 mls/min. The larger first dog (28.6kg) had a large output of 2000mls/min yielding a large standard deviation of 809 mls/min.

The slopes (PRSW) of the left and right ventricular stroke works during vena caval occlusions had high correlation coefficients (R2) of 0.94 and 0.95 respectively. This suggested that the methodology of geometric and pressure data acquisition was satisfactory although there was considerable deviation from the means; 46.4 erg.10^3 ± 13.2 and 17.71 erg.10^3 ± 10.66.

The x-intercept yielded some feedback regarding the estimation of freewall ventricular volumes at the end of the study. For the left ventricle the estimation would appear too large as the X-intercept was 5.2 mls ± 6.5 mls and more care was needed to remove all excess tissue and protruding papillary muscles. The right ventricle though had an underestimation of freewall volume giving an x-intercept of 17.7 mls ± 10.6 mls.

Table 2.

Summary of pressure / volume data from pilot studies.

Abbreviations.

LV PRSW; Left ventricular pre-load independent recruitable stroke work.

RV PRSW; Right ventricular pre-load independent recruitable stroke work.

Y(int); Intercept on Y axis for plot of stroke work vs end diastolic intraventricular volume.

X(int); Intercept on X axis for plot of stroke work vs end diastolic intraventricular volume.

R2; correlation coefficient of line plot of PRSW and data points on line. (Figure 5, 6).

SUMMARY OF PRESSURE / VOLUME DATA OF PILOT STUDIES

STUDY NO.	Time	LV PRSW	Y(int)	X(int)	R2	RV PRSW	Y(int)	X(int)	R2
	mins	*erg.1000*	*mmHg*	*mls*		*erg.1000*	*mmHg*	*mls*	
PILOT 1	Baseline	40.7	-516	12.7	0.95	10.6	-289	27.3	0.93
	15	.	.	.	.	8.7	-178	20.3	0.98
	45	43.4	-307	7.1	0.95	8.9	-184	20.8	0.60
	90	42.1	-494	11.7	0.95	7.2	-159	22.1	0.64
	120	35.5	-359	10.1	0.93	12.4	-313	25.2	0.87
PILOT 2	Baseline	61.5	-38	2.4	0.99	30.0	-175	6.3	0.94
	15	92.1	-285	5.3	0.96	33.7	-218	8.3	0.88
	45	49.4	-136	3.6	0.97	22.2	-121	6.5	0.94
	90	46.4	-130	2.8	0.57	20.7	-117	5.6	0.91
	120	53.9	-34	0.6	0.95	26.1	-162	6.2	0.96
PILOT 3	Baseline	37.0	-21	0.6	0.89	12.6	-501	39.9	0.99
	15	61.7	235	-3.8	0.95	48.1	-2304	47.9	0.92
	45	30.9	129	-4.2	0.87	9.6	-331	34.7	0.92
	90	34.3	29	-0.9	0.95	14.0	-581	41.5	0.92
	120	.	.	.	.	.	.	.	.

	Time	LV PRSW	Y(int)	X(int)	R2	RV PRSW	Y(int)	X(int)	R2
Means;	Baseline	46.4	-192	5.2	0.94	17.7	-322	24.5	0.95
	15	76.9	-25	0.7	0.95	30.2	-900	25.5	0.93
	45	41.2	-105	2.2	0.93	13.6	-212	20.7	0.82
	90	40.9	-198	4.6	0.82	14.0	-285	23.1	0.82
	120	44.7	-196	5.4	0.94	19.2	-237	15.7	0.92
Standard Deviations;	Baseline	13.2	281	6.5	0.05	10.7	165	17.0	0.03
	15	21.5	368	6.4	0.01	19.9	1216	20.3	0.05
	45	9.4	220	5.8	0.05	7.5	108	14.1	0.19
	90	6.1	268	6.5	0.22	6.8	257	17.9	0.16
	120	13.0	230	6.7	0.02	9.7	106	13.4	0.06

The impedance data analysis was more varied. The first animal had a large cardiac output so that subsequent calculations gave high values at the upper range of normal for hydraulic power (total power right ventricle 84.5 mW) and low values for both the pulmonary vascular resistance (641 dyn/s/cm^{-5}) and characteristic impedance (104 dyn/s/cm^{-5}). The oscillatory power was only 10% of total power (13.3 mW / 84.6mW) whereas it should be approximately 30%. The third animal had less than half the output (855 mls/min) giving values of hydraulic power at the lower end of the normal range. Oscillatory power was only 10% of the total (4.6 mW / 48.46 mW). Pulmonary vascular resistance and characteristic impedance had high values of 2383 and 266 dyn/s/cm^{-5} respectively.

This data underlined the variability between animals and the need for reasonable numbers of comparable animals to obtain satisfactory baseline data. These animals had also been used in previous medical experiments, the nature and effects of which were unknown.

The diverse nature of the animals and baseline data prohibited detailed analysis of results following brain death. However some interesting trends were noted. At 15 minutes following brain death there was an increase in PRSW for left and right ventricles (+65% and +76% respectively) presumably due to the inotropic effect from the residual elevation of serum catecholamines. For the remainder of the experiments left and right ventricular values fell to below baseline levels (87% and 76% of baseline), results complimented for the right ventricle from calculated stroke work (42% of baseline) derived from impedance data. This drop in stroke work was mirrored by a decrease in cardiac output (50% of baseline).

The Cushing Response

The Cushing response is the hyperdynamic response following an acute rise of intracranial pressure originally described by Cushing, 1903. It occurred in all three animals and is demonstrated graphically in figure 21. These six tracings, along with representative sections of the electrocardiograph, show the immediate response to inflation of the intracranial balloon.

Table 3.

Summary of impedance data from pilot studies.

Stroke work; this is the calculated PRSW for the right ventricle calculated from 500 Hz steady state files. In chapter 5 this calculation is shown to be defective.

Pulm. Vasc. Resistance; Pulmonary vascular resistance.

Charac. Impedance; Characteristic impedance, frequency 7-11Hz.

SUMMARY OF IMPEDANCE DATA FROM PILOT STUDIES

STUDY NO.	TIME	HEART RATE	STROKE WORK	CARDIAC OUTPUT	TOTAL POWER	MEAN POWER	OSCILLAT. POWER	PULM. VASC. RESISTANCE	CHARAC. IMPEDANCE
	mins	*beat/min*	*erg.1000*	*mls/min*	*milliwatts*	*milliwatts*	*milliwatts*	*dyn/s/cm5*	*dyn/s/cm5*
PILOT 1	Baseline	99	30.6	2001	84.6	71.3	13.3	641	104
	15	124	14.6	1385	44.5	37.2	7.3	699	132
	45	114	20.0	1116	34.4	28.9	5.6	834	109
	90	106	14.6	1081	31.2	26.2	5.0	807	86
	120	98	15.9	949	27.8	23.1	4.8	788	80
PILOT 2	Baseline	.	.	.	.	.	.	.	.
	15	.	.	.	.	.	.	.	.
	45	115	-2.0	460	22.8	19.9	2.9	3383	215
	90	114	5.2	670	28.3	24.7	3.7	1979	203
	120	110	4.5	467	22.9	21.2	1.7	3498	155
PILOT 3	Baseline	140	27.9	855	53.1	48.5	4.6	2384	266
	15	125	39.8	1125	43.3	37.9	5.4	1079	128
	45	130	18.7	477	16.9	14.8	2.0	2348	163
	90	122	18.1	486	18.8	16.4	2.4	2504	157
	120	.	.	.	.	.	.	.	.

	TIME	HEART RATE	STROKE WORK	CARDIAC OUTPUT	TOTAL POWER	MEAN POWER	OSCILLAT. POWER	PULM. VASC. RESISTANCE	CHARAC. IMPEDANCE
Means;	Baseline	119	29.2	1428	68.8	59.9	8.9	1513	185
	15	125	27.2	1255	43.9	37.6	6.3	889	130
	45	120	12.2	684	24.7	21.2	3.5	2188	162
	90	114	12.6	746	26.1	22.4	3.7	1763	149
	120	104	10.2	708	25.4	22.2	3.2	2143	117
Standard Deviations;	Baseline	29	1.9	810	22.3	16.2	6.1	1232	115
	15	1	17.8	184	0.9	0.5	1.4	268	3
	45	9	12.3	374	8.9	7.1	1.8	1282	53
	90	8	6.7	305	6.5	5.3	1.3	869	59
	120	8	8.0	341	3.5	1.3	2.2	1916	53

On inflation of the balloon intracranial pressure is elevated above 500mmHg and then remains above arterial systolic pressure. The response of the heart is an initial bradycardia for a few seconds. This is rapidly followed by a marked tachycardia with multifocal ventricular ectopics. Left ventricular systolic pressure became elevated to >300mmHg and right ventricular systolic pressure rose above 100mmHg. This is accompanied by a corresponding rise in cardiac output (more than 2litres/min) followed by the electroencephalogram becoming a flat trace. Cardiac rhythm, ventricular and peripheral pressures gradually decreased to resume baseline values and a sinus rhythm by twenty minutes post brain death. In the interim period the tachycardia became bigemini in two animals (figure 21) and in all three animals there was residual ST depression of over 2mm at 30 minutes post brain death. This resolved after a further 30 minutes.

Hormone Results

Overall the results of the hormone studies from the second pilot study showed that the collection and preparation of the samples allowed satisfactory analysis (table 4). They allowed some trends to be seen: catecholamines were all markedly elevated at 15 minutes with noradrenaline and dopamine showing a sustained rise, although the former stayed in normal limits: vasopressin immediately fell and remained at sub-physiological levels following brain death, from 42.5 to 1.0 pg/ml: cortisol showed a small peak at 15 minutes before declining to the low end of the normal range (10.6, 14.1 and 5.60 ug/dl) probably secondary to the rapid decline of adrenocorticotrophic hormone (84.4 fm/ml to 20.0 fm/ml): the thyroid hormones, tetra- and tri- iodothyronine remained within normal limits with a suggestion of declining values (1.42 to 1.26 ug/ml and 0.66 to 0.55 ng/ml respectively): insulin and glucagon levels peaked at 15 minutes post brain death and returned to physiological values subsequently.

The rapid decline of vasopressin levels was encouraging in that it suggested disruption of the hypothalamic - pituitary axis, and that with longer experiments diabetes insipidus should ensue. Hormonally this mimics the clinical picture of brain death.

Table 4.

Results of hormone studies in pilot study.

These results proved the techniques of sampling and analysis to be satisfactory. The trends demonstrated by ACTH and vasopressin implied disruption of the hypothalamic-pituitary axis, reinforcing the neuropathological findings that brain death was accomplished using inflation of the intracranial balloon.

Abbreviations.

ACTH; Adrenocorticotrophic hormone.

T4; Tetra-iodothyronine.

T3; Tri-iodothyronine.

RESULTS OF HORMONE STUDIES IN PILOT EXPERIMENT.

Time post brain death;		BASELINE	15	45	90	120
DOPAMINE						
pg/ml	PILOT 2	5	82	176	48	55
NORADRENALINE						
pg/ml	PILOT 2	350	881	869	876	790
ADRENALINE						
pg/ml	PILOT 2	26	52	115	12	23
VASOPRESSIN						
pg/ml	PILOT 2	42.5	7.9	3.2	0.7	1.0
ACTH						
fM/ml	PILOT 2	84.4	48.3	25.8	12.5	20.0
CORTISOL						
mcg/dl	PILOT 2	10.6	14.1	7.0	3.8	5.60
INSULIN						
uU/ml	PILOT 2	4.8	13.8	2.4	1.6	2.3
GLUCAGON						
pg/ml	PILOT 2	68.8	119.2	54.6	46.6	54.7
T4						
ug/ml	PILOT 2	1.42	1.49	1.38	1.40	1.26
T3						
ng/ml	PILOT 2	0.66	0.70	0.56	0.48	0.55

ß-Adreno Receptor Analysis.

These samples were inadequate in size and not frozen rapidly enough following sampling. Therefore no analysis was possible.

Myocardial Histology

The specimens harvested permitted adequate staining and microscopic evaluation. In each specimen there were localised areas of cardiac myocytes with irreversible ischaemic damage and contraction bands. Some of these damaged myocytes had progressed to focal necrosis, findings consistent with previous reports of myocardial injury following brain death, head injury and cerebrovascular accidents (Kolin and Norris, 1984).

Neuropathology

The initial inspection macroscopically of the resected cerebrum and brain stem showed changes consistent with raised intracranial pressure. The cerebral gyri were flattened and the superficial sulci narrowed. There was tentorial notching into the medial temporal lobe. The cerebellum was soft and swollen with the cerebellar tonsils disrupted. In each animal the brain stem was soft and swollen with the basis pontis and ventral surface of the medulla flattened, obscuring surface markings. These macroscopic findings suggested 'coning' of the brain with brain stem herniation and brain stem death.

Urine Output

In the 2 animals catheterised urine output was unaffected by brain death. Following catheterisation the residual volumes were 120 and 60 mls. The volumes passed during the experiments was 25 and 35 mls respectively (approximately 0.5-1.0ml/kg/hr) and it was observed that urine output increased during the hypertension of the Cushing response and reduced nearer the end of the study.

COMMENTS

The pilot studies were extremely rewarding in exposing the strengths and shortcomings of the model and allowed many refinements to be made to the experimental set-up. These will be discussed in the chronological order of the experiment.

Anaesthesia

Persistent hypertension following induction of anaesthesia in the dogs was disturbing. It suggested that either the anaesthesia was inadequate, causing endogenous catecholamine release and altering baseline measurements. To resolve this problem the veterinary school at the University of North Carolina, Chapel Hill, was consulted. Their advice concurred with the advice of the senior research analyst, George Quick; they suggested returning to the original form of anaesthesia used in earlier studies, relinquishing recent modifications and the polypharmacy that had resulted. Firstly the premedication was omitted as the dogs were cooperative and relaxed and removed the unreliable 'depot' preparation of intramuscular morphine and diazepam. The anaesthesia became based on Pentothal (20mg/kg) supplemented with Ketamine (20mg/kg) for induction, supplemented with Pentothal and Fentanyl (0.02mg/kg) where necessary, and retained the use of a short acting muscle relaxant, Succinylcholine (0.08mg/kg). This replaced the multiple drugs already used, simplifying the protocol and making it more reproducible. The omission of etomidate proved to be a major improvement and these changes resulted in reliable, steady state, anaesthesia with controlled blood pressure and heart rate. Moreover the baseline haemodynamic data became consistent.

Urinary Catheterisation

Published articles on animal models and brain death made no reference to urine output and the incidence of diabetes insipidus. This study aimed to document urine output in the canine model requiring reliable catheterisation. Initially regular rubber Foley catheters were used (size 12) but were difficult to insert creating false passages. Hence the Foley was replaced with a stiffer arterial perfusion cannula size 10F (type 1855 #10, USCI, CR Bard Inc, Billerica, MA). This catheter was

combined with modification of technique; the foreskin was fully retracted and the penis held taught with a haemostat to create a straight urethra. Occasionally, where this was unsuccessful, the foreskin was incised and the incision continued down the body of the penis to allow direct insertion of the catheter to the urethra. These improvements ensured routine urinary catheterisation. Where possible male dogs were used in this thesis to facilitate catheterisation, although females did not present any problems in the latter studies.

Surgical Preparation

Minor changes were made to the surgical preparation as a result of the pilot studies. Firstly the thymus gland was left intact as exposure to the ascending aorta and superior cava was adequate without excision of the gland.

The passing of nylon tapes around the aorta and pulmonary artery was abandoned as they did not assist the procedure and blunt dissection was of more importance to facilitate the application of the flow probes.

Silk sutures that were used to stabilize the micromanometers to the epicardium were dispensed with, except for the left atrial catheter. Positional adjustment of the manometers was frequent to obtain clean signals and the ligatures prevented repositioning and reinsertion. Without ligatures the catheters were easily manipulated and tended not to migrate. However the catecholamine surge after brain death caused profuse bleeding at the left atrial catheter site and so a purse string, with a snare, was used for this manometer.

As already alluded to, the application of the pulmonary artery flow probe was the most difficult maneuver of the study. In the early studies this was left to the end of the preparation often displacing the micromanometers and piezo-electric crystals. Therefore the flow probe was applied directly after fixation of the base crystal. At this point access to the pulmonary artery was optimal and only the base crystal could be disturbed.

Ventricular Biopsies

Three changes were required to the methodology of ventricular biopsy. Firstly the trucut needle technique did not yield samples of equal size, adequate size nor free of trauma. Therefore the method of choice was a suction drill biopsy. This provides excellent tissue samples with precision, and leaves a defect easy to close with minimal disturbance to the geometry of the ventricle. Therefore left ventricular biopsies during the study were always obtained using this method through a horizontal haemostatic suture during a brief period of inflow occlusion.

The preliminary results from the pilot studies showed probable impairment of right ventricular stroke work following brain death. The protocol was subsequently changed to include biopsies of the right ventricle, to undergo similar analysis as the left ventricle. Due to the relative thinness of the right ventricular free wall, compared to the left ventricle, drill biopsy was not appropriate. Therefore samples were directly excised (approximately 0.5ml) during inflow occlusion from the right ventricular free wall, caudal to the outflow tract, avoiding the axes of the crystals.

The failure to obtain myocardial ß-receptor and metabolite analysis required a more rapid method of tissue freezing. Dr. Jennings, Director of the Cardiovascular Pathology, advised that the samples be frozen in freon, that should be in a receptacle adjacent to the operating table. This would allow quicker immersion, and freezing, in a substance that adheres to the tissue longer than liquid nitrogen. Therefore subsequent tissue handling causes less surface thawing and hence better preservation.

These methods consistently gave good specimens with minimal disturbance to both the ventricles and the haemodynamic status of the animal.

Haemodynamic Data Acquisition

Data acquisition was cumbersome during the pilot studies in that collected data in the laboratory was copied to microdisk for analysis on a different personal computer. Each study collects 30 - 50 files involving 2 - 4 megabytes of information. During a study the assessment of the quality of the data was awkward with this 'manual' transfer of data. Therefore the personal computers were connected to the Department of Surgery computer network; this permitted collection of data on a central file that could be accessed from any networked computer. The central file was protected by code word access. The data therefore could be studied immediately following collection, to ascertain satisfactory recordings, and easily duplicated so that all data were 'backed-up' on separate computers.

This rapid assessment of data revealed how the ten channels required constant attention and adjustment: signals from the piezo-electric crystals would alter their size and their trigger levels; the micromanometers would shift in position giving poor tracings and temperature drift was significant (see below); and finally the ultrasonic flow probes would lose signal due to a lack of ultrasound jell. These pilot studies gave invaluable experience in the maintenance of all ten channels.

At the outset of the pig studies it was not routine to maintain the Millar micromanometers in saline at a temperature of 37°C. The calibration of each catheter was performed at room temperature and when recalibrated at the end of the study there was ± 3 - 10 mmHg of drift. This was unacceptable, so it then became routine to keep the manometers immersed in saline in a warm organ bath. Once the catheters were calibrated at a temperature of 37°C the recorded drift at termination was decreased to ± 1 - 3 mmHg.

Despite these modifications to data collection the results showed significant variation (table 2, 3) between each file at a given sample time. This work and previous studies stressed the necessity to take multiple data sets (4 - 6) of both impedance and occlusion files to calculate a representative mean with standard error for each sample time.

Body Temperature

In each animal the body temperature dropped during the experiment. After induction of anaesthesia the dogs were normothermic between 37.2°C and 37.5°C, but after sternotomy and particularly after brain death they became relatively hypothermic to 34.2, 34.8 and 35.0°C. Subsequent studies used a variety of methods to maintain normothermia: the room was maintained at a temperature of 19°C; the animal was placed on a warmed blanket: the sternotomy was approximated when possible; inhaled gases were prewarmed; the operating light was lowered to provide radiant heat and the animal was kept dry and thoroughly insulated with surgical drapes. These measures were usually successful, in the studies using this model, to maintain normothermia.

CONCLUSIONS

The pilot studies were performed to examine three main topics:

1. The suitability of the animal model.
2. The mechanisms of data collection.
3. The skills necessary to collect and interpret data.

The Animal Model.

These three animals suggested that inflation of an intracranial balloon would cause brain death and in particular compress the brain stem and cause brain stem death. This method had proved relatively straightforward producing similar Cushing responses in each animal. The drop in serum vasopressin levels supports this impression although clinical diabetes insipidus did not occur.

Inasmuch that these animals had been used for other experiments and were also markedly different in size any further conclusions would be invalid. However this complex animal model was successful in three consecutive studies and warranted further investigation.

The Mechanisms of Data Collection

The measuring instruments, computer hardware and software had proved reliable in collecting data. These six studies emphasised the need for 'networking' the data to allow on-line analysis as some data files were unusable due to a channel error. With on-line facilities, all channels can be scrutinised to ensure high quality of the signals.

The Skills Necessary to Collect and Interpret Data

The six studies had enabled the author to gain experience with the surgical and technical skills required. Modifications to the surgical technique were made rapidly to facilitate the procedure, and instrumentation of the heart became routine. Co-ordination of 13 instruments on the heart into 10 accurately calibrated channels of data was more complex. These required constant attention throughout the studies to avoid poor quality data as demonstrated by the failure to collect baseline impedance data in the second animal. Subsequent analysis of data for pressure / volume relationships and impedance data was well structured with satisfactory computer software.

Overall the pilot studies proved that the facilities and technical expertise associated with the laboratory provided a sound environment for the projected studies. They also showed that inflation of an intracranial balloon was a reproducible, straightforward method to induce brain death, the effects of which could be investigated in a validation study.

CHAPTER 5
VALIDATION OF BRAIN DEATH

Introduction

Advances in surgical techniques, organ preservation, immunosuppressive treatment, and life supporting technology have allowed organ transplantation to play a major role in the treatment of patients with end-stage disease. The shortage of suitable organ donors remains the single most important barrier to solid organ transplantation (Rapaport and Anaise, 1991). Restrictions on available donors have been relaxed to extend the donor pool. However it is estimated that 20% of potential donors are not used due to unexpected death and haemodynamic instability (Emery et al, 1986), and 50% will die within 24 hours if not appropriately resuscitated and supported (Bart et al, 1981). Therefore, donor management has received major attention among factors that can affect the organ supply favourably (Soifer and Gelb, 1989). This has led to a renewed interest in the investigation, prevention and treatment of the complications related to brain death. The 'brain dead heart beating cadaver' is known to be associated with donor organ dysfunction, metabolic and hormonal changes, which could contribute to early post transplant organ failure (Rose et al, 1988). The methods and interventions applied to preserve donor organ function are still experimental and controversial using a wide variety of unvalidated animal models. A principle concern remains that no study had confirmed brain stem death conclusively, which was assumed by indirect observations and measurements. These consisted of;

assessment of reflexes in the presence of recent general anaesthesia;

electroencephalography that is difficult to reproduce and may be unreliable,

and hormone measurements to evaluate the hypothalamic endocrine function.

Previous work done in this laboratory had preferred ligation of head and neck vessels in the swine model. However this produced a large volume of unperfused tissue; the upper body became rigid with rigor mortis, associated with hyperkalaemia and metabolic acidosis. A further problem existed in the canine model due to the extensive collateral blood supply to the brain (Finkelstein et al, 1987). Ligation of the first two aortic arch vessels will not reliably cause cerebral ischaemia and ligation of the jugular veins is also recommended. This maneuver would produce the large volume of ischaemic tissue that was encountered in the pig model.

Therefore, prior to investigating the effect of brain death on myocardial preservation it was important to validate a model of brain death itself. The method chosen to instigate brain death was inflation of an intracranial balloon. This method has been supported due to its relative simplicity, its reliability and because it only causes local damage to the intracranial contents without widespread tissue necrosis (Novitzky et al, 1984). Moreover this technique mimics 55% to 75% of all clinical donors, in that it causes a fatal increase of intracranial pressure.

The overall aim of these experiments was to establish a reliable model of brain death that would allow us to examine the effect of brain death on cardiac preservation in the canine model. More specifically the intentions of the validation studies were as follows;

(1) measure baseline haemodynamic, blood, metabolic and histological parameters in the anaesthetised animal,
(2) instigate brain death and repeat the previous measurements at preset time intervals,
(3) to obtain a histological specimen of brain stem at the conclusion of the experiment.

Materials and Methods

The study group consisted of 10 dogs in consecutive studies, their weights ranging from 23.3 to 28.1 kg (mean 25.3 kg, SEM 0.5 kg). All animals were anaesthetised, paralysed, and ventilated in the manner previously described with the modifications outlined on page 133. These changes were needed for more controlled anaesthesia, due to problems encountered in the pilot studies.

The experiment was designed to permit paired analysis with the animal acting as its own control, allowing suitable analysis of each dog before and after instigation of brain death. Measurements were to be recorded at baseline, 15, 45, 90, 120 and every subsequent 60 minutes following brain death. Fifteen minutes was chosen as the first data point as the computer software cannot handle

data from heart rates greater than 200 bpm. The Cushing response has been well documented in prior studies and so fifteen minutes following brain death provided data at a heart rate reliably less than 200bpm. The studies were terminated when the animals became haemodynamically unstable.

Procedure

All animals were surgically prepared in the same manner with no further changes than already described in the previous chapter. Likewise the cardiac dimensions, pressures and flows were acquired and analysed. All animals were successfully catheterised transurethrally.

When all dogs were stable under anaesthesia (appropriate heart rate, systemic pressure and suitable filling pressures of the left atrium and end diastolic right ventricular pressure), baseline data were recorded. As the baseline data were fundamental to the experiment, in terms of each dog acting as its own control, 6 files of 500Hz (steady state, 6 seconds each) and 6 files of 200Hz (caval occlusion, 16 seconds each) were taken at baseline and the pressure volume loops checked for consistency. These data were acquired over a half hour period with the ventilator disconnected during each file recording, and a stabilisation period after each caval occlusion.

Blood samples and biventricular biopsies were taken, and brain death was instigated. During the Cushing response the heart was carefully observed for any bleeding points.

Cushing Response

Brain death was accomplished with an average balloon inflation of 17.8ml (sem ±0.5). This triggered the Cushing response in each animal within 30 - 90 seconds of balloon inflation, during which period there were transient bradycardias and sinus arrest. Then the progressive tachycardia and a haemodynamic explosion followed with severe increases of blood pressure and cardiac output. This initial response of hypertension and tachycardia lasted for a period of 8 to 20 minutes (13.3 min, ±1.5) before measures declined to baseline values or below. During the peak of this phenomenon, in all animals, the heart rate, cardiac output, systemic systolic and diastolic pressures

increased to values greater than 250 beats/min, 4litre/min and 350/200 mmHg respectively. A representative haemodynamic response is depicted in figure 21.

In the subsequent hour the animals were unstable requiring an increased intravenous infusion rate to maintain intravascular volume and blood pressure, a temporary increase in ventilation to maintain pO2 and a bolus of intravenous bicarbonate to correct metabolic acidosis. This was routinely encountered with arterial blood gas analysis at 15 minutes post brain death. The arterial partial pressure of oxygen fell from a mean baseline value of 401 mmHg (sem 48, 167 - 564) to 278 mmHg* (sem 58, 79 - 506), and then returning to baseline values. This was accompanied by a metabolic acidosis represented by a normal partial pressure of carbon dioxide, a lowered pH of 7.31* (sem 0.02, 7.22 - 7.41) from 7.37 (sem 0.02, 7.30 - 7.49), and a decrease in base excess from -3.1 (sem 0.5, -1.2 to -5.1) to -6.7* (sem 0.7, -3.0 to -10.8). These three values at fifteen minutes post brain death were all statistically significant from baseline ($*p \leq 0.05$). Subsequently arterial oxygen concentration and acid - base equilibrium returned to normal, for the duration of the study, assisted with occasional intravenous bolus' of 8.4% sodium bicarbonate (see below).

Therapeutic Interventions

Intravascular volume was maintained with Ringer's lactate solution to maintain a mean systemic arterial pressure of 60 mmHg. If serum sodium concentrations became supra normal (>148mmol/L) the infusion was changed to 5% dextrose. The acid - base balance was maintained between a pH range of 7.3 to 7.4 using intravenous bolus' of sodium bicarbonate 8.4% to infuse 75% of the base deficit according to the formula;

Base deficit = (body weight kg) (0.4) X (desired $\{HCO_3\}$ in mEq/L) - actual $\{HCO_3\}$ mEq/L

Serum potassium was maintained between 4.0 and 4.5 mmol/L by slow injection through a peripheral vein of potassium chloride. At no point were inotropic agents or vasopressor agents used.

A record of temperature via an oesophageal thermometer was made. The animals were laid on a warming blanket and double wrapped in surgical drapes which were kept dry. Following instrumentation of the heart and at cessation of the Cushing response the sternotomy was narrowed as much as possible to conserve body heat. Despite these methods the core temperature did drop from baseline (mean 36.6°C sem 0.2) to 35.6°C (sem 0.5) at 7 hours post brain death although this did not reach statistical significance.

Experiment number 7 survived two hours after brain death; this was the only animal not to develop diabetes insipidus and haemodynamic function progressively declined despite adequate left and right sided filling pressures. Experiment 10 ceased at two hours post brain death due to haemorrhage from a left ventricular biopsy site. The other experiments were terminated due to haemodynamic instability and irreversible deterioration at 6 hours in 4 animals and 7 hours in 4 animals. There are no haemodynamic data for animal 4 at 15 minutes as the instruments were recalibrated to record the Cushing response, shown in figure 11.

Statistical Analysis

Statistical analysis was performed on a Zenith personal computer using the SAS statistical software package (Cary, North Carolina) and validated by the department of Surgical Statistics. All data were tested for normality prior to further analysis, performed with two tailed paired Student's t-test. In the text results are expressed as the mean, plus / minus one standard error of the mean (SEM = standard deviation / $\sqrt{n}$, where n = number of animals in study). Statistical significance was considered where $p \leq 0.05$.

RESULTS

HAEMODYNAMIC CHARACTERISTICS

Baseline Values

Mean values for parameters in 10 sets of control animals prior to brain death are summarised in tables 2 and 3, with the means of these values shown in the top row and 1 standard error of the mean in the second row.

The heart rate in the resting anaesthetised state was 115bpm (± 4); with a systolic/diastolic pressure of 128/84 mmHg (± 9/8). The mean pulmonary artery pressure was 13.5 mmHg (± 0.8) with a left atrial pressure at 6.2 mmHg (± 0.3).

Cardiac output in the resting animals was 1397 ml/min (± 76), systemic vascular resistance was calculated at 5691 dyne.sec.cm^{-5} (± 544) {SVR = ((MAP - RAP)/ CO) X 80}.

Pre-load Independent Recruitable Stroke Work (PRSW)

This assessment of systolic function is determined by plotting ventricular stroke work versus end diastolic volume during a vena caval occlusion. The slope (MM_W) of this linear regression represents load independent analysis of the ventricle's ability to work per unit volume of myocardium.

The mean MM_W for the left ventricle at baseline was 73.1 erg.10^3(± 4.4) and for the right ventricle 24.0 erg.10^3(± 2.96). This corresponds to the stroke power calculated from 500Hz steady state files of 23.6 erg.10^3(± 3.07).

Table 5.

Baseline values for haemodynamic data in validation of brain death studies. *±1sem.* (*n*=10).

Abbreviations;

Left atrial P.; mean left atrial pressure.

Mean PA pressure; mean pulmonary artery pressure

LV PRSW; Left ventricular pre-load independent recruitable stroke work.

RV PRSW; Right ventricular pre load independent recruitable stroke work.

Stroke work; PRSW for right ventricle calculated from 500Hz steady state files.

These baseline values were in the normal range, with relatively low standard errors from the mean. Animal 7 had markedly raised PRSW for both ventricles.

BASELINE VALUES FOR HAEMODYNAMIC DATA FOR VALIDATION OF BRAIN DEATH STUDIES (N=10)

STUDY NUMBER	HEART RATE *bpm*	CARDIAC OUTPUT *mls/min*	LEFT ATRIAL P. *mmHg*	MEAN PA PRESSURE *mmHg*	LV PRSW *erg.103*	RV PRSW *erg.103*	STROKE WORK *erg.103*
Mean	**115**	**1397**	**6.2**	**13.5**	**73.1**	**24.0**	**23.6**
SEM	**4**	**76**	**0.3**	**0.8**	**4.4**	**3.0**	**3.1**
1	103	1261	6.9	13.7	65.9	16.8	18.5
2	112	1137	4.4	13.7	87.2	26.3	17.6
3	103	1474	5.6	17.4	92.4	27.8	36.0
4	124	1836	7.1	18.0	67.8	21.6	29.4
5	125	1283	6.9	11.5	67.0	20.7	21.2
6	103	1454	6.7	10.3	65.8	23.4	17.7
7	144	1393	4.6	10.4	96.4	47.5	24.3
8	122	1791	6.3	11.7	78.2	28.0	44.6
9	110	1090	6.5	12.1	55.2	10.9	12.7
10	99	1247	6.8	15.8	55.3	16.8	14.0
Max	144	1836	7.1	18.0	96.4	47.5	44.6
Min	99	1090	4.4	10.3	55.2	10.9	12.7

Impedance Characteristics

The resistance to mean flow, or Input Resistance (Rin), averaged 788 dyne.sec.cm^{-5} (± 53). The average, or characteristic impedance(Zo) was calculated as the mean impedance modulus over the range 7 - 11 Hz and found to be 180 dyne.sec.cm^{-5} (± 10). This is just under 23% of the input resistance for this group. The pulmonary vascular resistance was calculated at 426 dyne.sec.cm^{-5} (± 49).

Hydraulic Power Analysis

The right ventricular hydraulic power developed averaged 59.1milliwatts (± 5.9). The term 'steady flow' is the hydraulic power that would be required to move blood through the pulmonary circulation in a steady non-pulsating stream at the observed average flow rate. This was calculated at 42.1 mwatts (± 4.1), comprising 71.4% of the total power output of the right ventricle. Steady-flow power is controlled primarily by the vascular resistance, and hence by the activity of the microcirculation. The term 'oscillatory' power represents the energy wasted in performing pulsatile flow and this averaged 17.0mwatts (± 1.9), 28.6% of the total power output.

Transpulmonary efficiency is a ratio between the power dissipated in the pulmonary vasculature and the pulmonary artery flow. Therefore if the value is higher then the pulmonary circulation is **less** efficient, inasmuch that there is less flow with more power wasted. The baseline value for these animals was 2.51 J/L (± 0.15).

Table 6.

Baseline values for impedance data from validation of brain death studies.

±1sem. (*n*=10).

Abbreviations;

Oscill. Power; Oscillatory power

Rin; Pulmonary input resistance at 0 Hz.

PVR; Pulmonary vascular resistance

'Zo; Characteristic impedance, between 7 and 11 Hz.

EFF; Transpulmonary efficiency, the ratio of hydraulic total power of the right ventricle to pulmonary artery flow.

BASELINE VALUES FOR IMPEDANCE DATA FOR VALIDATION OF BRAIN DEATH STUDIES (N=10)

STUDY NUMBER	STROKE WORK *erg.103*	TOTAL POWER *mW*	MEAN POWER *mW*	%MEAN POWER	OSCILL. POWER *mW*	%OscIII POWER	Rin *dyn.s.cm-5*	PVR *dyn.s.cm-5*	Z0 *dyn.s.cm-5*	EFF *J/L*
Mean	**23.6**	**59.1**	**42.1**	**71.4**	**17.0**	**28.6**	**788**	**426**	**181**	**2.51**
S.E.M.	**3.1**	**5.9**	**4.2**	**1.3**	**1.9**	**1.3**	**54**	**49**	**10**	**0.15**
1	18.5	56.6	38.4	68.0	18.2	32.0	874	434	175	2.69
2	17.6	44.5	34.7	78.0	9.8	22.0	967	661	145	2.35
3	36.0	77.4	57.1	73.9	20.4	26.1	949	644	236	3.15
4	29.4	105.1	73.6	70.0	31.5	30.0	786	475	220	3.44
5	21.2	50.1	32.7	64.1	17.4	35.9	717	286	122	2.35
6	17.7	50.4	33.2	65.8	17.3	34.2	565	198	189	2.08
7	24.3	43.8	32.1	73.3	11.7	26.7	596	334	176	1.89
8	44.6	65.1	46.8	71.8	18.3	28.2	525	244	166	2.18
9	12.7	39.5	29.3	74.2	10.2	25.8	888	411	169	2.18
10	14.0	58.6	43.7	74.5	14.9	25.5	1012	575	209	2.82
Max	44.6	105.1	73.6	78.0	31.5	35.9	1012	661	236	3.44
Min	12.7	39.5	29.3	64.1	9.8	22.0	525	198	122	1.89

Effect of brain death on haemodynamic characteristics

(1) Cushing Response

As already stated no data files were collected during the Cushing response as the computer software was not able to process information when the heart rate rises above 200 beats per minute. However from the direct tracings of peripheral arterial pressure and right atrial pressure the following observations were made.

The blood pressure increased from baseline to 402 mmHg (±15.5) systolic and 246 mmHg (±12.3) diastolic. In four animals the systolic pressure increased to greater than 500mmHg and so beyond the range of the monitor. Diastolic pressure in these four animals was 300 to 360 mmHg. Intracranial pressure was rapidly elevated to greater than 600 mmHg (±25.8) and remained greater than systolic arterial pressure.

Cardiac output increased to 4300 ml/min (±1200), while systemic vascular resistance also rose to 8840 dyne.sec.cm^{-5}(±700).

(2) Post Cushing Response

The intervention of brain death and the resultant Cushing response causes major alterations to the haemodynamic status of the animals. In reviewing the data it will be apparent that there are immediate effects followed by more gradual changes.

Peripheral Arterial Pressure and Cardiac Output

Peripheral arterial pressure drops below baseline values after the Cushing response (table 7, figure 22). Systolic, diastolic and mean pressure fall progressively to become statistically significant at two hours, and eventually to values of 50 - 60% below baseline. The cardiac output increased from 1397 mls/min (±75.7) to statistically significant levels at 5 and 6 hours, 2506 (±456.1) and 2158 (±349.9) mls/min respectively (table 8). There was wide variation in this response; animals 1,3,4 and 5 developed large cardiac outputs while animals 2,6 and 7 only modest increases. The tenth

Table 7.

(Above) Systolic peripheral arterial pressure. *±1sem.* (*n*=10).

(Middle) Diastolic peripheral arterial pressure. *±1sem.* (*n*=10).

(Lower) Mean peripheral arterial pressure. *±1sem.* (*n*=10).

Throughout the course of the experiments there is a progressive decline of systemic arterial pressures.(* denotes $p<0.05$)

SYSTOLIC, DIASTOLIC AND MEAN PERIPHERAL ARTERIAL PRESSURES (mmHg)

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)					SYTOLIC PRESSURE							
0	95	140	110	140	180	160	130	115	120	85	**128**	*8.7*
15	70	80	130	70	135	180	85	70	90	60	**100**	*13.3*
45	70	95	100	65	105	110	105	85	80	75	**87**	*5.3*
90	90	70	100	70	115	105	75	85	90	80	**89**	*5.1*
120	85	95	100	75	105	105	55	100	105	50	**81***	*8.2*
180	100	80	110	95	110			100	65	75	**92**	*6.2*
240	75	75	90	85	110	95		100	70		**87***	*5.3*
300	70	80	80	80	90	100		90	70		**83***	*4.0*
360	65	75	70	90	100	100		65	60		**78***	*5.7*
420		65	70	75		90					**75***	*5.4*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)					DIASTOLIC PRESSURE							
0	55	100	100	95	140	75	75	65	80	55	**84**	*7.7*
15	50	45	100	45	115	85	50	45	50	40	**64**	*9.3*
45	55	62	80	40	65	60	55	45	55	55	**57**	*3.9*
90	60	50	80	45	80	60	45	45	65	60	**61**	*4.4*
120	60	70	80	45	85	65	35	50	75	35	**59**	*7.8*
180	65	55	70	55	70			50	45	40	**56***	*4.2*
240	45	45	55	40	60	55		50	45		**49***	*2.6*
300	45	50	50	45	50	60		40	40		**47***	*2.5*
360	45	35	45	50	65	55		30	35		**45***	*4.1*
420		35	45	40		45					**41**	*2.4*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)					MEAN PRESSURE							
0	68	113	103	110	153	103	93	82	93	65	**99**	*7.6*
15	57	57	110	53	122	117	62	53	63	47	**76**	*10.1*
45	60	73	87	48	78	77	72	58	63	62	**67**	*4.0*
90	70	57	87	53	92	75	55	58	73	67	**70**	*4.4*
120	68	78	87	55	92	78	42	67	85	40	**66***	*7.9*
180	77	63	83	68	83			67	52	52	**68***	*4.6*
240	55	55	67	55	77	68		67	53		**62***	*3.3*
300	53	60	60	57	63	73		57	50		**59***	*2.7*
360	52	48	53	63	77	70		42	43		**56***	*4.5*
420		45	53	52		60					**53***	*3.1*

Table 8.

(Above) Cardiac output post brain death. *±1sem.* (*n*=10).

At five hours post brain death the cardiac output was significantly increased

(* denotes $p<0.05$)

(Below) Heart rate post brain death. *±1sem.* (*n*=10).

At fifteen minutes post brain death the residual effects of the Cushing response causes a significant tachycardia. Otherwise values are statistically unchanged from baseline.

CARDIAC OUTPUT AND HEART RATE FOLLOWING BRAIN DEATH

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)				CARDIAC OUTPUT (MLS/MIN)								
0	1261	1137	1474	1836	1283	1454	1393	1791	1090	1247	**1397**	*75.7*
15	2928	1228	1737		1669	1808	1445	1582	1513	1532	**1716**	*161.8*
45	2140	1208	1684	1714	1947	1590	2053	2452	1177	1338	**1730**	*126.3*
90	2527	1062	1458	1856	2042	1387	1719	1299	1197	839	**1539**	*151.1*
120	2366	1060	1954	1916	1830					831	**1660**	*239.9*
180	2649	1116	2573	2149	1997			1772	1410		**1952**	*215.3*
240	2511	1204	2863	1962	2198	1498		1778	1036		**1881**	*223.4*
300	3860	1359	2241	2299	2768						**2506***	*456.1*
360	3949	1460	2160	2437	2959	1422		1320	1561		**2158***	*349.9*
420		1335	2969	2603		1602					**2127**	*452.5*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)				HEART RATE (BEATS PER MINUTE)								
0	103	112	103	124	125	103	144	122	110	99	**115**	*4.3*
15	176	164	174		125	119	120	128	178	187	**152**	*9.6*
45	151	123	147	112	117	96	118	112	127	136	**124**	*5.0*
90	139	124	140	108	118	89	111	106	113	138	**119**	*5.1*
120	143	116	133	107	119					138	**126**	*5.7*
180	140	110	130	140	118			109	104		**122**	*5.7*
240	134	105	119	106	119	92		106	110		**111**	*4.4*
300	125	104	120	102	120						**114**	*5.3*
360	127	105	123	96	119	79		98	105		**106**	*6.0*
420		104	126	90		78					**99**	*11.9*

animal was exceptional due to the haemmorrhage causing low output, and animal 8 had a unique catecholamine response. This variation prevented uniform statistical significance although the graph in figure 23 displays the trend.

Systemic Vascular Resistance

The rise in cardiac output is not resultant from an increase in heart rate (table 8). At baseline the rate is 115 (±4.3) bpm and at 15 minutes there is a residual tachycardia from the catecholamine surge (152 (±9.6) bpm); subsequently there is no significant change in heart rate from baseline values (figure 22). The more likely reason for the increase in output is the fall in systemic vascular resistance (SVR) (table 9). The decrease occurred in all animals at 15 minutes from a mean baseline value of 5691 (±543) dyne.sec.cm^{-5} to 3581 (±464) dyne.sec.cm^{-5}. The SVR continues to fall (figure 23) to reach statistical significance at four hours post brain death with a value of 2709 (±320) dyne.sec.cm^{-5} and remains low until termination of the studies.

End diastolic pressures and dimensions

Two dimensional measurements of the left and right ventricles by the free wall to septal distances revealed interesting changes. At baseline the measurements were very consistent for all animals; 52.8 mm (±0.8) and 30.2 mm (±1.3) for LV freewall to septal distance and RV free wall to septal distance respectively (table 11). This inferred that placement of the septal crystal between the two free wall crystals was consistent, strengthening subsequent calculations of biventricular volumes. These dimensions increased in left and right ventricles throughout the experiment, with values of 56.2 mm (±2.4) and 34.8* mm (±1.3) respectively(Figure 24). This increase was significant in the right ventricle at 6 and 7 hours post brain death,

Table 9.

(Above) Systemic vascular resistance post brain death. *±1sem.* (*n*=10).

There is an immediate and persistent decrease in SVR post brain death, probably caused by denervation of the arterioles

(* denotes p<0.05)

(Below) Left atrial pressure. *±1sem.* (*n*=10).

Left atrial pressure significantly increases post brain death (* denotes p<0.05).

SYSTEMIC VASCULAR RESISTANCE AND LEFT ATRIAL PRESSURE FOLLOWING BRAIN DEATH

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)		SYSTEMIC VASCULAR RESISTANCE (DYN.SEC.10-5)										
0	4207	7748	5495	4759	9386	5595	5197	3612	6852	4061	**5691**	*543.8*
15	1472	3402	4952		5695	5061	3324	2584	3322	2413	**3581**	*464.1*
45	2160	4478	3968	2030	3119	3736	2688	1787	4215	3522	**3224**	*335.2*
90	2146	3867	4519	2144	3562	4181	2559	3469	4843	6155	**3876**	*423.1*
120	2225	5430	3417	2057	3997					3652	**3463**	*468.3*
180	2315	4541	2591	2544	3338			3011	2931		**3039**	*275.8*
240	1694	3196	1743	2057	2722	3511		2783	3968		**2709***	*320.5*
300	1105	3531	2142	1972	1830						**2116***	*340.1*
360	960	2231	1787	1923	1961	3760		1965	1993		**2072***	*275.2*
420		2240	1311	1421		2741					**1927***	*341.1*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)			LEFT ATRIAL PRESSURE (mmHg)									
0	6.9	4.4	5.6	7.1	6.9	6.7	4.6	6.3	6.5	6.8	**6.2**	*0.3*
15	8.8	8.1	7.2		7.1	7.4	5.7	4.1	5.2	5.8	**6.6**	*0.5*
45	7.6	8.6	6.0	6.8	8.0	8.8	9.4	6.1	5.8	6.3	**7.3**	*0.4*
90	7.7	6.9	9.9	6.4	8.0	8.1	7.6	4.0	6.2	5.2	**7.0**	*0.5*
120	7.5	13.1	11.0	7.1	7.9					5.7	**8.7**	*1.1*
180	7.9	11.9	13.8	7.2	7.8			6.0	12.3		**9.6***	*1.1*
240	6.5	11.8	13.2	7.3	7.4	7.4		6.4	16.3		**9.6***	*1.3*
300	9.9	13.2	9.4	7.4	7.9						**9.6***	*1.1*
360	9.4	13.3	8.4	8.7	8.5	8.7		10.0	9.7		**9.6***	*0.6*
420		11.2	8.9	9.2		9.7					**9.7***	*0.6*

Figure 22. (Above)

Heart rate and peripheral arterial pressures following brain death. (*n*=10).

The progressive fall in arterial pressure is depicted, with no change in heart rate compared to baseline.

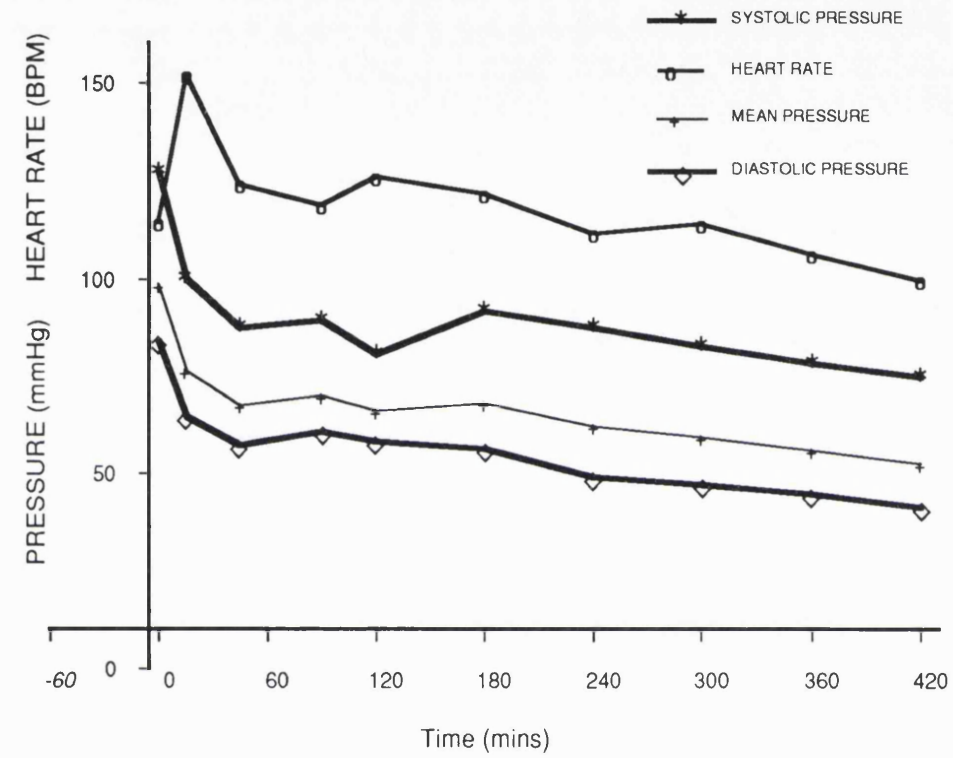
Figure 23. (Below)

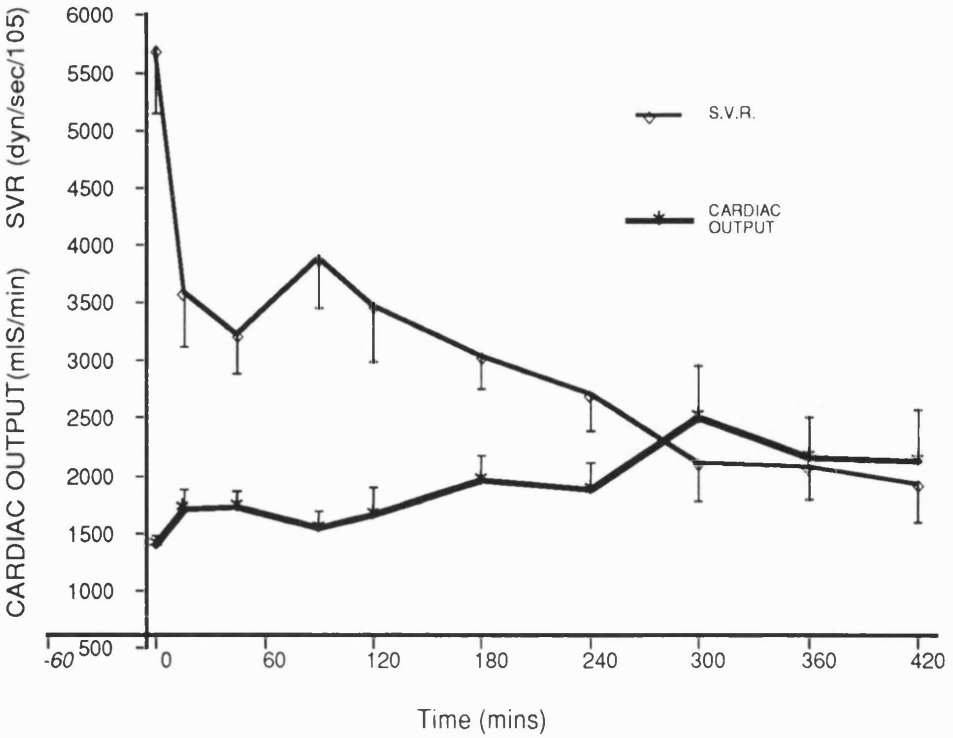
Systemic vascular resistance and cardiac output post brain death.

±1sem. (*n*=10).

SVR immediately decreases, whilst the cardiac output shows a progressive increase that becomes significant five hours post brain death.

HEART RATE, PERIPHERAL ARTERIAL PRESSURES, CARDIAC OUTPUT AND SYSTEMIC VASCULAR RESISTANCE FOLLOWING BRAIN DEATH





The change in left and right ventricular dimensions were mirrored by the changes in their respective end-diastolic filling pressures (LVEDP and RVEDP). The LVEDP increased from 7.3 mmHg (±1.1) to 10.4* mmHg (±1.3) at six hours with a similar rise in RVEDP from 1.8 mmHg (±0.3) to 5.3* mmHg (±1.7) (table 11, figure 24). The values for the LVEDP are supported by mean values of the left atrial pressure which rose from a mean baseline value of 6.2 mmHg (±0.3) to 9.6* mmHg (±0.6) in the same period (table 9).

Pressure Volume Relationships (PRSW)

These direct measurements of peripheral pressure, ventricular pressures and dimensions suggested impairment of myocardial contractility. This impression is supported by data derived from the pressure volume loops for left and right ventricles. Left ventricular preload independent recruitable stroke work (PRSW) was increased initially at 15 minutes to 80.9 erg.10^3 (±14.4) from a baseline value of 73.11 erg.10^3 (±4.11). From this point the LVPRSW declined to significant levels, at 2, 3 and 5 hours, of 49.2 erg.10^3 (±3.5), 56.7 erg.10^3 (±3.3) and 57.0 erg.10^3 (±5.9) respectively (table 12). In animal 8 the six hour PRSW was 158% of its baseline value, correlated to a high epinephrine level (table 17), preventing statistical significance at this time point. These changes in LVPRSW are displayed graphically in figure 25.

The change in X-intercept is shown in table 12. This data shows marked variation between studies especially animal 5 which remains negative throughout, ie., the theoretical left ventricular end diastolic volume at which stroke work is zero remained less than the baseline value throughout the experiment. All other studies show a trend of the X-intercept to become increasingly positive, indicating the PRSW slope is moving to the right. This correlates to a failing ventricle with increasing end-diastolic volumes and pressures. Due to the variance between animals these values do not reach statistical significance.

Table 10.

(Top) LVEDP; Left ventricular end-diastolic pressure. *±1sem.* (*n*=10).

(Second) RVEDP; right ventricular end-diastolic pressure. *±1sem.* (*n*=10).

Table 11.

(Third) Left ventricular free wall to septal distance. *±1sem.* (*n*=10).

(Fourth) Right ventricular free wall to septal distance. *±1sem.* (*n*=10).

The baseline data for septal / free wall distances is remarkably consistent at baseline, reflecting consistent piezo-electric crystal placement.

This data demonstrates similar trends for left and right ventricles with increasing diastolic pressures and ventricular dimensions post brain death.

(* denotes $p<0.05$)

LVEDP, RVEDP, LV FREE WALL-SEPTAL AND RV FREE WALL-SEPTAL DISTANCES FOLLOWING BRAIN DEATH

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)					LVEDP (mmHg)							
0	4.9	13.9	8.6	9.6	6.9	4.4	5.1	3.6	3.9	12.2	**7.3**	*1.1*
15	8.6	6.9	9.3		6.9	5.5	6.5	4.2	6.3	6.3	**6.7**	*0.5*
45	9.5	13.1	11.8	10.7	8.3	5.6	9.8	6.8	5.0	10.5	**9.0**	*0.9*
90	9.3	4.3	12.0	13.9	6.3	4.3		4.3	3.0	9.2	**7.4**	*1.3*
120	11.0	9.2	12.0	14.2	2.7		8.6			7.7	**9.3**	*1.4*
240	6.8	17.1	20.0	10.5	7.9	4.5		7.2	1.9		**9.5**	*2.2*
360	12.9	13.8	11.0	10.5	13.9	5.8		11.3	3.9		**10.4***	*1.3*
420		13.8	10.3	12.6		7.6					**11.1***	*1.4*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)					RVEDP (mmHg)							
0	2.0	3.2	2.1	0.8	2.9	1.6	2.8	0.8	0.0	1.7	**1.8**	*0.3*
15	2.8	4.4	2.5		2.9	2.3	1.6	2.2	0.5	0.4	**2.2**	*0.4*
45	2.2	5.4	3.1	4.8	2.4	2.4	2.7	3.6	1.3	2.8	**3.1**	*0.4*
90	2.2	5.3	4.3	3.6	0.8	2.5		2.0	0.9	2.1	**2.6**	*0.5*
120	2.5	6.4	3.2	5.7	0.2		2.7			2.1	**3.3**	*0.8*
240	1.9	6.9	4.3	4.6	1.9	2.6		4.8	1.9		**3.6**	*0.6*
360	4.3	7.6	5.1	4.8	4.1	3.2		9.3	4.5		**5.3***	*0.7*
420		7.6	4.7	5.4		5.1					**5.7***	*0.7*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)					LV FREE WALL - SEPTAL DISTANCE (mm)							
0	52.9	52.7	56.0	51.9	58.2	51.8	52.4	49.9	54.2	48.4	**52.8**	*0.8*
15	54.0	51.4	59.8		58.2	51.7	53.7	49.1	48.6	45.0	**52.4**	*1.6*
45	56.0	53.6	62.4	54.9	59.2	52.4	56.3	51.0	51.9	48.2	**54.4**	*1.5*
90	57.3	52.3	64.0	54.5	57.0	51.3		49.1	54.6	48.3	**54.3**	*1.6*
120	61.3	54.8	64.0	54.7	56.7		56.9			47.3	**56.5**	*2.0*
240	55.7	54.7	65.0	55.6	56.4	50.5		50.1	51.4		**54.9**	*1.7*
360	57.1	53.5	61.8	55.6	57.9	51.1		50.8	54.8		**55.3**	*1.3*
420		53.5	62.9	56.2		52.2					**56.2**	*2.4*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)					RV FREE WALL - SEPTAL DISTANCE (mm)							
0	32.2	34.2	32.7	27.4	23.5	32.3	26.5	37.3	29.2	26.4	**30.2**	*1.3*
15	29.3	36.2	33.0		24.1	31.9	27.1	36.4	28.0	27.1	**30.3**	*1.4*
45	28.3	36.5	32.9	29.6	24.1	33.1	26.7	37.8	28.9	28.1	**31.0**	*1.5*
90	30.2	35.4	32.0	30.2	22.7	32.8		37.3	28.6	27.7	**30.8**	*1.4*
120	31.0	35.8	32.1	30.6	22.9		28.0			27.4	**29.7**	*1.5*
240	30.8	36.7	34.3	29.1	23.8	33.5		40.0	30.2		**32.3**	*1.8*
360	33.2	36.7	33.9	29.9	24.7	34.9		41.7	32.8		**33.5***	*1.7*
420		36.7	34.4	31.1		36.9					**34.8***	*1.3*

Figure 24.

Left and right ventricular end-diastolic pressures, and left/right free wall to septal distances post brain death. *±1sem.* (*n*=10).

Shaded bar graph denotes septal to right ventricular free wall distance (Sept/RV-FW)

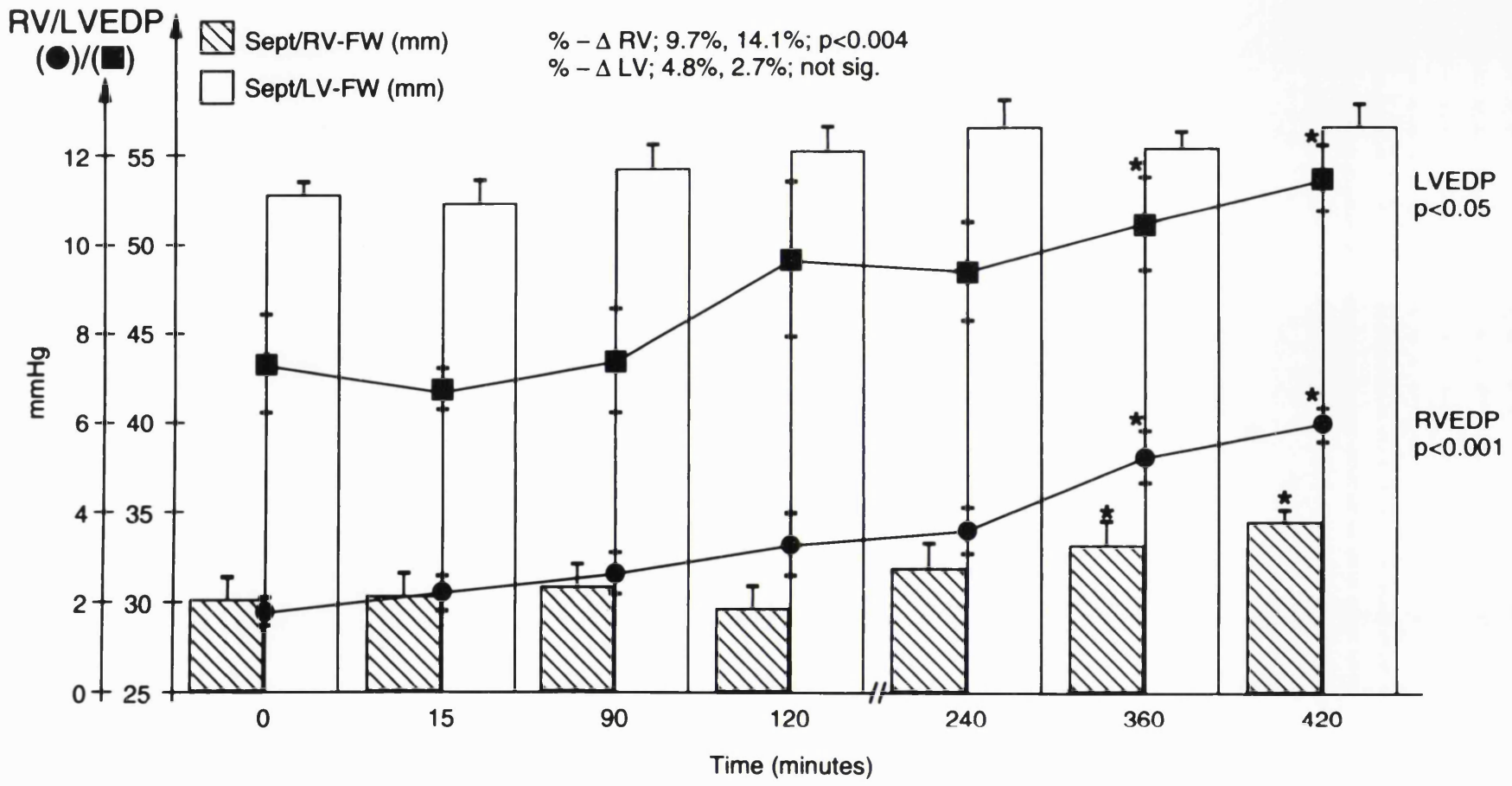
Plain bar graph denotes septal to left ventricular free wall distance (Sept/LV-FW)

Line graph with • denotes right ventricular end-diastolic pressure(RVEDP)

Line graph with¤ denotes left ventricular end-diastolic pressure(LVEDP)

X-axis is time post brain death, where 0 equals baseline.

At six hours post brain death there is a significant change in all parameters except the Sept/LV-FW distance.


RV/LVEDP
(●)/(■)
Sept/RV-FW (mm)
Sept/LV-FW (mm)
% – Δ RV; 9.7%, 14.1%; p<0.004
% – Δ LV; 4.8%, 2.7%; not sig.
mmHg
12
10
8
6
4
2
0
55
50
45
40
35
30
25
0
15
90
120
240
360
420
Time (minutes)
LVEDP
p<0.05
RVEDP
p<0.001

The changes evident in the LVPRSW and X-intercept are more pronounced in the **right** ventricle. The baseline mean value of 23.9 erg.10^3 (±3.0) remains constant at 15 minutes (23.5 erg.10^3 (±3.4)) before dropping to significant values at 2 hours {10.0 erg.10^3 (±2.2)} until the end of the study (table 13). The percentage change of RVPRSW is plotted against the percent change of LVPRSW in figure 25, displaying the greater significance of the right ventricular injury with regard to change from baseline. It is interesting to note that animal 7, which ceased 2 hours following brain death, had the most marked injury to left and right ventricles, even at 15 minutes.

The right ventricle has similar variance to the left ventricle for changes of the x-intercept. The trend is also similar with increasing values through the experiment to a mean of 11.4 mls (±3.5) at 6 hours post brain death.

Impedance Characteristics

The resistance to flow from the right ventricle is expressed as the input resistance (Rin) (table 14). This does not significantly change through the study (788 dyne.sec.cm^{-5} (±53.5) at baseline versus 667 dyne.sec.cm^{-5} (±68.) at 6 hours). Nor does the characteristic impedance (Zo) change (181 dyne.sec.cm^{-5} (±10) at baseline versus 148 dyne.sec.cm^{-5} (±15) at 6 hours) which represents the average of moduli between 7 and 11 Hz. These are reflections from the proximal and intermediate part of the vascular bed, and changes in Zo represent changes in the larger proximal branches of the pulmonary artery. However when left atrial pressure is subtracted from the calculation of input resistance, to obtain the pulmonary vascular resistance (PVR), there are significant changes. The PVR drops from 426 dyne.sec.cm^{-5} (±49.1) to 250 dyne.sec.cm^{-5} (±27.5) at six hours. This implies decreased resistance in the pulmonary vasculature which is offset by the increased resistance caused by a rising left atrial pressure. The percentage changes for input resistance, pulmonary vascular resistance and characteristic impedance are plotted on figure 26.

Table 12.

(Top) Actual values for left ventricular pre-load independent recruitable stroke work (LV PRSW). *±1sem.* (*n*=10).

(Middle) Percentage change of LV PRSW. *±1sem.* (*n*=10).

(Lower) X-Intercept for line plot of LV PRSW. *±1sem.* (*n*=10).

The PRSW for the left ventricle is significantly impaired at 2, 3 and 5 hours post brain death. The high value in animal 8 at six hours corresponds to a very high serum epinephrine level.

(* denotes $p<0.05$)

LEFT VENTRICULAR STROKE WORK, ACTUAL AND PERCENTAGE CHANGE, AND CHANGE IN X-INTERCEPT FOR LEFT VENTRICLE (mls) FOLLOWING BRAIN DEATH

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)		LEFT VENTRICULAR STROKE WORK (erg.103)										
0	65.9	88.5	92.4	67.8	67.0	65.8	96.4	78.2	55.2	55.3	**73.1**	*4.4*
15	50.7	89.9	51.2		48.7	79.8	55.9	64.4	184.0	103.2	**80.9**	*14.4*
45	48.4	53.1	37.5		54.5	60.8		67.4	83.4	57.3	**57.8**	*4.5*
90	48.8	67.8	50.0	48.0	53.7	59.1	48.8	69.8	63.2	58.4	**56.8**	*2.5*
120	37.7	56.8	49.3	57.1	57.1		51.2			35.0	**49.2***	*3.5*
180	42.9	54.6	54.4		59.7			64.3	64.4		**56.7***	*3.3*
240	44.3	47.2	57.0	60.9	61.8	91.6		65.9	72.4		**62.6**	*5.3*
300	49.8	43.0	51.2	75.3	65.9						**57.0***	*5.9*
360	51.4	45.0	53.4	72.3	66.1	74.2		124.0	41.2		**65.9**	*9.4*
420		46.4	44.9	69.7		75.1					**59.0**	*7.8*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)		% CHANGE OF LV PRELOAD RECRUITABLE STROKE WORK										
0	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	**100%**	*0%*
15	77.0%	101.6%	55.5%		72.7%	121.2%	58.2%	82.4%	333.5%	186.5%	**118.7%**	*30.4%*
45	73.4%	59.9%	40.5%		81.3%	92.4%		86.1%	151.2%	103.5%	**86.1%**	*10.9%*
90	74.1%	76.7%	54.1%	70.7%	80.0%	89.8%	53.4%	89.3%	114.5%	105.6%	**78.8%**	*7.1%*
120	57.2%	64.1%	53.4%	84.1%	85.2%		55.0%			63.4%	**63.2%***	*6.6%*
180	65.1%	61.7%	58.9%		89.1%			82.2%	116.8%		**79.0%***	*9.0%*
240	67.2%	53.3%	61.7%	89.8%	92.2%	139.2%		84.3%	131.3%		**89.9%**	*11.0%*
300	75.6%	48.6%	55.4%	111.0%	98.3%						**77.8%***	*12.0%*
360	78.0%	50.8%	57.8%	106.6%	98.5%	112.8%		158.6%	74.6%		**92.2%**	*12.3%*
420		52.4%	48.6%	102.7%		114.1%					**79.4%**	*16.9%*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)			CHANGE IN X-INTERCEPT (mls)									
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	*0.0*
15	17.1	6.8	34.7		-9.1	-4.6	5.5	4.5	-4.7	-1.5	**5.4**	*4.5*
45	25.4	8.6	38.3		-5.8	-4.6		6.7	-0.6	9.6	**9.7**	*5.1*
90	22.9	12.7	44.3	16.7	-9.8	-5.4	7.8	9.2	2.1	5.6	**10.6**	*4.6*
120	24.1	12.0	43.6	17.6	-11.1		6.1			9.8	**14.6**	*6.4*
180	23.0	12.7	40.9		-13.5			9.2	1.0		**12.2**	*7.6*
240	11.7	10.8	36.4	17.9	-14.2	-0.8		12.2	2.2		**9.5**	*5.2*
300	9.8	9.5	33.6	13.9	-14.6						**10.4**	*7.7*
360	12.1	11.4	35.2	12.2	-14.3	-1.1		42.8	15.0		**14.2**	*6.4*
420		10.9	35.9	12.9		1.5					**15.3**	*7.3*

Table 13.

(Top) Actual values for right ventricular pre-load independent recruitable stroke work (RV PRSW). *±1sem.* (*n*=10).

(Middle) Percentage change of RV PRSW. *±1sem.* (*n*=10).

(Lower) X-intercept for line plot of RV PRSW. *±1sem.* (*n*=10).

The PRSW for the right ventricle is significantly impaired after two hours of brain death for the duration of the experiments. The positive trend in the X-intercept mirrors the change seen in the left ventricle.

(* denotes $p<0.05$)

RIGHT VENTRICULAR STROKE WORK, ACTUAL AND PERCENTAGE CHANGE, AND CHANGE IN X-INTERCEPT FOR RIGHT VENTRICLE (mls) FOLLOWING BRAIN DEATH

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)		RIGHT VENTRICULAR STROKE WORK (erg.103)										
0	16.8	25.4	27.8	21.6	20.7	23.4	47.5	28.0	10.9	16.8	**23.9**	*3.0*
15	13.2	25.1	15.8		19.0	46.9	23.3	28.7	14.9	25.1	**23.5**	*3.4*
45	12.9	24.5	6.9		15.6	24.9		28.4	8.8	7.2	**16.1**	*2.9*
90	12.6	12.8	2.3	13.5	17.7	18.0	9.6	36.2	6.1	5.5	**13.4**	*2.8*
120	8.4	13.0	2.7	17.6	16.6		5.8			5.7	**10.0***	*2.2*
180	11.5	12.2	11.2		18.2			19.1	6.4		**13.1***	*1.9*
240	11.0	12.6	9.7	18.4	20.2	22.5		14.8	5.9		**14.4***	*2.0*
300	13.7	11.9	13.6	20.6	17.4						**15.4***	*1.6*
360	13.5	13.3	11.3	19.9	19.8	18.4		14.8	5.1		**14.5***	*1.8*
420		15.1	8.5	21.3		11.8					**14.2***	*2.7*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)		% CHANGE OF RV PRELOAD RECRUITABLE STROKE WORK										
0	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	**100%**	*0%*
15	78.3%	98.9%	56.6%		91.8%	200.7%	49.0%	102.3%	136.3%	149.8%	**107.1%**	*16.0%*
45	76.7%	96.2%	24.7%		75.3%	106.4%		101.4%	80.2%	42.8%	**75.5%**	*9.5%*
90	74.7%	50.3%	8.4%	62.2%	85.4%	77.1%	20.2%	129.2%	56.0%	32.7%	**59.6%**	*10.5%*
120	49.8%	51.0%	9.8%	81.4%	80.0%		12.2%			34.1%	**45.5%***	*10.9%*
180	68.6%	48.2%	40.3%		88.0%			68.0%	59.0%		**62.0%***	*6.9%*
240	65.6%	49.4%	34.9%	84.9%	97.7%	96.2%		52.7%	53.5%		**66.9%***	*8.3%*
300	81.3%	46.7%	49.0%	95.2%	84.1%						**71.3%***	*9.8%*
360	80.5%	52.4%	40.5%	92.2%	95.6%	78.8%		52.7%	46.2%		**67.4%***	*7.7%*
420		59.4%	30.4%	98.7%		50.6%					**59.8%***	*14.3%*

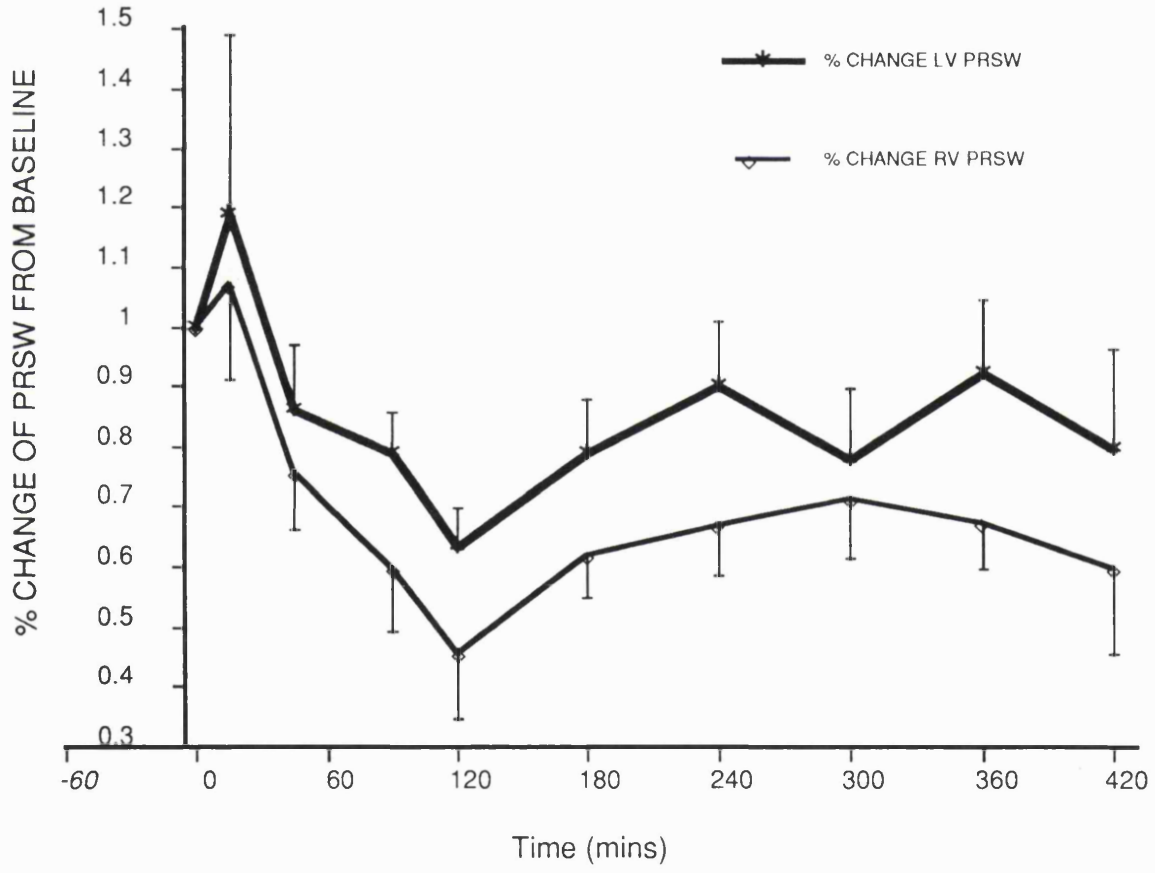
Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)			CHANGE IN X-INTERCEPT (mls)									
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	*0.0*
15	-0.4	9.3	6.5		-1.7	1.9	-3.8	2.5	-1.3	2.4	**1.7**	*1.4*
45	4.3	13.3	3.6		-0.4	1.2		6.3	0.5	5.1	**4.3**	*1.5*
90	8.2	11.9	-17.9	6.1	-0.3	-1.2	-0.6	9.5	2.1	0.6	**1.8**	*2.5*
120	8.3	13.2	-8.8	8.7	-2.0		-1.4			10.1	**4.0**	*3.0*
180	8.9	12.4	11.0		0.3			8.9	3.8		**7.6**	*1.9*
240	4.4	11.7	7.0	5.9	2.0	1.3		7.0	5.0		**5.5**	*1.2*
300	4.1	9.4	14.2	4.6	1.3						**6.7**	*2.3*
360	5.7	9.9	15.1	5.1	3.2	2.5		31.4	18.4		**11.4**	*3.5*
420		9.7	19.4	5.7		-0.1					**8.7**	*4.1*

Figure 25.

Percentage change of pre-load independent recruitable stroke work for left and right ventricles post brain death. *±1sem.* (n=10).

The impairment of right ventricular systolic function post brain death is significant, and has not previously been recognised. Compared to the left ventricle the right ventricle is more significantly impaired in its change from baseline.

PERCENTAGE CHANGE OF PRELOAD INDEPENDANT RECRUITABLE STROKE WORK FOR LEFT AND RIGHT VENTRICLES FOLLOWING BRAIN DEATH



Right Ventricular Hydraulic Power Analysis

The derivation of hydraulic power data depends appropriately on the values of pressure and flow. Pulmonary artery pressure remains constant through the studies (table 16) while pulmonary artery flow increases reflected by the cardiac output (table 8). Therefore hydraulic power increases incrementally following brain death with the increase in cardiac output. This change becomes significant at 3 to 7 hours post brain death with total power being 132.9 mW (±29.2), 117.1 mW (±23.9), 104.5 mW (±29.9) respectively, compared to a baseline value of 59.1 mW (5.9). The percentage contributions to total power from mean power and oscillatory power remain unchanged at the end of study; mean power contributing 71.4%(±1.3) at baseline versus 73.4%(±3.1) and oscillatory power the remainder (28.6% vs 26.6%). This implies the elasticity of the pulmonary vasculature remains unchanged. In the mid portion of the study this ratio changes to a maximum at 3 hours where mean power is 79%* (±1.4) and oscillatory power 21%* (±1.4), suggesting less power being dissipated into the vascular bed and more efficiently involved in the propulsion of blood.

However transpulmonary efficiency does not change following brain death as the ratio of oscillatory power to pulmonary artery flow does not significantly change (table 16).

The calculation of right ventricular stroke work from 500Hz steady state files is plotted as a percentage change against the percentage change of RVPRSW in figure 27. Until 4 hours post brain death these two measures of contractility in the right ventricle show identical changes. Following this the variance in the value derived from 500Hz files becomes large resulting in a trend back to baseline values.

Table 14.

Pulmonary Impedance profile post brain death.

(Top) Input Resistance. *±1sem.* (*n*=10).

(Middle) Pulmonary Vascular Impedance. *±1sem.* (*n*=10).

(Lower) Characteristic Impedance. *±1sem.* (*n*=10).

The pulmonary vascular resistance is significantly decreased at six and seven hours post brain death. There is a slight trend for input resistance and characteristic impedance to fall but does not become significant.

(* denotes $p<0.05$)

INPUT RESISTACE, PULMONARY VASCULAR RESISTANCE AND CHARACTERISTIC IMPEDANCE FOLLOWING BRAIN DEATH DYNES . SEC . cm-5

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean S.E.M
TIME (min)				INPUT RESISTANCE (Rin)							
0	874	967	949	786	717	565	596	525	888	1012	**788** *53.5*
15	532	1063	880		810	552	675	508	967	741	**748** *66.3*
45	599	967	836	619	605	586	610	412	1165	798	**720** *66.5*
90	538	993	983	595	624	648	574	605	920	1058	**754** *62.0*
120	576	1039	798	593	666					1104	**796** *93.1*
180	514	1108	716	575	643			514	932		**715** *85.7*
240	533	1165	659	553	655	627		541	1109		**730** *90.6*
300	428	1015	697	482	575						**639** *116.7*
360	421	950	687	463	563	655		827	769		**667** *68.4*
420		951	542	442		652					**647** *127.3*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean S.E.M
TIME (min)				PULMONARY VASCULAR RESISTANCE							
0	434	661	644	475	286	198	334	244	411	575	**426** *49.1*
15	291	533	548		472	224	359	302	691	436	**429** *49.6*
45	313	398	549	302	277	145	246	213	770	423	**364** *55.0*
90	294	477	442	318	310	181	220	357	507	564	**367** *37.8*
120	321	51	349	298	319					552	**315** *65.2*
180	275	257	285	306	331			243	233		**276** *13.2*
240	326	377	289	256	386	231		251	8		**265** *42.0*
300	222	236	361	225	347						**278** *34.8*
360	230	222	376	178	333	166		222	271		**250*** *27.5*
420		281	302	160		168					**228*** *43.0*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean S.E.M
TIME (min)				CHARACTERISTIC IMPEDANCE (Zo)							
0	175	145	236	220	122	189	176	166	169	209	**181** *10.3*
15	131	129	106		116	188	173	186	177	163	**152** *10.5*
45	85	182	190	168	153	206	150	133	210	207	**168** *11.9*
90	100	125	221	156	142	173	110	166	183	189	**157** *11.3*
120	90	136	221	182	152					173	**159** *18.2*
180	86	156	144	134	147			160	138		**138** *9.3*
240	131	141	179	185	146	166		150	162		**157** *6.6*
300	109	164	143	150	146						**142** *10.2*
360	103	192	84	154	142	166		148	199		**148** *15.0*
420		147	158	156		175					**159** *6.7*

Figure 26.

Pulmonary impedance profile post brain death.

Percentage change of input resistance, pulmonary vascular resistance and characteristic impedance post brain death. *±1sem.* (n=10).

The pulmonary vascular resistance is significantly decreased 6 and 7 hours post brain death. The resistance to flow from the right ventricle (ie the input resistance) remains unchanged.

PERCENTAGE CHANGE OF INPUT RESISTANCE, PULMONARY VASCULAR RESISTANCE AND CHARACTERISTIC IMPEDANCE FOLLOWING BRAIN DEATH

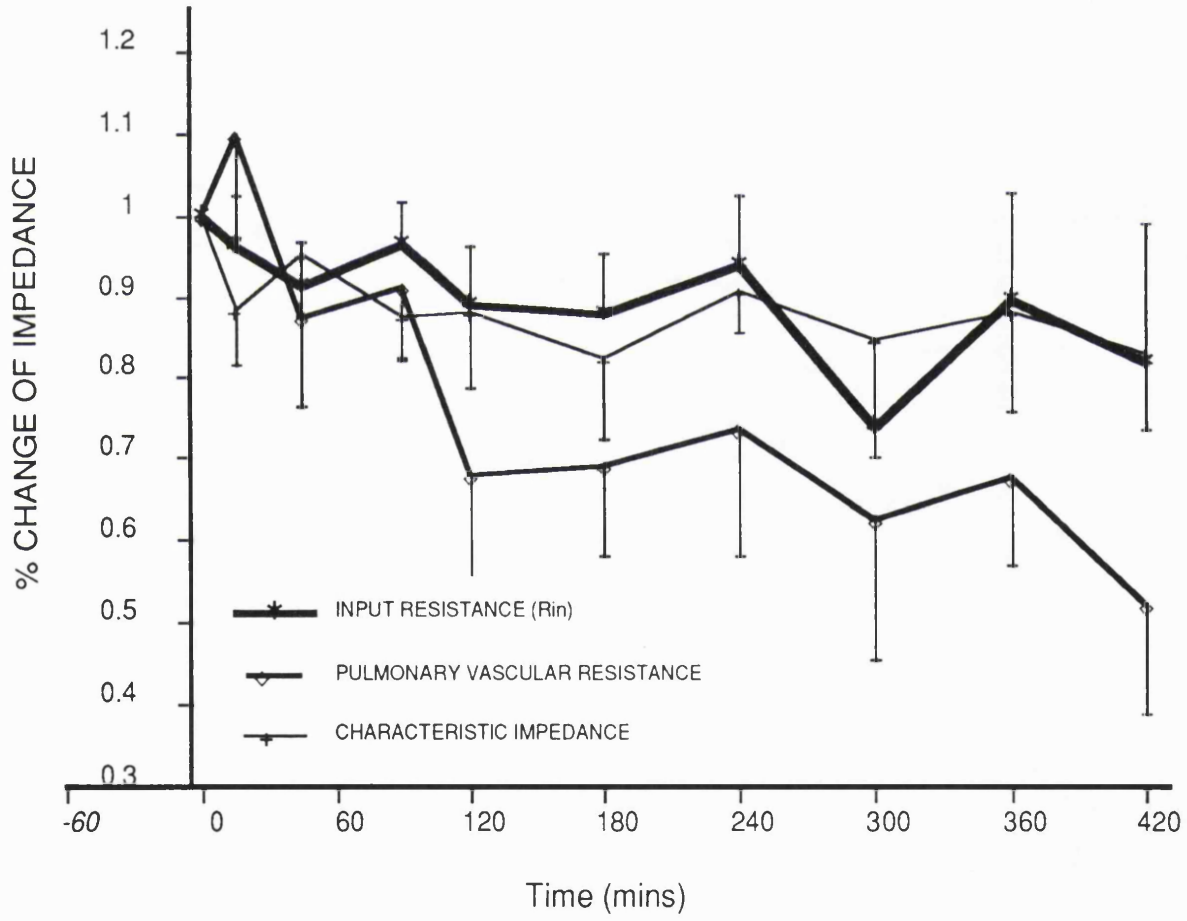


Table 15.

Hydraulic power analysis for the right ventricle post brain death;

(Top) Total hydraulic power from the right ventricle. *±1sem.* (*n*=10).

(Middle) Mean power including its percentage of total power.

±1sem. (*n*=10).

(Lower) Oscillatory power including its percentage of total power.

±1sem. (*n*=10).

Total hydraulic power from the right ventricle increases post brain death attaining statistical significance at three hours. In the mid portion of the study (2 and 3 hours) the percentage of total power dissipated in the pulmonary vasculature (oscillatory power) significantly decreases.

(* denotes $p<0.05$)

HYDRAULIC POWER ANALYSIS FOR THE RIGHT VENTRICLE FOLLOWING BRAIN DEATH

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)				TOTAL POWER (mW)								
0	56.6	44.5	77.4	105.1	50.1	50.4	43.8	65.1	39.5	58.6	**59.1**	*5.9*
15	148.0	52.6	97.5		84.5	73.6	49.1	50.2	81.2	61.2	**77.6**	*10.5*
45	90.5	47.3	88.3	63.4	86.0	60.3	85.5	92.8	58.6	49.6	**72.2**	*5.4*
90	115.3	37.3	78.8	71.8	97.2	51.6	57.4	42.7	48.9	28.7	**63.0**	*8.3*
120	107.2	40.1	109.3	75.7	84.3					30.1	**74.5**	*13.6*
180	119.9	47.4	164.3	90.6	96.0			60.6	65.3		***92.0**	*15.2*
240	116.5	58.6	193.5	72.5	114.5	57.7		63.9	41.9		***89.9**	*17.6*
300	217.1	66.7	127.3	94.3	159.0						***133**	*29.2*
360	228.0	72.8	118.7	100.9	175.8	55.6			68.2		***117**	*23.9*
420		60.5	174.2	113.0		70.5					***105**	*29.9*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M	Mean	S.E.M
TIME (min)				MEAN POWER (mW)									% OF TOTAL POWER	
0	38.4	34.7	57.1	73.6	32.7	33.2	32.1	46.8	29.3	43.7	**42.1**	*4.2*	**71.4**	*1.3*
15	126.7	44.6	73.7		62.7	50.1	39.2	35.3	61.5	48.3	**60.2**	*9.2*	**77.0**	*2.0*
45	76.2	39.2	65.8	50.5	63.7	41.1	71.5	68.9	44.8	39.7	**56.1**	*4.4*	**77.8**	*1.5*
90	95.3	31.1	58.1	57.0	72.3	34.6	47.1	28.4	36.6	20.7	**48.1**	*6.9*	**75.6**	*1.8*
120	89.6	32.4	84.6	60.4	62.0					21.2	**58.3**	*11.2*	***77.6**	*2.0*
180	100.3	38.3	131.6	73.8	71.3			44.8	51.3		**73.1**	*12.6*	***79.0**	*1.4*
240	93.4	46.9	149.9	59.2	87.9	39.1		47.5	33.0		**69.6**	*13.8*	**77.1**	*1.6*
300	177.1	52.1	97.2	70.8	122.4						**103.9**	*24.4*	**77.6**	*1.2*
360	182.3	56.2	89.0	76.4	137.0	36.8		40.0	52.1		**83.7**	*19.4*	**75.5**	*1.5*
420		47.1	132.8	83.2		46.5					**77.4**	*23.5*	**73.4**	*3.1*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M	Mean	S.E.M
TIME (min)				OSCILLATORY POWER (mW)									% OF TOTAL POWER	
0	18.2	9.8	20.4	31.5	17.4	17.3	11.7	18.3	10.2	14.9	**17.0**	*1.9*	**28.6**	*1.3*
15	21.3	8.0	23.8		21.8	23.5	10.0	14.9	19.7	12.9	**17.3**	*2.0*	**23.0**	*2.0*
45	14.3	8.1	22.4	12.9	22.3	19.1	14.0	23.9	13.8	9.9	**16.1**	*1.7*	**22.2**	*1.5*
90	19.9	6.2	20.7	14.8	24.9	17.0	10.3	14.3	12.3	8.0	**14.8**	*1.8*	**24.4**	*1.8*
120	17.6	7.7	24.7	15.4	22.4					8.9	**16.1**	*2.8*	***22.4**	*2.0*
180	19.6	9.1	32.7	16.9	24.7			15.7	14.0		**18.9**	*2.9*	***21.0**	*1.4*
240	23.2	11.7	43.6	13.3	26.5	18.6		16.4	8.9		**20.3**	*3.9*	**22.9**	*1.6*
300	40.1	14.6	30.1	23.5	36.6						**29.0**	*5.1*	**22.4**	*1.2*
360	45.7	16.6	29.7	24.6	38.7	18.8		12.9	16.1		**25.4**	*4.4*	**24.5**	*1.5*
420		13.4	41.4	29.8		24.0					**27.2**	*6.8*	**26.6**	*3.1*

Table 16.

(Top) Mean pulmonary artery pressure post brain death. *±1sem.* (*n*=10).

(Middle) Right ventricular stroke work post brain death derived from analysis of 500 Hz steady state files. *±1sem.* (*n*=10).

(* denotes p<0.05)

(Lower) Trans pulmonary efficiency post brain death. *±1sem.* (*n*=10).

Mean pulmonary artery pressure shows a trend post brain death to increase but does not reach statistical significance. In chapter 10 this trend is shown to be significant..

The RV stroke work mirrored the change in RV PRSW until three hours post brain death. The discrepancy between values prompted a review of these analyses - see figure 27.

There is no significant change in transpulmonary efficiency although the trend is to higher values implying less efficiency.

MEAN PULMONARY ARTERY PRESSURE, RIGHT VENTRICULAR STROKE WORK AND TRANSPULMONARY EFFICIENCY FOLLOWING BRAIN DEATH

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)		MEAN PULMONARY ARTERY PRESSURE (mmHg)										
0	14	14	17	18	11	10	10	12	12	16	**13.5**	*0.8*
15	19	16	19		17	12	12	10	18	14	**15.4**	*1.1*
45	16	15	18	13	15	12	16	13	17	13	**14.7**	*0.6*
90	17	13	18	14	16	11	12	10	14	11	**13.6**	*0.8*
120	17	14	19	14	15					11	**15.2**	*1.1*
180	17	15	23	15	16			11	16		**16.4**	*1.3*
240	17	18	24	14	18	12		12	14		**15.9**	*1.4*
300	21	17	20	14	20						**18.2**	*1.4*
360	21	17	19	14	21	12		14	15		**16.5**	*1.3*
420		16	20	14		13					**15.9**	*1.8*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)			RV STROKE WORK (ERG.103)									
0	18.5	17.6	36.0	29.4	21.2	17.7	24.3	44.6	12.7	14.0	**23.6**	*3.1*
15	18.6	11.5	21.2		21.6	26.5	14.2	34.3	10.8	7.9	**18.5**	*2.8*
45	7.6	12.1	18.5	16.2	23.5	24.0	14.6	52.3	6.1	5.0	**18.0**	*4.1*
90	6.6	4.3	10.6	17.3	20.0	20.8	14.9	34.5	3.4	2.2	**13.4***	*3.0*
120	3.6	7.3	7.6	20.4	18.6					0.3	**9.7***	*3.3*
180	7.5	7.7	23.6	20.0	19.8			38.6	5.9		**17.6**	*4.4*
240	9.6	13.2	29.5	27.2	22.2	22.5		36.0	2.6		**20.4**	*3.9*
300	28.8	17.2	16.5	36.5	29.2						**25.7**	*4.3*
360	26.7	20.2	14.6	41.1	31.1	22.8		20.8			**25.3**	*3.3*
420		17.0	13.1	48.7		25.4					**26.0**	*9.2*

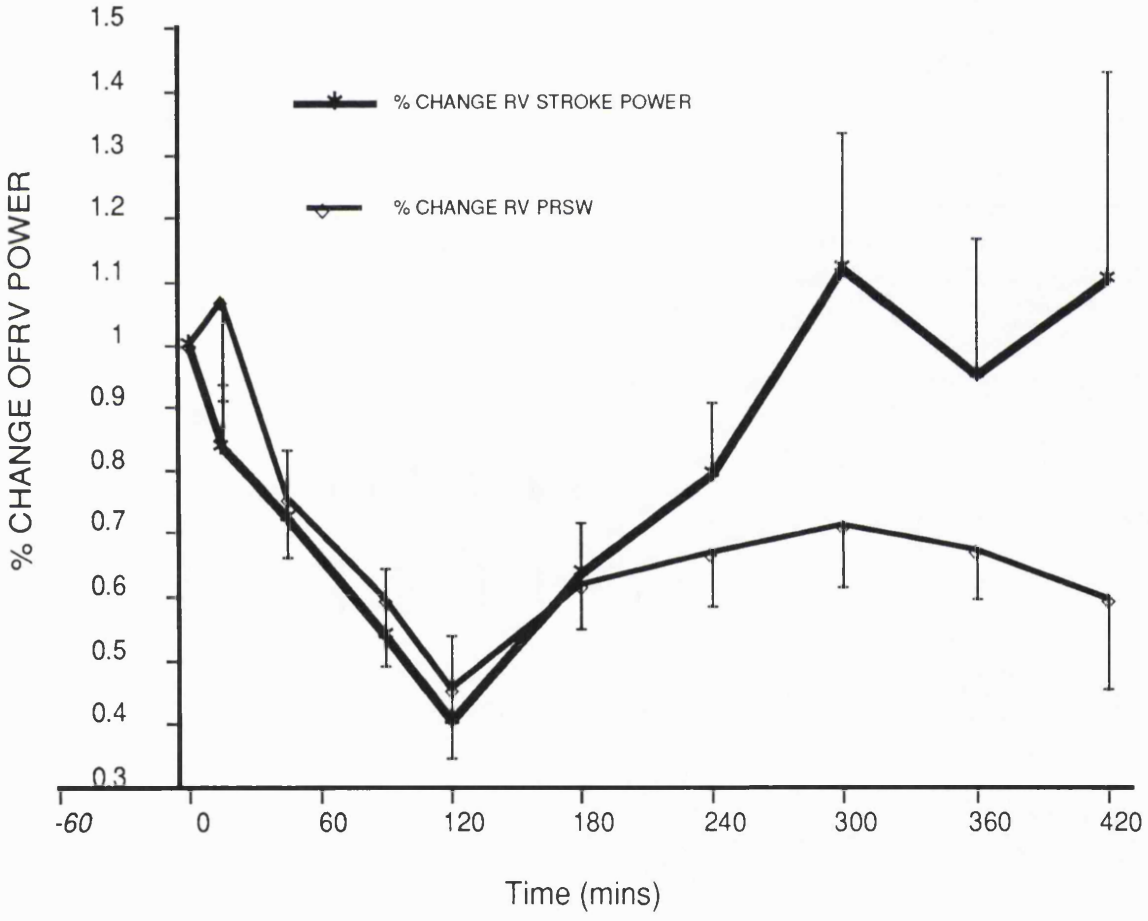
Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)				EFFICIENCY (J/L)								
0	2.69	2.35	3.15	3.44	2.35	2.08	1.89	2.18	2.18	2.82	**2.51**	*0.15*
15	3.04	2.57	3.37		3.04	2.44	2.04	1.91	3.22	2.40	**2.67**	*0.17*
45	2.54	2.35	3.14	2.22	2.65	2.27	2.50	2.27	2.99	2.23	**2.52**	*0.10*
90	2.74	2.11	3.25	2.32	2.86	2.23	2.00	1.97	2.46	2.05	**2.40**	*0.13*
120	2.72	2.27	3.36	2.38	2.77					2.17	**2.61**	*0.18*
180	2.72	2.55	3.83	2.53	2.89			2.05	2.79		**2.76**	*0.21*
240	2.79	2.92	4.06	2.22	3.13	2.31		2.16	2.43		**2.75**	*0.22*
300	3.38	2.95	3.41	2.46	3.45						**3.13**	*0.21*
360	3.47	3.00	3.30	2.49	3.57	2.34		2.41	2.62		**2.90**	*0.19*
420		2.72	3.52	2.61		2.64					**2.87**	*0.25*

Figure 27.

Comparison of percentage change of right ventricular pre-load independent recruitable stroke when calculated from a vena caval occlusion or from a steady state 500 Hz file. *±1sem.* (*n*=10).

The marked difference between these two values at the end of the studies prompted a review of the calculations required for these two values. The 500 Hz file analysis was found to be defective, whilst the 200 Hz caval occlusion retained a high degree of accuracy.

COMPARISON OF PERCENTAGE CHANGE OF RV PRSW AND RV STROKE WORK / HYDRAULIC ANALYSIS FOLLOWING BRAIN DEATH



HORMONE RESULTS

Serum Catecholamines (table 17, figure 28).

At baseline norepinephrine was 332 pg/ml (±31) and doubled at 15 minutes to 667 pg/ml (±131). The wide variation at this sample point is dependent on the duration of the Cushing response and the presumed peak concentration achieved (Novitzky et al, 1984). Norepinephrine concentrations at 15 minutes post brain death will be rapidly falling and the serum levels will reflect at which point on this steep curve the sample was taken. For the remainder of the study there was a trend for norepinephrine levels to fall but not differ from baseline / normal values.

The changes in epinephrine concentrations are more extreme and more varied than for norepinephrine. The mean baseline value was 76 pg/ml (±37), with animals 5 and 9 having high concentrations of 284 and 310 pg/ml respectively. At 15 minutes the variation was more extreme from 5 to 2430 pg/ml. The mean value at this time was 602 pg/ml (±219). This range of values continued through the remaining period of brain death maintaining the means at values higher than baseline. Of note is the concentration at 6 hours in animal 8, of 2009 pg/ml which correlates to a markedly elevated PRSW in the left ventricle. This agrees with the clinical study by Powner where wide variations in serum catecholamines were found, with the conclusion the levels of these hormones were not an accurate indicator of cardiac injury (Powner et al, 1992). These large peaks of epinephrine have been attributed to spinal reflex activity that occurs in the brain dead animal (Gramm et al, 1992).

Dopamine shows a slower response to brain death than the previous catecholamines. The baseline value of 15 pg/ml (±3) rises to a peak at 45 minutes of 272 pg/ml (±81) and then declines. As for the other catecholamines there is marked variation between animals and this variation does not correspond between the three hormones.

Table 17.

Serum Catecholamines Post Brain Death.

(Top) Norepinephrine. *±1sem.* (*n*=10).

(Middle) Epinephrine. *±1sem.* (*n*=10).

(Lower) Dopamine. *±1sem.* (*n*=10).

(* denotes $p<0.05$)

There is a statistically significant increase in catecholamines at 15 minutes post brain death. Dopamine remains significantly elevated for a further 75 minutes. The wide variance in epinephrine prevents significance at subsequent data points; this is exemplified by animals 1 and 8 where epinephrine >1000 pg/ml. In animal 8 this correlates with a high PRSW, and elevated insulin levels.

SERUM CATECHOLAMINES FOLLOWING BRAIN DEATH

(pg/ml)

Experiment no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.
TIME (mins)				NOREPINEPHRINE								
0	533	187	327	190	305	350	380	338	384	321	**332**	*31*
15	603	626	415	232	117	881	771	1446	387	1192	**667***	*131*
45	274	291	143	151	254	869	847	180	496	474	**398**	*68*
90	375	154	328	125	486	876	292	782	631	428	**448**	*64*
240	269	265	135	167	733	571		631	200		**361**	*62*
360	218		120	183	749	316		504	107		**308**	*71*
420		103	148	285		355					**223**	*28*

Experiment no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.
TIME (mins)				EPINEPHRINE								
0	5	24	15	26	284	53	5	35	310	5	**76**	*37*
15	837	604	280	52	410	85	5	882	433	2430	**602***	*219*
45	5	221	98	115	700	171	365		5	752	**270**	*89*
90	1323	5	48	12	305	110	890	470	5	401	**357**	*138*
240	108	326	5	23	929	109		320	251		**375**	*111*
360	1113		14	25	104	79		2009	185		**469**	*228*
420		96	15	703		211					**256**	*113*

Experiment no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.
TIME (mins)				DOPAMINE								
0	5	5	5	37	14	5	19	26	20	12	**15**	*3*
15	272	10	146	10	10	82	180	387		115	**135***	*41*
45	594	29	10	420	10	176	207	142	384	746	**272***	*81*
90	838	11	154	18	27	48	135	5	363	651	**225***	*93*
240	16	17	5	10	36	5		19	5		**56**	*30*
360	69		5	10	10	99		5	5		**70**	*38*
420		5	5	90		86					**47**	*15*

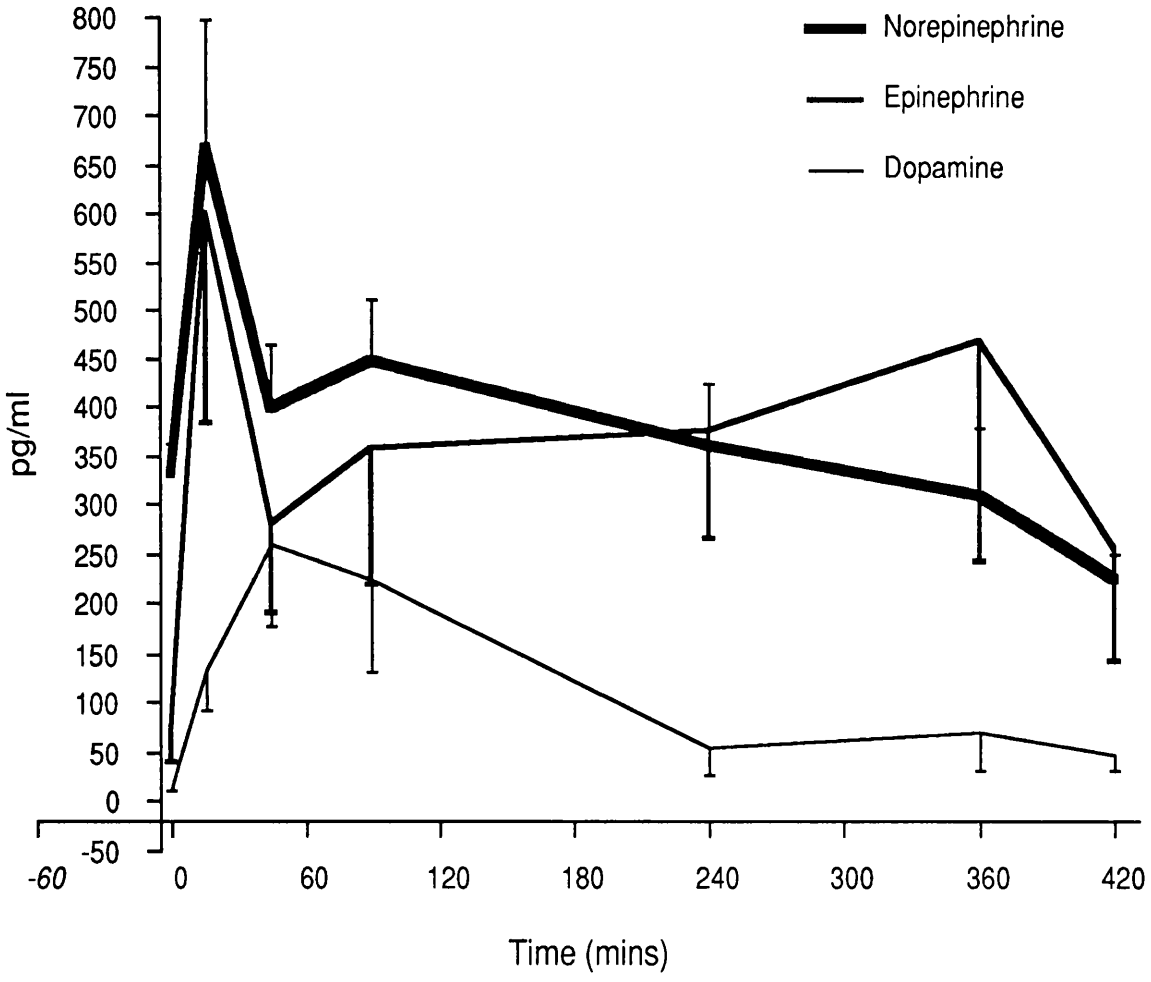
Figure 28.

Serum Catecholamines post brain death.

±1sem. (*n*=10).

At fifteen minutes post brain death norepinephrine, epinephrine and dopamine are significantly raised. Norepinephrine returns to baseline values whilst epinephrine remains elevated. The response of dopamine is slower with a more gradual decline to baseline values.

SERUM CATECHOLAMINES
FOLLOWING BRAIN DEATH



Vasopressin

This neuropeptide is made in the hypothalamus and secreted in the neurohypophysis (posterior pituitary gland) in a response primarily to hypovolaemia. Its has two main effects; firstly it promotes reabsorption in the distal renal tubules to conserve intravascular volume and secondly it is a potent vasoconstrictor. It has a short half life of less than 5 minutes and, when absent, will lead to the clinical state of diabetes insipidus, with fatal polyuria unless corrected. At baseline, vasopressin is at the high end of normal range, as would be expected from the normal response to surgery 51.1 pg/ml (±11) (Haas and Glick, 1978). Following brain death there is an immediate significant drop to 4.4 pg/ml (±1.1) suggesting disruption of the hypothalamic - pituitary axis (table 18, figure 29).

Adrenocorticotrophic Hormone (ACTH) and Cortisol.

ACTH is a polypeptide made and secreted from the adenohypophysis (anterior pituitary gland). Its release is cyclical, caused by the corticotrophic hormone releasing factor from the hypothalamus, with a peak in the morning. It has a half life of ten minutes and stimulates the adrenal cortex with subsequent hormone release, primarily the steroid, cortisol. ACTH has a baseline value of 47.7 fM/ml (±12.5) significantly dropping at 45 minutes to 17.5 fM/ml (±4.0) and falling further to negligible levels. This change in ACTH concentration may reflect the decline of cortisol from 15.7 mcg/ml (±1.6) to 8.6 mcg/ml (±0.8) at 45 minutes. This data reinforces the supposition that intracranial balloon inflation disrupts the hypothalamic - pituitary axis (table 18, figure 29).

Thyroid Hormones

Tri-iodothyronine (T3) and tetra-iodothyronine (T4) concentrations are shown in table 19 and figure 30. In summary these hormones decrease following brain death from baseline levels of 0.58 ng/ml (±0.04) and 2.22 mcg/ml (±0.14) respectively to significant lower levels at four hours post brain death, although these levels remain within the normal range (0.34 ng/ml (±0.03) and 1.42 mcg/ml (±0.29)).

Table 18.

Vasopressin, Cortisol and adrenocorticotrophic hormone blood levels post brain death.

(Top) Vasopressin. *±1sem.* (*n*=10).

(Middle) Cortisol. *±1sem.* (*n*=10).

(Lower) Adrenocorticotrophic hormone (ACTH). *±1sem.* (*n*=10).

(* denotes $p<0.05$)

All three hormones are significantly effected post brain death. Vasopressin is immediately reduced, whilst Cortisol and ACTH are decreased 45 minutes post brain death.

VASOPRESSIN, CORTISOL AND ADRENOCORTICOTROPHIC HORMONE POST BRAIN DEATH

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)				VASOPRESSIN (pg/ml)								
0	18.0	26.0	42.5	17.8	87.2	37.6	29.6	50.9	125.0	76.4	**51.1**	*11.0*
15	0.4	1.0	7.9	10.8	8.5	5.6	2.3	2.2	2.5	2.7	**4.4***	*1.1*
45	0.9	0.8	3.2	1.6	3.8	1.3	1.1	0.5	4.1	0.5	**1.8***	*0.4*
90	0.3	0.4	0.7	1.9	1.7	1.0	0.9	1.4	2.2	1.3	**1.2***	*0.2*
240	0.0	0.5	1.0	0.7	0.6	0.0		0.0	0.9		**0.4***	*0.1*
360	0.0	0.0	0.5	0.0	0.4	0.0		0.0	0.0		**0.1***	*0.1*
420		0.0	0.0	0.0		0.0					**0***	*0.0*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)				CORTISOL (mcg/dl)								
0	16.2	28.6	14.2	10.6	14.6	14.4	11.6	16.8	16.1	13.8	**15.7**	*1.6*
15	16.7	13.3	12.8	14.1	13.6	10.2	13.2	20.6	16.5	14.2	**14.5**	*0.8*
45	9.5	5.0	7.4	7.0	9.8	6.9	8.0	15.0	9.1	8.6	**8.6***	*0.8*
90	5.7	3.4	4.3	3.8	5.9	5.2	6.8	8.8	7.1	5.6	**5.6***	*0.5*
240	1.3	0.6	0.7	1.1	1.0	1.0		2.9	2.3		**1.4***	*0.3*
360	0.9	0.0	0.0	0.6	0.7	0.1		1.1	0.1		**0.6***	*0.1*
420		0.0	0.0	0.0		0.1					**0.0***	*0.0*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)				ACTH (fM/ml)								
0	27.8	14.8	45.9	12.8	6.6	81.4	18.1	51.3	84.4	133.4	**47.7**	*12.5*
15	29.8	30.0	18.6	19.5	12.2	23.2	25.6	108.6	48.3	68.4	**38.4**	*9.3*
45	12.0	11.8	12.1	8.4	4.2	10.8	17.0	48.1	25.8	24.6	**17.5***	*4.0*
90	8.1	4.7	3.1	3.0	1.7	5.8	6.3	16.7	12.5	11.9	**7.4***	*1.5*
240	6.5	2.1	1.1	2.3	1.7	1.2		2.2	1.1		**2.2***	*0.5*
360	3.1	2.1	1.3	2.6	1.3	0.8		0.5	0.5		**1.7***	*0.3*
420		1.4	0.9	2.9		0.9					**1.5***	*0.3*

Figure 29.

Vasopressin, cortisol and adrenocorticotrophic hormone post brain death.

±1sem. (*n*=10).

Vasopressin precipitously falls significantly below physiologic levels immediately post brain death. Cortisol displays a more gradual decline, as does adrenocorticotrophic hormone (ACTH), becoming statistically lower at 45 minutes post brain death.

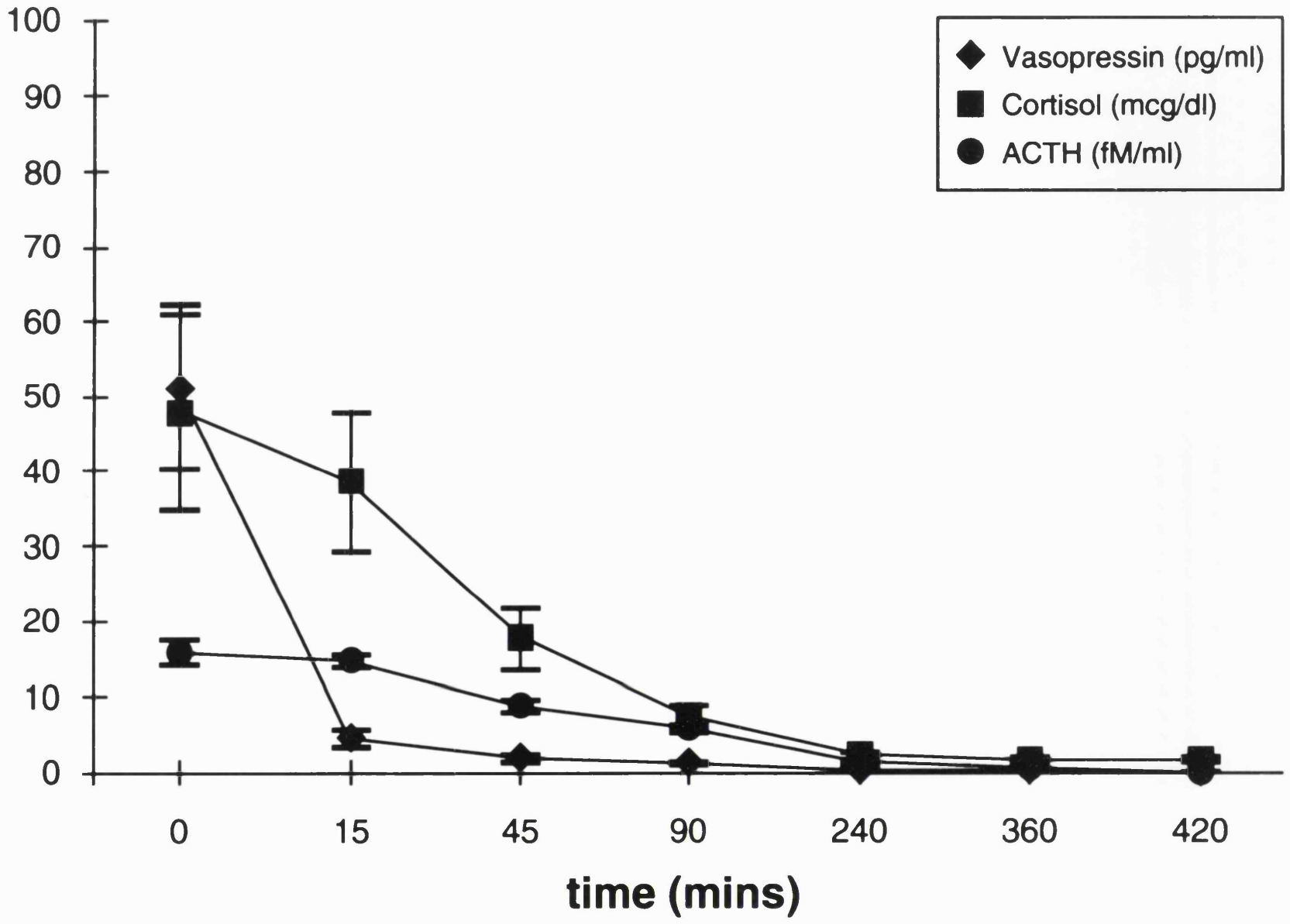

100
90
80
70
60
50
40
30
20
10
0
Vasopressin (pg/ml)
Cortisol (mcg/dl)
ACTH (fM/ml)
0
15
45
90
240
360
420
time (mins)

Table 19.

Tri-iodothyronine and tetra-iodothyronine post brain death.

±1sem. (*n*=10). (* denotes p<0.05)

Figure 30.

Tri-iodothyronine and tetra-iodothyronine post brain death.

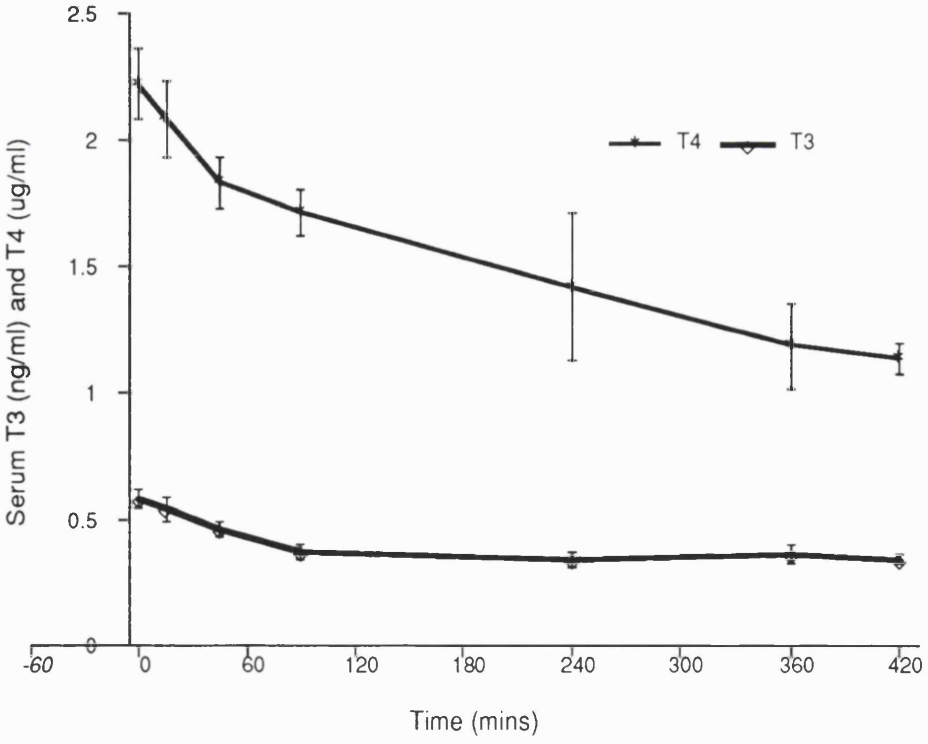
±1sem. (*n*=10).

The thyroid hormones remain in the normal range post brain death, although there is a statistically significant decrease from baseline for both T3 and T4 at four hours.

TRI-IODOTHYRONINE (T3) AND TETRA-IODOTHYRONINE (T4)
POST BRAIN DEATH

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)			TRI-IODOTHYRONINE (T3)									
0	0.66	0.36	0.79	0.61	0.56	0.32	0.75	0.68	0.58	0.51	**0.58**	*0.04*
15	0.70	0.25	0.68	0.44	0.55	0.29	0.60	0.76	0.63	0.53	**0.54**	*0.05*
45	0.56	0.30	0.63	0.36	0.44	0.31	0.53	0.53	0.48	0.47	**0.46**	*0.03*
90	0.48	0.25	0.46	0.25	0.35	0.22	0.50	0.41	0.42	0.35	**0.37**	*0.03*
240	0.55	0.25	0.42	0.28	0.25	0.16		0.34	0.43		**0.34***	*0.03*
360	0.57	0.26	0.26	0.30	0.25	0.28		0.39	0.37		**0.36***	*0.04*
420		0.28	0.27	0.38		0.43					**0.34***	*0.02*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)			TETRA-IODOTHYRONINE (T4)									
0	2.56	2.48	1.55		2.43	2.08	2.28	1.42	2.50	2.68	**2.22**	*0.14*
15	2.67	2.21	1.12	1.77	2.41	2.02	2.36	1.49	2.26	2.49	**2.08**	*0.15*
45	2.08	1.81	1.43	1.59	2.00	1.77	2.13	1.38	1.77	2.33	**1.83**	*0.10*
90	1.76	1.67	1.29	1.41	1.97	1.64	2.17	1.40	1.72	2.02	**1.71**	*0.09*
240	1.62	1.10	1.27	1.15	1.69	1.51		1.26	1.11		**1.42***	*0.29*
360	1.26	0.90	1.15	0.99		1.39		1.10	1.23		**1.19***	*0.17*
420		0.83	1.25	1.05		1.41					**1.14***	*0.06*



Insulin and Glucagon

Glucagon has a normal baseline value of 49.7 pg/ml (±9.0) which rises to 145.5 (±9.5) at 15 minutes. Insulin also increases significantly at fifteen minutes to 15.8 uIU/ml (±2.2). Glucagon concentration then falls below normal values at 6 hours to 9.8 pg/ml (±1.4) (table 20). These measurements of glucagon are relatively consistent, unlike insulin which shows marked variation. This variance of value prevents statistical conclusions but on inspection of the data there is a correlation of raised insulin levels in individual animals to raised levels of epinephrine. This is understandable as the gluconeogenic action of epinephrine would prompt increased secretion of insulin.

ß-Adrenergic Receptor Status

The results of the receptor analysis are displayed in table 21 and figure 31. The adenylyl cyclase activity EC 50 is the concentration of isoproterenol (isoprenaline) that causes 50% of the maximal adenylyl cyclase response. Pre brain death this value is 316 and 251 for right and left ventricles respectively, and post brain death it is 177 and 158. This indicates increased sensitivity of the ß-receptors post brain death and is reflected in the significantly increased percentage of adenylyl cyclase activity post brain death in response to isoproterenol; *34.1% (±1.7) vs 31.4% (±2.4) for the right ventricle, and *40.8% (±1.3) vs 31.8% (±1.4) for the left ventricle. Also the density of ß-receptors increases post brain death; 281 fmol/mg (±44) to 568 fmol/mg (±183) and 291 fmol/mg (±62) to *353 fmol/mg (±55) in right and left ventricles respectively. Due to large variance in the right ventricle this rise is only significant in the left ventricle. The basal unstimulated adenylyl cyclase activity as a percentage of the response to sodium fluoride is unchanged for both ventricles pre and post brain death.

These results show that in this model of brain death there is an 'up-regulation' of the ß-adrenergic receptors post brain death. There is an increase in density together with an increase in sensitivity. The receptor coupling to its signal transduction system is unchanged as demonstrated by the unaltered response to sodium fluoride post brain death.

Table 20.

Serum insulin and glucagon post brain death. *±1sem.* (*n*=10).

(* denotes p<0.05)

Glucagon increases to a significant peak post brain death and subsequently declines to significant levels at four hours post brain death. The response of insulin is more difficult to interpret due to wide variance in values; however the raised insulin levels do correlate to high epinephrine levels. The rise in insulin is significant at fifteen minutes, but subsequent variance prevents statistical significance.

SERUM INSULIN AND GLUCAGON
POST BRAIN DEATH

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)				INSULIN (uIU/ml)								
0	1.7	4.1	14.1		16.1	4.8	6.7	5.3	1.2	13.8	**7.5**	*1.8*
15	15.1	3.2	19.5		24.7	13.8	18.5	22.4	7.0	18.0	***15.8**	*2.2*
45	8.0	1.0	12.1		2.2	2.4	3.5	1.4	4.8	21.8	**6.4**	*2.1*
90	33.8	1.0	1.2		1.2	1.6	1.3	1.2	1.2	2.0	**4.9**	*3.4*
240	8.0	10.4	40.1		34.4	2.3		12.3	61.8		**20.5**	*6.1*
360	31.4	8.2	10.5		22.2	12.8		32.2	65.3		**28.1**	*7.8*
420		1.9	3.6			36.4					**14.0**	*0.4*

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)				GLUCAGON (pg/ml)								
0	64.7	5.0	69.5	55.8	5.0	68.8	60.1	33.6	39.1	95.3	**49.7**	*9.0*
15	123.2	89.8	139.0	93.8	105.1	119.2	97.1	144.1	255.4	288.0	**145.5***	*9.5*
45	1.9	5.0	38.9	5.0	28.4	54.6	35.8	56.1	35.9	68.6	**33.0**	*7.0*
90	5.0	5.0	39.2	5.0	28.5	46.6	20.8	84.1	26.6	52.0	**31.3**	*7.8*
240	5.0	5.0	5.9	5.0	5.0	54.7		24.7	13.0		**17.9***	*5.2*
360	5.0	5.0	5.0	5.0	14.0	28.6		16.0	12.0		**9.8***	*1.4*
420		5.0	5.0	5.0		12.6					**6.9***	*0.0*

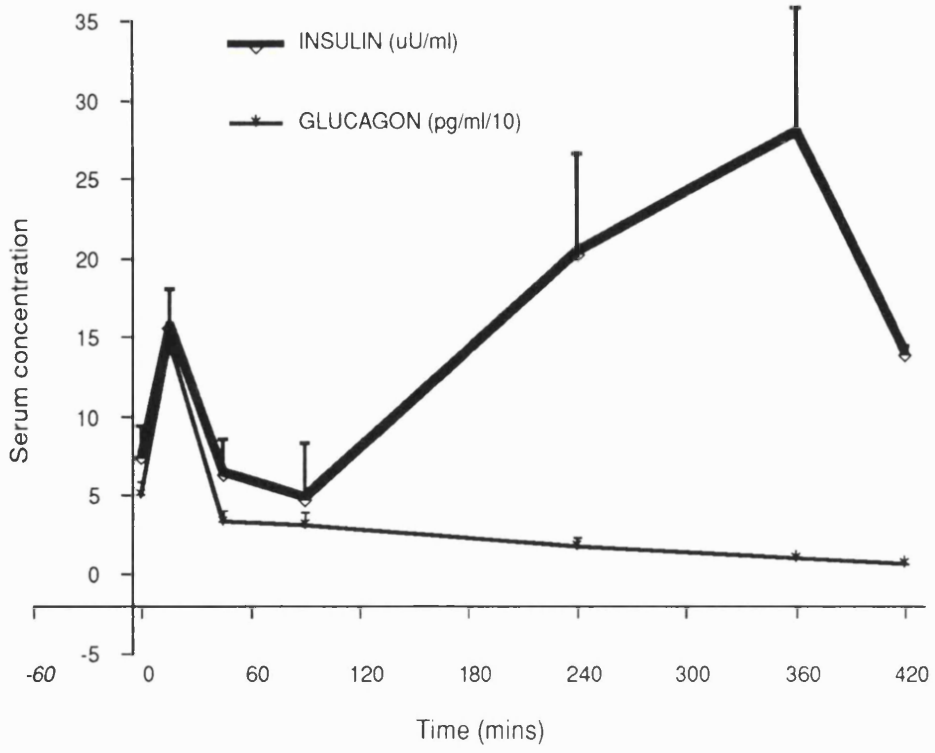


Table 21.

ß-adrenergic receptor function. *±1sem.* (*n*=10). (* denotes p<0.05)

Results for; myocardial ß-receptor density;

Isoproterenol stimulated adenylyl cyclase activity as a percentage of the maximum response (isoproterenol);

Basal unstimulated adenylyl cyclase activity as a percentage of the maximum response (sodium fluoride NaF) and

Adenylyl cyclase activity EC 50, which is the concentration of isoproterenol to achieve 50% of the maximum response.

Figure 31.

ß-adrenergic receptor function pre and post brain death. (*n*=10).

First two bars; ß-receptor density (fmol/mg/10).

Second two bars; Isoproterenol stimulated adenylyl cyclase activity (%).

Third two bars; Basal unstimulated adenylyl cyclase activity to sodium fluoride (%).

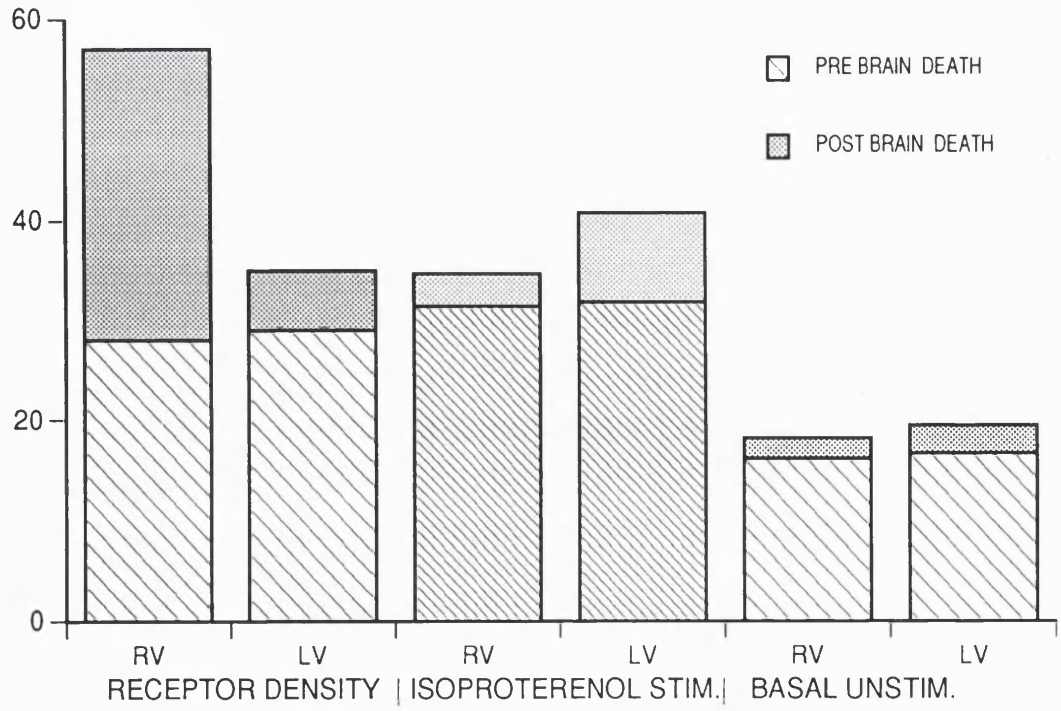
RV: right ventricle

LV: Left ventricle

These results show brain death to increase receptor density and increase sensitivity to adrenergic stimulation. There is no change in the signal transduction system.

B-ADRENERGIC RECEPTOR ANALYSIS POST BRAIN DEATH

	RIGHT VENTRICLE		LEFT VENTRICLE	
	BASELINE	BRAIN DEATH	BASELINE	BRAIN DEATH
Myocardial B-receptor density *fmol/mg*	281 *44*	568 *183*	291 *62*	*353 *55*
Isoproterenol-stimulated adenylyl cyclase activity *%*	31.4 *2.4*	*34.1 *1.7*	31.8 *1.4*	*40.8 *1.3*
Basal unstimulated adenylyl cyclase activity *% NaF*	16.1 *1.1*	18.1 *0.9*	16.6 *0.9*	19.4 *0.9*
Adenylyl cyclase activity EC50	316	177	251	158



Urine / Intravenous Fluid Balance

Diabetes insipidus was recorded in 9 of the 10 animals with an average urine output 12.3 mls/kg/min(±1.9). The onset varied between 45 minutes and 3 hours, with most animals polyuric at 90 minutes. This and the mean cumulative urine output is depicted on table 22. Also of note is the intravenous fluid replacement of these animals, a mean of 5.0 litres (±0.6), to maintain blood pressure and ventricular filling pressures.

Haematocrit

An unexpected finding from the study of the arterial blood gas analysis was the consistent change in haematocrit (table 23). This revealed a significant peak at 15 minutes to 46.6 % (±2.2) from a baseline value of 35.6% (±1.5). Haematocrit then falls to baseline levels and became significantly low at two hours 25.3% (±2.2). This drop is presumably haemodilution due to intravenous fluids. The haemoconcentration at fifteen minutes is probably a result of the severe hypertension in the Cushing response, forcing plasma into the extracellular compartment by hydrostatic pressure.

Histological Changes in the Myocardium

Greenshoot observed that the morphological changes of the myocardium post subarachnoid haemorrhage resembled changes induced by high dose catecholamines (Greenshoot, 1969). This injury is not solely specific for catecholamine injury and shares features of ischaemic injury such as the histological appearance of contraction bands within the myocardium (Todd et al, 1985). These lesions represent scattered focal areas of myocardial cell necrosis and are surrounded by mononuclear cells (Rose, 1988). Representative sections, taken from each animal just prior to the termination of the experiments, were histologically examined from the left and right ventricles. The significant features were as follows; areas of cardiac myocytes with irreversible ischaemic damage; contraction bands within these areas of ischaemic damage and changes with a progression to focal necrosis. The total area of affected myocardium in these specimens was subjectively quantified by a histopathologist, who was blinded as to the specimen's ventricular origin. The area affected in the left ventricular specimens was 5% (±0.9) compared to 18% (±2.9) in the right ventricle.

Table 22.

Urine output and intravenous fluid requirements post brain death.

±1sem. (*n*=10). (* denotes p<0.05)

In all animals except number 7, profound polyuria developed post brain death; the mean cumulative output is depicted in the graph. The rate of urine output for each animal is displayed at the bottom of the table; the mean is 12.3 ml/kg/min which is significantly higher than physiological baseline of 2 ml/kg/min.

Total intravenous fluid requirements per animal, from the commencement of the experiment, is shown on the bottom row of the table. Usually 1 litre was infused prior to brain death. (The fluid balance for 33 animals post brain death is discussed in chapter 10.)

**CUMULATIVE URINE OUTPUT, URINE OUTPUT (mls/kg/min)
AND TOTAL INTRAVENOUS INFUSION POST BRAIN DEATH**

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)			CUMULATIVE URINE OUTPUT (mls)									
0	30	125	20	0	150	50	20	10	50	50	**51**	*15*
15	30	150	80	0	150	50	25	60	50	80	**68**	*15*
45	30	160	85	0	150	60	25	70	90	900	**157**	*80*
90	350	250	150	20	400	140	30	130	120	1500	**309**	*131*
120	650	500	500	40	810	430	45	350	650	2000	**598**	*166*
180	800	800	850	100	1350	750		600	1100	3000	**1039**	*243*
240	1750	1350	1500	475	2000	1300		900	1250		**1316**	*142*
300	2200	1700	2000	850	2850	1900		1200	1500		**1775**	*185*
360	2600	2150	2200	1300	3400	2300		1450	1500		**2113**	*210*
420		2400	2200	2000		2800					**2350**	*103*
URINE (ml/kg/min)	16.0	17.0	13.4	12.0	21.0	20.0	2.0	10.0	6.3	5.4	**12.3**	*1.9*
TOT. IV INFUSION (L)	5.5	8.0	7.0	8.8	5.0	7.0	5.0	3.0	3.5	3.8	**5.7**	*0.6*

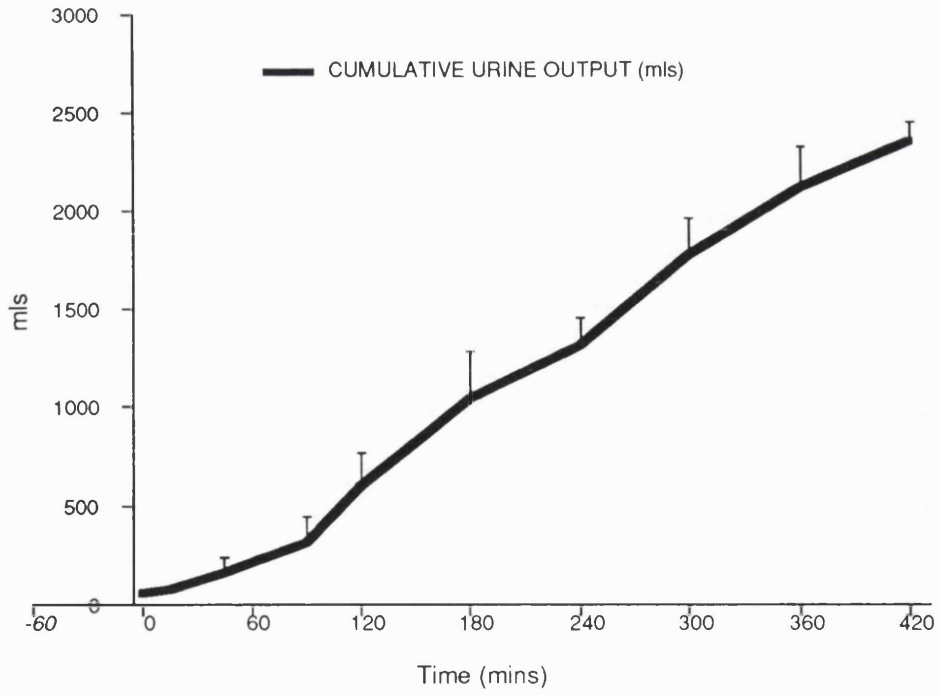


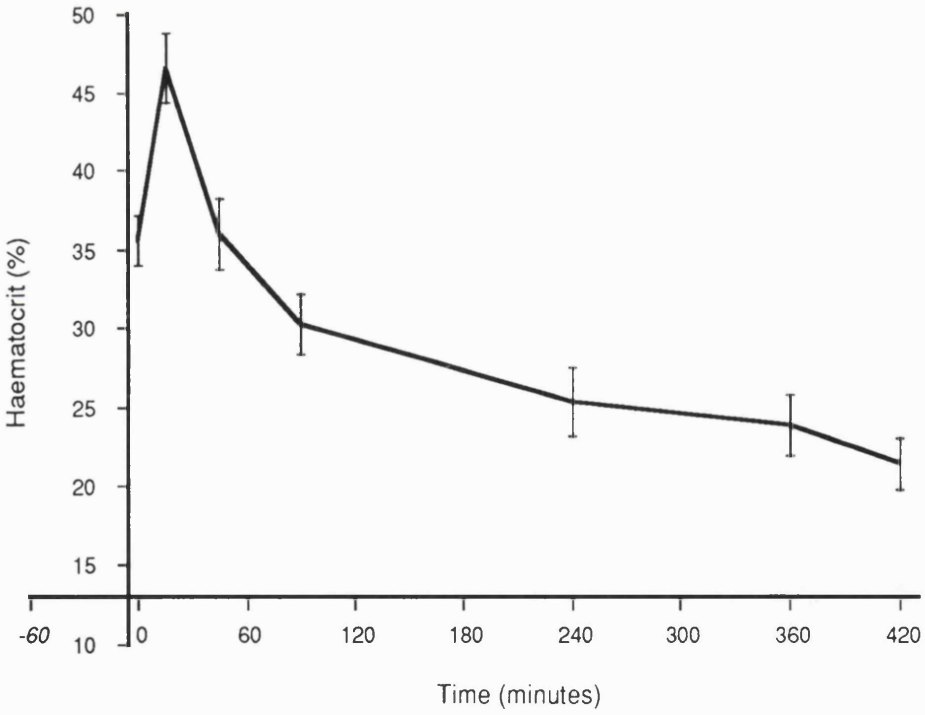
Table 23.

Haematocrit post brain death. *±1sem.* (n=10). (* denotes $p<0.05$)

The Cushing response causes an acute rise in haematocrit, a phenomenon not previously reported. Values gradually decline to reach significance at two hours post brain death, probably due to haemodilution.

HAEMATOCRIT POST BRAIN DEATH (%)

Expt no:	1	2	3	4	5	6	7	8	9	10	Mean	S.E.M
TIME (min)												
0	32	27	36	38	41	35	33	44	32	38	**35.6**	*1.5*
15	43	30	51	44	49	51	49	55	44	50	**46.6***	*2.2*
45	37	24	35	31	49	35	36	42	30	41	**36.0**	*2.2*
90	28	25	31	26	45	29	26	34	26	33	**30.3**	*1.9*
120	25	23	26	19	42	24	22	27	20	29	**25.3***	*2.2*
240	25	19	21		36	23		28	21		**23.9***	*1.9*
360	21				29			23	19		**21.4***	*1.8*
420		16	20	14		13					**15.9***	*1.8*



Neuropathological Assessment of the Excised Brain

The brains from all ten animals were excised at the end of the experiment and treated as described in chapter 4. The interval between the induction of brain death and removal of the brain ranged from 2.5 hours in two animals to 7.5 hours and 6.5 hours in the remaining four animals. External examination of the specimen revealed that all brains were diffusely swollen and boggy even after fixation, with a dusky grey discolouration. The cerebral gyri were flattened and the superficial sulci were compressed. In each specimen the temporal lobes were markedly swollen, and in some cases the temporal lobes contacted the cerebral peduncles. Symmetrically increased transtentorial notching was present on the medial temporal lobe in all cases. There was no subfalcine herniation. In three cases there were small (<0.4cm), superficial haemorrhagic lesions on the left parietal lobe that were secondary to damage from catheter placement. No other mechanical damage, epidural, subdural or subarachnoid haemorrhage was present. The cerebella were markedly soft and swollen with the cerebellar tonsils disrupted and haemorrhagic in all specimens. The brain stem was also swollen and soft. The basis pontis and ventral surface of the medulla were flattened and the external markings were obscured.

On cut section the cerebral hemispheres were significant for blurring of the grey - white junction as well as grey discolouration of the cortical ribbon and periventricular white matter. Similar discolouration was also present in the cerebellar white matter. Compression of the lateral ventricles and scattered petechial haemorrhages were seen in the cerebrum. Two dogs had intraventricular haematoma that were confined to the lateral ventricles. Flattening of the ventral surface of the brain stem, from the cerebral peduncles to the medullary pyramids, was easily appreciated on cut sections. The cerebral aqueduct and fourth ventricle were markedly compressed. Duret haemorrhages were identified in some cases.

Figure 32.

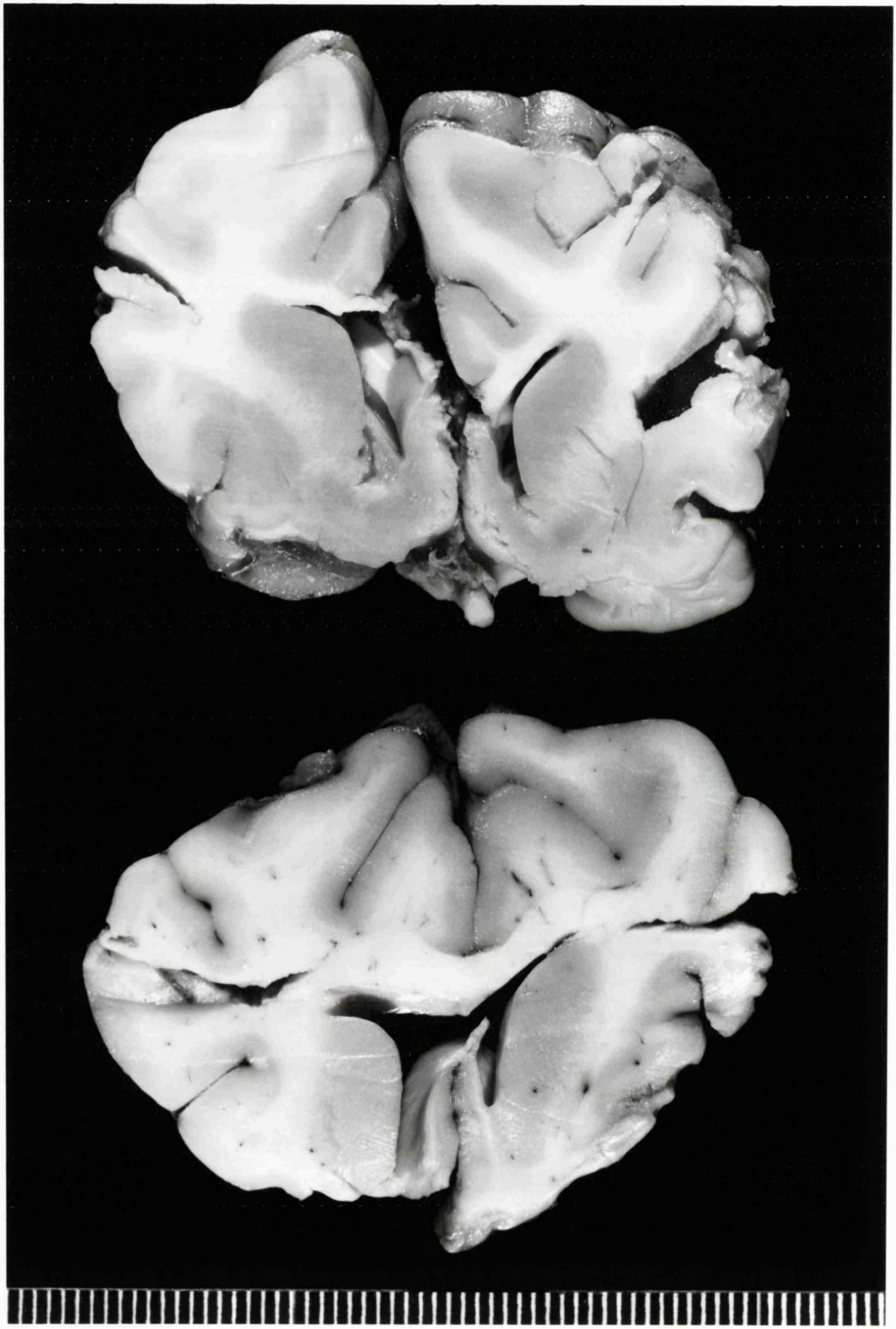
Coronal sections of cerebral cortex;

Upper section is control animal (no brain death, chapter 6).

Lower section is from animal number 6, seven hours post brain death.

The sections are at similar levels through the lateral ventricles.

Despite the inflation of an intracranial balloon the gross architecture of the lower specimen is remarkably preserved. The 'brain dead' specimen shows increased width and dusky discolouration of the cortical ribbon and the deep grey matter. The thrombosed veins cut transversely can be seen as small dark spots.



Examination of histologic sections revealed congestion and diffuse oedema of the cerebrum, cerebellum and brain stem. No area of tissue necrosis, neuron coagulative necrosis ('red neurons'), inflammatory infiltrate or gliosis was present.

All animals had neuropathological evidence of **brain stem death** and all brains showed early changes of irreversible global ischaemia consistent with pathologic findings in the heart beating brain dead cadaver (Moseley et al, 1976).

The determination of brain death was also made on examination and electroencephalographic monitoring. Neurologic and brain stem reflex examination was unreliable. Bilateral pupillary constriction at baseline changed to fixed dilatation immediately after brain death in all animals. Corneal reflexes were not present in three animals at baseline, probably dependent on the depth of anaesthesia. Spontaneous respiration was not tested off the ventilator as any resultant hypoxia or hypercapnia may have influenced myocardial function and blood / tissue analysis. In every animal there was cessation of neuronal - electrical brain activity on EEG monitoring between 8 and 20 minutes after balloon inflation, defined as the recorded unchanged oscillating noisy-spiked curve without high amplitude waves and spikes (figure 11).

DISCUSSION

To increase the donor pool for solid organ transplantation several researchers have employed a variety of animal 'brain death' models. The results from these models often differ and also do not correlate with the clinical picture of brain death (Gramm et al, 1992). Total cranial content evacuation(Blaine et al, 1984) and a variety of other methods have been used to induce brain death in animal models but two main methods have been applied; intracranial balloon inflation (Novitzky et al, 1984) and interruption of the cerebral blood supply (Wicomb et al, 1986b). This thesis selected to investigate the former method. Intracranial balloon inflation promised to cause less tissue damage and be more selective in its effects than ligation of the head and neck vessels, which may not achieve complete brain ischaemia due to a large collateral blood supply. Inflation of an

intracranial balloon also appeared more relevant to the clinical situation where 77% of organ donors are victims of raised intracranial pressure and not cerebral anoxia (Darby et al, 1989).

The haemodynamic response of these experiments is identical to the published reports using this method in the primate, canine, swine and rat models; a hyperdynamic Cushing response followed by a variable period of cardiovascular stability before circulatory collapse. However there are marked contrasts to the ischaemic method. Finkelstein's series of 10 dogs, using ligation of carotid and vertebral arteries, demonstrated no Cushing response or catecholamine surge, and remained cardiovascularly stable (Finkelstein al, 1987). There was a trend to a metabolic alkalosis rather than acidosis, with a slow rise in catecholamines over two hours. ACTH remained in normal limits while vasopressin levels dropped slowly, to an active level of 470 pg/ml. The change in T4 mimicked this study with a gradual decrease though remaining in the normal range. These results suggest attempted interruption of cerebral blood flow may abolish cortical function, as shown by the electroencephalogram, although some function in the medulla oblongata and brain stem may still be intact.

This study has satisfactorily validated brain stem death by neuropathological examination with consistent findings in all specimens. This is supported by measures of hypothalamic / pituitary function with an abolishment of vasopressin and adrenocorticotrophic hormones. This is accompanied by diabetes insipidus, not previously demonstrated in an animal model. The hormonal changes and the decrease in cortisol concur with the primate model of Novitzky (Novitzky, et al, 1984).

Thyroid hormone status following brain death has become a controversial topic. Our results show a trend of falling levels of both T3 and T4 but remaining in normal limits. This contradicts Novitzky's findings where T3/T4 fell below the normal range, but supports other studies where T3/T4 stayed constant (Schwartz et al, 1993). The benefit of thyroxine to the maintenance of organ donors is not clear with protagonists (Masson et al, 1990) (Wheeldon et al, 1992) and antagonists (Randell and

Hockerstedt, 1992)(Meyers et al, 1993).

The finding of diminished insulin levels prompted Novitzky and colleagues to introduce supplemental therapy to clinical donors. Our results showed marked variation, with the suggestion that feedback stimulation may be intact as insulin peaks coincided with raised epinephrine. Serum levels remained in the normal range. In contrast to the same study by Novitzky et al, who documented no change in glucagon, this canine model shows a significant decrease, possibly emphasising inter-species variation.

The alteration in the status of the ß-adrenergic receptor supports the findings of Sakagoshi et al who demonstrated an up-regulation of the receptors post brain death (Sakagoshi et al, 1992). The response also resembles the model of myocardial ischaemia in the dog by Mukherjee et al, 1982. Despite this observed increase in receptor density and sensitivity, the markedly elevated levels of epinephrine levels in animals 1 and 8 did not induce a hyperdynamic response. However the response of the hearts post transplantation in the group D animals (chapter 8) was very dramatic, and strongly suggestive of increased sensitivity. If the results of the ß-receptor analysis and the myocardial histology are combined it suggests the cardiac injury is an ischaemic one, occurring during the Cushing response, and the associated catecholamine storm. The extra demands on the heart during the hyperdynamic episode, with increased preload, afterload and in the presence of a metabolic acidosis, may predispose the myocardium to an ischaemic insult.

The injury to the myocardium is apparent from direct measurement, pressure volume relationships and histological studies. The immediate drop in systemic vascular resistance after brain death due to denervation of the arterioles decreases afterload and so protects the injured heart. This drop in SVR occurs despite the high levels of norepinephrine. The appearance of the myocardial injury histologically parallels the findings in the other animal models and also clinical studies (Koskelo et al, 1964); the nature of this is possibly ischaemic during the Cushing response due to poor subendocardial blood flow, coupled with relative hypoxia and acidosis. If this is true, it is difficult to

explain why the right ventricle suffers a more significant injury than the left, and studies of regional myocardial perfusion during the hyperdynamic state would be useful.

Documentation that brain death detrimentally effects right ventricular function is very relevant to cardiac transplantation. Early post cardiac transplant morbidity and mortality is related to right ventricular dysfunction and has been attributed to recognised, or unrecognised, raised pulmonary vascular resistance in the recipient (Kirklin et al, 1988). If right ventricular injury occurs in clinical brain death it would explain the susceptibility of the transplanted right ventricle to fail, particularly in the face of a raised PVR.

The discrepancy between RVPRSW and the stroke work calculated from impedance files at the latter stages of the study prompted a review of the computations in the computer software. The formatting of the PRSW from the pressure / volume relationship during an inflow occlusion was exact. However the calculations from steady state 500Hz files was inaccurate as the programme did not accurately assign start / finish points on each loop; thus over a series of files some estimations would be accurate and others invalid, explaining the discrepancy and the variation of these calculations.

Overall this group of experiments had been successful in validating a model of brain death. Furthermore they have quantified the myocardial and haemodynamic effects of brain death. This work has added to previous studies in demonstrating **right** ventricular dysfunction with load dependent and preload independent measures. Moreover this study gave experience with the brain dead animal model in preparation for the preservation studies and promoted further skills with the laboratory equipment for reliable data acquisition. The modifications applied from the pilot studies had proved dependable and no further changes were necessary.

For the purpose of planning transplantation studies the data was reviewed from this validation study and four hours post brain death was identified as the point where haemodynamic events, hormonal

and metabolic changes had reached a steady state, prior to the downward spiral. Therefore this time point was selected as the point cardioplegic arrest would be instigated in the preservation studies.

CONCLUSIONS

1. Inflation of an intracranial balloon in the canine model causes brain death, including brain stem death, due to global ischaemia. For the first time this has been proven neuropathologically, and supported by indirect measurements of pituitary hormones, examination of brain stem reflexes and electroencephalogram recordings.

2. Intracranial balloon inflation consistently causes the Cushing response, which is severe in all studies, although the degree of severity varies between animals.

3. This model of brain death causes injury to left and right ventricles. This study is the first to show that this impairment is more significant in the **right** ventricle, as measured by direct dimensions / pressures and also from pressure volume relationships (PRSW). This injury is masked by a significant drop in systemic vascular resistance reducing afterload on the left ventricle.

4. Histological evaluation and ß-adrenergic receptor analysis suggests the primary cause of the myocardial injury is ischaemic, presumed to occur during the Cushing response.

5. The changes in haematocrit were unexpected and not previously reported. The Cushing response causes a significant rise in the haematocrit immediately following brain death, which subsequently fell below baseline levels.

6. No previous animal study of brain death has documented urine output nor the occurrence of diabetes insipidus. The canine model in this thesis became polyuric in 9 of 10 animals coinciding with an absence of vasopressin.

CHAPTER 6

COMPLETE ATRIOVENTRICULAR TRANSPLANTATION

Introduction

The standard surgical technique for cardiac transplantation leads to distortion of the left and right atria in the donor heart to permit anastomosis to the recipient. The resultant cavities are large with discordant beating of donor and recipient atria. There are published reports of atrioventricular valvular insufficiency, formation of anastomotic thrombus, and theoretical impairment of diastolic atrial function (Angerman et al, 1990).

The hypothesis is that complete atrioventricular transplantation preserves normal atrial geometry and function, and therefore enhances cardiac function. This has prompted renewed interest in this technique, which involves 6 anastomoses of left pulmonary veins, right pulmonary veins, inferior vena cava, superior vena cava, pulmonary artery and aorta.

To date several transplant centres are using this technique, and modifications thereof where the left atrium is performed in standard fashion and the right atrium applied using bicaval anastomoses (Blanche et al, 1994). Comparative studies of standard and complete cardiac transplantation have not shown any advantages in the early post-operative period (Kendall et al, 1993)(Bizouarn et al, 1994). However, these clinical studies have demonstrated complete atrioventricular transplantation is technically feasible, without increasing morbidity or prolonging the duration of total ischaemic and cardiopulmonary bypass time.

The aim of these experiments was to compare these two techniques of implantation for cardiac transplantation; the standard technique, ventricular transplantation with atrioplasty, versus complete atrioventricular transplantation (figure 2). The haemodynamic measurements that are possible in the laboratory along with the subsequent data analysis presented an excellent opportunity to assess the potential benefit of this new surgical technique. Assessment of right ventricular function using pressure/volume analysis has not previously been accomplished in orthotopic transplantation. Also the ability to analyse atrial systolic function was fundamental to this study (chapter 2) and not previously described.

MATERIALS AND METHODS

Preparation of the Donor

The study group consisted of 40 dogs, their weights ranging from 22.4 to 29.8 kg (mean = 25.8 kg, SEM = 0.5 kg). All animals were anaesthetised, paralysed, and ventilated in the manner previously described with the modifications outlined in chapter 4. The studies were divided into two groups; group A to be ten transplants using the **standard** technique of implantation, and group B of ten transplants using **complete** atrioventricular transplantation. The technique was alternated with each experiment, and the heavier animal of the pair was used as the recipient. This was to avoid implanting a large heart into a small pericardium, and it was the experience of the technicians that larger dogs were more stable on cardiopulmonary bypass. All animals were surgically prepared as described in chapter 4.

Pre Transplant Data Acquisition

Baseline data were recorded when all dogs were stable under anaesthesia (stable heart rate, systemic pressure and suitable filling pressures). As in the validation study, the baseline data were of paramount importance with each dog acting as its own control. Therefore 6 files of 500Hz (steady state, 6 seconds each) and 6 files of 200Hz (caval occlusion, 16 seconds each) were taken at baseline and the pressure volume loops checked for consistency. These data were acquired over a half hour period with the ventilator disconnected during each file recording.

Donor Heart Preservation

The micromanometers were removed after data collection and the tips immersed in the organ bath at 37°C. The flow probes were also removed before the animal was fully anticoagulated with systemic injection of heparin (350 units/kg). The piezo-electric crystals were disconnected from the sonomicrometer and the leads wrapped together, and protected from immersion, by securing them in a surgical latex glove.

The superior vena cava was ligated at the junction of innominate, right internal jugular and right subclavian veins. The inferior vena cava was ligated at its emergence from the diaphragm followed promptly by cross clamping of the ascending aorta, at the origin of the innominate artery. The ventilation was discontinued. One litre of St. Thomas's cardioplegia at 4°C was infused to the aortic root via a 16 gauge cannula, and the heart was vented by incising the superior vena cava and right pulmonary veins distally. Topical normal saline, at 4°C, was poured over the heart which was kept immersed in this solution while the cardioplegia was infused.

The heart was excised in an identical manner for both groups. The aorta was transected as distal as possible, next to the cross clamp. Likewise the pulmonary artery was transected distally, at its bifurcation. Transection of the superior vena cava was at the tributary of the right supreme intercostal vein and the inferior cava transected at its emergence from the pericardium. The left and right pulmonary veins were transected at their pleural aspect outside the pericardium. The heart was then placed in 4°C normal saline.

Preparation of the Recipient

The recipient dog had immunosuppression therapy 2 hours prior to induction, using triple therapy of oral cyclosporin (10mg/kg), oral azathioprine (2mg/kg) and intravenous solumedrol (25mg/kg). The dog was then prepared for surgery in the same manner as for the donor except peripheral arterial cannulation used the left femoral artery. Following anticoagulation with heparin (350 units/kg), the animal was cannulated for cardiopulmonary bypass. The right femoral artery was exposed and a single arterial cannula (16 French) was inserted and secured. Venous drainage was via bicaval cannulation using a 28 French cannula for the inferior vena cava and a 24 French, right angled cannula for the superior vena cava. These were inserted through 2/0 Prolene purse strings into the extrapericardial segments of the inferior and superior vena cavae (Ethicon Inc, Johnson & Johnson Co, Somerville, NJ).

Cardiopulmonary Bypass

The heart lung machine was a Sarns 5000 (Ann Arbor, MI) using a COBE VPCML membrane oxygenator (COBE Laboratories Inc, Lakewood, CO). The circuit was primed with 1500ml of crystalloid solution using the formula described below;

Normosol pH 7.4	1000ml
Sterile Free Water	405ml
Sodium Bicarbonate 8.4%	50ml
Calcium Chloride	10ml
Mannitol 12.5%	50ml
Heparin 20000 u	2ml
Potassium Chloride	5ml
Frusemide 5mg	0.5ml
Final Osmolarity:	290 mOsm.

On bypass the temperature of the animal was allowed to drift to 32^{o}C, before rewarming was initiated during anastomosis of the pulmonary artery. The flow rate was kept at 80 - 100 ml/min/kg, and mean arterial pressure maintained in the range 60 to 80 mmHg. Occasionally, when pressure or circulating volume was low, a bolus of norepinephrine (1 - 2mg maximum) was administered. Regular arterial blood gas analysis was carried out, and hypokalaemia (<4.0 mmol/dl) or metabolic acidosis (pH<7.35) was corrected with potassium chloride and sodium bicarbonate supplements respectively, injected to the circulating volume.

Circulating post oxygenator blood gas analysis was performed immediately after the commencement, and immediately prior to cessation, of cardiopulmonary bypass, with intermediate samples every 20 minutes. Partial pressure oxygen was remained in normal limits with adjustment to the air/oxygen ratio, and only where necessary was anaesthesia maintained with 5% isoflurane. Throughout cardiopulmonary bypass the lungs were maintained on full ventilation so as to prevent

the pulmonary cellular injury associated with total circulatory support in dogs.

Before release of the cross clamp 20 ml of 12.5% mannitol, 10mg of frusemide and 50mg of lignocaine were administered into the circulating volume. The mannitol and frusemide were used to promote a diuresis that would help prevent lung injury and keep the haematocrit as high as possible following dilution with the prime. The role of the lignocaine was prophylactic to aid defibrillation of the heart and prevent subsequent ventricular dysrhythmias. Eighteen of the twenty experiments required a single 20 Joule DC trans cardiac shock to convert ventricular fibrillation to the underlying rhythm.

Weaning from Cardiopulmonary Bypass

Thorough de-airing of the heart was performed with needle aspiration through the apices of all the chambers. The cardioplegia site in the aorta was left open until after discontinuation of bypass to monitor escape of residual air. The micromanometers and flow probes were reapplied, and the piezo-crystals reconnected prior to weaning from bypass.

Weaning from cardiopulmonary bypass was done gradually monitoring left atrial pressure and systemic pressures, before removal of the venous cannula. Mean systemic pressure greater than 50 mmHg were acceptable with a mean left atrial pressure of 5 - 12 mmHg. **No inotropic agents were used nor any low dose dopamine**.

Once the heart had resumed control of the circulation the venous cannula were promptly removed to prevent any hindrance of blood flow through the cavae.

Data Acquisition Post Transplantation

One hour after discontinuation of bypass, data were collected as for baseline values over a period of half an hour with six steady state 500Hz files and six vena caval occlusions at 200 Hz. The experiment was then terminated and the heart excised. The anastomoses were inspected, the

depth of the septal crystal from the right ventricular endocardium was measured and the right and left ventricular free wall volumes measured by saline displacement.

Group A Recipients; Standard Technique

Explantation and Transplantation

The vena cavae were snared around the venous cannulae, the heat fibrillated and the aorta cross clamped. The aorta and pulmonary artery were transected as proximal to the base of the heart as possible before incising the right atrium at the base of the right atrial appendage. This incision was extended superiorly to the roof of the left atrium and inferiorly to the coronary sinus. The inter-atrial septum was incised and the left atrium transected at the mid atrial level at the base of the left atrial appendage. The aorta and pulmonary artery were dissected free from each other to facilitate anastomosis. On the donor heart the superior vena cava was ligated **avoiding the sinu-atrial junction**, and the aorta mobilised from the pulmonary aorta. The right atrium was fashioned to fit the recipient by an incision from the inferior cava along the crista terminalis and if necessary turning anterior toward the atrial appendage. Care was taken to avoid injury to the sinu-atrial junction. Finally the left atrium was laid open, using the pulmonary vein orifices, so as to match the recipient.

All four anastomoses were performed using 4/0 monofilament Prolene suture in a continuous fashion. On completion of the aortic anastomosis, and after thorough de-airing the cross clamp would be released. Ultrasonic flow probes were reapplied and the micromanometers reinserted. In all but one of these studies the heart was in slow ventricular rhythm. Isoprenaline was avoided due to its effects on pulmonary vasculature and minor cardiac effects. Atrial pacing wires were applied and worked successfully in all animals so that cardiopulmonary bypass could be discontinued after 25 minutes of reperfusion.

Group B Recipients; Complete Technique

Explantation and transplantation

The vena cavae were snared, the heat fibrillated and the aorta cross clamped. The pulmonary artery and aorta were transected and dissected free as for group A. The superior vena cava was transected at its most proximal intrapericardial portion. The inferior vena cava was transected at its junction with the right atrium. The right atrium was carefully dissected free from the right pulmonary artery before incising the left atrium at mid atrial level. This incision was taken circumferentially around the atrium as for group A. The anatomy of the pulmonary veins was delineated before dividing the posterior wall of the atrium into two Carrel patches containing the left and right pulmonary veins. Excess atrial tissue was then excised from the edges of these two patches so that minimal atrial tissue remained in the recipient.

On the donor heart the transected ends of the pulmonary artery and aorta were dissected from each other. The pulmonary vein orifices were identified and left and right orifices were fashioned for anastomosis to the pulmonary veins. All the anastomoses were performed using 4/0 prolene suture in a continuous fashion and executed in the following order; left pulmonary veins, right pulmonary veins, inferior vena cava, pulmonary artery, aorta and superior vena cava. The latter anastomosis was done having de-aired the heart and removed the cross clamp. Care was taken not to let the heart distend during this time with prompt defibrillation and avoidance of cross clamping the donor superior vena cava.

Statistical Analysis

The experiment were designed to permit analysis between the two groups, with each animal acting as its own control, allowing suitable analysis of each dog before and after transplantation. Statistical analysis was performed on a Zenith personal computer using the SAS statistical software package (Cary, North Carolina) and validated by the Department of Surgery Statistician. All data were tested for normality prior to further analysis, and having been found satisfactory the percentage

Figure 33.

Cardiopulmonary bypass in the canine model, during orthotopic cardiac transplantation.

The arterial cannula is exposed entering the right common femoral artery.
The venous cannulae are exiting the mediastinum on the right side down to the oxygenator, with a pump sucker entering the wound from top left.
The cannula for arterial pressure monitoring is inserted to the left femoral artery.

The author is being assisted by Dr. Hartmuth Bittner MD and Mr. George Quick, with Mr. Kurt Campbell operating the Sarns 5000 heart-lung machine.

change from baseline values were used in comparison with unpaired Student's t-test. between groups. To test significance of change from pre transplant levels paired Student's t-test was employed. In the text results are expressed as the mean, plus / minus one standard error of the mean. Statistical significance was considered where $p \leq 0.05$.

RESULTS

Baseline (table 24)

The 20 animals in the complete transplant group had a mean weight of 25.6 kg (±0.5) compared to the 20 animals in the standard group of 26.6 kg (±0.6). At baseline the two groups of donor animals were comparable for their haemodynamic profile with no significant differences; the following data are presented with the complete group listed first. Heart rate was 109 (±6) versus 111 (±6) beats per minute and mean arterial pressure 97 mmHg (±4) versus 89 mmHg (±4). Pulmonary artery pressure and left atrial pressure were similar at 15.1 (±0.6) and 7.1 (±0.8) mmHg versus 13.7 mmHg (±0.7) and 7.0 (±0.5) mmHg respectively. Cardiac outputs at baseline were 1550 (±94) mls/min and 1613 mls/min (±124) with comparable resistance to flow for both the left ventricle (SVR 5258 (±472) dyne.sec.cm^{-5} and 4746 (±481) dyne.sec.cm^{-5}) and the right ventricle (input resistance 840 (±81) dyne.sec.cm^{-5} and 752 dyne.sec.cm^{-5} (±101). The pulmonary vasculature had similar impedance profiles demonstrated by the characteristic impedance and the pulmonary vascular resistance, 153 (±14) and 436 (±73) versus 146 (±17) and 388 (±76) dyne.sec.cm^{-5} respectively. Likewise right ventricular hydraulic power analysis was unremarkable, with a total power of 71.8 (±4.0) and 71.0 (±5.8) mW comprised of mean power, at 71.5 %(±1.6) and 68.7%(±2.7), and oscillatory power, at 28.5%(±1.6) and 31.3%(±2.7). There were small non significant differences in the pre-load independent recruitable stroke work for both ventricles. The values for the left ventricle were 73.2 (±2.8) and 66.2 (±3.4) ergx10^3, and for the right ventricle 21.0 (±2.1) and 23.6 (±1.7) ergx10^3.

In comparing baseline values it was gratifying that these two groups of ten dogs were not only strongly comparable to each other but also to the ten animals in the brain death validation study

Table 24.

Comparison of baseline values for three experimental groups

(n=10 for each group) *±1sem*

There are no statistical differences between the following three groups at baseline:

Complete; donor animals that were to be transplanted using

complete atrioventricular transplantation

Standard; donor animals that were to be transplanted using

ventricular transplantation with atrioplasty

Validation; animals used in chapter 5 in the validation of brain death.

Parameter abbreviations;

wt; body weight. **HR**; heart rate. **Sys**; systolic pressure.

Dias; diastolic pressure. **MAP**; mean arterial pressure.

PAP; mean pulmonary artery pressure. **LAP**; mean left atrial pressure

CO; cardiac output. **SVR**; systemic vascular resistance.

EFF; transpulmonary efficiency. **Z0**; characteristic impedance

PVR; pulmonary vascular resistance. **Rin**; input resistance.

%OP; % of total power that is oscillatory. **OP**; oscillatory power.

%MP; % of total power that is mean. **MP**; mean power.

TP; total power.

LVPRSW; left ventricular pre load independent recruitable stroke work.

RVPRSW; right ventricular pre load independent recruitable stroke work.

COMPARISON OF BASELINE VALUES FOR THE EXPERIMENTAL GROUPS (N=10 FOR EACH GROUP)

	Wt *kg*	HR *b/min*	Sys *mmHg*	Dias *mmHg*	MAP *mmHg*	PAP *mmHg*	LAP *mmHg*	CO *ml/min*	SVR *dyn.s.cm-5*	EFF *J/L*
COMPLETE	25.6 *0.5*	109 *6*	126 *4*	83 *6*	97 *5*	15.1 *0.6*	7.1 *0.8*	1550 *94*	5258 *472*	2.82 *0.12*
STANDARD	26.6 *0.6*	111 *6*	119 *4*	74 *4*	89 *4*	13.7 *0.7*	7.0 *0.5*	1613 *124*	4746 *481*	2.69 *0.15*
VALIDATION	25.3 *0.5*	115 *4*	128 *9*	84 *8*	99 *8*	13.5 *0.8*	6.2 *0.3*	1397 *76*	5691 *544*	2.51 *0.15*
	LVPRSW *erg.103*	**RVPRSW** *erg.103*	**TP** *mW*	**MP** *mW*	**%MP**	**OP** *mW*	**%OP**	**Rin** *dyn.s.cm-5*	**PVR** *dyn.s.cm-5*	**Z0** *dyn.s.cm-5*
COMPLETE	73.2 *2.8*	21.0 *2.1*	71.8 *4.0*	51.2 *2.7*	71.5 *1.6*	20.6 *1.8*	28.5 *1.6*	840 *81*	436 *73*	153 *14*
STANDARD	66.2 *3.4*	23.6 *1.7*	71.0 *5.8*	49.3 *4.8*	68.7 *2.7*	21.7 *2.4*	31.3 *2.7*	752 *101*	388 *76*	146 *17*
VALIDATION	73.1 *4.4*	24.0 *3.1*	59.1 *5.9*	42.1 *4.2*	71.4 *1.3*	17.0 *1.9*	28.6 *1.3*	788 *53*	426 *49*	181 *10*

(table 24). This reinforced that the techniques utilised for instrumentation of the heart and subsequent data acquisition and analysis were highly consistent.

Baseline Data for Atrial Systole (table 25)

The computer software used for the analysis of data points during atrial systole on a 500 Hz steady state file had not previously been used (chapter 3). This program was specifically designed and written for this study. Following semi-automated sequencing of the cardiac cycles and correction by the author, the program would assemble the average of the ten raw channels of data, the two volume channels for left and right ventricle and the five derivative channels. These latter channels were namely left ventricular dP/dt, dV/dt, right ventricular dP/dt, dV/dt and left atrial dP/dt. The program would plot clean traces for each channel, particularly important for the 'noisy' channels of ventricular volumes and their derivatives. The period of atrial systole was then defined as starting at 0 dP/dt for the left atrium prior to atrial systole and finishing at end diastole (chapter 3). With atrial systole defined, the program would document details of left atrial pressure, left ventricular volume, right ventricular pressure and volume and the timing of the changes in these parameters.

Table 25 shows samples of baseline data from 16 outcomes of a total of 52 outcomes. Each number is the mean of 6 files of data, each file the mean of approximately 10 cardiac cycles. The results are remarkably consistent; the duration of atrial systole was 0.11 sec (±0.01) versus 0.09 (±0.00) sec, which was 19.4%(±1.0) and 16.9%(±0.9) of the cardiac cycle. The left atrial pressure at the beginning of atrial systole was 6.3 (±0.9) and 6.0 (±0.4) mmHg with an increase of pressure during atrial systole of 3.9 (±0.7) and 4.0 (±0.4) mmHg. The time to reach this maximum left atrial pressure was 0.064 (±0.003) versus 0.058 (±0.002) seconds. As a percentage of atrial systole this was similar at 56.7 % (±2.1) and 64.2 % (±2.4) demonstrating neither group had significant first degree heart block. With a similar pressure rise over a similar time course it is not surprising that the maximum left atrial dP/dt was comparable, with values of 115 (±25) and 124 (±15) mmHg/sec. As atrial systole is defined as starting when left atrial pressure is static (ie dP/dt = 0) the increase in left atrial dP/dt is almost identical to the maximum values (110 (±26) and 119 (±14) mmHg/sec).

Table 25.

Pre - transplant data during atrial systole.

Comparison of sixteen outcomes, of a possible fifty two, in analysis of atrial systole at baseline. There are no significant differences between hearts to be used for complete or standard methods of transplantation *±1sem.* (n=10 for each group).

Parameter abbreviations are:

Duration; duration of atrial systole. **% cycle**; % of cardiac cycle that is atrial systole.

Max LVV; maximum left intraventricular volume during cardiac cycle.

Max RVV; maximum right intraventricular volume during cardiac cycle.

LVEDP; left ventricular end diastolic pressure.

RVEDP; right ventricular end diastolic pressure.

RVEDV; right ventricular end diastolic volume.

(The reason for RVEDV being smaller than MaxRVV is explained in 'Results'.)

Incr. LAP; actual increase of left atrial pressure during atrial systole.

LAP O; left atrial pressure at start of atrial systole.

Max LAP; maximum left atrial pressure during atrial systole.

Min LAP; minimum left atrial pressure during atrial systole.

t max; time to achieve maximum left atrial pressure from start of atrial systole.

% t max; time to achieve maximum left atrial pressure from start of atrial systole expressed as a percentage of the duration of atrial systole.

Max LAdP; Maximum rate of change for left atrial pressure during atrial systole.

Incr. LAdP; Increase in rate of change for left atrial pressure during atrial systole.

PRE - TRANSPLANT DATA DURING ATRIAL SYSTOLE

	Duration *sec*	% Cycle	Max Lvv *ml*	Max Rvv *ml*	LVEDP *mmHg*	LVEDV *ml*	RVEDP *mmHg*	RVEDV *ml*
COMPLETE	0.11 *0.01*	19.4 *1.0*	51.3 *8.2*	24.2 *3.0*	6.5 *0.7*	45.1 *7.8*	1.9 *0.3*	22.5 *3.3*
STANDARD	0.09 *0.00*	16.9 *0.9*	52.5 *3.4*	30.8 *4.1*	8.1 *0.8*	44.2 *3.2*	2.7 *0.3*	28.0 *4.0*
	Incr LAP *mmHg*	**LAP O** *mmHg*	**Max LAP** *mmHg*	**Min LAP** *mmHg*	**t max** *sec*	**% t max**	**Max LAdP** *mmHg/sec*	**Incr LAdP** *mmHg/sec*
COMPLETE	3.9 *0.7*	6.3 *0.9*	10.2 *0.9*	5.4 *1.0*	0.064 *0.003*	56.7 *2.1*	115 *25*	110 *25.8*
STANDARD	4.0 *0.4*	6.0 *0.4*	10.0 *0.6*	5.4 *0.5*	0.058 *0.002*	64.2 *2.4*	124 *15*	119 *14.4*

End diastolic pressures for left and right ventricles were similar with respective values of 6.5 (±0.7) and 1.9 (±0.3) mmHg in the complete group, and 8.1 (±0.8) and 2.7 (±0.3) mmHg in the standard group. Likewise volume calculations for each ventricle showed only minor differences; maximum LV volume was 51.3 (±8.2) versus 52.5 (±3.4) mls and maximum RV volume was 24.2 (±3.0) versus 30.8 (±4.1) mls.

The data derived from atrial systole was reassuring that the computer program was correctly sampling atrial systole, and analysing the data therein. The high correlation between the two groups at baseline, coupled with reliable computation, would permit a detailed comparison of atrial systole between the two surgical methods of cardiac transplantation.

Post Transplantation

Ten studies in each group were successful, in that they were weaned from cardiopulmonary bypass and yielded reliable data. Two studies from each group were unsuccessful: in the complete group one recipient had a left sided superior vena cava and a second recipient aspirated gastric contents on induction of anaesthesia resulting in a very low arterial oxygen concentration off bypass (<100mmHg); two recipients in the standard group suffered pulmonary injury on cardiopulmonary bypass of unknown aetiology, resulting in poor gas exchange off bypass. Low arterial partial pressures of oxygen caused significant physiologic changes in the anaesthetised dog, with metabolic acidosis and haemodynamic instability, resulting in any recorded haemodynamic data being invalid.

In the complete group all hearts resumed sinus rhythm with no external pacing (figure36).

In the standard group nine hearts had only a slow ventricular rate (<60bpm) and all required pacing via right atrial epicardial leads at 120 bpm; none of these hearts developed sinus rhythm by the end of the study, which was 1.5 to 2 hours after cessation of cardiopulmonary bypass. The remaining heart in this group had immediately resumed sinus rhythm.

The total ischaemic time in the complete group was 86 minutes (±5) with the duration of cardiopulmonary bypass being 82 minutes (±7). These times were almost identical to the standard group which were 85 (±3) and 82 (±6) minutes respectively.

The peripheral arterial pressure was higher in the complete group, though not significantly {mean arterial pressure 74 mmHg(±3) versus 64 (±3) in the standard group} (figure 37). This reflects the minor elevation of the systemic vascular resistance {7340 dyne.sec.cm^{-5} (±847) versus 5976 dyne.sec.cm^{-5} (±546)} with similar cardiac outputs {917 mls/min (±98) versus 934 mls/min (±88)} (figures 38 and 39). Despite the higher afterload the mean left atrial pressure was lower in the complete group at 4.8 mmHg (±0.5) than the standard group, 6.3 mmHg (±0.4), (figure 41). There is a trend towards superior haemodynamics in the complete group, although none of the differences reached statistical significance.

Despite comparatively brief periods of ischaemia and cardiopulmonary bypass, the cardiac output and mean peripheral arterial pressure are only 60% and 75% of their pre transplantation levels. This underlines the species intolerance of complete circulatory support with increased vasomotor tone (SVR up 40%).

This is emphasised by the impedance data for the pulmonary vasculature. Mean pulmonary artery pressure is similar between groups at 12.0 (±0.7) mmHg and 13.2 (±0.7) mmHg and unchanged from baseline levels. However, input resistance increases to 1223 (±155) and 1257 (±118) dyne.sec.cm^{-5} for the complete and standard groups, a change mirrored by the pulmonary vascular resistance and the characteristic impedance (table 26, figure 40). The proportional decrease in oscillatory power and pulmonary artery flow in both groups gives no statistical change in transpulmonary efficiency (pre transplantation 2.82 J/L (±0.12) and 2.69 J/L (±0.15), post transplant 2.54 J/L (±0.18) and 2.62 J/L (±0.11).

Table 26.

Complete vs. standard orthotopic cardiac transplantation: duration, rhythm, pressures and impedance post transplantation *±1sem.* (n=10 each group).

Complete atrioventricular transplantation does not prolong ischaemic time nor duration of cardiopulmonary bypass.

Standard transplantation does not preserve sinus rhythm, requiring atrial pacing in 9 cases ($p<0.001$, chi^2 test).

Peripheral arterial pressures are higher with a lower left atrial pressure in the complete group, but this is not statistically significant.

There is no difference in the other parameters between the two groups.

COMPLETE VS STANDARD ORTHOTOPIC CARDIAC TRANSPLANTATION: DURATION, RHYTHM, PRESSURES AND IMPEDANCE POST-TRANSPLANT

		COMPLETE		STANDARD	
ISCHAEMIC TIME	*min*	86	*5*	85	*3*
CARDIOPULMONARY BYPASS TIME	*min*	82	*7*	82	*6*
SINUS RHYTHM		*10		1	
SYSTOLIC PRESSURE	*mmHg*	102	*3*	91	*3*
DIASTOLIC PRESSURE	*mmHg*	60	*2*	50	*3*
MEAN ARTERIAL PRESSURE	*mmHg*	74	*3*	64	*3*
LEFT ATRIAL PRESSURE	*mmHg*	4.8	*0.5*	6.3	*0.4*
CARDIAC OUTPUT	*ml/min*	917	*98*	934	*88*
SYSTEMIC VASCULAR RESISTANCE	*dyn.sec.10-5*	7340	*847*	5976	*546*
MN PULMONARY ARTERY PRESSURE	*mmHg*	12.0	*0.7*	13.2	*0.7*
INPUT REISTANCE	*dyn.sec.10-5*	1223	*155*	1257	*118*
PULMONARY VASCULAR RESISTANCE	*dyn.sec.10-5*	719	*89*	656	*81*
CHARACTERISTIC IMPEDANCE	*dyn.sec.10-5*	208	*29*	267	*29*
TRANSPULMONARY EFFICIENCY	*J/L*	2.54	*0.18*	2.62	*0.11*

Figure 34. (Above)

Frequency of sinus rhythm pre and post transplantation for complete and standard techniques. (n=10 each group).

Only one heart in the standard group resumed sinus rhythm after implantation. All remaining hearts required atrial pacing with only a slow underlying ventricular rhythm until termination of the study.

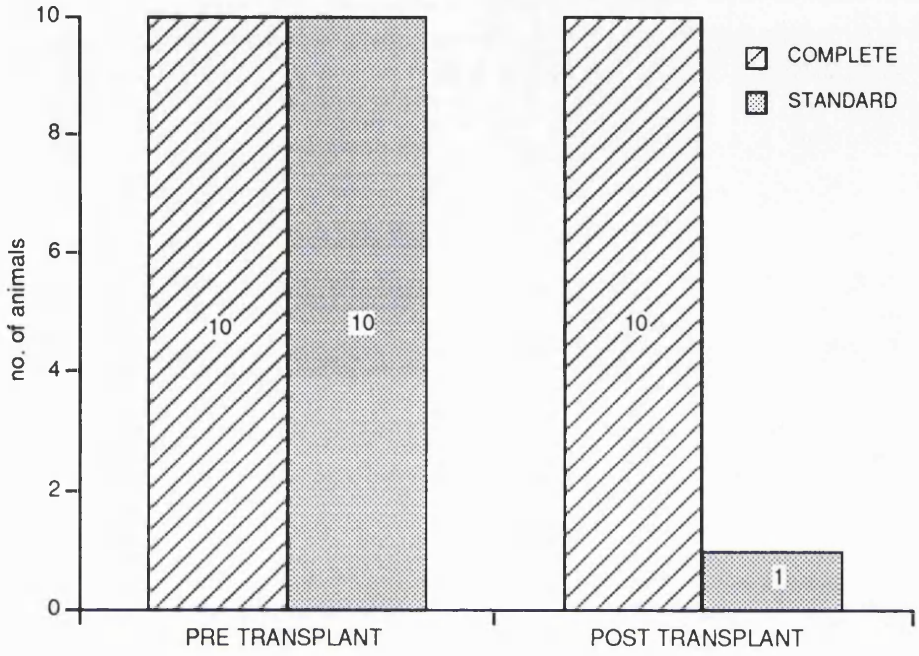
All hearts in the complete group spontaneously resumed sinus rhythm. This difference between groups is statistically significant.

Figure 35. (Below)

Mean peripheral arterial pressure pre and post transplant in complete and standard methods of implantation *±1sem.* (n=10 each group).

As for the cardiac output there is no difference between groups pre and post transplant, with pressures in the complete group slightly higher. After transplantation values from both groups are 70% of their baseline pressure.

FREQUENCY OF SINUS RHYTHM



MEAN PERIPHERAL ARTERIAL PRESSURE

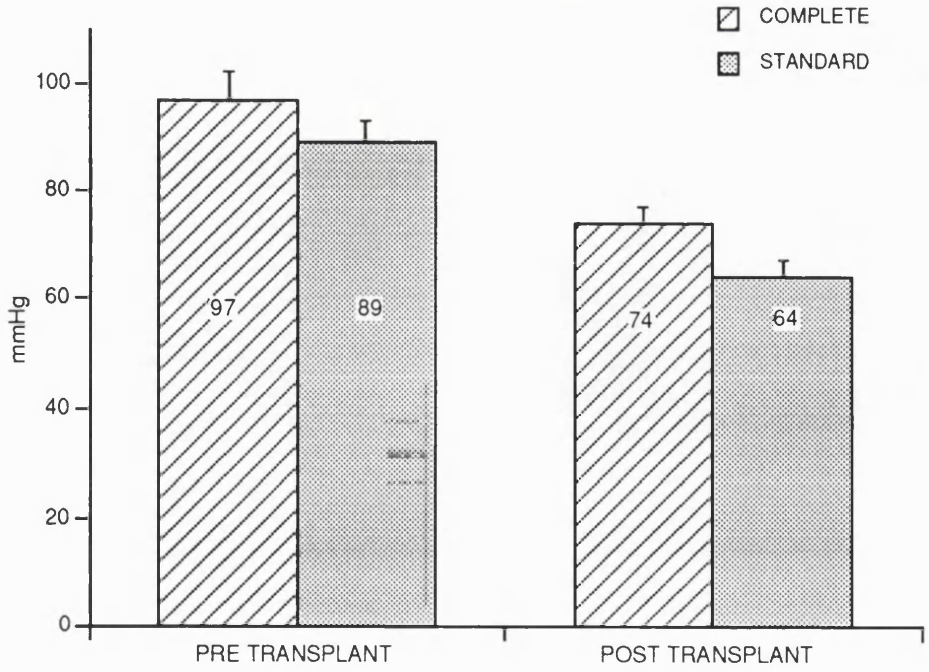


Figure 36. (Above)

Systemic vascular resistance pre and post transplant for complete and standard transplantation *±1sem.* (n=10 each group)

Systemic vascular resistance is similar for the two methods of transplantation. Post transplantation SVR is raised 40% and 26% in the recipients of the complete and standard techniques respectively.

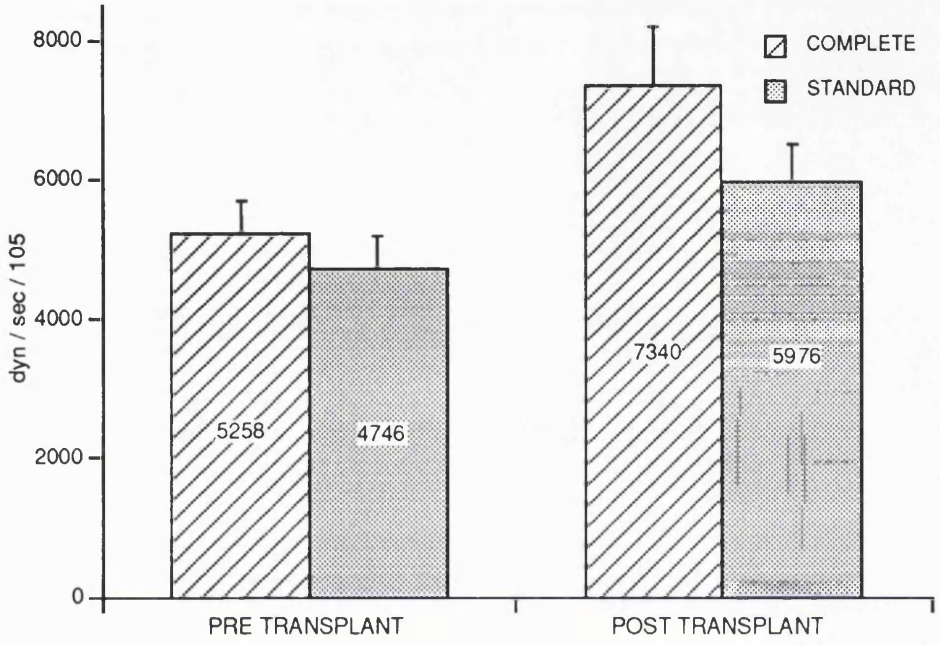
Figure 37. (Below)

Cardiac output pre and post transplant for complete and standard transplantation.

±1sem (n=10 each group).

As for SVR there is no difference between groups in their cardiac output pre and post transplantation. Post transplantation the cardiac output is 60% of its baseline value in both groups.

SYSTEMIC VASCULAR RESISTANCE



CARDIAC OUTPUT

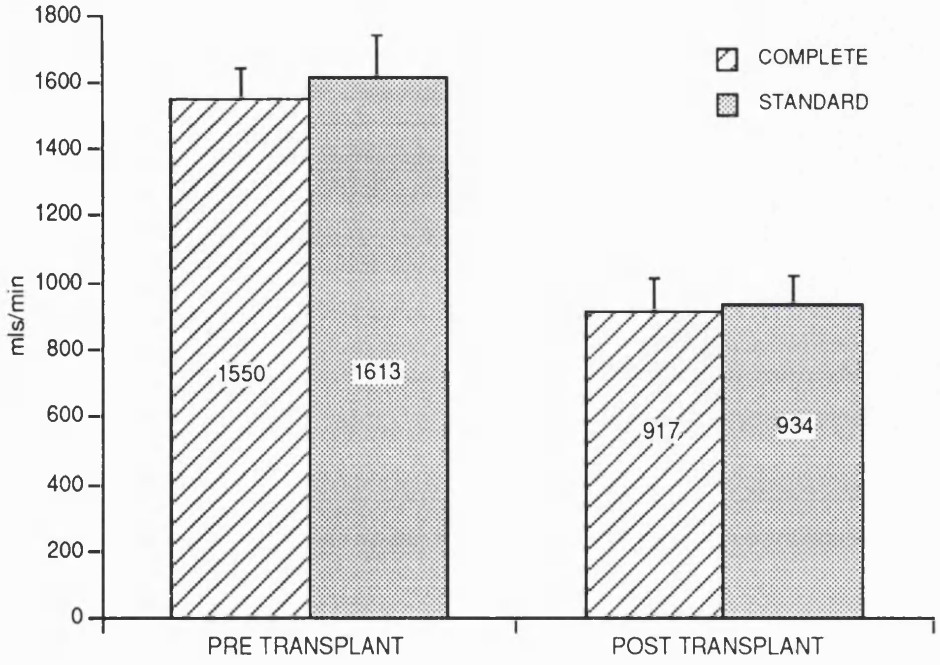
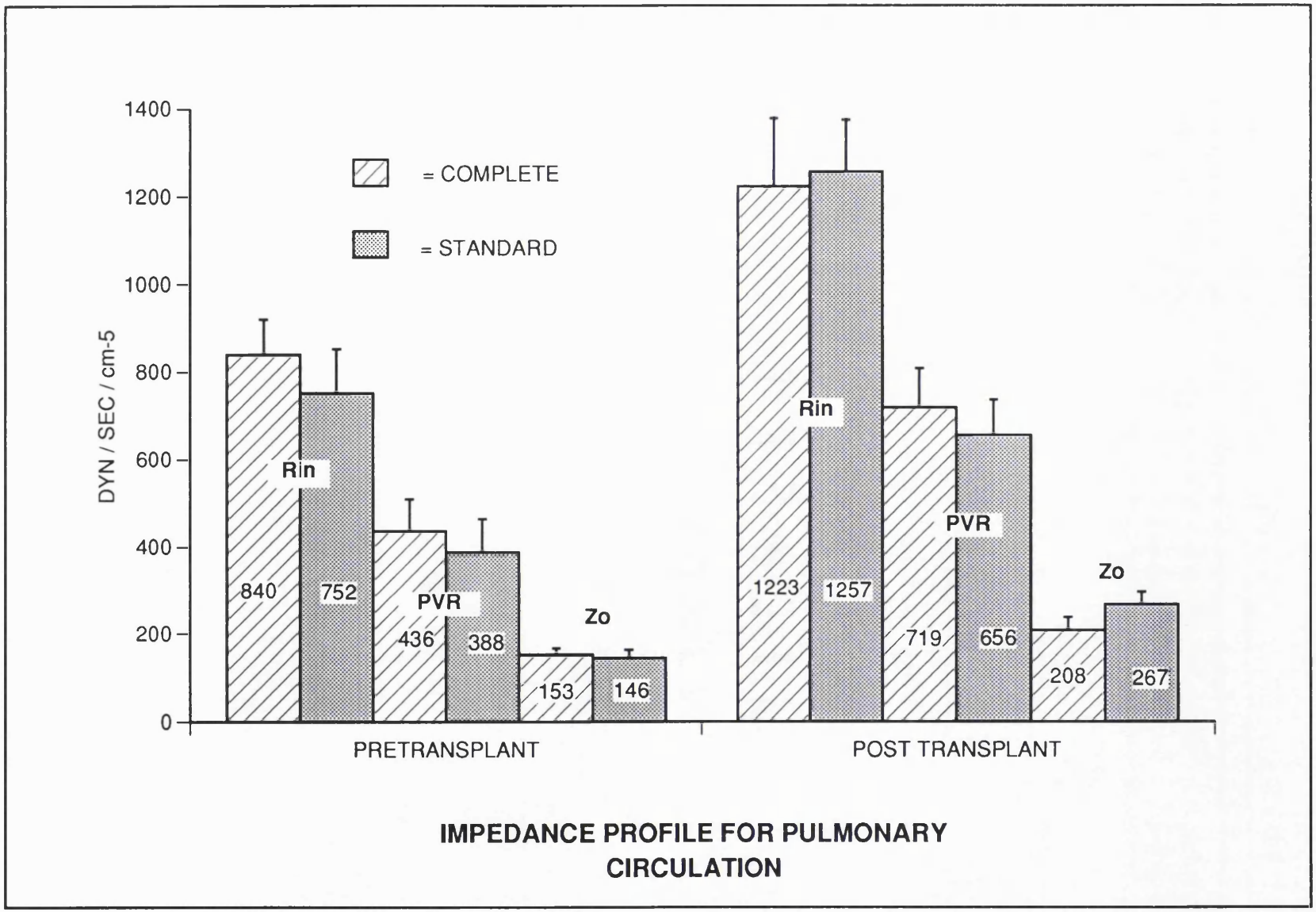


Figure 38.

Impedance profile for pulmonary circulation.

Input resistance (Rin), pulmonary vascular resistance (PVR) and characteristic impedance (Zo) pre and post transplant, for complete and standard methods of implantation *±1sem.* (n=10 for each group).

As would be expected the impedance profile of the pulmonary circulation is similar for both groups of animals pre and post transplantation. However, following cardiopulmonary bypass and transplantation there is at least a 60% increase of impedance for Rin, PVR and Zo.



IMPEDANCE PROFILE FOR PULMONARY CIRCULATION

Preload Independent Recruitable Stroke Work (PRSW)

PRSW is a measure of power per unit volume of myocardium. In the conscious, closed chest, animal this relationship of intraventricular pressure and volume is linear, hence being independent of the influence of preload and afterload. In the anaesthetised open chested preparation the pressure volume relationship remains relatively linear although there is some influence of preload (chapter 2).

After transplantation complete and standard methods of implantation conserve left ventricular PRSW; 70.1 ergx10^3 (±2.9) and 68.5 ergx10^3(±2.2), with baseline values of 73.2 ergx10^3 (±2.8) and 66.2 ergx10^3(±3.4) respectively. In percentage terms LV PRSW is 95% (±3.4) and 107% (±7.3) conserved with the two methods and this is not statistically different. (table 27, figure 35)

Right ventricular PRSW is conserved in the complete group (103%(±4.4)) but there is a significant drop in the standard group; 13.5 ergx10^3 (±1.3) post transplant compared to a pre transplant value of 23.6 ergx10^3. As a percentage this is 61% of baseline and is highly significant statistically ($p<0.001$) (table 27, figure 34).

In both groups the X-intercept for left and right ventricles became positive. The intercept for the left ventricle was +1.7 ml (±4.2) and +4.6 ml (±2.8) for complete and standard groups respectively, suggesting a tendency of the left ventricular free wall volume to increase slightly. The right ventricular X-intercept became larger in the standard group (+7.9 ml (±2.1)) unlike the complete group (+0.9 ml (±4.2)), implying right ventricular myocardium increasing in volume for the standard method of implantation.

Table 27. (Above)

Pre load Independent recruitable stroke work for left and right ventricles pre and post orthotopic transplantation *±1sem.* (n=10 for each group)

Left ventricular stroke work is conserved after transplantation in both groups. Right ventricular stroke work is conserved in complete transplantation but drops to 60% of its baseline value in the standard group. This is statistically significant with $p<0.001$.

The X-intercept for left and right ventricles increases insignificantly after transplantation.

Table 28. (Bottom)

Right ventricular hydraulic power analysis pre and post orthotopic transplantation *±1sem.* (n=10 for each group).

Total power reflects the changes in cardiac output. There is no difference between groups, but there is a decrease in power after transplantation. The contribution to total power of mean power and oscillatory power remain proportionally unaltered.

PRE-LOAD INDEPENDANT RECRUITABLE STROKE WORK
FOR LEFT AND RIGHT VENTRICLES
PRE AND POST ORTHOTOPIC TRANSPLANTATION

	PRE-TRANSPLANT				POST-TRANSPLANT			
	COMPLETE		STANDARD		COMPLETE		STANDARD	
LV PRSW erg.103	73.2	*2.8*	66.2	*3.4*	70.1	*2.9*	68.5	*2.2*
RV PRSW erg.103	21.0	*2.1*	23.6	*1.7*	21.3	*1.9*	*13.5	*1.3*

		POST-TRANSPLANT			
		COMPLETE		STANDARD	
% CHANGE OF LV PRSW		95.2	*3.4*	106.8	*7.3*
% CHANGE OF RV PRSW		102.9	*4.4*	*60.7	*8.0*
Change of LV X-intercept		1.7	*4.2*	4.6	*2.8*
Change of RV X-intercept		0.9	*2.4*	7.9	*2.1*

RIGHT VENTRICULAR HYDRAULIC POWER ANALYSIS
PRE AND POST ORTHOTOPIC TRANSPLANTATION

	PRE-TRANSPLANT				POST-TRANSPLANT			
	COMPLETE		STANDARD		COMPLETE		STANDARD	
TOTAL POWER mW	71.8	*4.0*	71.0	*5.8*	37.5	*4.3*	41.0	*4.0*
MEAN POWER mW	51.2	*2.7*	49.3	*4.8*	24.4	*3.0*	27.6	*2.8*
% MEAN POWER	71.5	*1.6*	68.7	*2.7*	64.4	*3.5*	67.0	*1.6*
OSCILLATORY POWER mW	20.6	*1.8*	21.7	*2.4*	13.1	*2.1*	13.4	*1.4*
% OSCILLATORY POWER	28.5	*1.6*	31.3	*2.7*	35.6	*3.5*	33.0	*1.6*

Figure 39. (Above)

Right ventricular pre load independent recruitable stroke work *±1sem.*

(n=10 for each group)

Right ventricular PRSW is conserved post transplant in complete transplantation. In standard transplantation there is a significant reduction of RV PRSW to 13.5 ergx10^3 from 23.6 ergx10^3 at baseline.

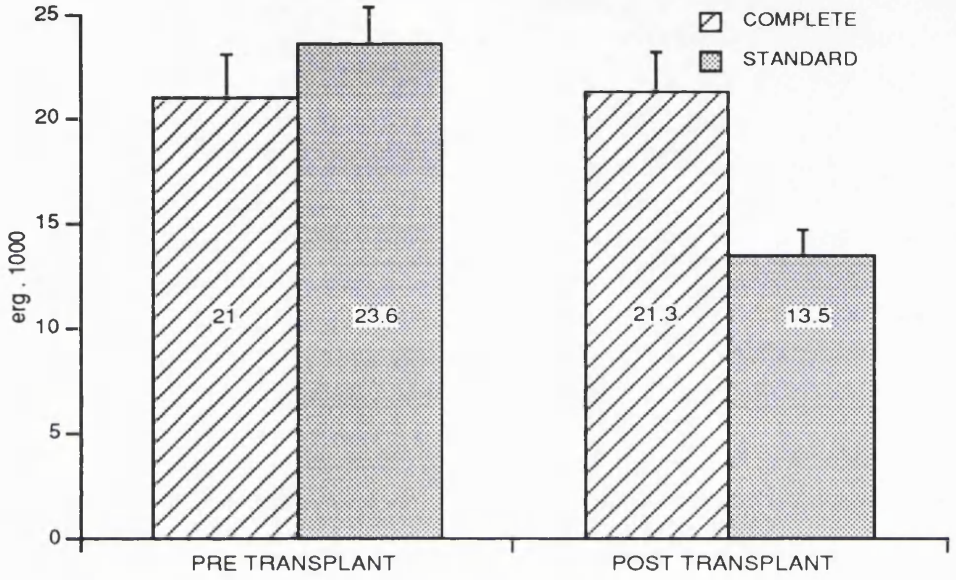
Figure 40. (Below)

Left ventricular pre load independent recruitable stroke work *±1sem.*

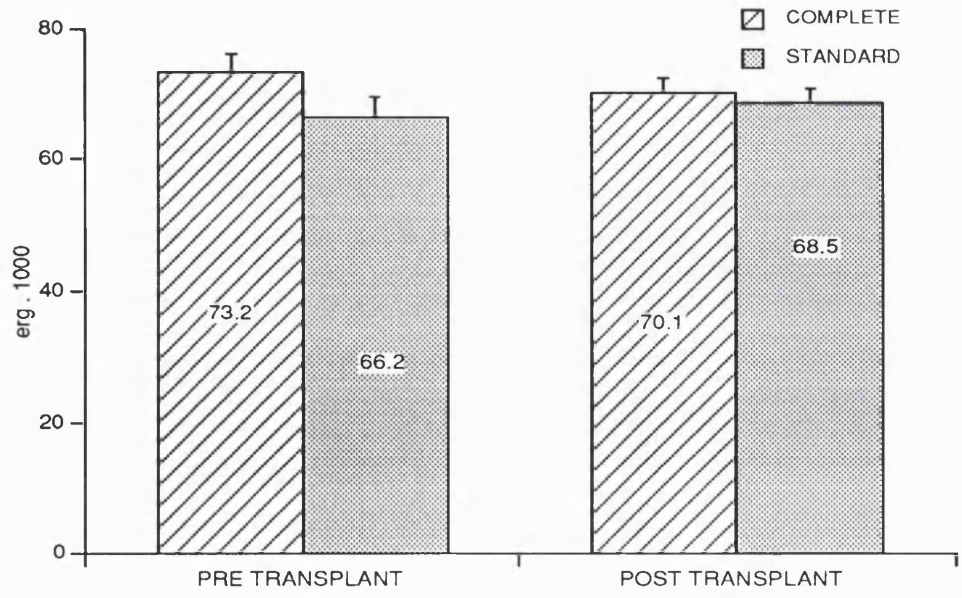
(n=10 for each group).

In both methods of implantation left ventricular PRSW is conserved following transplantation.

RIGHT VENTRICULAR STROKE WORK



LEFT VENTRICULAR STROKE WORK



Right Ventricular Hydraulic Power Analysis

Post transplant the total power decreases in both groups in accordance with the decrease in cardiac output ; 37.5 mW (±4.3) and 41.0 mW (±4.0) compared to 71 mW (±5) pre transplant (table 28). The proportions of mean power and oscillatory power making up the total power remain consistent before and after transplantation. Mean power is 64.4% (±3.5) vs. 67.0% (±1.6) post transplant as opposed to 71.5% (±1.6) vs. 68.7% (±2.7) pre transplant, while oscillatory power is 35.6% (±3.5) vs. 33.0% (±1.6) post transplant as opposed to 28.5% (±1.6) vs. 31.3% (±2.7) pre transplant.

Analysis of Atrial Systole

The analysis for the left atrium and the left ventricle during atrial systole is summarised in table 29.

The duration of atrial systole was unaffected by transplantation. In the complete method atrial systole was 0.086 sec (±0.008), or 18.4% of the cardiac cycle, and in the standard group 0.104 sec (±0.010), or 20.2% of the cardiac cycle. The shorter duration in the complete group is due to the faster sinus rhythm of 131 bpm (±4) compared to the atrially paced standard group at 118 (±3). However, the similar values as a percentage of the cardiac cycle suggest similar propagation of the paced beat. The duration of atrial systole is also comparative to baseline values showing that there is no evidence of first degree heart block such that there is no delay of the atrial beat at the atrio-ventricular node. It was important that the duration of atrial systole was comparative between groups, with one group in sinus rhythm and one group atrially paced, to allow meaningful interpretation of subsequent data from atrial systole.

The left ventricular and right ventricular end diastolic pressures (LVEDP and RVEDP) were unaltered in both groups following transplantation. In the complete group the LVEDP was 6.5 mmHg (±0.7) pre transplant and 5.9 mmHg (±1.3) post transplant, and in the standard group the LVEDP was 8.1 mmHg (±0.8) pre transplant and 6.3 mmHg (±1.0) post transplant. The RVEDP was 1.9 mmHg (±0.3) and 2.7 mmHg (±0.3) pre transplant, and 1.6 mmHg (±0.2) and 2.3 mmHg (±0.2)

post transplant, for complete and standard groups respectively.

The comparison of intraventricular volumes was interesting. The LV volumes remained similar between groups, although transplantation caused these volumes to become smaller. At baseline the volumes were 51.3 ml (±8.2) and 52.5 ml (±3.4), and post transplant were 42.0 ml (±7.7) and 39.9 ml (±2.6). The RV volumes show opposite trends between groups. For the complete group RV volume is reduced post transplant, from 24.2 ml (±3.0) to 19.9 ml (±2.1) a similar proportional decrease to the LV. However in the standard group RV volume increased from 30.8 ml (±4.1) to 40.0 ml (±5.5). These differences are statistically significant, and are evaluated in the 'Discussion'.

Also of note in the results of intracavitary volumes is the discrepancy between end-diastolic volume and maximum volumes. In theory intracavitary volume should be at maximum at end-diastole when ventricular filling is complete. However it is a consistent finding, using these mathematical models to calculate volume, that maximum volumes are obtained after end diastole, prior to ejection. It appears that during the period of isovolumetric contraction there is geometric rearrangement of the ventricles, altering the relative positions of the piezo-electric crystals, that the mathematical model interprets as an increase in intraventricular volume. In table 29 this discrepancy is shown as approximately 5.5 mls in the left ventricle and 1.5 mls for the right ventricle.

To address the data during atrial systole the three measured chambers will be scrutinised in turn; left atrium, left ventricle and finally right ventricle.

Atrial Systole; Left Atrium

Left atrial pressure (LAP) at the beginning of atrial systole was less post transplant in both groups; at baseline the LAP was 6.29 mmHg (±0.86) and 5.98 mmHg (±0.42), and 3.67 mmHg (±0.41) and 3.63 mmHg (±0.45) post transplant. During atrial systole the actual increase in pressure was 3.01 mmHg (±0.47) and 2.71 mmHg (±0.39) (figure 43). The time to achieve this pressure is similar in both groups at 0.061 sec (±0.010) and 0.074 sec (±0.009).

The increase of the pressure derivative for the LAP (LAdP/dt) was significantly less in the standard group compared to pre transplant levels, although there was no difference between groups. The values of LA dP/dt in the complete group were 109 mmHg/sec (±26) pre transplant and 81 mmHg/sec (±17) post transplant, whereas in the standard group they were 119 mmHg/sec (±14) pre transplant and 69 mmHg/sec (±13) post transplant (figure 44). Also the minimum LAdP/dt was significantly less in the standard group; -38 mmHg/sec (±8) versus -126 mmHg/sec (±18) pre transplant compared to the complete group with values of -81 mmHg/sec (±26) post transplant versus -124 mmHg/sec (±25) pre transplant. These data suggest that the left atrium in the complete group conserves its ability to contract and relax, more consistently than the standard group.

Atrial Systole; Left Ventricle

The data for LV volume in table 29 shows the volume at the start of atrial systole, the maximum volume achieved during atrial systole, with the difference between these values as the actual increase in volume during atrial systole. As already mentioned above, the LV volume is decreased post transplantation but otherwise there is no difference between the groups. However, the time taken to achieve this volume is significantly reduced in the standard group when expressed as a percentage of atrial systole. Pre transplant this value is 96.4% (±2.0), compared to 96.5% (±1.2) in the complete group, and post transplant it falls to 75.4% (±7.2), compared to 88.4% (±4.5) in the complete group. The reason for this more rapid filling compared to baseline, and compared to the complete group, may in part be due to the standard group having a higher baseline of ventricular filling at the start of atrial systole (see below).

Table 29.

Data analysis of atrial systole: left atrium and left ventricle *±1sem*

Comparison of pre and post transplant data for complete and standard transplantation; POST TRANSPLANT LISTED IN MIDDLE COLUMN (n=10 for each group). * indicates statistical significance from baseline, $p<0.05$.

Duration of atrial systole, and its percentage of the cardiac cycle, is listed in the first two rows.

First section;

LVEDP; left ventricular end diastolic pressure

RVEDP; right ventricular end diastolic pressure

Max LV volume is the maximum left intraventricular volume during cardiac cycle

Max RV volume is the maximum right intraventricular volume during cardiac cycle

Third section lists analysis of left atrial pressure.

Fourth section lists analysis of the derivative of left atrial pressure.

Fifth section lists analysis from left intraventricular volume.

Sixth section lists analysis of the derivative of left intraventricular volume.

DATA ANALYSIS OF ATRIAL SYSTOLE: LEFT ATRIUM AND LEFT VENTRICLE

		POST TRANSPLANT				PRE TRANSPLANT			
		COMPLETE		STANDARD		COMPLETE		STANDARD	
Duration atrial systole	*sec*	0.086	*0.008*	0.104	*0.010*	0.115	*0.006*	0.092	*0.003*
As % of cardiac cycle		18.4	*1.3*	20.2	*1.7*	19.4	*1.0*	16.9	*0.9*
Max LV volume	*ml*	42.0	*7.7*	39.9	*2.6*	51.3	*8.2*	52.5	*3.4*
Max RV volume	*ml*	19.9	*2.1*	*40.0	*5.5*	24.2	*3.0*	30.8	*4.1*
LVEDP	*mmHg*	5.9	*1.3*	6.3	*1.0*	6.5	*0.7*	8.1	*0.8*
LVED volume	*ml*	37.2	*7.7*	33.4	*2.9*	45.1	*7.8*	44.2	*3.2*
RVEDP	*mmHg*	1.6	*0.2*	2.3	*0.2*	1.9	*0.3*	2.7	*0.3*
RVED volume	*ml*	38.4	*2.2*	*38.3	*5.4*	22.5	*3.3*	28.0	*4.0*
LAP pre atrial systole	*mmHg*	3.67	*0.41*	3.63	*0.45*	6.29	*0.86*	5.98	*0.42*
LAP Max	*mmHg*	6.18	*0.64*	6.34	*0.57*	10.18	*0.90*	9.97	*0.61*
Increase LAP in atr. sys	*mmHg*	3.01	*0.47*	2.71	*0.39*	3.88	*0.67*	3.99	*0.43*
Time to reach max LAP	*sec*	0.061	*0.010*	0.074	*0.009*	0.064	*0.003*	0.058	*0.002*
As % of atrial systole		69.2	*6.1*	70.5	*5.3*	56.7	*2.1*	64.2	*2.4*
Min LAP during atr. sys	*mmHg*	2.4	*0.7*	3.5	*0.4*	5.4	*1.0*	5.4	*0.5*
LA dP/dt pre atr. sys	*mmHg/sec*	2.1	*0.5*	2.2	*0.5*	5.1	*1.2*	5.5	*1.4*
Max LA dP/dt	*mmHg/sec*	83	*17*	71	*13*	115	*25*	124	*15*
Increase of LA dP/dt	*mmHg/sec*	81	*17*	*69	*13*	110	*26*	119	*14*
Time to Max LA dP/dt	*sec*	0.036	*0.003*	0.043	*0.005*	0.043	*0.003*	0.038	*0.002*
As % of atrial systole		43.3	*2.7*	44.6	*5.5*	37.2	*1.9*	41.8	*2.3*
Min LA dP/dt in atr. sys	*mmHg/sec*	-81.0	*26.4*	*-37.8	*7.9*	-124.1	*25.8*	-125.5	*17.6*
LV volume pre atr. sys	*ml*	34.2	*7.3*	30.7	*3.3*	39.9	*7.0*	37.9	*2.8*
Max LV volume	*ml*	37.3	*7.8*	33.6	*2.9*	45.1	*7.8*	44.2	*3.2*
Increase LV volume	*ml*	3.15	*0.60*	2.96	*0.53*	5.21	*1.08*	6.32	*0.63*
Time to reach Max LV vol	*sec*	0.072	*0.003*	0.079	*0.012*	0.110	*0.005*	0.089	*0.004*
As % of atrial systole		88.4	*4.5*	*75.4	*7.2*	96.5	*1.2*	96.4	*2.0*
LV dV/dt pre atr. sys	*mls/sec*	38.2	*7.6*	69.7	*11.8*	43.9	*9.5*	70.2	*14.0*
Max LV dV/dt	*mls/sec*	91.9	*20.8*	84.7	*10.7*	92.0	*14.9*	123.2	*14.2*
Increase of LV dV/dt	*mls/sec*	53.7	*24.0*	*15.0	*5.0*	48.1	*9.4*	53.0	*13.9*
Time to Max LVdV/dt	*sec*	0.027	*0.006*	0.015	*0.004*	0.067	*0.009*	0.043	*0.008*
As % of atrial systole		35.1	*8.2*	15.5	*4.0*	60.6	*8.3*	46.4	*7.9*

Figure 41. (Above)

Mean left atrial pressure *±1sem.*

Mean left atrial pressure pre and post transplant for complete and standard techniques. (n=10 for each group).

The groups are comparable at baseline, but after transplant the complete group has a lower mean left atrial pressure. This approaches statistical significance (p=0.0593)

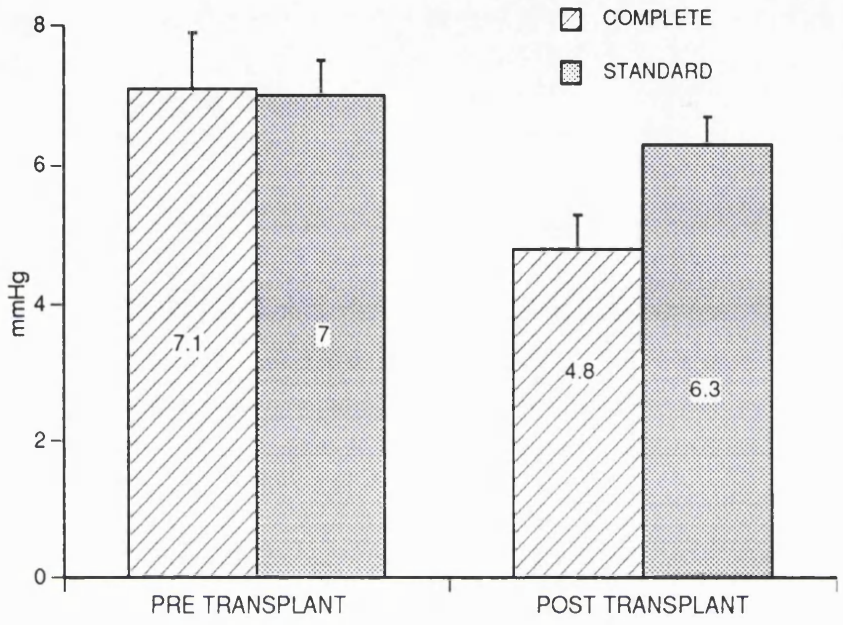
Figure 42. (Below)

Maximum dV/dt for the left ventricle during atrial systole *±1sem*

Comparison of maximum dV/dt during atrial systole for the left ventricle, pre and post transplant for complete and standard techniques. (n=10 for each group).

There is a significant decrease in rate of ventricular filling in the standard group post transplant.

MEAN LEFT ATRIAL PRESSURE



MAXIMUM dV/dt FOR THE LEFT VENTRICLE DURING ATRIAL SYSTOLE

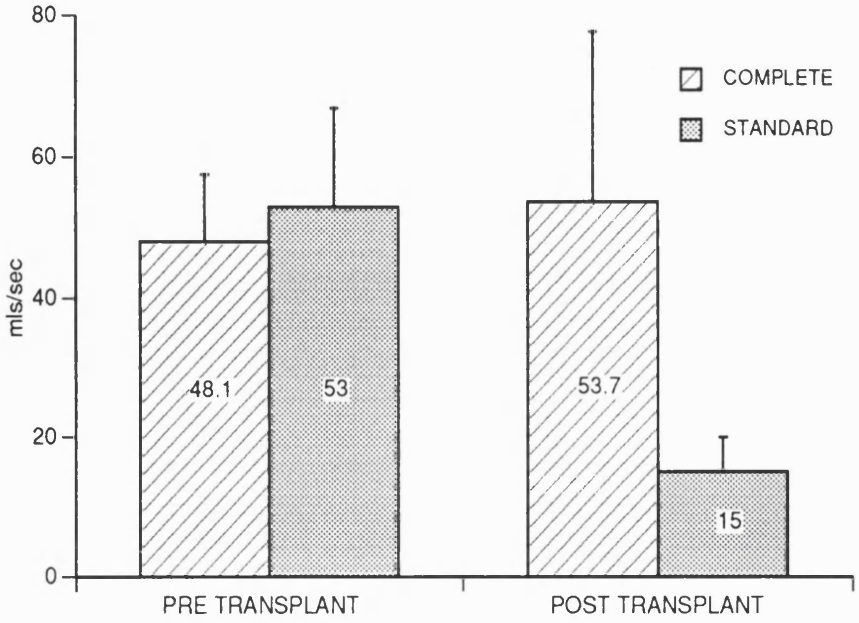


Figure 43. (Above)

Actual rise in left atrial pressure during atrial systole *±1sem.*

Comparison of the rise in left atrial pressure during atrial systole pre and post transplant for complete and standard techniques (n=10 for each group).

There is no difference between groups, although the gain in left atrial pressure is decreased following transplantation.

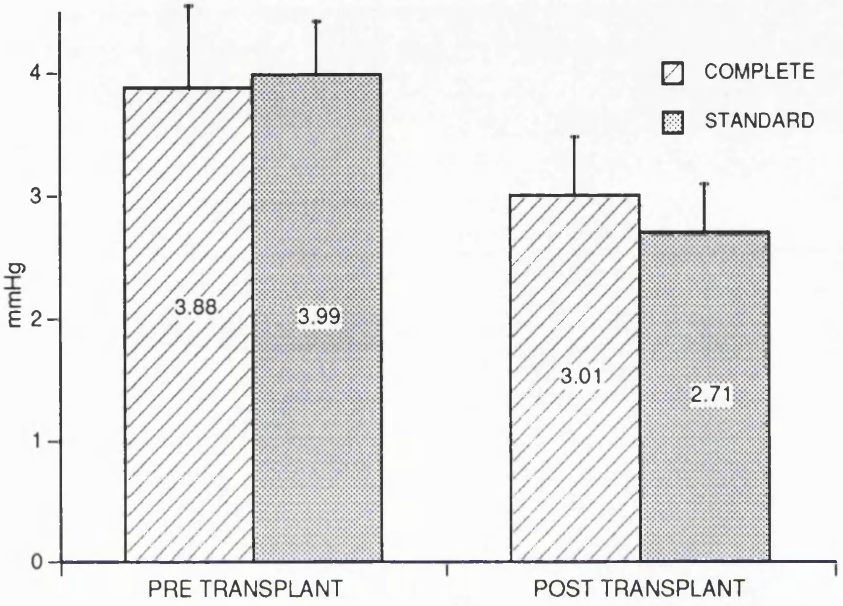
Figure 44. (Below)

Maximum dP/Dt for left atrial pressure during atrial systole *±1sem.*

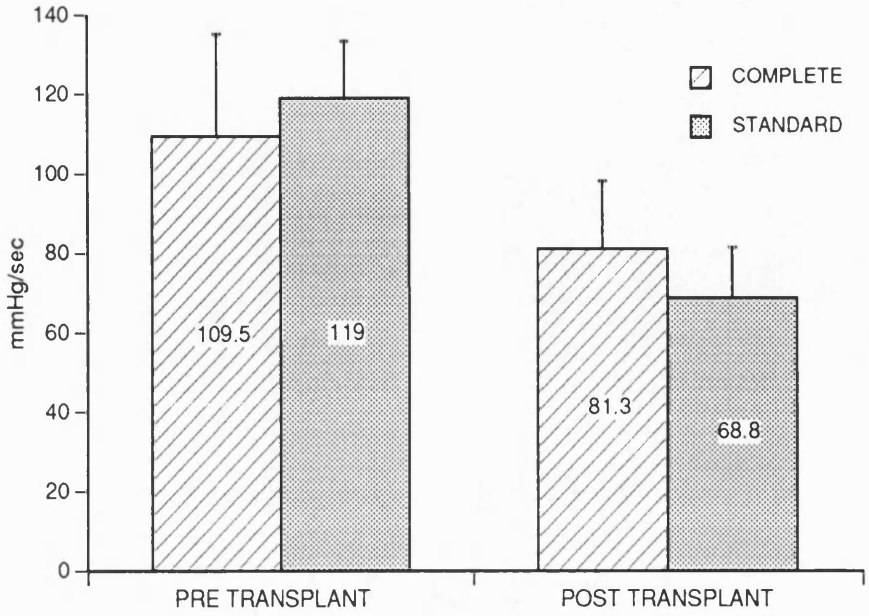
Comparison of rate of change of pressure in the left atrium during atrial systole, following transplantation with the complete or standard method. (n=10 for each group).

There is a significant decrease in left atrial dP/dt in the standard group post transplantation.

ACTUAL RISE IN LEFT ATRIAL PRESSURE DURING ATRIAL SYSTOLE



INCREASE dP/dt FOR LEFT ATRIAL PRESSURE DURING ATRIAL SYSTOLE



The derivative of LV volume (LVdV/dt) at the start of atrial systole shows no change for both groups pre and post transplant, but the standard group having higher flows. Pre and post transplant values are 43.9 ml/sec (±9.5) and 38.2 ml/sec (±7.6) for the complete group and 70.2 ml/sec (±14.0) and 69.7 ml/sec (±11.8) for the standard group. The maximum LVdV/dt is reduced significantly in the standard group post transplant, reducing the increase to 15.0 ml/sec (±5.0) compared to a pre transplant level of 53.0 ml/sec (±13.9). The complete group maintains its rate of LV filling with 53.7 ml/sec (±24) compared to 48.1 ml/sec (±9.4) pre transplant (figure 42).

Atrial Systole; Right Ventricle

The change in RV volume pre and post transplant for the two groups makes further interpretation of volume changes difficult (see above).

In the complete group RV volume, at the start of atrial systole, decreases slightly from 20.1 ml (±2.9) to 16.2 ml (±2.5) post transplant. The increase of volume during atrial systole remains constant (2.6 ml (±0.8) versus 2.4 ml (±0.5) post transplant) but the time taken to achieve this rise is reduced, as a percentage of atrial systole, from 89.8% (±2.1) to 74.1% (±6.5) post transplant. The derivative of RV volume (RVdV/dt) is maintained, regarding baseline values, maximum values and time to achieve these values (table 30).

The standard method of transplantation decreases the increase in RV volume during atrial systole from 4.3 ml (±0.6) to 2.2 ml (±0.4) post transplant. As for the complete method this rise in pressure is reached in less time, expressed as a percentage of atrial systole; 68.8 % (±8.2) versus 88.6% (±4.6) pre transplant. The derivative of volume shows the same changes as for the LV. Maximum RVdV/dt is decreased from 113.2 ml/sec (±13.8) to 68.7 ml/sec (±6.2) post transplant causing a reduction in the absolute increase of RVdV/dt from 83.3 ml/sec (±12.5) to 43.0 ml/sec (±8.3) post transplant. These changes are statistically significant. The time to reach the maximum RVdV/dt is reduced in the same proportion as the time to reach maximum volume; 0.037sec (±0.007) versus 0.053 sec (±0.005) pre transplant.

Table 30.

Right ventricular pressure and volume during atrial systole.

Listing of data during atrial systole pre and post transplant for complete and standard techniques *±1sem.* (n=10 for each group).

* indicates statistical significance $p<0.05$ in comparison to baseline.

First section is analysis of right intraventricular volume. Whereas RV volume decreases in the complete group, as in the LV, post transplant, the RV volume increases in the standard group.

Second section shows derivative data of right ventricular volume. The maximum, and increase of, RVdV/dt in the standard group is significantly decreased post transplant.

Third section lists data from right ventricular pressure during atrial systole.
There are no significant differences between groups pre and post transplant.

Fourth section lists data from the derivative of RV pressure during atrial systole. This shows a reverse trend to LA dP/dt, with a decrease of dP/dt in the complete group post transplantation. This is probably the influence of pacing in the standard group.

RIGHT VENTRICLULAR PRESSURE AND VOLUME DURING ATRIAL SYSTOLE

		POST TRANSPLANT				PRE TRANSPLANT			
		COMPLETE		STANDARD		COMPLETE		STANDARD	
RV volume pre atr. sys	*ml*	16.2	*2.5*	*36.8	*5.4*	20.1	*2.9*	24.0	*4.2*
Max RV volume	*ml*	18.6	*2.3*	*39.0	*5.3*	22.7	*3.3*	28.3	*4.0*
Increase RV volume	*ml*	2.4	*0.5*	2.2	*0.4*	2.6	*0.8*	4.3	*0.6*
Time to Max RV volume	*sec*	0.060	*0.004*	0.070	*0.009*	0.102	*0.005*	0.080	*0.004*
As % of atrial systole		74.1	*6.5*	68.8	*8.2*	89.8	*2.1*	88.6	*4.6*
RV dV/dt pre atr. sys	*ml/sec*	38.3	*13.2*	25.7	*5.8*	34.1	*13.2*	30.0	*6.5*
Max RV dV/dt	*ml/sec*	79.2	*11.9*	*68.7	*6.2*	78.9	*14.5*	113.2	*13.8*
Increase of RV dV/dt	*ml/sec*	40.9	*13.8*	*43.0	*8.3*	44.9	*5.0*	83.3	*12.5*
Time to max dV/dt	*sec*	0.033	*0.008*	0.037	*0.007*	0.053	*0.005*	0.053	*0.005*
As % of atrial systole		43.0	*10.4*	*38.3	*6.5*	47.8	*4.4*	59.9	*4.8*
RVP pre atrial systole	*mmHg*	1.3	*0.2*	1.8	*0.2*	1.6	*0.3*	2.4	*0.3*
Max RVP	*mmHg*	1.8	*0.2*	2.4	*0.2*	2.4	*0.3*	2.9	*0.3*
Increase of RVP	*mmHg*	0.5	*0.1*	0.6	*0.1*	0.8	*0.2*	0.5	*0.1*
Time to Max RVP	*sec*	0.050	*0.008*	0.073	*0.010*	0.068	*0.006*	0.055	*0.009*
As % of atrial systole		57.0	*6.0*	69.4	*7.4*	58.9	*4.7*	58.6	*9.5*
RV dP/dt pre atrial sys.	*mmHg/sec*	10.4	*2.0*	9.8	*3.9*	4.0	*1.0*	4.3	*0.7*
Max RV dP/dt	*mmHg/sec*	17.7	*2.4*	23.4	*3.5*	27.5	*6.5*	18.8	*2.6*
Increase of RV dP/dt	*mmHg/sec*	*7.4	*1.4*	13.6	*2.1*	23.5	*6.8*	14.5	*2.6*
Time to max RV dP/dt	*sec*	0.028	*0.008*	0.049	*0.010*	0.054	*0.008*	0.050	*0.007*
As % of atrial systole		29.2	*6.1*	51.0	*9.7*	47.8	*7.1*	55.1	*7.5*

Figure 45. (Above)

Actual rise in right ventricular pressure during atrial systole.

Comparison of the rise in right ventricular pressure during atrial systole pre and post transplant in complete and standard methods of implantation *±1sem.* (n=10 for each group).

There is no statistical difference between the groups pre and post transplant.

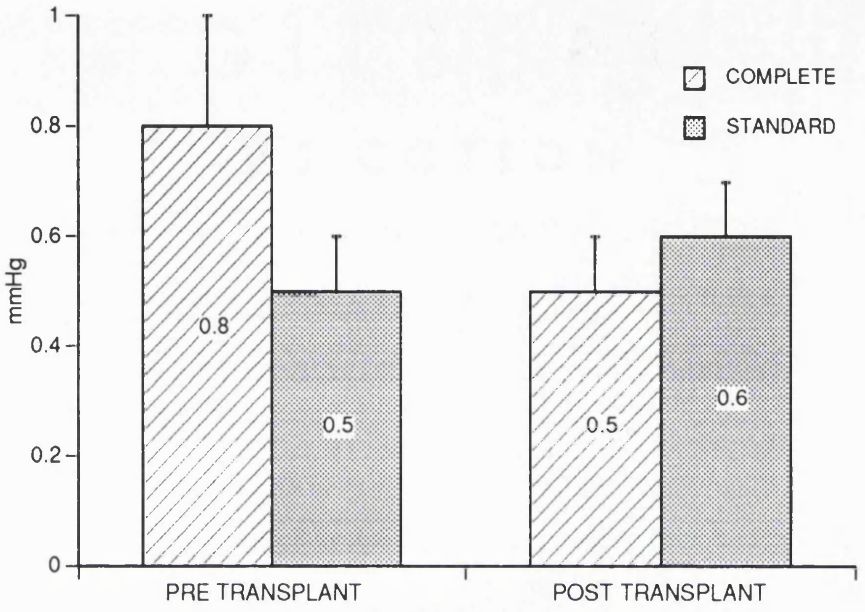
Figure 46. (Below)

Maximum dP/dt for right ventricular pressure during atrial systole.

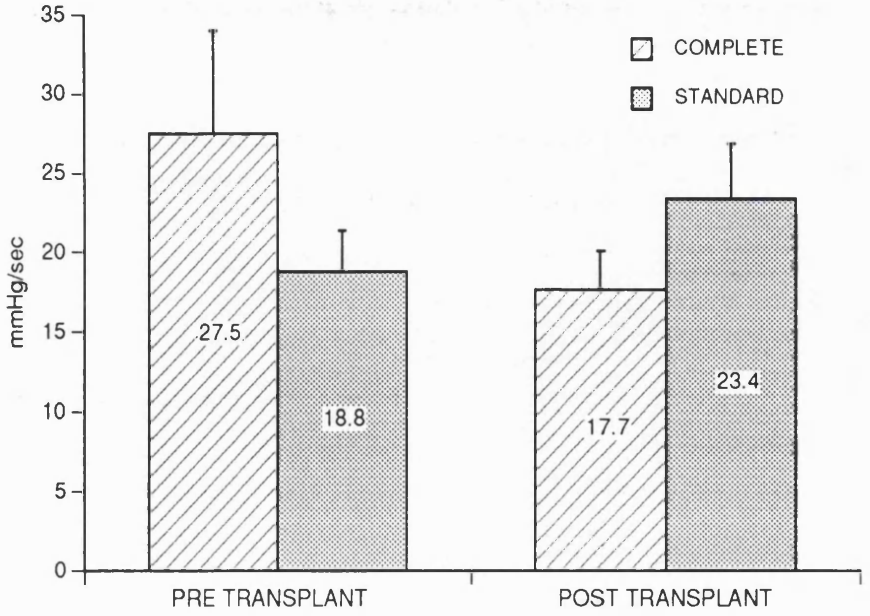
Comparison of the maximum dP/dt in the right ventricle during atrial systole pre and post transplant for complete and standard transplantation *±1sem.* (n=10 for each group).

Complete transplantation has a decrease in dP/dt following transplant compared to a mild increase in the standard group. This may be related to atrial pacing.

ACTUAL RISE IN RIGHT VENTRICULAR PRESSURE DURING ATRIAL SYSTOLE



MAXIMUM dP/dt FOR RIGHT VENTRICULAR PRESSURE DURING ATRIAL SYSTOLE



Right ventricular pressure (RVP) during atrial systole is not altered by transplantation nor by the surgical method employed. Prior to transplant the RVP at the start of atrial systole is 1.6 mmHg (±0.3) and 2.4 mmHg (±0.3) in complete and standard groups respectively, with values post transplant of 1.3 mmHg (±0.2) and 1.8 mmHg (±0.2). The increase of RVP in atrial systole is 0.8 mmHg (±0.2) and 0.5 mmHg (±0.1) at baseline, and 0.5 mmHg (±0.1) and 0.6 mmHg (±0.1) post transplant (figure 45). The time to achieve this pressure remains constant for both groups pre and post transplant at approximately 60% of atrial systole.

The derivative of RVP (RVdP/dt) shows a reverse trend to LA dP/dt, in that rate of pressure change is decreased post transplantation in the complete group while conserved in the standard group. For standard implantation the increase of RVdP/dt is 14.5 (±2.6) mmHg/sec pre transplant versus 13.6 mmHg/sec (±2.1) post transplant. In the complete group the value is 23.5 (±6.8) mmHg/sec pre transplant versus 7.4 mmHg/sec (±1.4) post transplant (figure 46). This is significant statistically.

Post Transplant Comparison of Sinus Rhythm versus Atrial Pacing in the Complete Group

Having completed 6 transplants in each group it was apparent that complete atrioventricular transplantation conserved sinus rhythm compared to the standard method, where all animals to that time had required external pacing. If differences were found between groups, they might be attributable to external pacing alone. Therefore the last four complete transplants had data recorded in sinus rhythm and also with atrial pacing over riding the intrinsic sinus rhythm.

The data for these four animals post transplantation is displayed in tables 31, 32 and 33.

The heart rate is faster in the paced group as it required a faster paced rhythm to overcome the intrinsic atrial rhythm. Otherwise no changes occur due to pacing with regard to direct measurements, ie. pressures and output, nor to indirect measures such as systemic and pulmonary impedance, hydraulic power and the preload independent recruitable stroke work (table 31). It is noteworthy that RV PRSW is still conserved with atrial pacing.

Table 31.

Comparison of sinus rhythm vs. atrial pacing. Pressures, preload independent recruitable stroke work (PRSW), hydraulic power and impedance profiles *±1sem.* (n=4).

Comparison of sinus rhythm and atrial pacing post transplant in animals that underwent complete implantation of the donor heart.

The heart rate is faster in the paced group due to the need for over pacing.

There are no significant differences between sinus rhythm and atrial pacing. RV PRSW is maintained with pacing.

SINUS RHYTHM VS ATRIAL PACING. PRESSURES, PRSW, HYDRAULIC POWER AND IMPEDANCE PROFILE

		POST TRANSPLANT			
		PACED		SINUS RATE	
Heart rate	*bpm*	149	*7*	136	*10*
Systolic pressure	*mmHg*	105	*3*	107	*3*
Diastolic pressure	*mmHg*	60	*0*	60	*0*
Mean arterial pressure	*mmHg*	75	*1*	76	*1*
Left atrial pressure	*mmHg*	5.8	*1.5*	6.0	*1.7*
Mean pulmonary art. pr.	*mmHg*	11.5	*1.9*	11.4	*2.1*
Cardiac output	*ml/min*	921	*413*	884	*313*
Systemic vascular resist.	*dyn.s.cm-5*	9029	*2824*	8364	*2153*
LV PRSW	*erg.1000*	86.4	*8.9*	93.9	*12.4*
RV PRSW	*erg.1000*	20.9	*5.7*	23.8	*5.6*
% LV PRSW		97.9%	*3.6%*	105.7%	*4.1%*
% RV PRSW		94.6%	*6.9%*	109.1%	*10.2%*
Change in LV X-intercept	*ml*	-0.6	*1.5*	-4.0	*2.0*
Change in RV X-intercept	*ml*	-0.7	*3.5*	1.1	*5.1*
Total power	*mW*	29.9	*6.0*	29.8	*3.7*
Mean power	*mW*	20.4	*5.4*	20.3	*4.2*
% mean power		66.2	*4.4*	66.5	*6.8*
Oscillatory power	*mW*	9.5	*0.8*	9.5	*1.5*
% Oscillatory power		33.8	*4.4*	33.5	*6.8*
Input resistance	*dyn.s.cm-5*	1584	*642*	1419	*534*
Characteristic impedance	*dyn.s.cm-5*	153	*6*	161	*15*
PVR	*dyn.s.cm-5*	761	*284*	658	*217*
EFF	*J/L*	2.37	*0.47*	2.37	*0.55*

Table 32.

Sinus rhythm vs. atrial pacing. Data analysis of atrial systole; left atrium and left ventricle.

Comparison of atrial pacing vs. sinus rhythm post transplant in animals who have undergone complete implantation of the donor heart. The data listed are analyses of atrial systole, as in table 29, for left atrium and left ventricle *±1sem.* (n=4)

Atrial pacing causes no deterioration of atrial function compared to sinus rhythm. It enhances LV filling as seen in the maximum, and increase of, LV dV/dt. Atrial pacing insignificantly decreases left atrial dP/dt but also enhances left atrial relaxation by decreasing the minimum LA dP/dt.

SINUS RHYTHM VS ATRIAL PACING.
DATA ANALYSIS OF ATRIAL SYSTOLE:
LEFT ATRIUM AND LEFT VENTRICLE

		POST TRANSPLANT			
		PACED		SINUS RATE	
Duration atrial systole	*sec*	0.083	*0.004*	0.076	*0.007*
As % of cardiac cycle		20.8	*2.3*	17.2	*0.1*
Max LV volume	*ml*	39.5	*4.8*	39.7	*3.7*
Max RV volume	*ml*	15.4	*1.7*	16.0	*3.0*
LVEDP	*mmHg*	2.2	*0.2*	2.4	*0.0*
LVED volume	*ml*	33.6	*4.2*	33.2	*3.1*
RVEDP	*mmHg*	2.3	*0.5*	2.6	*0.2*
RVED volume	*ml*	14.5	*2.4*	14.1	*3.5*
LAP pre atrial systole	*mmHg*	6.09	*1.99*	4.91	*1.45*
LAP Max	*mmHg*	8.72	*0.15*	9.22	*0.53*
Increase LAP in atr. sys	*mmHg*	2.62	*2.14*	4.31	*1.97*
Time to reach max LAP	*sec*	0.029	*0.024*	0.066	*0.007*
As % of atrial systole		32.9	*26.9*	86.8	*0.9*
Min LAP during atr. sys	*mmHg*	5.5	*1.5*	4.9	*1.4*
LA dP/dt pre atr. sys	*mmHg/sec*	1.2	*1.0*	1.4	*0.8*
Max LA dP/dt	*mmHg/sec*	81.6	*63.8*	118.2	*65.3*
Increase of LA dP/dt	*mmHg/sec*	80.4	*62.9*	116.8	*64.5*
Time to Max LA dP/dt	*sec*	0.049	*0.022*	0.038	*0.001*
As % of atrial systole		61.1	*29.8*	51.0	*4.1*
Min LA dP/dt in atr. sys	*mmHg/sec*	-99.4	*45.9*	-45.7	*30.8*
LV volume pre atr. sys	*ml*	30.0	*5.2*	31.4	*2.3*
Max LV volume	*ml*	33.6	*4.2*	33.2	*3.0*
Increase LV volume	*ml*	3.60	*0.98*	1.85	*0.69*
Time to reach Max LV vol	*sec*	0.082	*0.005*	0.068	*0.001*
As % of atrial systole		98.7	*1.1*	89.3	*7.5*
LV dV/dt pre atr. sys	*ml/sec*	8.0	*6.5*	25.9	*18.3*
Max LV dV/dt	*ml/sec*	*95.3	*4.3*	54.8	*3.5*
Increase of LV dV/dt	*ml/sec*	*87.2	*10.8*	28.9	*21.8*
Time to Max LVdV/dt	*sec*	0.053	*0.011*	0.031	*0.015*
As % of atrial systole		64.0	*9.8*	42.3	*24.8*

Table 33.

Sinus rhythm vs. atrial pacing. Right ventricular pressure and volume during atrial systole.

Comparison of sinus rhythm and atrial pacing post transplant in animals who have undergone complete implantation of the donor heart. Listed is analysed data from atrial systole, as in table 30, for the right ventricle *±1sem.* (n=4).

Atrial pacing causes enhancement of right ventricular filling in terms of actual increase in RV volume during atrial systole and an increase of RV dV/dt.

It is interesting that atrial pacing increases the rise of dP/dt in the right ventricle, which was the one advantage of the standard technique over the complete technique, suggesting it may be attributable to pacing itself.

SINUS RHYTHM VS ATRIAL PACING. RIGHT VENTRICULAR PRESSURE AND VOLUME DURING ATRIAL SYSTOLE

		POST TRANSPLANT			
		PACED		SINUS RATE	
RV volume pre atr. sys	*ml*	8.5	*2.4*	12.8	*4.5*
Max RV volume	*ml*	14.6	*2.3*	14.2	*3.5*
Increase RV volume	*ml*	6.2	*0.0*	1.4	*1.0*
Time to Max RV volume	*sec*	0.072	*0.001*	0.049	*0.012*
As % of atrial systole		87.7	*5.4*	66.5	*23.7*
RV dV/dt pre atr. sys	*ml/sec*	73.5	*28.2*	30.8	*25.1*
Max RV dV/dt	*ml/sec*	*126.3	*0.6*	60.1	*1.5*
Increase of RV dV/dt	*ml/sec*	52.8	*27.6*	29.4	*23.6*
Time to max dV/dt	*sec*	0.029	*0.008*	0.038	*0.031*
As % of atrial systole		34.7	*8.1*	45.4	*36.5*
RVP pre atrial systole	*mmHg*	1.5	*0.1*	1.9	*0.1*
Max RVP	*mmHg*	2.3	*0.5*	2.7	*0.2*
Increase of RVP	*mmHg*	0.8	*0.4*	0.8	*0.3*
Time to Max RVP	*sec*	0.064	*0.003*	0.061	*0.009*
As % of atrial systole		75.7	*1.1*	78.9	*3.5*
RV dP/dt pre atrial sys.	*mmHg/sec*	4.6	*3.8*	15.0	*6.4*
Max RV dP/dt	*mmHg/sec*	25.1	*6.9*	22.3	*7.0*
Increase of RV dP/dt	*mmHg/sec*	*20.6	*3.2*	7.3	*0.6*
Time to max RV dP/dt	*sec*	0.034	*0.010*	0.037	*0.012*
As % of atrial systole		41.4	*13.8*	46.7	*10.8*

The analysis of atrial systole for these four animals showed minimal changes due to pacing. The duration of atrial systole remained constant in actual time, and as a percentage of the cardiac cycle (20.8% (±2.3) paced versus 17.2 (±0.1) non paced). Atrial pacing reduced the increase of LAdP/dt from 117 mmHg/sec (±64) to 80 mmHg/sec (±63) but this was not significant. Paradoxically it decreased the minimum LAdP/dt from -46 mmHg/sec (±31) to -99 mmHg/sec (±46) (table 32).

There were no changes in LV volume due to pacing, although the derivative channel revealed that pacing increased the gain of LVdV/dt from 29 ml/sec (±22) to 87 ml/sec (±11). This is the opposite to the influence of standard transplantation.

Right ventricular changes in atrial systole due to pacing were as follows. Atrial pacing enhanced RV filling, raising the increase of RV volume from 1.4 ml (±1.0) to 6.2 ml (±0.0), and accomplishing the filling in a time close to baseline values (88% of the duration of atrial systole). As a result the increase of RVdV/dt was nearly doubled to 53 ml/sec (±28) from 29ml/sec (±24). This trend is also opposite to the standard transplant group.

Right ventricular pressure is unaltered by external pacing. The increase of RVdP/dt is trebled due to pacing, from 7.3 mmHg/sec (±0.6) to 20.6 mmHg/sec (±3.2) and this mimics the results from the standard group . This implies atrial pacing is the cause for the more rapid rise in RV pressure in the standard group.

DISCUSSION

Complete atrioventricular transplantation has now been successfully accomplished in the animal model with no increase in ischaemic nor cardiopulmonary bypass time. This overcomes the original need for the standard technique that was devised to avoid technical difficulties and the subsequent prolonged ischaemic and bypass times (Lower and Shumway, 1960). For complete transplantation the dog is technically easier than the human, due to a long intrathoracic inferior vena cava. However in the 30 recipients at Papworth hospital, England, that received complete implants, the

inferior vena cava did not present any problems (Kendall et al, 1993).

A reservation of surgeons is whether there will be sufficient atrium on the donor heart to permit the completion of the anastomoses, particularly in donors where the lungs are sent to different transplant centres. The experience at Papworth showed there was always enough left atrium, superior vena cava and inferior vena cava, on hearts from lung donors, liver donors and also heart-lung recipients that donated their heart in a 'Domino' procedure. The donor heart should be inspected prior to resection of left atrium and right atrium, to ascertain if there is sufficient tissue available for complete implantation. If there is a deficiency then the standard technique may be employed for one or both atria.

The direct measurements of pressure and flow show no advantages for the complete technique. Peripheral arterial pressure, ventricular filling pressures, left atrial pressure and pulmonary artery pressure are similar between both techniques although the trend towards better haemodynamics in the complete group was obvious. In this controlled environment, with similar weight donors and recipients coupled with minimum ischaemic times, there is the optimal opportunity to compare transplant techniques. Therefore it is not surprising that in clinical studies, of small numbers using heterogeneous donors, recipients and ischaemic times, the parameters outlined above show no differences (Bizouarn et al, 1994).

Right ventricular pre-load independent recruitable stroke work (RVPRSW) has not previously been evaluated in orthotopic transplantation. The decrease of RVPRSW following standard implantation was unexpected, as this should be a measure of myocardial contractility per unit volume that is pre-load independent, and not dependent on atrial function. The explanation lay in the perceived increase of right ventricular volume by the computer program; RV volume increased dramatically post transplant in the standard group and is attributable to distortion of the right free wall piezo-electric crystal away from the septal crystal. The sono-micrometer and subsequent calculation would interpret this as an increase in volume. This observation reinforces a criticism of the standard

technique; that distortion of the atria disturbs cardiac geometry predisposing to tricuspid and mitral reflux (Stevenson et al, 1987). With the complete technique RVPRSW was conserved as for the LVPRSW, making this the better technique for experimental transplantation measuring recruitable stroke work in the right ventricle.

The assessment of atrial systole using the computer software specifically written for this study was rewarding. The quality of baseline data, and high correlation between the two groups, reinforced the accuracy in the selection of atrial systole and subsequent analysis. Atrial systolic function in the standard group post transplant was fair and gross impairment was not found. Complete implantation does preserve left atrial dP/dt, left ventricular dV/dt and right ventricular dV/dt, better than the standard technique during atrial systole.

When performing the standard transplant **meticulous** care was taken to avoid damage to the sinu-atrial junction. Despite this only one heart resumed sinus rhythm, with the remainder having a normal atrio-ventricular conduction system that required atrial pacing. No isoprenaline was used in this study due to its cardiovascular effects (other than chronotropism) which was probably the drug needed to attain satisfactory rhythm. The complete group was remarkable for the consistent resumption of normal sinus rhythm, significantly different to the standard group.

The difference between groups of the origin of heart beat necessitated the comparison of sinus rhythm versus atrial pacing in the last four complete transplants. This was to ascertain if the differences between groups may be attributable to atrial pacing. In these four animals the contrary was found; atrial pacing caused no detriment to function but actually enhanced right and left ventricular filling during atrial systole. It also improved RV dP/dt during atrial systole, that was the only advantage standard transplantation had over complete transplantation, suggesting the pacing was responsible for this finding.

Complete atrioventricular transplantation is a feasible alternative to ventricular transplantation with

atrioplasty, the 'standard' technique. In the canine model it confers advantages that may be important when assessing interventions such as preservation solutions. The role of complete transplantation in clinical practice remains unclear.

CONCLUSIONS

1. The technique of complete atrioventricular transplantation is technically possible in the canine model.

2. Complete atrioventricular transplantation is accomplished in the canine model without prolongation of either the ischaemic time nor the duration of cardiopulmonary bypass. This concurs with data from clinical studies.

3. In the canine model complete atrioventricular transplantation conserves sinus rhythm in the immediate post-ischaemic and post-bypass period.

4. The pre-load independent recruitable stroke work for the right ventricle can be measured before and after orthotopic cardiac transplantation using the shell subtraction model, not previously accomplished.

5. The pre-load independent recruitable stroke work for the right ventricle is more reliably measured using the complete method of implantation. This may be attributable to conservation of ventricular geometry maintaining alignment of the piezo-electric crystals.

6. Complete atrioventricular transplantation has superior results to standard implantation in atrial systole with regard to; the increase of left ventricular dV/dt; the increase of right ventricular dV/dt and also the maximum and minimum left atrial dP/dt. Standard transplantation is superior only for the increase in right ventricular dP/dt during atrial systole, and this may be due to atrial pacing.

7. External pacing of the right atrium post transplantation with epicardial leads causes no significant alterations in haemodynamics with regard to either direct measurements such as pressure and dimension, or indirect measures such as PRSW, impedance and hydraulic power.

8. Atrial pacing in complete transplantation has no detrimental effects to atrial systole as assessed in this thesis. Atrial function in complete transplantation, is affected by atrial pacing for the following parameters; increasing left ventricular dV/dt; increasing right ventricular dV/dt and increasing right ventricular dP/dt. The changes in volume derivatives are the opposite effects that occur with standard transplantation.

This study of 20 transplants has shown that cardiac function, with particular regard to atrial systole and pressure volume relationships in both ventricles, may be accurately assessed following transplantation. Complete atrioventricular transplantation in this model conserves the intrinsic sinus rhythm of the heart, and better preserves atrial systolic function as quantified in this thesis. Complete transplantation would appear to conserve normal geometry of the ventricles and so allows accurate measurement of pre load independent stroke work post transplant.

These advantages of the complete method influenced the author to use it for all subsequent transplants in this thesis.

For the vast majority of heart transplant recipients the standard technique is adequate. The advantages of the complete method would be useful in those recipients who required maximum work from their heart ie young, active patients. At times of allograft malfunction, such as acute rejection, the reserve of atrial systole may be beneficial, although these insults are often associated with dysrhythmias that negate any atrial contribution.

CHAPTER 7

MYOCARDIAL FUNCTION FOLLOWING TRANSPLANTATION FROM A BRAIN DEAD DONOR

Introduction

Research in cardiac transplantation has utilised hearts from a variety of animal models. The majority of this work focused on explants from healthy animals that are merely anaesthetised. These studies have repeatedly shown successful organ preservation for up to 24 hours and beyond, as measured in the isolated preparation and in orthotopic implantation. Despite these impressive results, clinical transplantation achieves best outcomes when the total ischaemic time is below four hours. With longer ischaemic times there is a significant increase in morbidity and mortality, particularly if prolonged over six hours. The reason for this discrepancy between research and clinical practice is not understood, although brain death in humans and animals is known to cause cardiovascular instability and eventual circulatory collapse.

At present there is little known about myocardial preservation in the hearts from brain dead animals. To date there are three studies published on this subject; Wicomb et al studied swine hearts, from animals that were presumed brain dead from carotid ligation, on an isolated preparation (Wicomb et al, 1986b); Galinanes used the rat model, made brain dead by inflation of an intracranial balloon, and assessed post preservation function with an auto perfused isolated preparation (Galinanes and Hearse, 1992), and Shivalkar et al compared the effects of rapid and slow inflation of an intracranial balloon on cardiac function in the canine orthotopic transplant model (Shivalkar et al, 1993).

These three models produced conflicting results. Galinanes showed brain death to impair left ventricular function which resolved after 6 hours preservation. Wicomb found that brain death impaired function post preservation, although storage reduced the anaerobic metabolism that had developed in the myocardium of the brain dead animal. In contrast to Galinanes, Shivalkar found it near impossible to wean hearts from cardiopulmonary bypass in the rapid inflation model, unless there were large doses of inotropes infused. These studies used load dependent analysis of ventricular function and there was no assessment of right ventricular function.

The aim of this thesis was to investigate the effect of brain death on myocardial preservation. In chapter 5 a reliable model of brain death was validated, with consistent detrimental effects on left and right ventricular function. In this chapter and chapter 8 this model would be used to assess preservation as gauged by post transplant function, using the complete transplant group in chapter 6 as controls.

An experimental design protocol was designed to allow 2-way multivariate analysis.

		BRAIN DEAD DONOR	
		NO	YES
FOUR HOURS OF MYOCARDIAL PRESERVATION	NO	GROUP A CHAPTER 6 *n=10*	GROUP B CHAPTER 7 *n=8*
	YES	GROUP C CHAPTER 8 *n=8*	GROUP D CHAPTER 8 *n=8*

This would permit comparison of the **main effects** of brain death and four hours preservation, and also compare the **interaction** of brain death and preservation.

In this chapter the experiments in group B are described and compared to group A. These experiments would use the minimum ischaemic time and the complete method of implantation in an attempt to guarantee successful outcomes with valid data acquisition. Were these experiments successful it would allow progression to groups C and D.

MATERIALS AND METHODS

Preparation of the Donor

The study group consisted of 16 dogs, their weights ranging from 23.3 to 29.9 kg (mean = 26.8 kg, SEM = 0.5 kg). The heavier animal of the pair was used as the recipient, with a mean weight of 27.8kg ±0.4. These animals were used in eight consecutive orthotopic cardiac transplants with no failed studies. All animals were anaesthetised, paralysed, and ventilated in the manner previously described, and were surgically prepared as in chapter 4. These studies were designed to complete group B, for subsequent two way multivariate analysis.

Pre Brain Death Data Acquisition

Baseline data were recorded when all dogs were stable under anaesthesia. Six files of 500Hz (steady state, 6 seconds each) and six files of 200Hz (caval occlusion, 16 seconds each) were taken at baseline, and the pressure volume loops checked for consistency. These data were acquired over a half hour period with the ventilator disconnected during each file recording.

Instigation of Brain Death

Following satisfactory collection of baseline data, brain death was instigated as described in chapter 4, 'Preliminary Studies'. The eight donor animals had a Cushing response as described previously, varying in duration from 6.5 minutes to 12 minutes. This hyperdynamic response was promptly followed by haemodynamic instability, that was observed in the validation study, requiring rapid intravenous fluid replacement and correction of metabolic acidosis. Blood pressure, filling pressures and serial arterial blood gas analysis became stable after approximately 30 minutes.

The validation study had showed greatest impairment two hours post brain death to recruitable stroke work for both ventricles. At four hours there was still impairment of function although the majority of animals were stable, having reached a 'steady state' as shown by haemodynamic and hormonal parameters. Beyond four hours there was progression to haemodynamic instability. Therefore data acquisition was timed at 2 hours and 4 hours post brain death, after which point the

heart was arrested and explanted for immediate transplantation. At these data points, 4 steady state 50Hz files and 4 caval occlusion 200Hz files were recorded.

Donor Heart Preservation

For the hearts in group B, donor heart preservation was identical to group A. The ischaemic time was kept as brief as possible so that any observations might be attributable to brain death, and the influence of hypothermic preservation kept to a minimum. The micromanometers were removed after data collection and the tips immersed in the organ bath at 37°C. The flow probes were also removed before the animal was fully anticoagulated with systemic injection of heparin (350 units/kg). The piezo-electric crystals were disconnected from the sonomicrometer and the leads wrapped together, and protected from immersion.

The heart was prepared for cardioplegic arrest and explantation as for group A, before the ventilation was discontinued. A cross clamp was applied to the distal ascending aorta and one litre of St. Thomas's cardioplegia at 4°C was infused to the aortic root, via a 16 gauge cannula. The heart was vented by incising the superior vena cava and right pulmonary veins distally. Topical normal saline, at 4°C, was poured over the heart which was kept immersed in this solution while the cardioplegia was infused.

Explantation of the donor heart was similar to the method used for the donors in group A; the aorta was transected as distal as possible, next to the cross clamp. Likewise the pulmonary artery was transected distally, at its bifurcation. Transection of the superior vena cava was at the tributary of the right supreme intercostal vein, and the inferior cava transected at its emergence from the pericardium. The left and right pulmonary veins were transected at their pleural aspect outside the pericardium. The heart was then placed in 4°C normal saline.

Preparation of the Recipient

The recipient animal was prepared according to the description in chapter 6. The availability of an adjacent operating theatre allowed the recipient to be anaesthetised and surgically prepared 20 minutes before arrest of the donor. The donor heart was explanted and the operating table prepared, so that the recipient could be moved into the main laboratory. With the sternotomy already performed and the femoral vessels exposed, the delay between applying the cross clamp on the donor aorta and commencing cardiopulmonary bypass in the recipient was an average of 26 minutes (±3). This delay was consistent in all transplant studies where ischaemic times were to be as short as possible.

Cardiopulmonary Bypass

The heart lung machine, oxygenator and crystalloid prime were unchanged from the previous transplants. Homeostasis of the animal was maintained on bypass with steady pressure and flow, coupled with regular circulating arterial blood gas analysis. The interventions and their timing, to maintain the *status quo,* was as described in chapter 6.

The surgical technique for **complete** transplantation was used in each animal. There were no technical difficulties encountered, nor were any modifications of the technique required.

Thorough de-airing of the heart was performed with needle aspiration through the apices of all the chambers, after which six hearts required a single 20 Joule DC trans cardiac shock to convert ventricular fibrillation to sinus rhythm. The micromanometers and flow probes were reapplied, and the piezo-crystals reconnected prior to weaning from bypass.

No inotropes nor low dose dopamine were necessary; all eight hearts were weaned from bypass without difficulty, while monitoring systemic and left atrial pressures. As soon as cardiopulmonary bypass was discontinued the venous cannula were removed to prevent any obstruction to blood flow through the cavae.

Data Acquisition Post Transplant

One hour after discontinuation of bypass, data was collected as for baseline values over a period of half an hour with six steady state 500Hz files and six vena caval occlusions at 200 Hz. The experiment was then terminated and the heart excised.

Statistical Analysis

Baseline data was tested for normality prior to further analysis and found to be entirely satisfactory. The unpaired Student's t-test was used for comparison with the complete group post transplant and also for comparison to the validation group for post brain death changes. In the text results are expressed as the mean, plus / minus one standard error of the mean (SEM = standard deviation / $\sqrt{n}$, where n = number of animals in study). Statistical significance was taken where $p \leq 0.05$.

RESULTS

Haemodynamic Measurements; Baseline.

At baseline the heart rate was 109 bpm (±5) with a mean arterial pressure of 84 mmHg (±4) and a left atrial pressure of 5.8 mmHg (±0.5). The cardiac output was 1692 ml/min (±133) and the calculated systemic vascular resistance 4068 dyne.sec.cm^{-5} (±325) (table 34). These were not significantly different to group A nor to the validation group, although the higher output with decreased pressure suggested group B animals were more vasodilated.

The pulmonary vasculature was correspondingly less resistant to flow than the other groups; input resistance was 585 dyne.sec.cm^{-5} (±32), pulmonary vascular resistance 307 dyne.sec.cm^{-5} (±35) and characteristic impedance 175 dyne.sec.cm^{-5} (±19). The former value was significantly reduced compared to the other groups.

Table 34.

Baseline data for group A, group B and the validation group *±1sem.*

In group B the systemic pressure, systemic vascular resistance and pulmonary impedance are decreased compared to the other groups. These differences are not significant when tested for normality.

PRSW; pre load independent recruitable stroke work.

BASELINE DATA FOR GROUP A, GROUP B AND VALIDATION GROUP

		GROUP B n=8		GROUP A n=10		VALIDATION n=10	
Mean Weight	*kg*	26.8	*0.4*	25.6	*0.5*	25.3	*0.5*
Heart rate	*bpm*	109	*5*	109	*6*	115	*4*
Systolic pressure	*mmHg*	109	*5*	126	*4*	128	*9*
Diastolic pressure	*mmHg*	71	*5*	83	*6*	84	*8*
Mean arterial pressure	*mmHg*	84	*4*	97	*5*	99	*8*
Left atrial pressure	*mmHg*	5.8	*0.5*	7.1	*0.8*	6.2	*0.3*
Mean pulmonary artery pr.	*mmHg*	12.0	*0.2*	15.1	*0.6*	13.5	*0.8*
Cardiac output	*ml/min*	1692	*133*	1550	*94*	1397	*76*
Systemic vascular resist.	*dyn.s.cm-5*	4068	*325*	5258	*472*	5691	*544*
Total power	*mW*	69.1	*5.4*	71.8	*4.0*	59.1	*5.9*
Mean power	*mW*	45.5	*4.3*	51.2	*2.7*	42.1	*4.2*
% Mean power		65.7	*2.1*	71.5	*1.6*	71.4	*1.3*
Oscillatory power	*mW*	23.6	*1.7*	20.6	*1.8*	17.0	*1.9*
% Oscillatory power		34.3	*2.1*	28.5	*1.6*	28.6	*1.3*
Input resistance	*dyn.s.cm-5*	585	*32*	840	*81*	788	*53*
Pulmonary vascular resist.	*dyn.s.cm-5*	307	*35*	436	*73*	426	*49*
Characteristic impedance	*dyn.s.cm-5*	175	*19*	153	*14*	181	*10*
Transpulmonary efficiency	*J/L*	2.45	*0.07*	2.82	*0.12*	2.51	*0.15*
Left ventricular PRSW	*erg.103*	74.2	*2.4*	73.2	*2.8*	73.1	*4.4*
Right ventricular PRSW	*erg.103*	21.3	*1.5*	21.0	*2.1*	24.0	*3.1*

Load independent analysis of recruitable stroke work (PRSW) showed normal baseline values; the PRSW of the left ventricle was 74.2 erg.10^3 (±2.4) and for the right ventricle 21.3 erg.10^3 (±1.5). These results reinforced that the PRSW is relatively independent of pre-load and afterload.

Right ventricular hydraulic power total was 69.1 mW (±5.4) of which 65.7% was steady (mean) power and the remaining 34.3% oscillatory power. The ratio of flow to power yielded a transpulmonary efficiency of 2.45 J/L (±0.07).

Haemodynamic Measurements; Post Brain Death

In each of the eight donor animals the Cushing response was immediate, following inflation of the intracranial balloon. The blood pressure increased from baseline to over 350 mmHg systolic and 200 mmHg diastolic. Intracranial pressure was rapidly elevated above systolic arterial pressure. Cardiac output increased to above 4000 ml/min, with the systemic vascular resistance also increasing, to 8000 dyne.sec.cm^{-5}. The hyperdynamic response was accompanied by relative hypoxia and a metabolic acidosis as in the validation group, that responded to therapeutic interventions.

At two hours post brain death the heart rate increased insignificantly to 120 bpm (±6) with mean arterial pressure constant at 79 mmHg (±2) and a slight decrease in cardiac output to 1574 ml/min (±132), despite a decrease in systemic vascular resistance to 3684 dyne.sec.cm^{-5}. After four hours there was no further change; heart rate 121 bpm (±7), mean arterial pressure 71 mmHg (±4), cardiac output 1707 ml/min (±184) and systemic vascular resistance 3497 dyne.sec.cm^{-5} (table 35). The left atrial pressure remained comparatively low at 4.8 mmHg (±0.9) which was significantly lower than the validation group, 9.6 mmHg (±1.3). Otherwise these values were comparable.

Table 35.

Comparison of haemodynamic parameters post brain death in two experimental groups *±1sem.* (n=8 group B, n=10 validation group)

At two hours and four hours post brain death the groups are comparable for heart rate and systemic pressures.

The left atrial pressure in group B is significantly lower than the validation group.

Cardiac output and systemic vascular resistance is similar.

The changes in left and right ventricular pre load independent recruitable stroke work are discussed with figures 47 and 48.

PRSW; pre load independent recruitable stroke work.

COMPARISON OF HAEMODYNAMIC PARAMETERS POST BRAIN DEATH IN TWO EXPERIMENTAL GROUPS

		BASELINE				2 HOURS				4 HOURS			
		GROUP B		VALIDATION		GROUP B		VALIDATION		GROUP B		VALIDATION	
Heart rate	*bpm*	109	*5*	115	*4*	120	*6*	126	*6*	121	*7*	111	*4*
Systolic pr.	*mmHg*	109	*5*	128	*9*	107	*3*	81	*8*	96	*5*	88	*5*
Diastolic pr.	*mmHg*	71	*5*	84	*8*	65	*2*	59	*8*	58	*3*	49	*3*
Mean arterial pr.	*mmHg*	84	*4*	99	*8*	79	*2*	66	*8*	71	*4*	62	*3*
Left atrial pr.	*mmHg*	5.8	*0.5*	6.2	*0.3*	4.8	*0.8*	8.7	*1.1*	*4.8	*0.9*	9.6	*1.3*
Mean PA pr.	*mmHg*	12.0	*0.2*	13.5	*0.8*	11.6	*0.6*	15.2	*1.1*	12.7	*0.7*	15.9	*1.4*
Cardiac output	*ml/min*	1692	*133*	1397	*76*	1574	*132*	1660	*240*	1707	*184*	1881	*223*
Sys. Vasc. Resist.	*dn.s.cm-5*	4068	*325*	5691	*544*	3684	*388*	3463	*468*	3497	*323*	2709	*320*
LV PRSW	*erg.103*	74.2	*2.4*	73.1	*4.4*	56.5	*3.5*	49.2	*3.5*	57.4	*4.0*	62.6	*5.3*
RV PRSW	*erg.103*	21.3	*1.5*	24.0	*3.1*	15.2	*1.3*	10.0	*2.2*	16.2	*1.5*	14.4	*2.0*
%LV PRSW		100.0%	*0.0%*	100.0%	*0.0%*	75.4%	*3.3%*	63.2%	*6.6%*	77.2%	*4.4%*	89.9%	*11.0%*
%RV PRSW		100.0%	*0.0%*	100.0%	*0.0%*	73.5%	*4.2%*	*45.5%	*10.9%*	77.1%	*5.6%*	66.9%	*8.3%*
Left X-intercept	*ml*	0.0	*0.0*	0.0	*0.0*	17.6	*3.6*	14.6	*6.4*	9.9	*2.7*	9.5	*5.2*
Right X-intercept	*ml*	0.0	*0.0*	0.0	*0.0*	6.1	*1.8*	4.0	*3.0*	5.9	*1.0*	5.5	*1.2*

Figure 47. (Above)

Comparison of validation group vs group B: percentage change of left ventricular pre load independent recruitable stroke work post brain death *±1sem.* (n=8 group B, n=10 validation group)

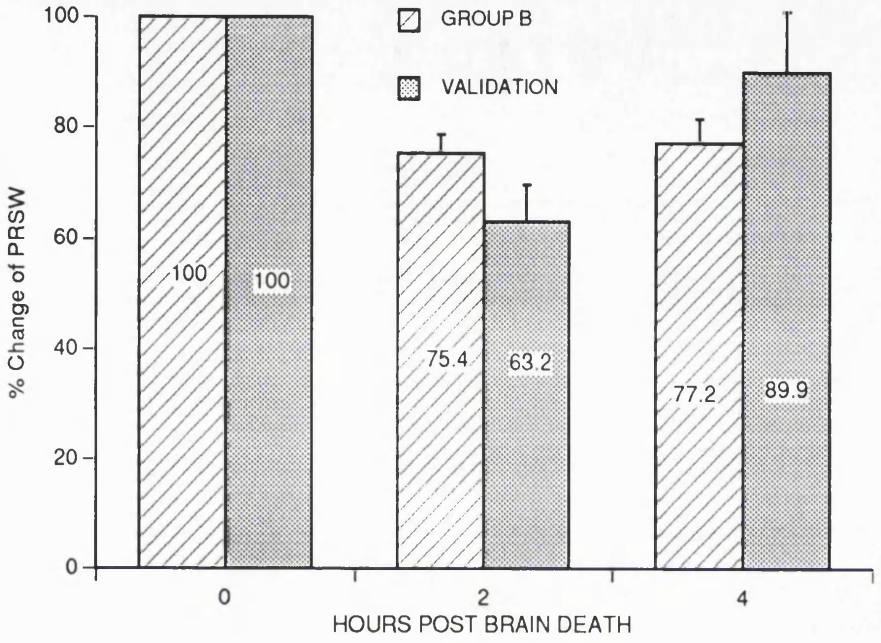
The impairment of PRSW in the left ventricle is similar in the two groups two hours post brain death. At four hours the decrease in PRSW remains constant in group B although there is some improvement in function in the validation group. There is no statistical difference between groups, but group B remains significantly impaired at four hours compared to baseline.

Figure 48. (Bottom)

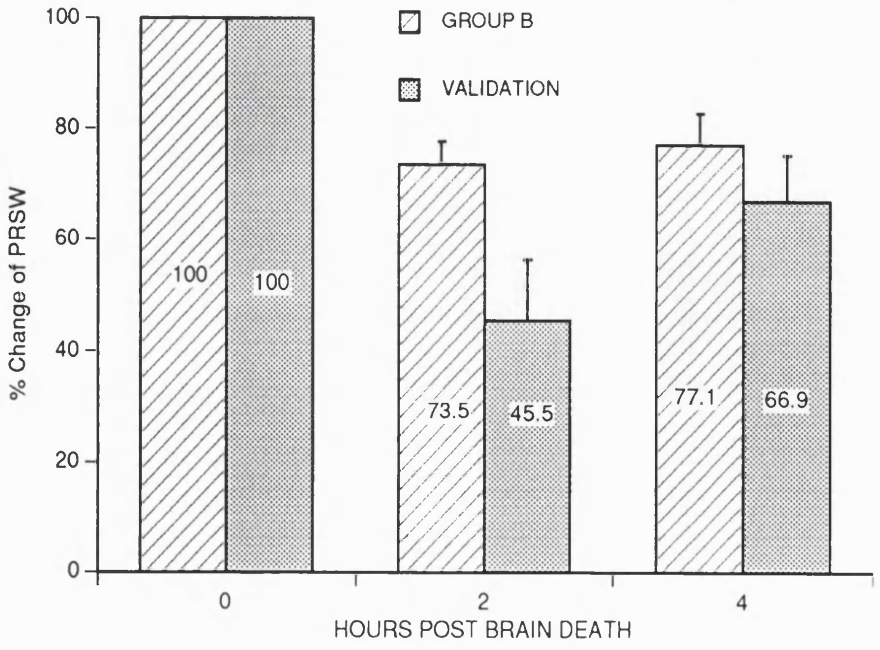
Comparison of group B vs validation group: percentage change of right ventricular pre load independent recruitable stroke work post brain death *±1sem.* (n=8 group B, n=10 validation group).

The impairment of right ventricular PRSW is less significant in group B after two hours and remains unchanged at four hours. The injury is also similar to the values for the left ventricle, unlike the validation group where the left ventricle was less impaired by brain death.

COMPARISON OF VALIDATION GROUP VS GROUP B
% CHANGE OF LEFT VENTRICULAR PRSW
POST BRAIN DEATH



% CHANGE OF RIGHT VENTRICULAR PRSW
POST BRAIN DEATH



The maintenance of higher systemic pressures without elevation of left atrial pressure in group B inferred less damage to myocardial contractility. In this group the percentage drop in left ventricular stroke work at two hours and four hours was 75.4% (±3.3) and 77.2% (±4.4) respectively, whilst in the validation group these values were 63.2% (±6.6) and 89.9% (±11.0) (figure 47). The right ventricle in group B suffered a similar reduction, to 73.5% (±4.2) at two hours and 77.1% (±5.6) at four hours post brain death. In the validation group this reduction was 45.5% (±10.9) and 66.9% (±8.3) respectively (figure 48).

The X-intercept for left and right ventricles became more positive after brain death. After two hours there was no further significant change, and the increases were very similar to the validation group.

The total hydraulic power of the right ventricle remained constant in group B; at baseline it generated 69.1 mW (±5.4) reducing to 55.1 mW (±5.9) at two hours and 64.4 mW (±9.4) at four hours. The fluctuations in total power were not significant and reflected the alteration in cardiac output. The proportion of total power that resulted in the movement of blood (mean power) increased from 65.7% (±2.1) at baseline to 74.0% (±1.6) and 76.7% (±2.1) at two and four hours post brain death. Correspondingly the power dissipated in the pulmonary vasculature (oscillatory power) decreased from 34.3% to 26.0% and 23.3% after brain death. The total power in the validation group increased with the cardiac output but the proportions of mean and oscillatory power were identical to group B, suggesting similar responses of the pulmonary vasculature after brain death (table 36). The ratio of power to flow decreased with a transpulmonary efficiency of 2.21 J/L (±0.1) implying more efficiency, achieving more flow at the expense of less power.

Table 36.

Comparison of hydraulic power analysis and pulmonary impedance profile post brain death in two experimental groups *±1sem.* (n=8 group B, n=10 validation group)

The oscillatory and steady components of total hydraulic power remain proportionally the same in both groups at two hours and four hours post brain death. There is no significant difference between total power in the two groups a value which reflects the changes in cardiac output.

Following brain death the impedance profile for the pulmonary circulation is the same between groups. Input resistance remains similar to baseline, while characteristic impedance decreases. Pulmonary vascular resistance has opposite trends between groups due to the low baseline value in group B and the persistent low left atrial pressure.

Total intravenous fluid infused after four hours brain death is 1.5 litres less in group B; this does not decrease urine output but lessens the degree of haemodilution seen in the haematocrit.

PVR; pulmonary vascular resistance **TP;** transpulmonary **IV;** intravenous.

COMPARISON OF HYDRAULIC POWER ANALYSIS AND PULMONARY IMPEDANCE POST BRAIN DEATH IN TWO EXPERIMENTAL GROUPS

		BASELINE				2 HOURS				4 HOURS			
		GROUP B		VALIDATION		GROUP B		VALIDATION		GROUP B		VALIDATION	
Total power	*mW*	69.1	*5.4*	59.1	*5.9*	55.1	*5.3*	74.5	*13.6*	64.4	*9.4*	89.9	*17.6*
Mean power	*mW*	45.5	*4.3*	42.1	*4.2*	41.0	*4.3*	58.3	*11.2*	49.6	*7.5*	69.6	*13.8*
% Mean power		65.7	*2.1*	71.4	*1.3*	74.0	*1.6*	77.6	*2.0*	76.7	*2.1*	77.1	*1.6*
Oscillatory power	*mW*	23.6	*1.7*	17.0	*1.9*	14.1	*1.2*	16.1	*2.8*	14.8	*2.3*	20.3	*3.9*
% Oscill. power		34.3	*2.1*	28.6	*1.3*	26.0	*1.6*	22.4	*2.0*	23.3	*2.1*	22.9	*1.6*
Input Resist.	*dn.s.cm-5*	585	*32*	788	*53*	611	*45*	796	*93*	619	*39*	730	*91*
PVR	*dn.s.cm-5*	307	*35*	426	*49*	369	*59*	315	*65*	391	*50*	265	*42*
Characteristic Imp.	*dn.s.cm-5*	175	*19*	181	*10*	141	*15*	159	*18*	141	*11*	157	*7*
TP efficiency	*J/L*	2.45	*0.07*	2.51	*0.15*	2.09	*0.07*	2.61	*0.18*	2.21	*0.10*	2.75	*0.22*
Cumulative Urine	*ml*	102	*25*	51	*15*	729	*135*	598	*166*	1421	*97*	1361	*142*
IV fluid total	*ml*	986	*83*			2543	*382*			3357	*380*	5000	*955*
						15 MINUTES POST DEATH							
Haematocrit	%	37	*1*	36	*2*	45	*2*	47	*2*	30	*2*	25	*2*

Figure 49. (Above)

Comparison of validation group vs group B: cumulative urine output post brain death *±1sem.* (n=8 group B, n=10 validation group)

The total amount and rate of urine production is similar between groups.

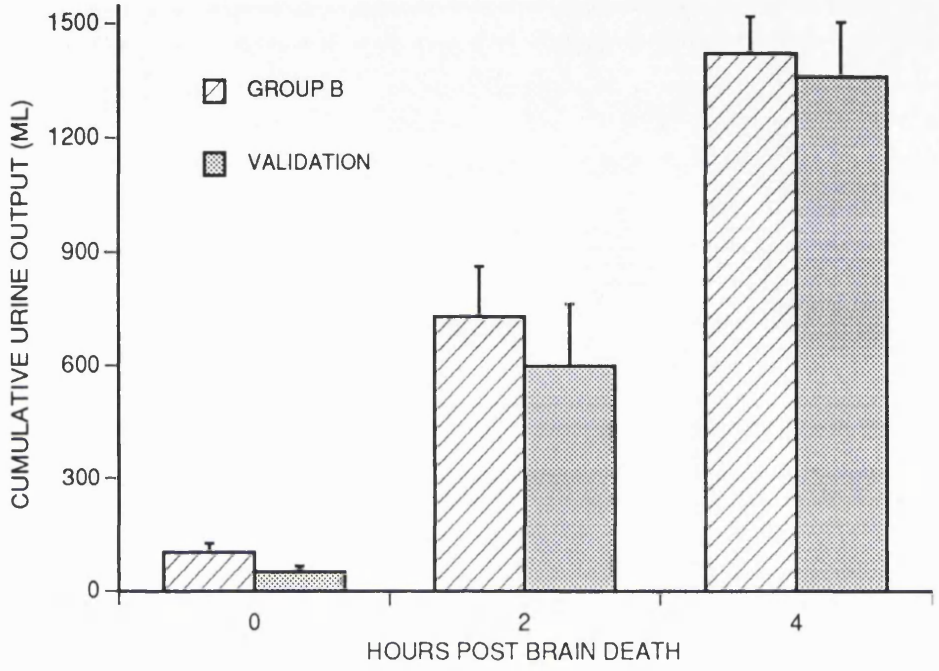
Figure 50. (Bottom)

Comparison of validation group vs group B: haematocrit post brain death *±1sem.* (n=8 group B, n=10 validation group).

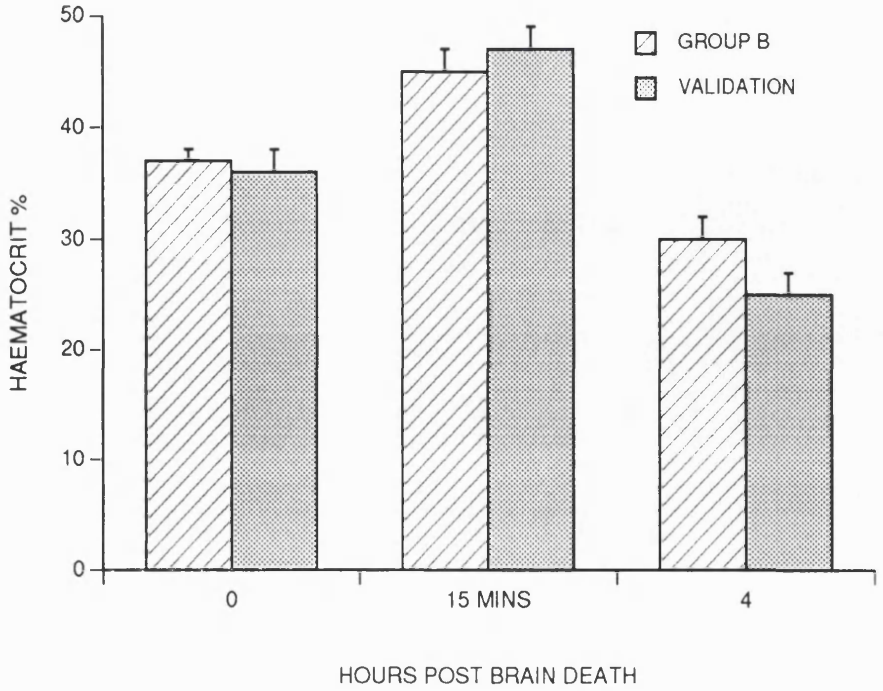
Haematocrit shows a significant rise in both groups 15 minutes post brain death. This has dropped at 4 hours to below baseline levels, although to a lesser extent in group B.

COMPARISON OF VALIDATION GROUP VS GROUP B

CUMULATIVE URINE OUTPUT POST BRAIN DEATH



HAEMATOCRIT POST BRAIN DEATH



The input resistance to the pulmonary circulation stayed constant at 611 dyne.sec.cm^{-5} (±45) and 619 dyne.sec.cm^{-5} (±39). The persistent low left atrial pressure maintained the pulmonary vascular resistance at 369 dyne.sec.cm^{-5} (±59) and 391 dyne.sec.cm^{-5} (±50) whereas in the validation group the pulmonary vascular resistance decreased. The characteristic impedance, an indicator of resistance in the proximal pulmonary vasculature, mimics the validation group in reducing post brain death from 175 dyne.sec.cm^{-5} (±19) to 141 dyne.sec.cm^{-5} (±11).

Post Brain Death; Urine output and Haematocrit

The animals in group B all became polyuric and the cumulative urine output (1421 mls (±97) at four hours) matches the output from the validation group (figure 49). This is diabetes insipidus, in the absence of vasopressin, and occurred at the same time. The mean total of intravenous fluid replacement was 3357 mls (±380) at four hours, 1.5 litres less than the validation group at 5000 mls (±955). This decrease in intravenous fluid requirements is possibly the result of less ventricular injury at brain death, and hence less need for increased ventricular filling pressures. Moreover the haemodilution is therefore lessened and haematocrit remained at 30% (±2) in group B compared to 25% (±2) in group A (table 36). The acute rise in haematocrit post brain death occurred to the same degree as the validation group, and is depicted in figure 50.

Post Transplant

The total ischaemic time for group B was 84 minutes (±4) with the duration of cardiopulmonary bypass being 83 min (±4) compared to group A at 86 min (±5) and 82 min (±7) respectively. Cardiopulmonary bypass was discontinued in all eight animals with the hearts able to fully support the recipient circulation. They all resumed sinus rhythm and there was no need for inotropes, isoprenaline nor low dose dopamine. Data collection was satisfactory, following which the experiments were terminated between 1.5 and 2 hours from weaning off circulatory bypass.

Table 37.

Group B vs group A pre and post transplant for haemodynamic parameters and pre load independent recruitable stroke work (PRSW)

±1sem. (n=10 group A, n=8 group B).

Ischaemic time and cardiopulmonary bypass time were near identical between the two groups. The complete method of implantation conserved sinus rhythm in all hearts.

There was no significant differences between groups for the measured pressures, but there was a significant reduction in systemic pressure post transplant.

Cardiac output and pre load independent recruitable stroke work are depicted in figures 52, 53 and 54.

Systemic vascular resistance in both groups increases by similar proportions post transplant; there is no difference between groups.

GROUP A VS GROUP B PRE AND POST TRANSPLANT FOR HAEMODYNAMIC PARAMETERS AND PRSW

		BASELINE				4 HOURS		POST TRANSPLANT			
		GROUPB		GROUP A		GROUP B		GROUP B		GROUP A	
Ischaemic time	*min*							84	*4*	86	*5*
Bypass time	*min*							83	*4*	82	*7*
Sinus rhythm		8		10		8		8		10	
Heart rate	*bpm*	109	*5*	109	*6*	121	*7*	138	*8*	132	*10*
Systolic pr.	*mmHg*	109	*5*	126	*4*	96	*5*	94	*2*	102	*3*
Diastolic pr.	*mmHg*	71	*5*	83	*6*	58	*3*	54	*2*	60	*2*
Mean arterial pr.	*mmHg*	84	*4*	97	*5*	71	*4*	67	*2*	74	*3*
Left atrial pr.	*mmHg*	5.8	*0.5*	7.1	*0.8*	4.8	*0.9*	4.8	*0.4*	4.8	*0.5*
Mean PA pr.	*mmHg*	12.0	*0.2*	15.1	*0.6*	12.7	*0.7*	12.6	*1.1*	12.0	*0.7*
Cardiac output	*ml/min*	1692	*133*	1550	*94*	1707	*184*	1100	*148*	917	*98*
Sys. Vasc. Resist.	*dn.s.cm5*	4068	*325*	5258	*472*	3497	*323*	5511	*819*	7340	*847*
LV PRSW	*erg.103*	74.2	*2.4*	73.2	*2.8*	57.4	*4.0*	66.2	*2.5*	70.1	*2.9*
RV PRSW	*erg.103*	21.3	*1.5*	21.0	*2.1*	16.2	*1.5*	15.3	*1.9*	21.3	*1.9*
%LV PRSW		100.0%	*0.0%*	100.0%	*0.0%*	77.2%	*4.4%*	89.4%	*2.7%*	95.2%	*3.4%*
%RV PRSW		100.0%	*0.0%*	100.0%	*0.0%*	77.1%	*5.6%*	73.1%	*8.2%*	102.9%	*4.4%*
Left X-intercept	*ml*	0.0	*0.0*	0.0	*0.0*	9.9	*2.7*	6.6	*3.1*	1.7	*4.2*
Right X-intercept	*ml*	0.0	*0.0*	0.0	*0.0*	5.9	*1.0*	4.5	*2.2*	0.9	*2.4*

Figure 51. (Above)

Group B vs group A: mean arterial pressure pre and post transplant *±1sem.* (n=10 group A, n=8 group B).

Although group B hearts have suffered four hours of brain death the values for mean arterial pressure post transplant are similar to group A hearts, explanted from anaesthetised animals.

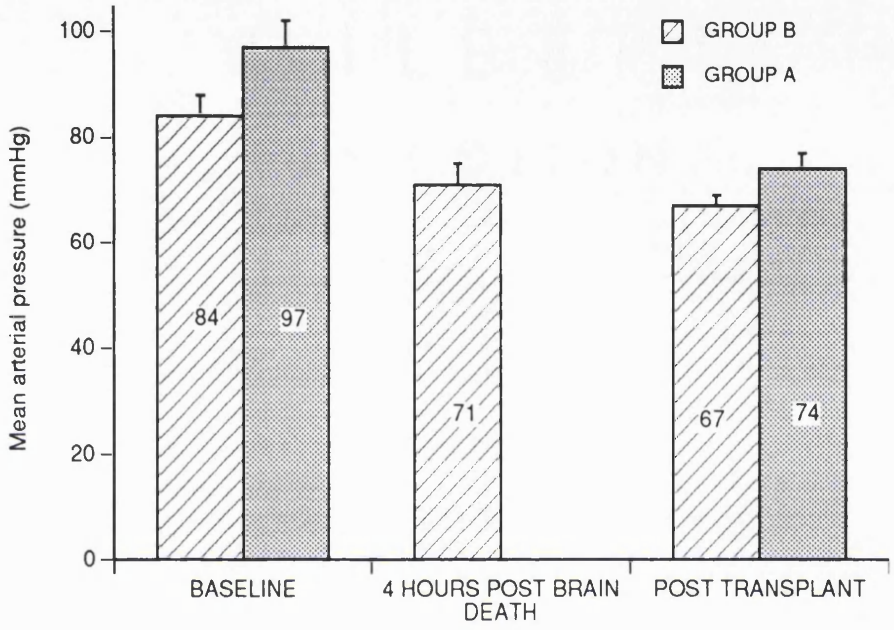
Figure 52. (Bottom)

Group B vs group A: cardiac output, pre and post transplant *±1sem.* (n=10 group A, n=8 group B).

Post transplant group B hearts produce similar cardiac outputs to group A.
The proportional change to baseline is the same.

GROUP B VS GROUP A

MEAN ARTERIAL PRESSURE



CARDIAC OUTPUT

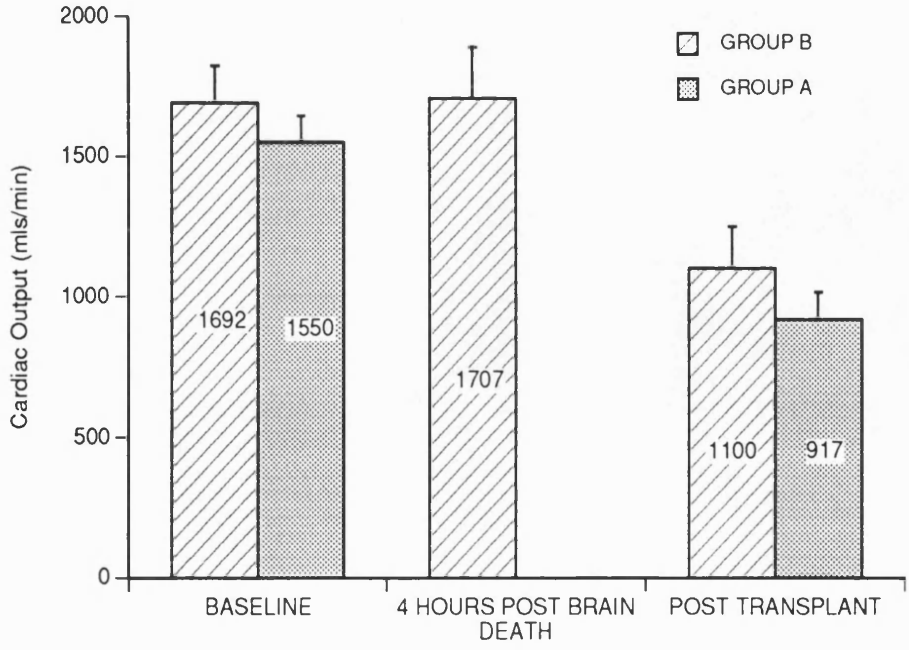


Figure 53. (Above)

Group B vs group A: percentage change of left ventricular pre load Independent recruitable stroke work, pre and post transplant *±1sem.* (n=10 group A, n=8 group B).

Despite a decrease of left ventricular PRSW after brain death to 77.2% of baseline, the hearts in group B recover to 89.4% after transplantation. This recovery is non significant statistically.

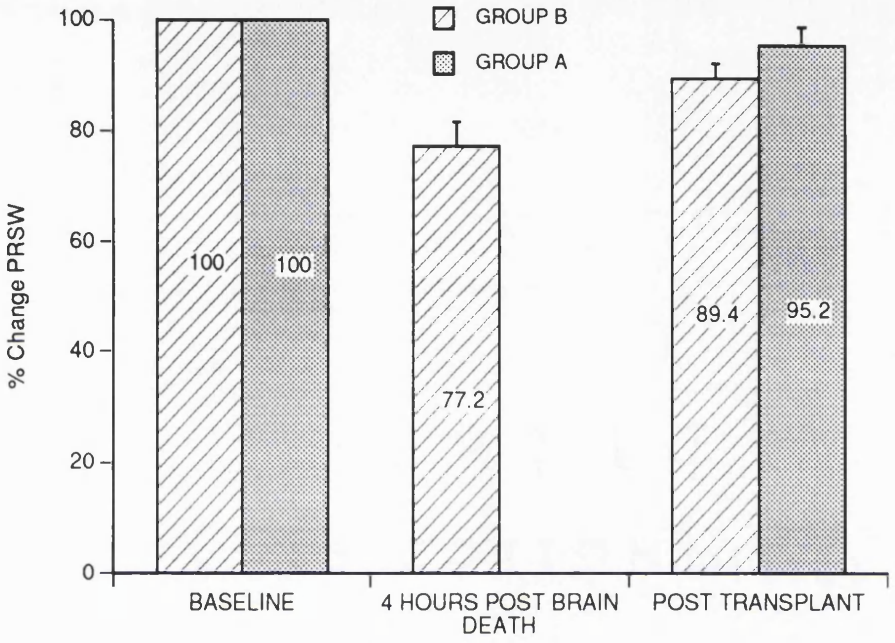
Figure 54. (Bottom)

Group B vs group A: percentage change of right ventricular pre load Independent recruitable stroke work, pre and post transplant *±1sem.* (n=10 group A, n=8 group B).

In group B there is no further decrease in right ventricular PRSW following transplantation. As with group A the PRSW is conserved.

GROUP B VS GROUP A

% CHANGE OF LEFT VENTRICULAR PRSW



% CHANGE OF RIGHT VENTRICULAR PRSW

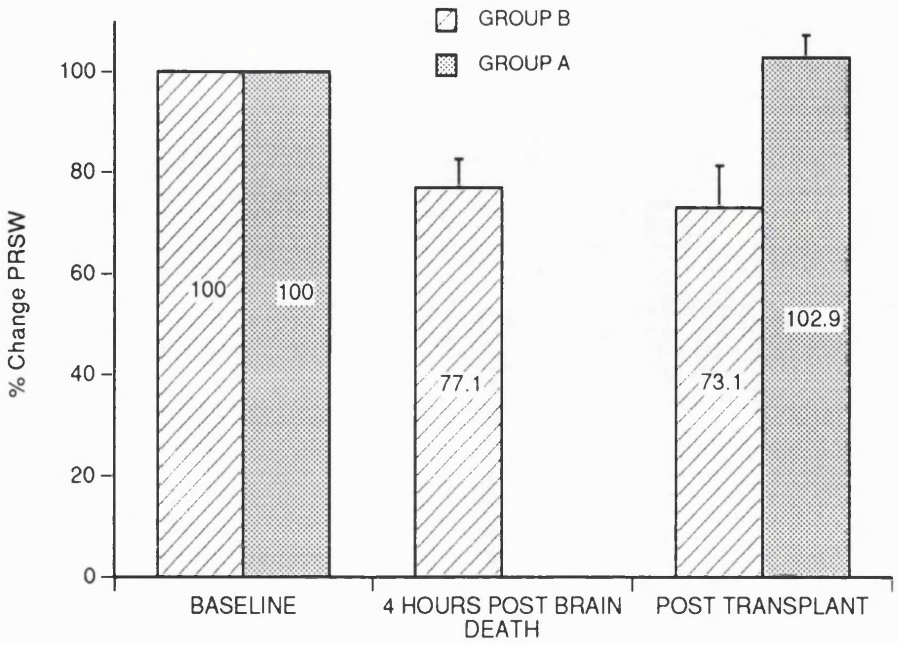


Table 38.

Group A vs group B pre and post transplant for right ventricular hydraulic power analysis and pulmonary impedance profile *±1sem.* (n=10 group B, n=8 group B)

Post transplant the right ventricles are presented with similar impedance profiles in group A and group B, which are significantly raised compared to baseline levels.

The values of total hydraulic power from the right ventricle are similar between groups post transplant, and the proportions of oscillatory and mean power are likewise similar.

The ratio of flow produced to power dissipated remains constant to cause no change to transpulmonary efficiency.

PVR; pulmonary vascular resistance **TP;** Trans pulmonary.

GROUP A VS GROUP B PRE AND POST TRANSPLANT FOR RV HYDRAULIC POWER ANALYSIS AND PULMONARY IMPEDANCE PROFILE

		BASELINE				4 HOURS		POST TRANSPLANT			
		GROUPB		GROUP A		GROUP B		GROUP B		GROUP A	
Total power	*mW*	69.1	*5.4*	71.8	*4.0*	64.4	*9.4*	46.0	*7.3*	37.5	*4.3*
Mean power	*mW*	45.5	*4.3*	51.2	*2.7*	49.6	*7.5*	30.9	*4.6*	24.4	*3.0*
% Mean power		65.7	*2.1*	71.5	*1.6*	76.7	*2.1*	67.9	*2.1*	64.4	*3.5*
Oscillatory power	*mW*	23.6	*1.7*	20.6	*1.8*	14.8	*2.3*	15.0	*3.0*	13.1	*2.1*
% Oscill. power		34	*2*	29	*2*	23	*2*	32	*2*	36	*4*
Input Resist.	*dyn.s.cm5*	585	*32*	840	*81*	619	*39*	1052	*186*	1223	*155*
PVR	*dyn.s.cm5*	307	*35*	436	*73*	391	*50*	631	*111*	719	*89*
Characteristic Imp.	*dyn.s.cm5*	175	*19*	153	*14*	141	*11*	190	*14*	208	*29*
TP efficiency	*J/L*	2.45	*0.07*	2.82	*0.12*	2.21	*0.10*	2.49	*0.22*	2.54	*0.18*

The hearts had an intrinsic sinus rhythm of 138 bpm (±8), producing a mean arterial pressure of 67 mmHg (±2) (table 37). The cardiac output was 1100 ml/min (±148) against a systemic vascular resistance of 5511 dyne.sec.cm^{-5} (±819) (figures 51 and 52). These measurements show no significant difference from group A post transplant with both groups displaying a 35% increase in systemic vascular resistance post transplant. Left atrial and pulmonary artery pressures at 4.8 mmHg (±0.4) and 12.6 mmHg (±1.1) were unchanged from baseline and near identical to group A.

Following transplantation there was no further insult to the left and right ventricular pre load independent recruitable stroke work. The left ventricle actually improved to 89.4% (±2.7) of its baseline PRSW compared to 77.2% after four hours of brain death (figure 53). The right ventricle remained impaired with a PRSW of 73.1% (±8.2) post transplant versus 77.1% (±5.6) at four hours post brain death (figure 54). The change in X-intercepts remained positive post transplant with statistically unchanged values compared to four hours; +6.6ml (±3.1) and +4.5 ml (±2.2) for left and right ventricles respectively.

The total power was 46.0 mW (±7.3) from the hydraulic energy of the right ventricle. This was significantly less than the 64.4 mW (±9.4) four hours post brain death and an equal reduction to that in group A. The ratio of oscillatory power to mean power altered post transplant indicating the right ventricle to be propelling blood through the **recipient** pulmonary vasculature. Thus oscillatory power increases from 23.3% (±2.1) at four hours post brain death to 32.1% (±2.1), a value similar to group A which was 35.6% (±3.5). Mean power correspondingly decreases from 76.7% to 67.9%. Trans pulmonary efficiency for blood flow through the recipient lungs was similar to baseline values at 2.49 J/L (±0.22) and 2.54 J/L (±0.18) in groups B and A respectively (table 38).

The impedance profile of the pulmonary vasculature presented values in group B similar to group A. The input resistance was 1052 dyne.sec.cm^{-5} (±186), the pulmonary vascular resistance was 631 dyne.sec.cm^{-5} (±111) and the characteristic impedance 190 dyne.sec.cm^{-5} (±14). Therefore the right ventricles in group A and group B were confronted with similar resistances to flow, but the

relative pulmonary vasodilatation at baseline in group B resulted in proportionally larger increases in post transplant pulmonary impedance in this group (table 38).

DISCUSSION

The aim of these experiments was to establish the feasibility of using hearts from brain dead donors in the canine model of orthotopic transplantation. Shivalkar had used this model and required large doses of inotropes to wean the recipients from cardiopulmonary bypass. These hearts had been explanted approximately one hour after brain death, and preserved for four hours (Shivalkar et al, 1993). Following the validation study in chapter 5, the period of brain death chosen before cardiac explantation was four hours; by this time the hormonal levels that had been evaluated had reached a steady state and the haemodynamic status of the animal was stable. One hour post brain death the animal is still recovering from the hyperdynamic response with ensuing metabolic acidosis and relative hypoxia. In Shivalkar's study there was no option but to harvest the heart at this point as it was not possible to maintain the animals any longer, following 'rapid' inflation of the intracranial balloon. The rapidity of the balloon inflation in his study was slower than the rate employed in this thesis and yet the eight consecutive animals in group B survived four hours post brain death.

Therefore with this one canine study for comparison, it was gratifying to obtain reliable data from eight consecutive experiments and, furthermore, no inotropes were necessary to support the hearts post transplant.

The baseline data for group B was similar to group A, and also the validation group. The lower systemic pressures and resistance to flows for left and right ventricles did not achieve significance statistically. The mean values at baseline for group B inferred there was a degree of vasodilatation, and therefore interpretation of percentage changes to baseline, for systemic and pulmonary impedance, was done with caution. These differences in afterload caused no alteration in the baseline pre load independent recruitable stroke work for left and right ventricles. The linearity of the pressure volume relationship was maintained, reinforcing the independent nature of this

measure of ventricular contractility from pre load and after load.

The donor animals in group B confirmed the majority of observations made in the animals from the validation study. The Cushing response occurred in all animals with similar variation in its duration and severity. The observed rise in haematocrit 15 minutes after brain death was consistent, to a mean of 45%. In group B this value did not decrease to the level of the validation group in the ensuing four hours, probably due to the decreased intravenous fluid requirements of these animals. Despite this drop in intravenous infusion, the rate and volume of urine excreted from these animals remained equal to the validation group; each donor in group B developed polyuria, almost certainly attributable to diabetes insipidus.

Group B donors did differ from the validation group in the impairment of ventricular contractility as measured by pre-load independent recruitable stroke work (PRSW). In group B two hours post brain death, the PRSW was decreased by 25% for left and right ventricles and remained at this level four hours post brain death. The injury was equal for both ventricles, unlike the validation group where the right ventricle was more significantly impaired. Furthermore, the significantly lower left atrial pressure and decrease in intravenous fluid requirements to maintain similar pressure and flows, compared to the validation studies, inferred less ventricular injury.

Interpretation of the pulmonary impedance profile is complicated by two factors; the baseline vasodilatation and the persistent low left atrial pressure. The input resistance remains constant in both groups, whilst the differing left atrial pressures cause opposite trends in the pulmonary vascular resistance (PVR); in group B the PVR slightly rises and in the validation group it decreases. The characteristic impedance, measuring resistance to flow between 7 and 11 Hz, decreases in both groups reflecting vasodilatation post brain death in the proximal pulmonary vasculature.

The complete method of implantation conserved sinus rhythm post transplant as in group A. The consistency of baseline data, total ischaemic time and cardiopulmonary bypass time allowed valid comparisons between groups A and B post transplant. This was coupled with comparable impedance profiles in the recipients; the SVR, input resistance, PVR and characteristic impedance were alike for both groups, providing the hearts with a similar environment in which to function. Although the PRSW is load independent the similarity in impedance also permitted comparisons of load dependent parameters. Therefore it is remarkable that the group B hearts achieved similar cardiac outputs and pressures post transplant, compared to group A, having suffered injury at brain death, and that no inotropic support was required.

In contrast Wicomb showed in the swine heart that carotid ligation significantly impaired left ventricular pressure and cardiac output when the ischaemic time was minimal. These hearts had been maintained in the donor with dobutamine and intravenous fluids, or by intravenous fluid alone, and subsequently tested on a Langendorff apparatus (Wicomb et al, 1986b). The different species and the different method to try and induce brain death makes comparisons to this study difficult.

The success of group B, in providing reliable data, provided the basis to progress to groups C and D to investigate the main effect of prolonged four hour preservation, and the interaction between brain death and preservation.

CONCLUSIONS

1. The canine model of brain death used in this thesis provides consistent haemodynamic changes and effects on cardiac function. The hearts from these animals may be used in orthotopic transplantation to obtain reliable load independent analysis of pressure / volume relationships for left and right ventricles post transplant.

2. The hearts from this model of brain death can adequately support the recipient, without the aid of inotropes, when the total preservation time is less than 90 minutes.

3. When compared to hearts transplanted from an anaesthetised animal, the 'brain dead' hearts achieve similar values for cardiac output, systemic and pulmonary pressures post transplant.

4. The recipient's systemic and pulmonary vasculature consistently present the left and right ventricles with a significant increase in resistance to flow, compared to baseline values in the donor animal.

5. As described in chapter 5, brain death caused by inflation of an intracranial balloon impairs pre load independent recruitable stroke work for left and right ventricles. In this second group of animals there is no difference in the degree of impairment between left and right ventricles.

6. A relatively short myocardial preservation time of less than 90 minutes causes no superimposed decrease in left and right ventricular PRSW after transplantation.

Overall the studies in group B provided further evidence of a consistent injury to ventricular contractility post brain death. Despite this injury these hearts were able to maintain the recipient circulation at a similar level to the hearts from an anaesthetised donor. The aim of the next group of experiments was to evaluate the effect of prolonged preservation on myocardial function in hearts from a brain dead donor.

CHAPTER 8

MYOCARDIAL FUNCTION FOLLOWING TRANSPLANTATION USING A BRAIN DEAD DONOR AND PROLONGED TRANSPLANTATION

Introduction

The studies in group A (chapter 6) were control experiments of complete orthotopic transplantation, with no brain death in the donor nor prolonged preservation of the donor heart. Moreover they had shown complete implantation to be superior to the standard technique for the assessment of pressure volume relationships in the right ventricle. In group B (chapter 7) the model of brain death validated in chapter 5 was used as the donor animal for complete orthotopic cardiac transplantation. These donors further substantiated the deleterious effects of brain death on left and right ventricular function and in eight consecutive studies did not show any superimposed effects of an ischaemic time less than ninety minutes. In fact these 'brain dead' hearts achieved cardiac outputs, systemic and pulmonary pressures equal to the control hearts in group A.

Group C and group D are described in this chapter. Group C consists of eight orthotopic transplants using anaesthetised donors with subsequent preservation of the donor heart for four hours. In effect these experiments study the effect of preservation alone, whilst group D combines brain death in the donor with four hours preservation of the heart, also in eight transplants.

Therefore the sixteen transplants reported below will complete the experimental design protocol that will permit two way analysis of the results.

		BRAIN DEAD DONOR	
		NO	YES
FOUR HOURS OF MYOCARDIAL PRESERVATION	NO	GROUP A CHAPTER 6 *n=10*	GROUP B CHAPTER 7 *n=8*
	YES	GROUP C CHAPTER 8 *n=8*	GROUP D CHAPTER 8 *n=8*

MATERIALS AND METHODS

Preparation of the Donor

Group C consisted of 16 dogs, their weights ranging from 23.4 kg to 29.9 kg (mean 26.9 kg ±0.4), and group D also 16 dogs 23.4 kg to 29.6 kg (mean 26.8 kg ±0.4). All animals were anaesthetised, paralysed and ventilated in the manner previously described, and in each pair of animals the heavier dog was used as the recipient. Surgical preparation of the donor was identical to the previous studies. The only discrepancy between groups was group D animals had a transurethral catheter inserted whereas group C had no catheter.

Pre Transplant Data Acquisition

Having completed full instrumentation of the donor heart and calibration of all instruments, baseline data were collected with the animal in a steady state of anaesthesia. Six files of 200 Hz with caval occlusion, and six files of 500 Hz steady state were collected. In all data acquisition these recordings were done in pairs. Firstly a pair of 500 Hz data sets would be recorded sequentially (total 12 seconds with ventilator disconnected), followed by a pair of 200 Hz data sets (each with 16 seconds of ventilator disconnection). The interval between the occlusion files was two to three minutes, followed by a five to ten minute interval before the next 500Hz pair during which time the ten channels would be rechecked and pressure / volume loops studied. This timing and sequence of file acquisition was chosen to ensure the cardiovascular system to be steady during the 500Hz files with no proximity to a caval occlusion file, that temporarily upsets the equilibrium of haemodynamic stability. Moreover this protocol ensured a minimum of thirty minutes to complete data collection, and hence avoid misleading data taken during any transient changes to the haemodynamics.

Instigation of Brain Death Group D

When the satisfactory baseline data had been recorded brain death was instigated in the eight donors. The Cushing response reliably occurred within seconds of inflation of the intracranial balloon. The duration of this response was 4.5 to 11 minutes and the ensuing metabolic acidosis

and haemodynamic instability was promptly treated. The 'brain dead' donors each survived with stable haemodynamics for four hours, with data acquisition at 2 hours and 4 hours post brain death.

Donor Heart Preservation

The cardioplegia / preservation solution was formulated in Duke Medical Center pharmacy, based on an extracellular preparation with high osmolarity. The final concentrations are listed below;

Sodium chloride	138 mEq/L	8.064 g/L
Potassium chloride	25 mEq/L	1.864 g/L
Calcium chloride	0.7 mM/L	0.103 mg/L (dihydrate)
Magnesium chloride	7.56 mEq/L	
Glucose		15 g/L (13.6 anhydrous)
Mannitol		20 g/L
Hetastarch		6%
Tromethamine	to pH 7.4 to 7.5	
Effective osmolarity	420 mOsm	

The magnesium chloride solution assayed at 768 mg/ml of $MgCl_2$-$6H_20$,

has molecular weight of 203.3 therefore 1 mEq = 101.65 mg.

Hence concentration = $^{768.8}/_{101.65}$ = 7.56 mEq/L

The solution was made in 19 litre batches in the following manner. At room temperature potassium chloride, calcium chloride and magnesium chloride were added incrementally to one litre of 8.8% hetastarch solution placed in a 3.5 litre container on a magnetic mixer. The dextrose and mannitol was added until the sugars no longer dissolved at which point the solute was further diluted to 2.5 litres with more hetastarch. The pH was adjusted with tromethamine to a pH of 7.4 to 7.5. The solution is then poured into a 20 litre container and the procedure repeated until 19 litres is prepared. Filtration is performed through a 0.22 micron filter and packaged by weight into 1 litre

viaflex bags, to be stored at 4°C.

The resulting preservation solution was hyperosmolar in an attempt to prevent cellular oedema during storage. For this purpose mannitol and hetastarch (used in University of Wisconsin solution) were employed including a glucose concentration approaching the level used by Watson (Watson et al, 1979).

The potassium concentration was to ensure satisfactory cardioplegic arrest; this was tested on the swine model in three animals already used for teaching purposes, prior to their euthanasia. The solution caused immediate arrest of the heart, with resumption of coarse ventricular fibrillation following removal of the aortic cross clamp.

The surgical preparation and technique for explanting the heart was the same for both groups. To arrest the heart, 500 ml of preservation solution at 4°C was infused to the aortic root at a pressure of 80 mmHg, and topical 4°C normal saline poured over the heart. After explantation the heart was placed in a zip lock polythene bag and the remaining 500 ml of preservation solution infused to the bag around the heart. The bag was then placed in a bowl of normal saline that was kept refrigerated at 4°C.

Preparation of the Recipient

Immunosuppression was administered to the recipient dog using the same doses as the previous transplants. General anaesthesia was induced 2.5 hours after the arrest of the donor heart. The four hour preservation period allowed the recipient to be prepared in the main laboratory without use of the adjacent theatre; in group D a 16 French gauge intravenous catheter was inserted to the left femoral vein in anticipation of the need for dopamine infusion post transplant. There were no other differences in preparation between groups.

Cardiopulmonary Bypass

No alterations were required to the running of complete circulatory support in these sixteen transplants. Maintenance of arterial blood gases and acid base balance utilised similar interventions, based on serial arterial blood gas analysis as described in chapter 6.

The protocol to wean the recipient from cardiopulmonary bypass was retained in group C; these hearts had sufficient function to resume support of the circulation. Group D hearts were more varied; if left atrial pressure was greater than 10 mmHg and mean systemic pressure less than 50 mmHg on cessation of cardiopulmonary bypass, dopamine was infused at a renal dose of 5 mcg/min/kg.

Explantation and Transplantation

The technique for complete atrioventricular transplantation, described in chapter 6, was used in all studies. No technical problems were encountered and no modifications to the method were necessary.

Data Acquisition Post Transplant

Three hearts in group D were weaned from bypass with an infusion of dopamine. Data were recorded at 15 minutes, 45 minutes and 70 minutes following discontinuation of bypass. After each data set the dose of dopamine was reduced, to 2.5 mcg/kg/min at 15 minutes and stopped at 30 minutes. Six steady state 500Hz and six 200Hz caval occlusion files were accumulated at each data set; in group C, and group D hearts not on dopamine support, data acquisition was identical to the previous transplants.

Statistical Analysis

Two-way multivariate analysis was implemented to assess the **main effects** of the factors brain death and four hour preservation, and also the **interaction** between them. In studies based on observational data, multifactorial analysis permits a ready evaluation of interaction effects and

economizes on the number of cases required for analysis (Neter et al, 1985). Due to three groups having zero requirements for inotropes, two way Anova analysis was not satisfactory, and the Mantel-Haenszel test used. In essence this test introduces inotrope as a third factor and performs two way analysis on eight cells rather than four. In the text results are expressed as the mean and one standard error of the mean. A difference was considered statistically significant when $p \leq 0.05$.

RESULTS

Baseline Controls

The baseline data for groups C and D were comparable to each other and also comparable to groups A and B. With regard to systemic and pulmonary resistance the values for C and D were midway between A and B, with no persistence of mild vasodilatation at baseline that was encountered in group B (tables 39 and 40).

The following values are presented with group C listed first. Mean arterial pressure was 100 mmHg (±2) and 98 mmHg (±2) with a left atrial pressure of 6.4 mmHg (±0.4) and 6.6 mmHg (±0.2). Cardiac outputs were similar at 1726 ml/min (±91)and 1731 ml/min (±132) making the calculation of systemic vascular resistance alike at 4744 dyne.sec.cm^{-5} (±287) and 4768 dyne.sec.cm^{-5} (±513). Total input resistance to the pulmonary circulation was 683 dyne.sec.cm^{-5} (±45) and 597 dyne.sec.cm^{-5} (±30) whilst group C had a higher pulmonary vascular resistance at 383 dyne.sec.cm^{-5} (±41) versus 275 dyne.sec.cm^{-5} (±17). However characteristic impedance was unaffected at 153 dyne.sec.cm^{-5} (±15) and 155 dyne.sec.cm^{-5} (±16).

With the cardiac output and pulmonary impedance profile similar in these two groups the hydraulic power analysis of the right ventricle was insignificantly different; total power was 85.5 mW (±6.8) and 74.5 mW (±8.6), broken down to its components of 65.7% and 66.4% mean power, and 34.3% and 33.6% oscillatory power. The slightly higher power to flow ratio in group C results in less transpulmonary efficiency of 2.96 J/L (±0.16) versus 2.53 J/L (±0.13).

Table 39.

Pre and post transplant data for group C *±1sem.* (n=8).

Each heart was weaned from cardiopulmonary bypass without inotropic support. After four hours preservation the haemodynamic parameters post transplant are similar to groups A and B.

Pre load independent recruitable stoke work was conserved in the left and right ventricles.

* denotes $p<0.05$.

PRE AND POST TRANSPLANT DATA FOR GROUP C

Weight	*kg*	26.9	*0.4*		
Ischaemic time	*min*	236	*4*		
Cardiopulmonary bypass	*min*	72	*3*		
		PRE TRANSPLANT		**POST TRANSPLANT**	
Heart rate	*bpm*	112	*5*	132	*5*
Systolic pressure	*mmHg*	128	*3*	*98	*3*
Diastolic pressure	*mmHg*	86	*2*	*58	*3*
Mean arterial pressure	*mmHg*	100	*2*	*71	*3*
Left atrial pressure	*mmHg*	6.4	*0.4*	*3.4	*0.7*
Pulmonary artery pressure	*mmHg*	14.5	*0.9*	13.6	*1.6*
Cardiac output	*ml/min*	1726	*91*	*1225	*157*
Systemic vascular resistance	*dyn.s.cm-5*	4744	*287*	5034	*506*
Total power	*mW*	85.5	*6.8*	*54.2	*7.8*
Meanpower	*mW*	56.3	*5.1*	37.6	*6.3*
% mean power		65.7	*2.7*	67.6	*3.5*
Oscillatory power	*mW*	29.2	*3.0*	16.6	*2.4*
% oscillatory power		34.3	*2.7*	32.4	*3.5*
Input resistance	*dyn.s.cm-5*	683	*45*	*963	*150*
Pulmonary vascular resistance	*dyn.s.cm-5*	383	*41*	*756	*161*
Characteristic Impedance	*dyn.s.cm-5*	153	*15*	191	*20*
Transpulmonary efficiency		2.96	*0.16*	2.64	*0.25*
Left ventricular PRSW	*erg.103*	66.1	*5.4*	70.9	*7.4*
Right ventricular PRSW	*erg.103*	18.6	*1.4*	20.5	*3.1*
% change of LV PRSW		100.0%	*0.0%*	108.8%	*9.9%*
% change of RV PRSW		100.0%	*0.0%*	107.3%	*11.1%*

Left ventricular pre load independent recruitable stroke work (PRSW) was 66.1 erg.10^3 (±5.4) and 76.7 erg.10^3 (±5.9), and for the right ventricle 18.6 erg.10^3 (±1.4)and 22.7 erg.10^3 (±1.2).

Groups A to D, and also the validation group were tested for normality prior to further assessment. All parameters were satisfactory to permit comparisons between groups following interventions.

Post Brain Death; Group D

Cushing Response

From the previous experience of group B and the validation group the Cushing response was predictable in its onset, and range of severity and duration. It occurred immediately following inflation of the intracranial balloon with a rise in systemic blood pressure to over 350 mmHg systolic and 200 mmHg diastolic. Likewise cardiac output increased to 4000 ml/min and systemic vascular resistance increased to 8000 dyne.sec.cm^{-5}. The relative hypoxia and metabolic acidosis, that had followed the Cushing response in the previous groups, occurred in all animals and responded to the therapeutic interventions described in chapter 5. The hyperdynamic response was timed as the interval where systemic pressure was higher than baseline values, and its duration was 4.5 to 11 minutes in this group.

Haemodynamic Measurements; Post Brain Death

A detailed analysis of changes in haemodynamic parameters following brain death is addressed in chapter 10, with the summation of results from thirty two experiments. Therefore this section is a summary of changes in the animals of group D alone (table 40).

Table 40.

Data for baseline, post brain death and post transplant in group D.

±1sem. (n=8).

Post transplant 4 of the 8 hearts required dopamine to be weaned from cardiopulmonary bypass. One of these hearts still failed to support the recipient circulation before data collection; therefore the post transplant data are from seven studies.

Haemodynamic parameters are similar post transplant to groups A, B and C.

The significant difference is the further decrease in right ventricular PRSW post transplant following four hours preservation.

* denotes $p<0.05$.

PRE; pre brain death, **2 hrs**; two hours post brain death,

4 hrs; four hours post brain death, and **POST**; post transplant.

LA press. left atrial pressure

Pulm. artery press. mean pulmonary artery pressure

SVR systemic vascular resistance **PVR** pulmonary vascular resistance

Characteristic Imp. characteristic impedance

Transpulm. efficiency transpulmonary efficiency

PRSW pre load independent recruitable stroke work **IV** intravenous

DATA FOR BASELINE, POST BRAIN DEATH AND POST TRANSPLANT IN GROUP D

Weight	*kg*	26.8	*0.4*				
Ischaemic time	*min*	232	*1*				
Bypass time	*min*	68	*3*				
		PRE		**2 hrs**		**4 hrs**	**POST**
Heart rate	*bpm*	120	*6*	121	*4*	113 *5*	142 *8*
Systolic pressure	*mmHg*	123	*3*	98	*6*	103 *6*	*97 *3*
Diastolic press.	*mmHg*	85	*3*	67	*5*	67 *4*	*53 *2*
Mean arterial press.	*mmHg*	98	*2*	77	*5*	79 *4*	*68 *2*
LA press.	*mmHg*	6.6	*0.2*	7.1	*0.8*	7.2 *1.1*	6.0 *1.0*
Pulm. artery press.	*mmHg*	12.6	*0.6*	12.1	*0.9*	13.1 *0.5*	13.6 *1.1*
Cardiac output	*ml/min*	1731	*132*	1678	*140*	1549 *107*	*942 *158*
SVR	*dyn.s.cm-5*	4768	*513*	3927	*505*	4183 *306*	6910 *1204*
Total power	*mW*	74.5	*8.6*	65.2	*8.0*	65.8 *7.8*	*45.2 *8.8*
Meanpower	*mW*	49.5	*5.6*	46.2	*6.1*	45.5 *4.0*	29.8 *5.9*
% mean pwr.		66.4	*1.9*	70.3	*2.5*	71.3 *3.4*	65.4 *3.6*
Oscillatory pwr.	*mW*	25.0	*3.4*	19.0	*2.6*	20.2 *4.6*	15.4 *3.8*
% oscillatory pwr.		33.6	*1.9*	29.7	*2.5*	28.7 *3.4*	34.6 *3.6*
Input Resist.	*dyn.s.cm-5*	597	*30*	605	*60*	700 *53*	*1377 *269*
PVR	*dyn.s.cm-5*	275	*17*	263	*52*	333 *67*	*773 *131*
Characteristic Imp.	*dyn.s.cm-5*	155	*16*	145	*19*	148 *19*	187 *22*
Transpulm. efficiency		2.53	*0.13*	2.30	*0.14*	2.49 *0.14*	2.82 *0.28*
LV PRSW	*erg.103*	76.7	*5.9*	57.6	*4.3*	55.8 *3.4*	58.7 *5.6*
RV PRSW	*erg.103*	22.7	*1.2*	14.6	*1.7*	15.6 *1.2*	11.3 *0.9*
% LV PRSW		100.0%	*0.0%*	*75.8%	*4.4%*	*76.3% *7.7%*	81.4% *9.7%*
% RV PRSW		100.0%	*0.0%*	*67.4%	*10.3%*	*71.8% *8.9%*	*51.5% *3.9%*
Cumulative IV fluid	*ml*	963	*135*	2431	*175*	3713 *378*	
Total urine	*ml*	206	*26*	619	*75*	1600 *131*	
Haematocrit	*%*	34.6	*1.2*	*43.5	*2.1*	*27.8 *1.6*	
				15 MINS			

Heart rate was maintained at 113 bpm (±5) while mean arterial pressure dropped to 79 mmHg (±4) at four hours, unchanged from two hours at 77 mmHg (±5). Left atrial pressure rose insignificantly to 7.2 mmHg (±1.1) four hours post brain death, an increase also reflected in the mean pulmonary artery pressure (13.5mmHg (±0.5)) (figure 55). Cardiac output decreased slightly to 1549 ml/min (±107) despite that the systemic vascular resistance reduced to 4183 dyne.sec.cm^{-5} (±306)(figures 56 and 57). The impedance profile for the pulmonary vasculature remained essentially unaltered; input resistance 700 dyne.sec.cm^{-5} (±53), pulmonary vascular resistance 333 dyne.sec.cm^{-5} (±67) and characteristic impedance 148 dyne.sec.cm^{-5} (±19) (figure 58).

Total hydraulic power decreased, proportional to the cardiac output, to 65.8 mW (±7.8) with less power wasted in pulsatile flow (oscillatory power 28.7% (±3.4)) and a corresponding increase of power conserved in blood flow (mean power 71.3% (±3.4)). These changes were insignificant (figure 60).

The impairment of pre load independent recruitable stoke work for left and right ventricles was significant. Furthermore the degree of injury mirrored the observations in group B. At two hours post brain death the PRSW for the left ventricle was 75.8% (±4.4) of baseline and at four hours remained static at 76.3% (±7.7) (figure 59). The right ventricular PRSW was 67.4% (±10.3) and 71.8% (±8.9) at two and four hours respectively (figure 61). These values were all statistically significantly different from baseline.

Post Brain Death; Urine output and Haematocrit

The volume of intravenous fluid infused was consistent with group B, with a total of 3.7 litres (±0.4) infused, 2.8 litres administered during the period of brain death. The urine output was 1600 ml (±131) similar in rate and volume to group B (1420 ml (±97)). All the animals became polyuric, assumed to be due to diabetes insipidus due to vasopressin decrease post brain death. The close correlation in intravenous fluid requirements to group B corresponds to similar impairments to ventricular performance, resulting in lower ventricular filling pressures. The haematocrit displayed

the characteristic peak at fifteen minutes post brain death of 43.5% (±2.1) dropping to 27.8% (±1.6) at four hours post brain death.

Post Transplant

In group C the mean ischaemic time was 236 minutes (±4) and the mean duration of cardiopulmonary bypass 72 minutes (±3). Four hearts required right ventricular pacing to be weaned from bypass, but within fifteen minutes sinus rhythm had resumed in each of these four studies. No inotropes were required in this group. One study was unsuccessful due to persistent tachycardia greater than 190 bpm preventing satisfactory data collection; this study is not included in the results leaving eight completed studies.

Group D hearts underwent an average ischaemic time of 232 minutes (±1) and the recipients were on complete circulatory support for 68 minutes (±3). Three hearts required right ventricular epicardial pacing, at 120 bpm, which behaved like hearts in group C in that they resumed sinus rhythm within 15 minutes. The first four studies did not need inotropic intervention to support cardiac function. However the latter four did not achieve adequate mean arterial pressure (>50mmHg) and renal dose dopamine was commenced. This is statistically significant compared to groups A, B and D using the Mantel-Haenszel test ($p<0.025$). The hearts responded dramatically with an obvious inotropic and chronotropic response both to direct observation and measured parameters of cardiac function. This response prompted a thorough review of the concentration and dosage of the dopamine infusion, which was found to be accurate at 5 mcg/kg/min. The dose response to this low dose of dopamine is discussed below and tabulated in table 41.

In the final study of group D the heart temporarily responded vigorously to the dopamine but rapidly failed to support the recipient. This study was terminated with no post transplant data collected; it is not included in the statistical analysis even though it was a clear demonstration of the detrimental effects of brain death combined with four hour preservation.

Figure 55. (Above)

Cardiac output in groups A, B, C and D at baseline, post brain death and post transplant. *±1sem.*

(group A *n*=10, group B *n*=8, group c *n*=8 and group D *n*=8).

Cardiac output is decreased in all groups post transplant. There is no significant difference between groups.

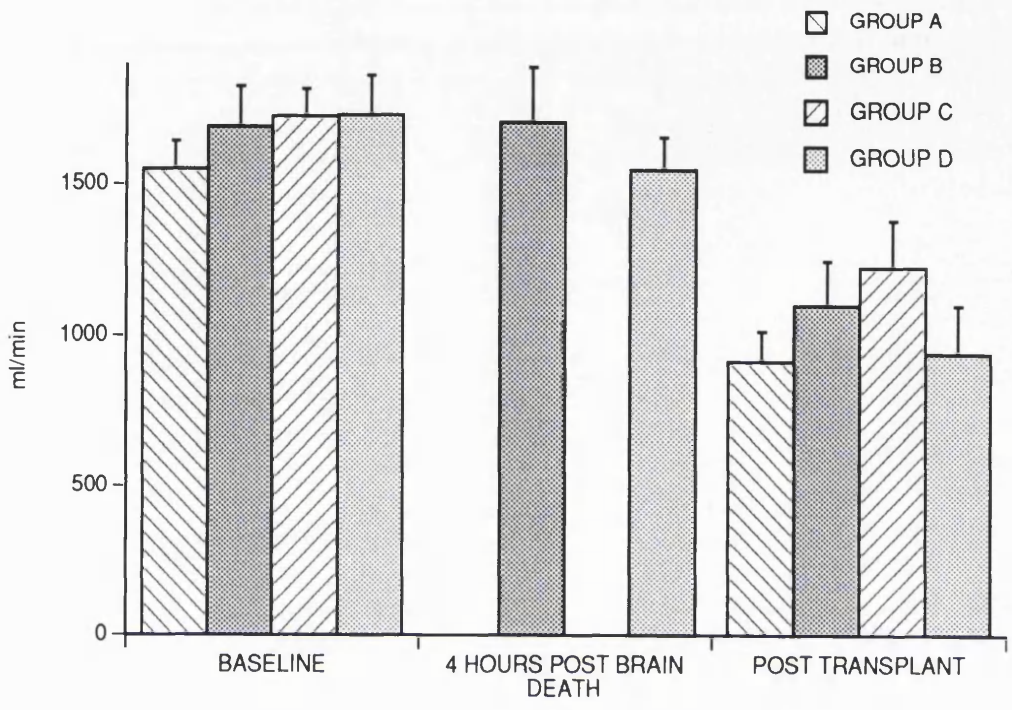
Figure 56. (Bottom)

Mean arterial pressure for groups A, B, C and D at baseline, post brain death and post transplant. *±1sem.*

(group A *n*=10, group B *n*=8, group c *n*=8 and group D *n*=8).

The mean arterial pressure decreases post brain death and remains at a similar level post transplant. There is no significant difference between groups.

CARDIAC OUTPUT



MEAN ARTERIAL PRESSURE

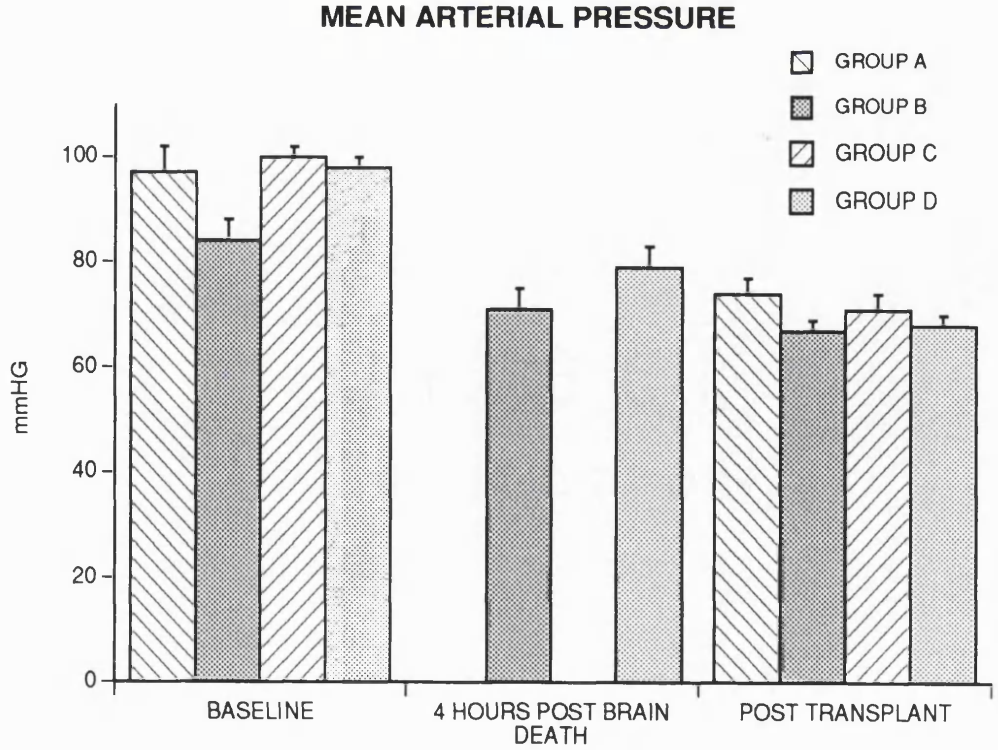


Figure 57. (Above)

Systemic vascular resistance in groups A, B, C and D at baseline, post brain death and post transplant. *±1sem.*

(group A *n*=10, group B *n*=8, group c *n*=8 and group D *n*=8).

There is a slight decrease in SVR post brain death; this is replaced post transplant by a raised SVR in the recipient systemic vasculature. There is no difference between groups.

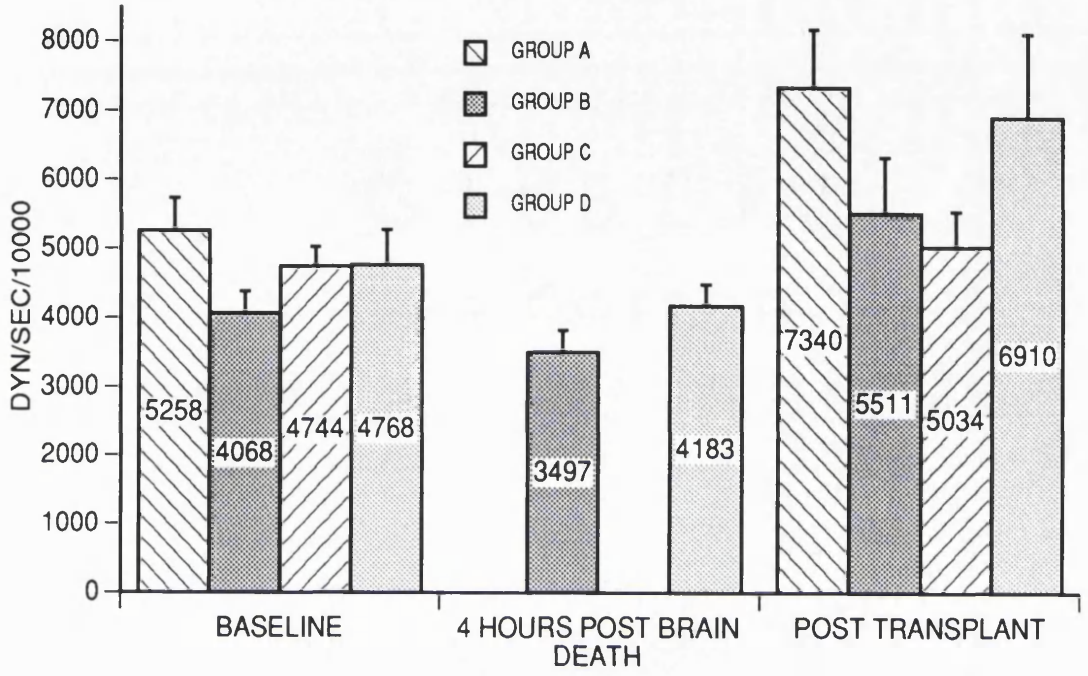
Figure 58. (Bottom)

Pulmonary input resistance (Rin) for groups A, B, C and D at baseline, post brain death and post transplant. *±1sem.*

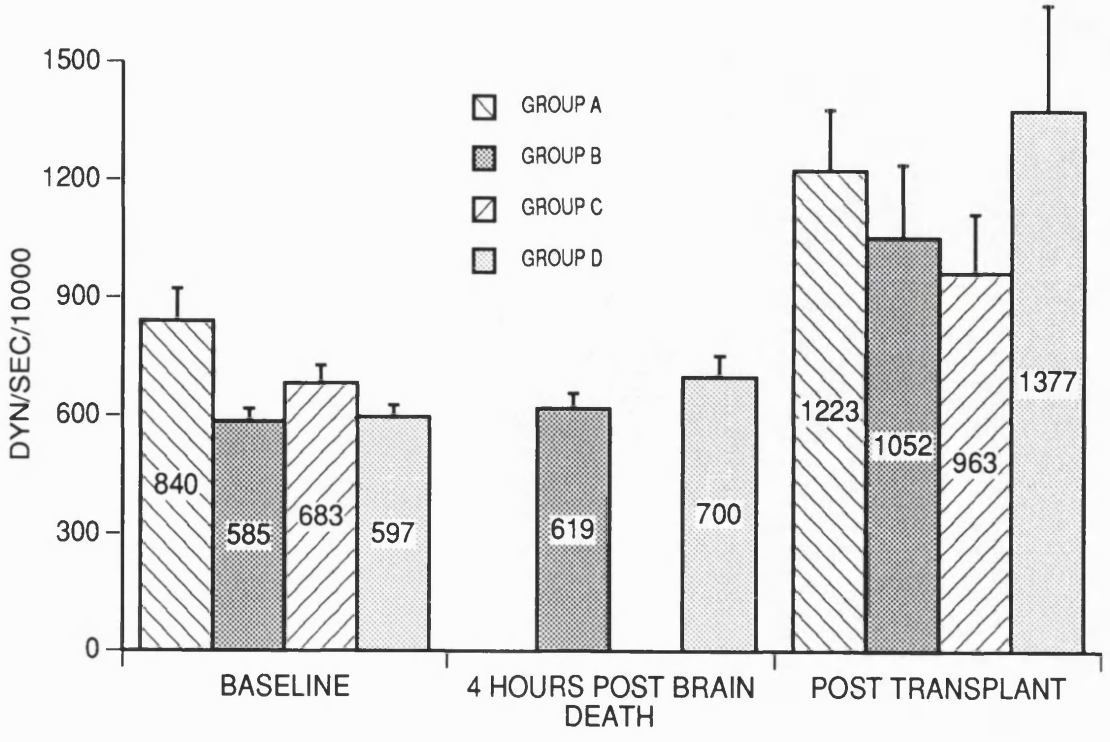
(group A *n*=10, group B *n*=8, group c *n*=8 and group D *n*=8).

Pulmonary input resistance does not change post brain death. In all groups the resistance to flow from the right ventricle is increased post transplant.

SYSTEMIC VASCULAR RESISTANCE



PULMONARY INPUT RESISTANCE



The data displayed in table 39 post transplant for group C underlines that the preservation solution formulated from the pharmacy had effectively conserved cardiac function satisfactorily. Moreover the function was conserved to the same degree as in the group A hearts that were preserved for less than 90 minutes. Post transplant group C hearts had a mean rate of 132 bpm (±5), a mean arterial pressure of 71 mmHg (±3) and a left atrial pressure of 3.4 mmHg (±0.7) (figure 56). The cardiac output was 1225 ml/min (±157) and the systemic vascular resistance 5034 dyne.sec.cm^{-5} (±506) (figure 55 and 57). The pulmonary impedance had increased to an input resistance of 963 dyne.sec.cm^{-5} (±150), pulmonary vascular resistance 756 dyne.sec.cm^{-5} (±161) and characteristic impedance 191 dyne.sec.cm^{-5} (±20) (table 58). Hydraulic power was greater than in the other groups post transplant at 54.2 mW (±7.8) due to the higher cardiac output, but consisted of similar proportions of oscillatory power (32.4% (±3.5)) and mean power (67.6% (±3.5)) (figure 60).

The prolongation of preservation time did not effect the pressure volume relationship in either ventricle. The left ventricle had a PRSW of 108.8% (±9.9) and the right ventricle 107.3% (±11.1) (figure 59 and 61).

Overall the group C hearts performed equal to the group A hearts in terms of rate, pressure and flow generated. The recipient systemic and pulmonary vasculature provided comparable environments, that had been subjected to equal lengths of artificial circulatory support.

The post transplant data in group D applied to the seven studies where data were successfully recorded. In the animals that required dopamine the analysed data were those which were recorded when dopamine had been discontinued. It is interesting that four hearts in this group could not support the circulation unaided, and that one of these was still unable to manage even with a dopamine infusion.

Figure 59. (Above)

Percentage change of left ventricular pre load independent recruitable stroke work in groups A, B, C and D at baseline, post brain death and post transplant. *±1sem.*

(group A *n*=10, group B *n*=8, group c *n*=8 and group D *n*=8).

Left ventricular PRSW is significantly impaired post brain death, but no further impairment occurs during 90 minutes or 240 minutes preservation (groups b and D). The PRSW is conserved post transplant in the 'non-brain dead' hearts (groups A and C) .

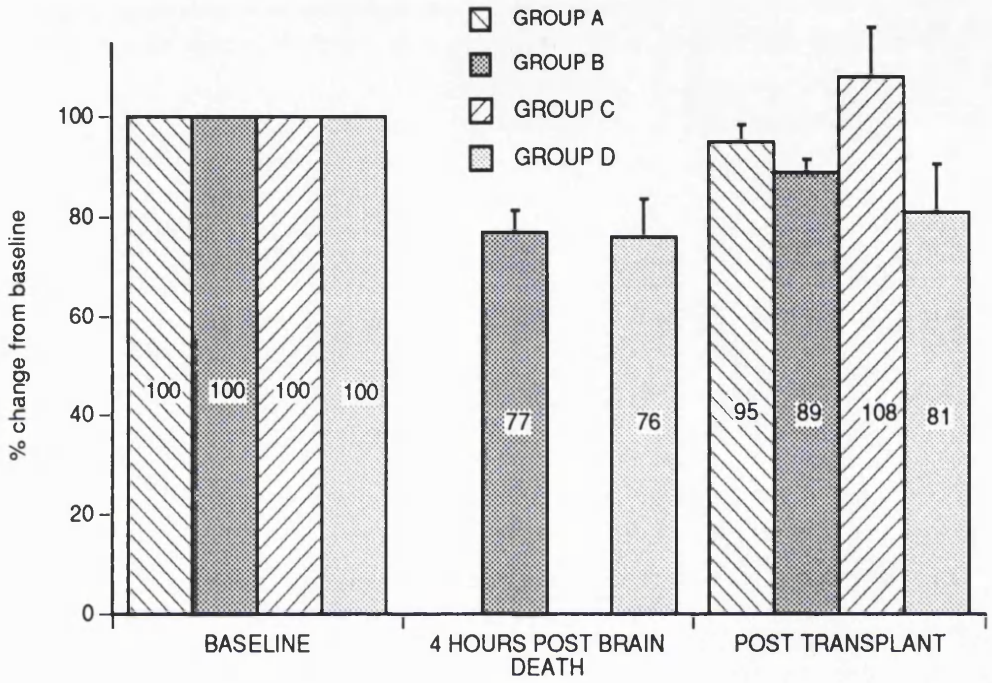
Figure 60. (Bottom)

Hydraulic power analysis for the right ventricle in groups A, B, C and D at baseline post brain death and post transplant. *±1sem.*

(group A *n*=10, group B *n*=8, group c *n*=8 and group D *n*=8).

Total power decreases post transplant proportional to cardiac output. The percentage components of mean power and oscillatory power post transplant are consistent in all groups. The percentage power dissipated as oscillatory power decreases post brain death, and is discussed in chapter 10.

PERCENTAGE CHANGE OF LEFT VENTRICULAR PRE LOAD INDEPENDENT RECRUITABLE STROKE WORK



HYDRAULIC POWER ANALYSIS

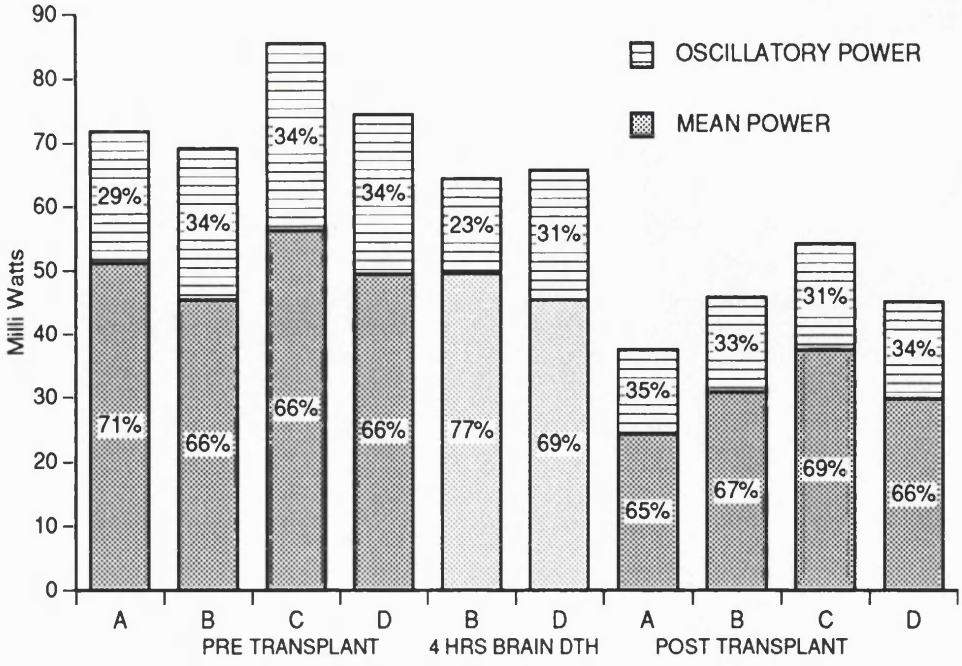


Figure 61. (Above)

Percentage change of pre load independent recruitable stoke work in the right ventricle for groups A, B, C and D at baseline, post brain death and post transplant. *±1sem.*

(group A *n*=10, group B *n*=8, group c *n*=8 and group D *n*=8).

In group D the significant impairment to right ventricular PRSW post brain death is further significantly impaired after four hours preservation. In contrast group C conserves function after four hours preservation.

Group B which has undergone brain death and a short ischaemic period suffers a significant reduction post brain death, as in group D, but no further impairment post transplant.

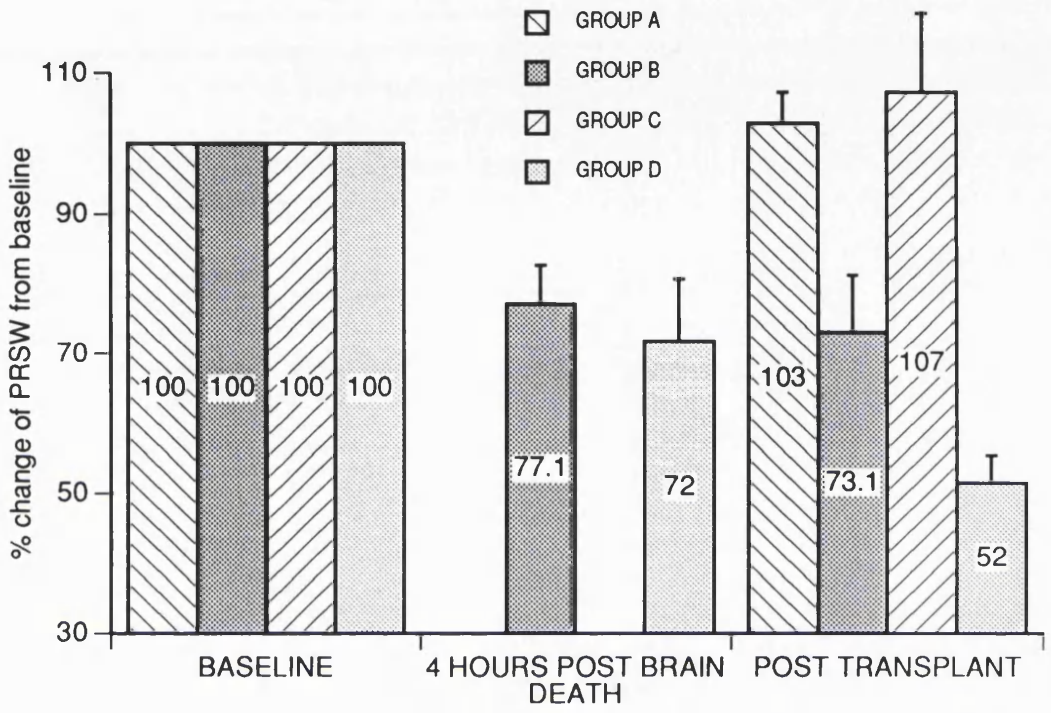
Figure 62. (Bottom)

Right ventricular pre load independent recruitable stroke work for groups A, B, C and D at baseline post brain death and post transplant. *±1sem.*

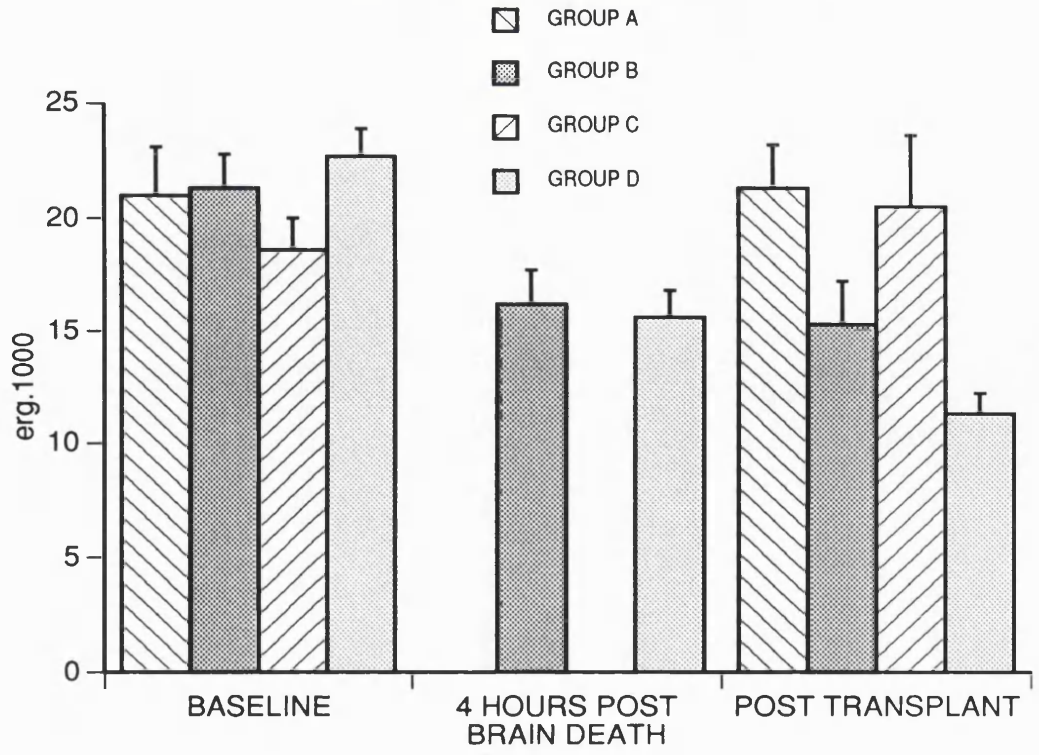
(group A *n*=10, group B *n*=8, group c *n*=8 and group D *n*=8).

At baseline the values for the PRSW of the right ventricle are similar permitting comparison of percentage change due to interventions.

PERCENTAGE CHANGE OF RIGHT VENTRICULAR PRE LOAD INDEPENDENT RECRUITABLE STROKE WORK



RIGHT VENTRICULAR PRSW



Post transplant the group C hearts had a rate of 142 bpm (±8), a mean arterial pressure of 68 mmHg (±2) and a left atrial pressure of 6.0 mmHg (±1.0) (table 40, figure 56). The cardiac output was 942 ml/min (±158) with a mean systemic vascular resistance of 6910 dyne.sec.cm^{-5} (±1204). Pulmonary impedance was also high; input resistance 1377 dyne.sec.cm^{-5} (±269), pulmonary vascular resistance 773 dyne.sec.cm^{-5} (±131) and characteristic impedance 187 dyne.sec.cm^{-5} (±22) (figure 57 and 58). None of these values are significantly different from the other groups.

Hydraulic power from the right ventricle has a total power 45.2 mW (±8.8) of which 65.4%(±3.6) is steady power and 34.6% (±3.6) is lost in oscillatory power (figure 60).

The most significant finding in this group of experiments relates to the values of PRSW post transplant. Following transplantation the PRSW for the left ventricle remains at 81.4% (±9.7) of its baseline function, unchanged from the 76.3% (±7.7) at four hours post brain death (figure 59). However the right ventricular PRSW reduces further post transplant to 51.5% (±3.9) from 71.8% (±8.9) after brain death (figure 61). This is a highly significant difference ($p<0.0001$) and reflects the need in this group for inotropic support to withdraw cardiopulmonary bypass.

Dopamine Response

The response of the hearts in group C to a low 'renal' dose of dopamine was remarkable. The dramatic response is tabulated in table 41. At 5 mcg/kg/min the heart rate was 159 bpm (±15), mean arterial pressure 73 mmHg (±9) and left atrial pressure 6.7 mmHg (±1.5). Cardiac output was elevated to 1531 ml/min (±486) against a systemic resistance of 4816 dyne.sec.cm^{-5} (±1734) and pulmonary input resistance of 1061 dyne.sec.cm^{-5} (±397). The change in PRSW is pronounced in both ventricles; increased to 97.2% (±3.4) against a background of 52.3% (±7.0) in the left ventricle and 112.2% (±14.5) versus an underlying 45.2% (±1.9) in the right ventricle. The dose response to dopamine for heart rate, cardiac output, PRSW, systemic and pulmonary resistance is depicted in figures 63, 64 and 65.

Table 41.

Effect of low dose dopamine post transplant in group D.

±1sem. (n=3).

Haemodynamic parameters are listed for three intravenous doses of dopamine;

5 mcg/kg/min, 2.5 mcg/kg/min, dopamine discontinued

The dramatic response of the three hearts in group C to low dose dopamine is demonstrated in the data; in particular the cardiac output, systemic vascular resistance, input resistance and pre load independent recruitable stroke work for the left and right ventricles reflect this dose related response.

LA press: left atrial pressure

Pulm. artery press: mean pulmonary artery pressure

SVR: systemic vascular resistance PVR: pulmonary vascular resistance

Characteristic Imp: characteristic impedance

Transpulm: efficiency transpulmonary efficiency

PRSW: pre load independent recruitable stroke work IV: intravenous

EFFECT OF LOW DOSE DOPAMINE POST TRANSPLANT IN GROUP D (n=3)

		5 mcg/kg/min		2.5 mcg/kg/min		No dopamine	
Heart rate	*bpm*	159	*15*	153	*12*	140	*4*
Systolic pressure	*mmHg*	98	*6*	93	*6*	103	*4*
Diastolic press.	*mmHg*	60	*10*	53	*6*	52	*4*
Mean arterial press.	*mmHg*	73	*9*	67	*6*	69	*3*
LA press.	*mmHg*	6.7	*1.5*	8.0	*0.9*	8.3	*0.6*
Pulm. artery press.	*mmHg*	15.5	*0.8*	15.2	*0.5*	15.1	*0.5*
Cardiac output	*ml/min*	1531	*486*	1233	*354*	1003	*272*
SVR	*dyn.s.cm-5*	4816	*1734*	5350	*1834*	6785	*2402*
Total power	*mW*	88.6	*31.2*	70.4	*22.0*	56.0	*14.4*
Meanpower	*mW*	54.4	*19.2*	42.7	*13.0*	33.3	*8.5*
% mean pwr.		60.2	*5.1*	60.4	*2.0*	59.1	*3.7*
Oscillatory pwr.	*mW*	34.1	*12.7*	27.7	*9.3*	22.7	*6.7*
% oscillatory pwr.		39.8	*5.1*	39.6	*2.0*	40.9	*3.7*
Input Resist.	*dyn.s.cm-5*	1061	*397*	1250	*455*	1574	*650*
PVR	*dyn.s.cm-5*	624	*290*	566	*187*	682	*248*
Characteristic Imp.	*dyn.s.cm-5*	205	*77*	242	*87*	203	*48*
Transpulm. efficiency		3.48	*0.28*	3.35	*0.17*	3.45	*0.31*
LV PRSW	*erg.103*	97.2	*3.4*	71.8	*8.4*	52.3	*7.0*
RV PRSW	*erg.103*	20.6	*3.1*	16.3	*2.5*	10.2	*0.8*
% LV PRSW		112.2%	*14.5%*	83.5%	*15.8%*	61.0%	*12.7%*
% RV PRSW		93.0%	*17.4%*	71.4%	*8.8%*	45.2%	*1.9%*

Figure 63. (Above)

Dose response to dopamine in group D post transplant; cardiac output and heart rate *±1sem.* (n=3).

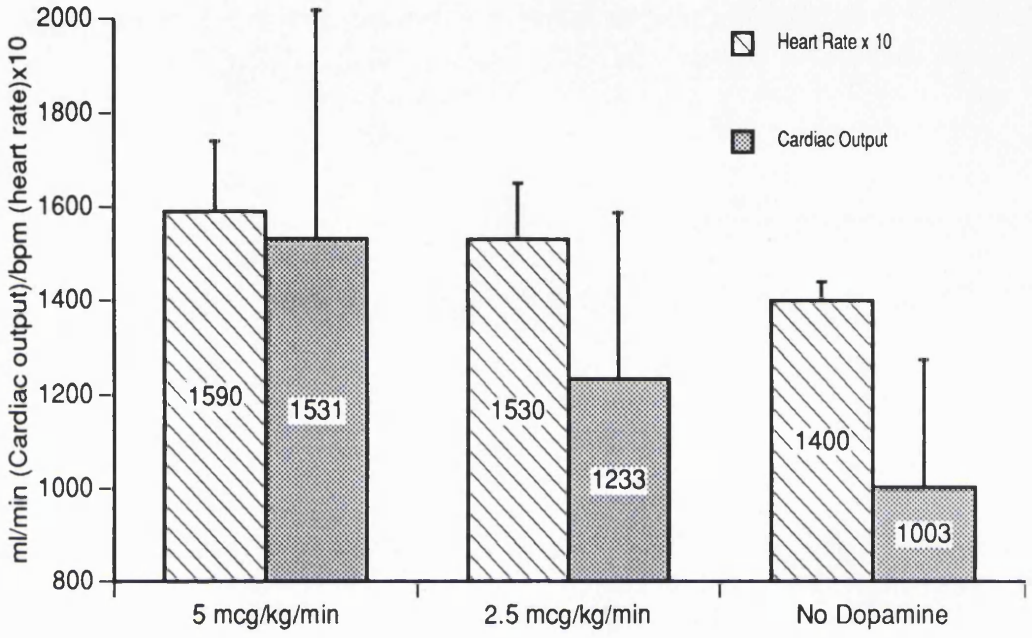
The chronotropic effect of low dose dopamine is less pronounced than the inotropic effect on cardiac output.

Figure 64. (Bottom)

Dose response to dopamine in group D post transplant; systemic and pulmonary input resistance *±1sem.* (n=3).

The resistance to flow from the left and right ventricles increases proportionally as dopamine is reduced.

DOSE RESPONSE TO DOPAMINE IN GROUP D POST TRANSPLANT; CARDIAC OUTPUT AND HEART RATE



DOSE RESPONSE TO DOPAMINE IN GROUP D POST TRANSPLANT; SYSTEMIC AND PULMONARY RESISTANCE

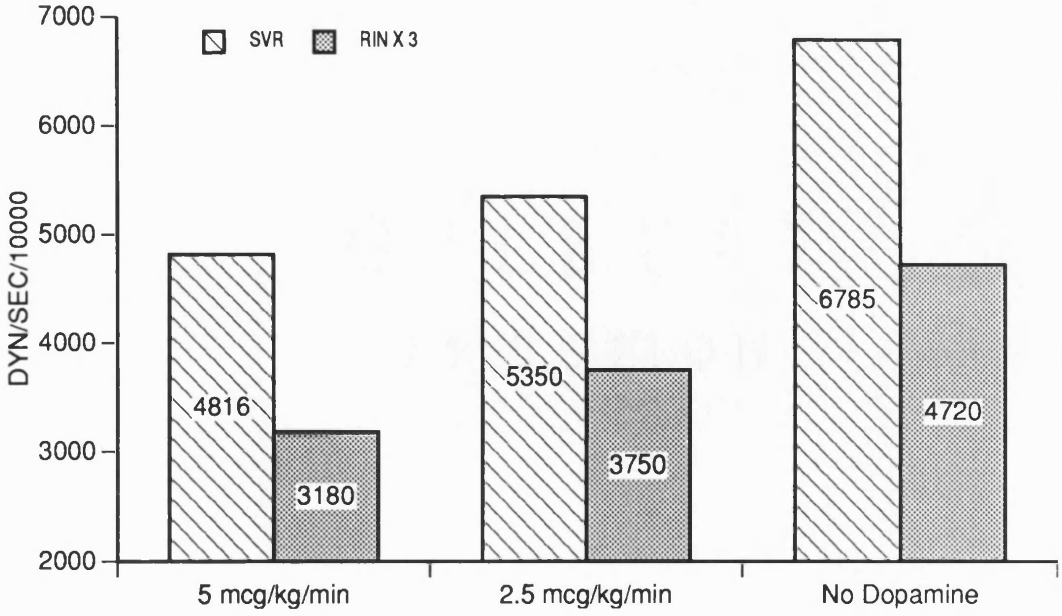
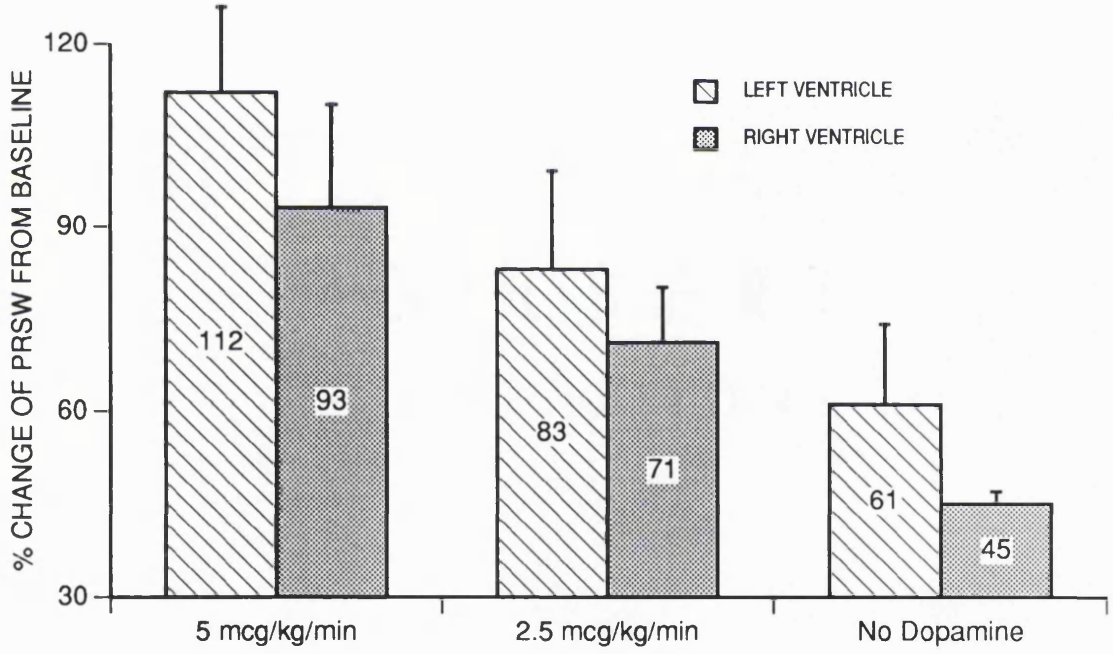


Figure 65.

Dose response to dopamine in group D post transplant; percentage change of pre load independent recruitable stroke work for left and right ventricles. *±1sem.* (n=3).

The change in PRSW for left and right ventricles is more characteristic of higher inotropic doses of dopamine (Krukencamp et al, 1987). This data suggests that in group D the effect of brain death and four hour preservation may increase the sensitivity of ß-adrenergic receptors.

DOSE RESPONSE TO DOPAMINE IN GROUP D POST TRANSPLANT; PERCENTAGE CHANGE OF PRSW FOR LEFT AND RIGHT VENTRICLES



DISCUSSION

In the hearts from non brain dead donors, four hours of total ischaemic time (group C) did not alter the myocardial function compared to a preservation time of less than ninety minutes (group A). This corresponds to results from other studies where the donor heart is from the anaesthetised animal. In fact four hours preservation is brief in comparison to twenty four hours' preservation, which was routine with resumption of adequate function (Watson, 1977). The results of group C also confirmed the efficacy of the new formula for the preservation fluid, and acted as control values for group D where brain death was superimposed as a second main effect.

To some extent the difficulty to wean hearts in group D from cardiopulmonary bypass concurred with the findings of Shivalkar et al (Shivalkar et al, 1993). They succeeded in two of four hearts to withdraw circulatory support, and that was only possible with large dose infusions of sympathomimetic amines. Group D hearts functioned very well compared to this study in that only half required support, and low dose dopamine was adequate to provide that support when necessary. These discrepancies between results may be attributed to the timing of donor organ harvest. Shivalkar was forced to harvest at one hour post brain death due to haemodynamic instability when presumably the hearts were under perfused, and under the influence of the insult from the hyperdynamic Cushing reflex with the ensuing metabolic acidosis. In contrast the hearts in group D were harvested at a time of haemodynamic stability, normal acid base balance and after four hours of brain death, that would allow any hormonal effects on the myocardium to take effect. This is more akin to the clinical situation where the donor is maintained for some hours before organ retrieval.

In terms of the heart's ability to resume control in the recipient circulation there is a significant interaction between brain death and prolonged preservation, such that inotropes were **only** necessary in group D. Subsequent data analysis when the dopamine had been discontinued reveals a statistically significant reduction in the PRSW of the right ventricle post transplant, that is superimposed on the reduction already caused by brain death.

The reasons for this observation are not clear. However, it may be a reasonable hypothesis that brain death causes impaired cardiac myocyte membrane function, that is less able to conserve cellular integrity during periods of hypothermic storage. The results of group B, where function is conserved post transplant despite brain death in the donor, shows that a period of ischaemia less than ninety minutes is not sufficient to expose this underlying dysfunction.

The left ventricle conserves contractility post transplant in all groups. Brain death itself causes a 25% reduction of PRSW but there is no added detriment to function following a short or prolonged period of preservation. There is little evidence from this study of any recovery of ventricular function post transplant, that was found in the rat model by Galinanes (Galinanes and Hearse, 1992), suggesting that rat and canine models are not comparable.

The results from these studies have clinical implications if this animal model of brain death represents changes in the human organ donor. It is well recognised that right ventricular dysfunction post cardiac transplantation is a major cause of early morbidity and mortality in clinical series. This has not been attributed to the donor heart, but to the recipient and a presumed elevation of pulmonary vascular resistance caused by chronic left ventricular failure. There is no doubt many recipients do have an elevated pulmonary vascular resistance, but the demonstration in this thesis of impaired right ventricular function post brain death, that is further damaged by prolonged preservation, gives a different perspective to the cause of this early morbidity / mortality.

The decision to use complete atrioventricular transplantation in groups B, C and D was justified. Group A had demonstrated significant conservation of sinus rhythm in all hearts compared to the standard technique and this was borne out by each of the twenty four subsequent transplants beating in sinus rhythm. After four hours preservation seven of sixteen hearts required initial ventricular pacing but after fifteen minutes this could be stopped with resumption of sinus rhythm. The increased need for pacing following prolonged preservation may explain the similarity in pacing

requirements between complete and standard transplantation in clinical series where ischaemic times are generally four hours. Furthermore the use of complete implantation conferred reliable pressure volume analysis of the right ventricle; in each group the deviation from the mean remained lower post transplant than in the standard method of implantation, and right ventricular volumes remained consistent compared to pre transplant values.

The supranormal response of group D hearts to dopamine post transplant was unexpected. The change in PRSW for left and right ventricles resembles dose response curves where large inotropic doses have been used (Krukencamp et al, 1987). These three hearts were extremely sensitive to low doses of dopamine which reinforces the ß-adrenergic receptor analysis post brain death in chapter 5. This analysis showed ß-adrenergic receptor changes to mimic ischaemic changes, with increased sensitivity. This supersensitivity has been described in clinical transplantation (Yusef et al, 1987) but is still controversial whether it is pre-synaptic or receptor mediated (Gilbert et al, 1989).

CONCLUSIONS

1. The hearts transplanted from an anaesthetised donor and preserved for four hours conserve function, and maintain the recipient circulation with equal pressures and flow compared to hearts with a minimum ischaemic time. No inotropic support was necessary for these experiments.

2. The pre load independent recruitable stroke work for left and right ventricles is conserved in donor hearts from anaesthetised animals, when preserved for four hours.

3. The hearts from a brain dead donor and preserved for four hours, may support the recipient adequately, although inotropic support was necessary in 50% of experiments to wean from cardiopulmonary bypass.

4. When the heart is explanted from a brain dead donor, myocardial preservation for four hours further impairs the pre load independent recruitable stroke work of the right ventricle .

5. Four hour preservation of hearts from brain dead donors causes no added impairment to the pre load independent recruitable stroke work of the left ventricle.

6. Complete atrioventricular transplantation conserved sinus rhythm in thirty five transplants. After preservation for four hours epicardial right ventricular pacing was required in seven hearts, for a maximum of twenty minutes, before resumption of sinus rhythm.

7. Load independent analysis of right ventricular systolic function may be consistently measured post transplant using complete atrioventricular transplantation and the shell subtraction model to calculate intraventricular volume.

In summary, brain death in the donor and four hour preservation of the heart interact significantly to impair right ventricular systolic function post transplant.

CHAPTER 9

HAEMODYNAMIC FUNCTION IN THE BRAIN DEAD MODEL FOLLOWING AN ACUTE INCREASE IN PULMONARY IMPEDANCE.

Introduction

Right ventricular dysfunction is a major cause of early morbidity and mortality in clinical orthotopic cardiac transplantation (Kirklin et al, 1988). The effect of chronic left ventricular failure in the recipient prior to transplantation is to alter the pulmonary vasculature, resulting in an increased resistance to flow from the right ventricle. This may be a permanent change with such high resistance to flow that the recipient is suitable only for heart-lung transplantation, or heart transplantation combined with single lung transplant. In patients with an intermediate increase of pulmonary input resistance there is always concern as to the ability of the donor right ventricle to overcome this resistance. if the right ventricle succeeds acutely in the early post operative period, there is some resolution of the pulmonary vascular disease, and a decrease in input resistance (Bhatia et al, 1987). When there is acute right ventricular failure post cardiac transplant, it is usually attributed to the recipient's pulmonary vasculature presenting a raised pulmonary impedance, that was underestimated in the pre transplant evaluation.

This thesis has shown there to be a substantial injury to right ventricular contractility in the canine model occurring after brain death. Furthermore, this injury increases during a period of prolonged preservation, reflecting the clinical scenario where the heart is harvested from a brain dead donor and preserved until implantation. This provides evidence of a different cause of right ventricular failure post transplantation not previously recognised.

The aim of the experiments in this chapter was to assess the ability of the right ventricle to pump blood against an acute rise of pulmonary impedance, before and after brain death. This is an attempt to create a model similar to clinical transplantation, where the heart from a brain dead donor is implanted into a recipient with an elevated pulmonary impedance. However, these experiments would remove the variable interaction of cardiopulmonary bypass and myocardial preservation on the recipient's haemodynamic status, to solely study the main effect of an acute rise in pulmonary impedance.

MATERIALS AND METHODS

The study group consisted of 9 dogs, their weights ranging from 21.7 to 29.9 kg (mean = 25.8 kg, ±0.4). All animals were anaesthetised, paralysed, and ventilated in the manner previously described.

The experiment was designed to permit paired analysis with the animal acting as its own control, allowing suitable analysis of each dog before and after banding of the pulmonary artery, and also pre and post instigation of brain death. The studies were terminated when the animals became haemodynamically unstable.

Procedure

All animals were surgically prepared in the same manner as previously described in chapters 4 and 5. The additional surgical maneuvers were as follows.

Following opening of the pericardium careful sharp dissection was performed to delineate the course and anatomy of the pulmonary artery and its bifurcation to left and right branches. The ligamentum arteriosum was divided and a 5 mm diameter nylon tape passed around the pulmonary artery at its bifurcation. This tape was brought out through a 0.5 cm^2 window fashioned in the pericardium and intrathoracic pleura, just above the left main pulmonary artery. The ends of the tape were passed through narrow polythene tubing that could act as a temporary snare. The pericardial window prevented the tape migrating to a more proximal aspect on the pulmonary artery. Two 18 gauge polythene cannulas were introduced and secured; one to the pulmonary artery proximal to the band, and one to the left pulmonary artery at its emergence from the left hilum of the lung. These were connected to pressure transducers to ascertain good pressure trace waveforms, before securing their position with 2/0 silk sutures. The application of the remaining instruments to the heart was unchanged from the prior studies, with the pulmonary artery flow probe uncompromised by the presence of the nylon band.

Data Acquisition

When all dogs were stable under anaesthesia baseline data were recorded. Six files of 500Hz (steady state, 6 seconds each) and six files of 200Hz (caval occlusion, 16 seconds each). The data were assessed for consistency and quality of the pressure volume loops. The band on the pulmonary artery was then tightened, while monitoring proximal and distal pulmonary artery systolic pressures, until the pressure drop across the band became 15 mmHg. This value was chosen to create a pressure drop above the maximum level accepted in cardiac transplant programmes (10mmHg), that is measured by subtracting left atrial pressure from mean pulmonary artery pressure. After a delay of fifteen minutes, to allow stabilisation of haemodynamics, a further set of data was acquired over a period of half an hour. The band was fully released while the quality of this data was checked, to permit resumption of a normal cardiovascular status. Brain death was instigated with inflation of an intracranial balloon as in the prior studies. Further data was acquired at two hours post brain death, without pulmonary artery banding, and then at four hours and six hours post brain death, firstly with no pulmonary artery banding and then with banding.

Cushing Response

The volume infused to the intracranial balloon was 18.2ml (±0.4). This reliably caused the Cushing response in each animal within 90 seconds of balloon inflation. The subsequent change in haemodynamics was predictable compared to the previous studies, with severe tachycardias and hypertension. The response persisted for 8 to 15 minutes before returning to baseline values, and in the subsequent hour the animals were cardiovascularly unstable; they required an increased intravenous infusion rate, a temporary increase in ventilation to maintain pO2 and a bolus of intravenous bicarbonate to correct metabolic acidosis. This was routinely encountered with arterial blood gas analysis at 15 minutes post brain death, and did not differ from the metabolic changes encountered in group B, group D and the validation group.

Therapeutic Interventions

Intravascular volume was maintained with Ringer's lactate solution to maintain a mean systemic arterial pressure of 60 mmHg. If serum sodium concentrations became supra normal (>148mmol/L) the infusion was changed to 5% dextrose. The acid - base balance was maintained between a pH range of 7.3 to 7.4 using intravenous bolus' of sodium bicarbonate 8.4% to infuse 75% of the base deficit. Serum potassium was maintained between 4.0 and 4.5 mmol/L by slow injection to a peripheral vein of potassium chloride. At no point were inotropic agents or vasopressor agents used.

Statistical Analysis

The experiment was designed to permit paired analysis with each animal acting as its own control, allowing suitable analysis of each dog before and after pulmonary artery banding. The response to pulmonary artery banding at four and six hours post brain death was compared the response at baseline using a paired Student's t-test. The changes at two hours and four hours post brain death were compared to groups B, D and the validation group using Anova, unpaired Student's t-test, to ascertain any influence of banding pre brain death on subsequent changes post brain death. Statistical analysis was performed on an IBM personal computer using the SAS statistical software package (Cary, North Carolina) and validated by the department of Surgical Statistics. All data was tested for normality prior to further analysis, performed with unpaired Student's t-test. In the text results are expressed as the mean, plus / minus one standard error of the mean. Statistical significance was considered where $p \leq 0.05$.

Table 42.

Haemodynamic data pre and post pulmonary artery banding after instigation of brain death *±1sem.* (*n*=9).

The response to brain death at two hours and four hours shows no statistical difference from group B, group D and the validation group.

Pulmonary artery banding causes similar changes in the systolic pulmonary artery gradient, but no change to systemic arterial pressures. However the cardiac output shows a significantly decreased response post brain death.

* denotes $p < 0.05$

HAEMODYNAMIC DATA PRE AND POST PULMONARY ARTERY BANDING
AFTER INSTIGATION OF BRAIN DEATH

		BASELINE		PA BAND		2 HOURS		4 HOURS		4HRS BAND		6 HOURS		6HRS BAND	
Heart rate	*bpm*	125	*5*	136	*6*	143	*5*	134	*5*	142	*4*	143	*6*	140	*7*
Systolic pressure	*mmHg*	134	*3*	132	*2*	97	*9*	80	*7*	80	*6*	78	*5*	80	*3*
Diastolic pressure	*mmHg*	92	*3*	87	*2*	63	*7*	47	*5*	47	*5*	45	*4*	47	*2*
Mean arterial press.	*mmHg*	106	*3*	102	*2*	74	*7*	58	*6*	58	*5*	56	*4*	58	*2*
Left atrial pressure	*mmHg*	8.6	*0.4*	8.0	*0.7*	10.3	*0.5*	12.3	*1.9*	8.4	*1.8*	9.3	*1.5*	8.8	*1.0*
Cardiac output	*ml/min*	1577	*196*	1407	*163*	1373	*167*	1490	*330*	*1096	*114*	1690	*326*	*1177	*257*
SVR	*dyn.s.cm-5*	5925	*773*	6290	*702*	4695	*667*	3311	*566*	4438	*624*	3013	*582*	4386	*1095*
Right atrial pressure	*mmHg*	2.1	*0.3*	3.3	*0.4*			5.0	*0.8*	6.3	*1.0*	6.8	*0.9*	5.3	*0.3*
Prox.PA sys. pressure	*mmHg*	25.6	*1.3*	41.0	*1.4*			25.8	*0.6*	37.0	*1.5*	28.6	*1.5*	38.3	*2.2*
Distal PA sys. pressure	*mmHg*	22.0	*1.4*	25.0	*2.7*			22.2	*1.0*	20.2	*1.9*	25.0	*0.7*	20.3	*1.9*
Gradient sys PA pressure	*mmHg*	3.6	*0.5*	16.0	*1.7*			3.1	*0.9*	16.8	*2.6*	3.6	*1.2*	18.0	*2.0*
Mean prox PA pressure	*mmHg*	15.3	*0.9*	19.3	*0.9*			15.7	*1.1*	19.7	*0.7*	18.4	*1.4*	21.0	*1.2*
Mean distal PA pressure	*mmHg*	13.7	*1.0*	13.7	*1.3*			14.7	*1.1*	12.7	*0.7*	16.6	*1.0*	14.7	*1.5*
Gradient mean PA pressure	*mmHg*	1.4	*0.5*	4.9	*1.0*			0.9	*0.4*	7.0	*0.7*	1.8	*0.7*	6.3	*0.3*
Change of distal mean press.	*mmHg*			0.0	*0.5*					-2.0	*1.4*			-1.7	*0.3*
Weight	*kg*	25.8	*0.4*												
Urine total	*ml*	0	*0*			464	*83*	1143	*146*						
Total intravenous fluid	*ml*	0	*0*			1886	*242*	3486	*451*						
						15 MINS POST BD									
Haematocrit	*%*	35.9	*1.6*			*49	*1.5*	*28.7	*2.1*						

Table 43.

Hydraulic power, pulmonary input resistance and biventricular load independent recruitable stroke work, pre and post pulmonary artery banding, after instigation of brain death. *±1sem* (*n*=9).

The response to brain death at two hours and four hours shows no statistical difference from group B, group D and the validation group. The impairment to pre load independent recruitable stroke work is comparable to the previous brain death experimental groups and is unaffected by pulmonary artery banding.

Pulmonary artery banding causes similar changes to the impedance profile of the pulmonary vasculature. However the response of the right ventricle, in terms of total hydraulic power, is significantly impaired post brain death.

* denotes $p < 0.05$

HYDRAULIC POWER, PULMONARY INPUT RESISTANCE AND BIVENTRICULAR PRE LOAD INDEPENDENT RECRUITABLE STROKE WORK PRE AND POST PULMONARY ARTERY BANDING AFTER INSTIGATION OF BRAIN DEATH

		BASELINE		PA BAND		2 HOURS		4 HOURS		4HRS BAND		6 HOURS		6HRS BAND	
Total power	*mW*	73.2	*10.5*	97.6	*10.0*	57.3	*7.2*	71.8	*15.7*	*72.9	*3.4*	81.9	*16.6*	*77.2	*14.9*
Mean power	*mW*	47.5	*7.3*	55.7	*6.8*	43.1	*5.9*	55.1	*13.2*	46.6	*3.9*	64.1	*14.4*	52.1	*12.2*
% mean power		64.4	*2.5*	57.1	*3.9*	75.0	*3.6*	74.3	*1.8*	63.2	*2.8*	77.2	*1.5*	66.4	*2.7*
Oscillatory power	*mW*	25.7	*3.8*	41.9	*5.5*	14.2	*2.2*	16.7	*2.6*	26.4	*1.6*	17.8	*2.3*	25.2	*2.7*
% oscillatory power		35.6	*2.5*	42.9	*3.9*	25.0	*3.6*	25.7	*1.8*	36.8	*2.8*	22.8	*1.5*	33.6	*2.7*
Input resistance	*dyn.s.cm-5*	727	*80*	1123	*138*	903	*129*	859	*108*	1496	*158*	917	*167*	1497	*363*
Pulmonary vascular resist.	*dyn.s.cm-5*	255	*47*	610	*69*	251	*63*	264	*72*	857	*179*	443	*128*	829	*181*
Characteristic impedance	*dyn.s.cm-5*	210	*15*	483	*41*	207	*10*	225	*22*	548	*64*	178	*16*	516	*85*
Transpulmonary efficiency	*J/L*	2.76	*0.16*	4.35	*0.40*	2.53	*0.14*	3.01	*0.16*	4.09	*0.21*	2.91	*0.10*	4.01	*0.29*
LV PRSW	*erg.1000*	79.5	*8.3*	92.0	*8.7*	66.8	*4.8*	52.2	*7.3*	63.6	*6.4*	60.3	*6.0*	70.3	*10.1*
RV PRSW	*erg.1000*	24.2	*1.8*	25.3	*2.0*	14.3	*0.7*	14.0	*0.9*	14.6	*0.7*	13.3	*2.2*	12.8	*0.3*
%LV PRSW		100.0%	*0.0%*	117.8%	*6.7%*	86.9%	*5.7%*	68.9%	*10.8%*	77.7%	*10.0%*	71.3%	*4.3%*	72.0%	*12.0%*
%RV PRSW		100.0%	*0.0%*	105.2%	*6.2%*	60.6%	*4.6%*	59.3%	*5.1%*	61.0%	*4.2%*	53.9%	*7.3%*	50.5%	*5.6%*
Change of X-intercept LV	*ml*	0.0	*0.0*	-3.6	*1.5*	19.3	*2.6*	15.0	*2.9*	17.0	*2.8*	17.0	*2.7*	17.7	*3.4*
Change of X-intercept RV	*ml*	0.0	*0.0*	1.6	*1.1*	11.4	*1.7*	12.3	*2.2*	17.3	*3.4*	12.2	*3.4*	18.2	*6.5*

RESULTS

Seven animals survived four hours yielding reliable data pre and post pulmonary artery banding. Six animals went on to survive six hours although only four tolerated banding sufficiently to allow collection of data. The experiments were terminated once the data had been checked. Two of the nine animals progressively deteriorated after the Cushing response despite therapeutic interventions, with decreasing systemic pressure and flow. These two animals died at 90 and 110 minutes post brain death.

Baseline Controls

Response to Pulmonary Artery Banding

At baseline all nine animals tolerated banding of the pulmonary artery (PA). The mean gradient of systolic pulmonary artery pressure across the band rose from 3.6 mmHg (±0.5) to 16.0 mmHg (±1.7). Therefore the proximal systolic pulmonary artery pressure rose from 25.6 mmHg (±1.3) to 41.0 mmHg (±1.4), with the mean pressure increasing from 15.3 mmHg (±0.9) to 19.3 mmHg (±0.9) (table 42). This response compensated for the band so that mean distal pulmonary artery pressure was unchanged at 13.7 mmHg (±1.3).

The heart rate increased from 125 bpm (±5) to 136 bpm (±6), while mean arterial pressure and left atrial pressure decreased from 106 mmHg (±3) and 8.6 mmHg (±0.4) to 102 mmHg (±2) and 8.0 mmHg (±0.7) respectively. Likewise there was a small reduction in cardiac output to 1407 ml/min (±163) from a pre-banding value of 1577 ml/min (±196), but none of these changes were significant. The application of the PA band increased the right atrial pressure from 2.1 mmHg (±0.3) to 3.3 mmHg (±0.4) and also increased the systemic vascular resistance to 6290 dyne.sec.cm^{-5} (±702) from 5925 dyne.sec.cm^{-5} (±773); both changes were statistically insignificant.

Figure 66. (Above)

Influence of pulmonary artery banding post brain death;

Systolic pressure gradient across pulmonary artery band. *±1sem.* (*n*=9).

The pulmonary artery band increased the systolic pulmonary artery gradient by equal amounts at each sample interval.

Figure 67. (Bottom)

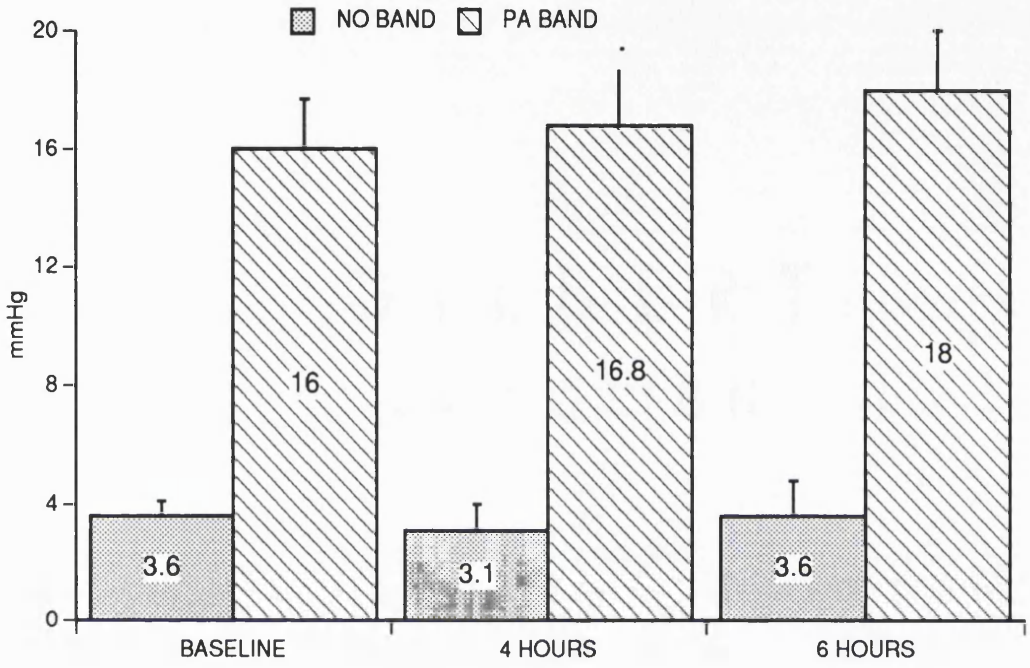
Influence of pulmonary artery banding post brain death;

CARDIAC OUTPUT. *±1sem.* (*n*=9).

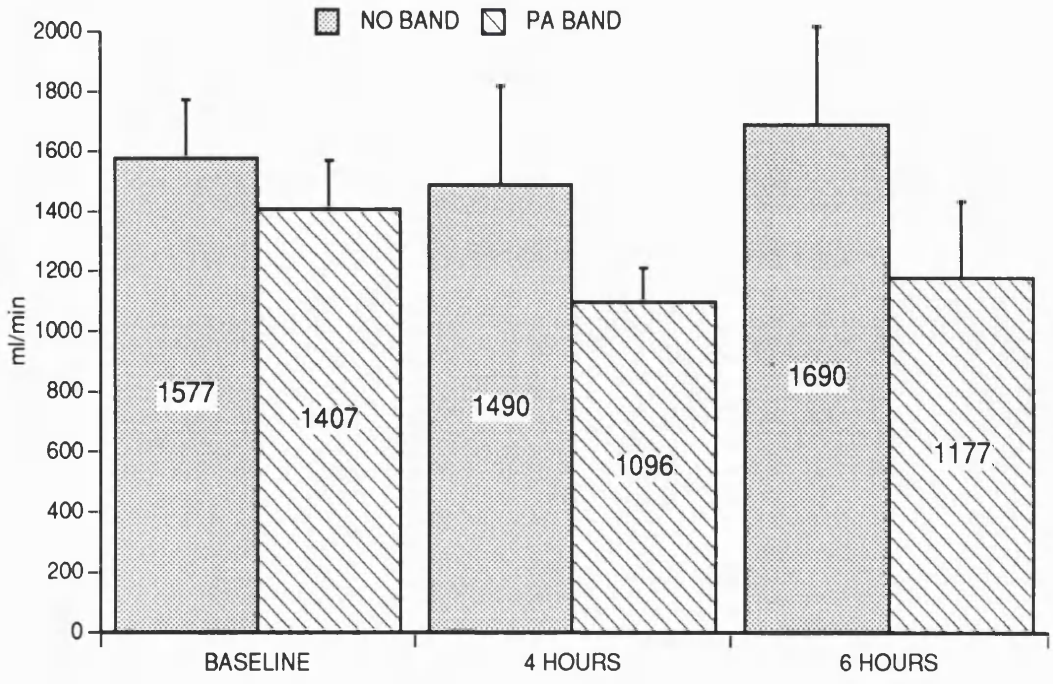
The change in cardiac output due to the pulmonary artery band post brain death was significantly different to the baseline response.

INFLUENCE OF PULMONARY ARTERY BANDING POST BRAIN DEATH

SYSTOLIC PRESSURE GRADIENT ACROSS PULMONARY ARTERY BAND



CARDIAC OUTPUT



The application of the pulmonary artery band caused an appropriate increase in pulmonary impedance. The input resistance changed from 727 dyne.sec.cm^{-5} (±80) to 1123 dyne.sec.cm^{-5} (±138), the pulmonary vascular resistance from 255 dyne.sec.cm^{-5} (±47) to 610 dyne.sec.cm^{-5} (±69) and the characteristic impedance from 210 dyne.sec.cm^{-5} (±15) to 483 dyne.sec.cm^{-5} (±41) (table 43). These changes are comparable to the changes in impedance pre and post cardiac transplantation in the canine model as displayed in figure 38.

There was a significant increase of total right ventricular hydraulic power from 73.2 mW (±10.5) to 97.6 mW (±10.0) associated with a disproportional increase of oscillatory power; 35.6% (±2.5) to 42.9% (±3.9), and a corresponding decrease in mean power from 64.4% (±2.5) to 57.1% (±3.9). The increase in total power and almost unchanged cardiac output decreased the transpulmonary efficiency, represented by the increase of power to flow ratio from 2.76 J/L (±0.16) to 4.35 J/L (±0.40).

There was no significant change in the pre load independent recruitable stroke work (PRSW) for left and right ventricles; the left ventricular PRSW increased post PA banding to 117.8% of baseline (±6.7) and the right ventricular PRSW increased to 105.2% (±6.2). As expected the X-intercept for the left ventricle decreased by 3.6 ml (±1.5) and increased for the right ventricle by 1.1 ml (±1.6) due to the increase in pulmonary impedance.

Therefore, at baseline the application of the pulmonary artery band to increase the systolic pressure by 13 mmHg caused a compensatory rise in right ventricular power to maintain pressure and flow in the pulmonary vasculature. The PRSW for the right ventricle remained unchanged reaffirming the load independent nature of this assessment of right ventricular contractility.

Figure 68. (Above)

Influence of pulmonary artery banding post brain death;

Input resistance to pulmonary circulation. *±1sem.* (*n*=9).

The pulmonary artery band causes a comparable rise in input resistance to the pulmonary vasculature at pre and post brain death. (This trend applies to the pulmonary vascular resistance and the characteristic impedance)

Figure 69. (Bottom)

Influence of pulmonary artery banding post brain death;

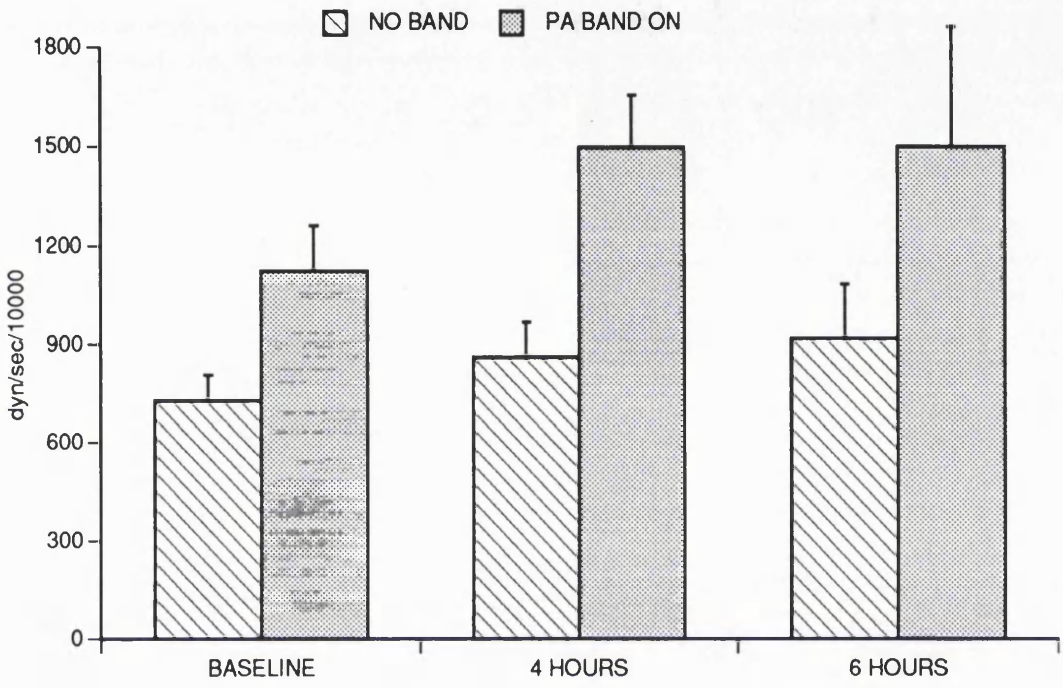
Right ventricular hydraulic power analysis. *±1sem.* (*n*=9).

At baseline the pulmonary artery band promotes an increase in right ventricular total power to overcome the increased resistance to flow. After brain death the response is significantly abolished, inferring decreased reserve of hydraulic power in the right ventricle.

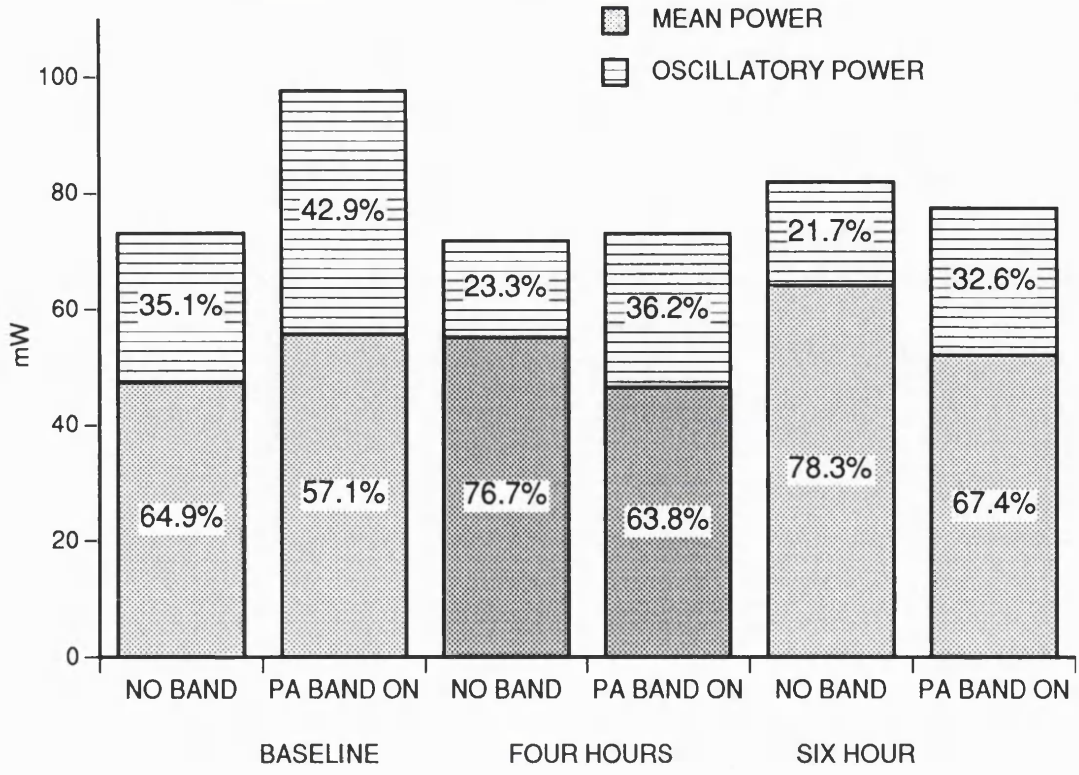
As in the other brain death groups the proportion of oscillatory power decreases post brain death, but the proportional increase in response to banding remains constant.

INFLUENCE OF PULMONARY ARTERY BANDING POST BRAIN DEATH

INPUT RESISTANCE (Rin) TO PULMONARY CIRCULATION



RIGHT VENTRICULAR HYDRAULIC POWER ANALYSIS



Post Brain Death

Post brain death the alterations in haemodynamics were similar to the changes recorded in group B, group D and the validation group. The comparison was made using an unpaired student's t-test, and no statistical differences were encountered.

Heart rate increased to 143 bpm (±5) and 134 bpm (±5) at two and four hours respectively. Mean arterial pressure dropped to 74 mmHg (±7) and 58 mmHg (±6) while left atrial pressure increased to 10.3 mmHg (±0.5) and 12.3 mmHg (±1.9) (table 42). As with the other brain death groups there was little change in cardiac output to 1490 ml/min (±330) whilst systemic vascular resistance decreased to 3311 dyne.sec.cm^{-5} (±566).

The pulmonary impedance profile remained unchanged after brain death; at four hours the input resistance was 859 dyne.sec.cm^{-5} (±108), pulmonary vascular resistance 264 dyne.sec.cm^{-5} (±72) and characteristic impedance 225 dyne.sec.cm^{-5} (±22). Hydraulic power from the right ventricle was also unchanged at four hours post brain death at 71.8 mW (±15.7) with a decrease in the proportion of oscillatory power to 25.7 % (±1.8), responses that were recorded in group B, group D and the validation group. Transpulmonary efficiency increased insignificantly to 3.01 J/L (±0.9).

Systolic performance of left and right ventricles was impaired by similar proportions to the other groups. In the left ventricle the PRSW decreased to 86.9% (±5.7) at two hours and 68.9 % (±10.8) at four hours. The right ventricular PRSW went down to 60.6 % (±4.6) and 59.3 % (±5.1) at two and four hours respectively. The X-intercept at four hours had increased by 15.0 ml (±2.9) and 12.3 ml (±2.2) for left and right ventricles respectively.

Aside from the haemodynamic changes, the fluid balance for these animals and the changes in haematocrit mirrored the previous brain death groups. The rate and volume of urine excreted post brain death was similar with a total of 464 ml (±83) excreted at two hours and 1143 ml (±146) at

four hours, with intravenous fluid infusions totalling 1886 ml (±242) and 3486 ml (±451) at the same time intervals. The haematocrit peaked at fifteen minutes post brain death at 49.0 % (±1.5) from a baseline value of 35.9 % (±1.6) declining to a level of 28.7 % (±2.1) at four hours.

The consistency of these results post brain death compared to the prior experimental groups suggested that the application of the pulmonary artery band prior to brain death was not affecting the effects of intracranial balloon inflation on subsequent changes in the canine model.

Effect of Pulmonary Artery Banding Post Brain Death

The pulmonary artery band achieved increases of the proximal systolic pulmonary artery pressure from 25.8 mmHg (±0.6) and 28.6 mmHg (±1.5) to 37.0 mmHg (±1.5) and 38.3 mmHg (±2.2) at four and six hours post brain death. In effect the systolic gradient across the band increased to 13.7 and 14.4 mmHg respectively (figure 66). This caused the right atrial pressure to increase at four hours from 5.0 mmHg (±0.8) to 6.3 mmHg (±1.0) and decrease at six hours, 6.8 mmHg (±0.9) to 5.3 mmHg (±0.3). The PA band also presented a similar impedance profile at four hours and six hours (figure 68); input resistance 1496 dyne.sec.cm^{-5} (±167) and 1497 dyne.sec.cm^{-5} (±363), pulmonary vascular resistance 857 dyne.sec.cm^{-5} (±179) and 829 dyne.sec.cm^{-5} (±181) and characteristic impedance 548 dyne.sec.cm^{-5} (±64) and 516 dyne.sec.cm^{-5} (±85). This change in impedance profile was not different statistically from the change at baseline.

The mean arterial pressure remained constant at 58 mmHg at four and six hours with or without pulmonary artery banding, whilst the left atrial pressure fell at four hours from 12.3 mmHg (±1.9) to 8.4 mmHg (±1.8) and at six hours from 9.3 mmHg (±1.5) to 8.8 mmHg (±1.0) when the band was applied. The rise in systemic vascular resistance noted at baseline with the PA band was more pronounced after brain death; at four hours it changed from 3311 dyne.sec.cm^{-5} (±566) to 4438 dyne.sec.cm^{-5} (±624) and at six hours 3013 dyne.sec.cm^{-5} (±582) to 4386 dyne.sec.cm^{-5} (±1095). These responses were statistically similar.

Figure 70. (Above)

Influence of pulmonary artery banding post brain death;
Percentage change of left ventricular pre load independent recruitable stroke work. *±1sem.* (*n*=9).

Left ventricular PRSW is impaired post brain death as in the previous brain death experimental groups. Pulmonary artery banding causes no significant change pre and post brain death.

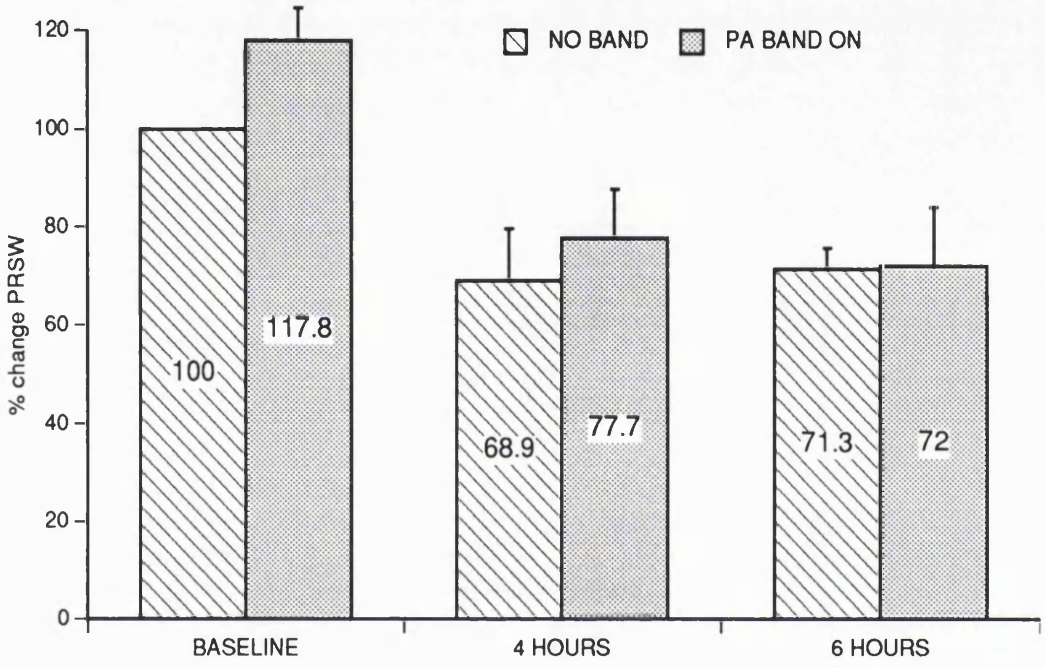
Figure 71. (Bottom)

Influence of pulmonary artery banding post brain death;
Percentage change of right ventricular pre load independent recruitable stroke work. *±1sem.* (*n*=9).

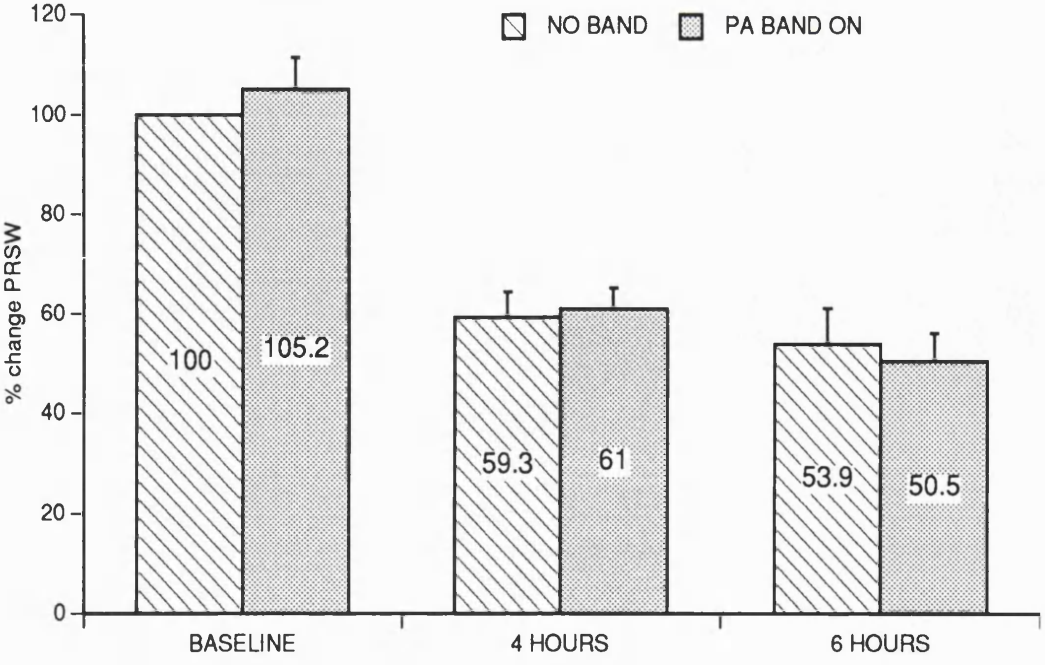
The impairment to right ventricular PRSW post brain death is statistically similar to the previous brain death experimental groups. Pulmonary artery banding causes no significant change, reinforcing the load-independent nature of this analysis of systolic function.

INFLUENCE OF PULMONARY ARTERY BANDING POST BRAIN DEATH

PERCENTAGE CHANGE OF LEFT VENTRICULAR PRE LOAD INDEPENDENT RECRUITABLE STROKE WORK



PERCENTAGE CHANGE OF RIGHT VENTRICULAR PRE LOAD INDEPENDENT RECRUITABLE STROKE WORK



However the change in the cardiac output was significantly changed after brain death (figure 67). Whereas at baseline there was minimal change in cardiac output, at four hours it decreased from 1490 ml/min (±330) to 1096 ml/min (±114) and likewise at six hours from 1690 ml/min (±326) to 1177 ml/min (±257).

This lack of compensation in response to banding is also seen in the distal mean pulmonary artery pressure. At baseline distal pressure is conserved but at four hours mean pressure falls by 2.0 mmHg (±1.4) and 1.7 mmHg (±0.3) at six hours, but this does not reach statistical significance

The ability of the right ventricle to propel blood against elevated pulmonary impedance is significantly impaired post brain death. Total hydraulic power remained constant at four hours post banding {72.9 mW (±3.4) vs 71.8 mW (±15.7)} and at six hours it decreased {77.2 mW (±14.9) vs 81.9 mW (±16.6)}. This is in marked contrast to the rise in total power at baseline following pulmonary artery banding (figure 69). As would be expected the proportion of this power used to propel blood is less post banding, 63.2% (±2.8) versus 74.3 % (±1.8) at four hours and 66.4% (±2.7) versus 77.2 % (±1.5) at six hours. There is a corresponding rise in oscillatory power.

The pre load independent recruitable stoke work for left and right ventricles was unaffected by banding of the pulmonary artery. Indeed the PRSW for the right ventricle remained very consistent underlining the true load-independent nature of this measure of systolic function. The percentage PRSW of baseline values was 68.9 % (±10.8) and 71.3 % (±4.3) for the left ventricle at four and six hours post brain death respectively, changing to 77.7 % (±10.0) and 72.0 % (±12.0) post banding (figure 70). In the right ventricle the PRSW was 59.3 % (±5.1) and 53.9 % (±7.3) at four and six hours post brain death respectively, changing to 61.0 % (±4.2) and 50.5 % (±5.6) post banding (figure 71). The trend in change of X-intercept for the right ventricle due to banding is more pronounced after brain death. At four hours the right ventricular X-intercept increases 5.0 ml at four hours and 6.0 mls at six hours reflecting the increased preload in the right ventricle required to overcome the outflow resistance. The left ventricular X-intercept increases 2.0 ml and 0.7 ml at four

and six hours respectively; none of these changes are significant.

These results of the haemodynamic responses to pulmonary artery banding reveal a decrease in the reserve of the right ventricle to cope with an acute rise in pulmonary impedance, as demonstrated by the fall in cardiac output and total hydraulic power.

DISCUSSION

The conclusions from chapter 8 regarding right ventricular function suggested that brain death interacted with prolonged preservation to impair systolic function. Indeed following combination of these main effects there was a significant inotropic requirement to wean the recipients from cardiopulmonary bypass.

Post transplantation the recipients in this thesis have had an elevated pulmonary vascular impedance profile (figure 38). This pertains to clinical transplantation where chronic left ventricular failure causes changes to the pulmonary vasculature and a subsequent increase in pulmonary vascular resistance. However the detrimental influence of myocardial preservation in the transplant model precludes assessment of right ventricular function alone, when confronted with a raised outflow resistance.

Therefore the model used in this chapter was designed to represent the clinical scenario where a heart from a brain dead donor is implanted to a recipient with an elevated pulmonary impedance. With the omission of preservation and implantation, and the associated changes due to cardiopulmonary bypass, it was hoped that the function of the right ventricle from a brain dead donor might be accurately assessed.

The response to banding at baseline by the right ventricle was appropriate to overcome the applied resistance to flow; cardiac output was maintained by a significant increase of total hydraulic power, which also preserved the mean pressure in the distal pulmonary artery.

The consistent increase in systolic pulmonary artery gradient and pulmonary impedance profile that the pulmonary artery band induced pre and post brain death was indicative that the right ventricle was presented with similar stress. Moreover the elevated impedance profile was not significantly different from impedance in the recipient animals, further justifying this model for creating a post -transplant environment for the right ventricle.

One concern for this model was the effect of the pulmonary artery band at baseline on the subsequent haemodynamic changes post brain death. Statistical comparison to group B, group C and the validation group, the previous brain death experiments, revealed no differences between groups at two and four hours post brain death. This permits the results from these experiments using pulmonary artery banding to be related to the transplant experiments and also clinical practice.

It was interesting, therefore, that the right ventricle post brain death could not increase its total hydraulic power to compensate for the acute rise in outflow resistance, resulting in a lower cardiac output. This response supports the observation that the right ventricular PRSW post brain death is decreased, which only becomes clinically apparent when extra demand is placed on the right ventricle.

The implication of this study is that pressure and flow in the pulmonary artery may be maintained post brain death in the donor, but should the heart be implanted to a recipient with an elevated pulmonary vascular resistance then the underlying injury to the right ventricle will predispose to right ventricular failure.

The raised impedance profile post transplant in the canine model may explain the need for inotropes to support the circulation (Shivalkar et al, 1993). Only when brain death is combined with four hour preservation is there sufficient injury to the right ventricle to prevent attainment of

satisfactory haemodynamics, otherwise the right ventricle has enough reserve to overcome the resistance. In the isolated rat and swine models only the left ventricle is studied, and the effect of pulmonary impedance on right ventricular function is excluded (Galinanes and Hearse, 1992)(Wicomb et al, 1986b).

This study has reinforced the observation that PRSW in the right ventricle decreases after brain death, and shows how this injury may remain clinically inconspicuous until extra demands are placed on the right ventricle, such as an increase in pulmonary impedance.

CONCLUSIONS

(1). Pulmonary artery banding for less than one hour does not influence subsequent haemodynamic changes post brain death in the canine model.

(2). At baseline the right ventricle increases hydraulic power to overcome the increased pulmonary impedance, maintaining cardiac output and mean distal pulmonary artery pressure.

(3). Pulmonary artery banding post brain death causes similar changes to the pulmonary impedance profile compared to pre brain death and the canine recipient. In effect it provides a suitable model to assess right ventricular function against a raised pulmonary vascular resistance.

(4). The right ventricular pre load independent recruitable stroke work is unaffected by pulmonary artery banding, exemplifying the load-independence of this method to assess right ventricular systolic function.

(5). Brain death impairs right ventricular function. Not only is the PRSW decreased but also its ability to pump blood against an increased pulmonary impedance is diminished, reflected by the decrease of total hydraulic power and cardiac output.

These experiments have attempted to simulate the environment of clinical cardiac transplantation, where a heart from a brain dead donor may be implanted to a recipient with a raised pulmonary vascular resistance. In utilising a pulmonary artery band around the distal pulmonary artery in this validated canine model of brain death, the effects of myocardial preservation and cardiopulmonary bypass have been eliminated. This has allowed an analysis of right ventricular function that has revealed a significant decrease of hydraulic power reserve when confronted with an acute rise in pulmonary vascular impedance.

CHAPTER 10

DISCUSSION

Brain Death; General Considerations

Validation

Several different animal models have been studied in relation to brain death, utilising different techniques to attempt to cause brain death. These investigations assumed brain death by indirect measures of serum hormones, reflex testing and electroencephalogram recording. However the relevance of such studies is questionable unless brain stem death is thoroughly validated, to allow comparison to clinical donor management and donor organ preservation, where brain stem death is confirmed in the absence of cerebral depressants.

This thesis initially aimed to validate brain stem death in an animal model; for this purpose intracranial balloon inflation was selected as it is minimally invasive, with least tissue destruction (Cushing, 1901). This method mimics clinical donors, to some extent, where the majority become brain dead secondary to an acute rise in intracranial pressure after a head injury or a subarachnoid haemmorrhage. In comparison, ligation of head and neck vessels leaves a large volume of tissue under perfused with resultant release of toxic agents. Furthermore, due to extensive cerebral collaterals, this may not reliably cause brain stem death (Finkelstein et al, 1987). The confirmation of brain stem death by neuropathological assessment was fundamental to the remainder of the studies in this thesis and has not been reported previously. Indeed in ten consecutive studies brain stem death was evident with surprisingly little damage to the gross cerebral architecture, despite the inflation of a 15-20 ml intracranial balloon (figure 32).

This method to instigate brain death consistently causes a hyperdynamic response, known as the Cushing reflex. This is a severe episode with supranormal systemic pressures, pulmonary pressures, heart rate and afterload for the ventricles (figure 11). Clinically, raised intracranial pressure is associated with hypertension, but not to the extent seen in this experimental situation; this was the concern of Shivalkar, prompting a comparison of sudden balloon inflation to gradual

balloon inflation (Shivalkar et al, 1993). Gradual balloon inflation caused less acute systemic changes, but decreased the more chronic responses post brain death.

Therefore sudden intracranial balloon inflation was selected in this thesis to promote measurable effects that are associated with severe head injury; the hormonal changes, haemodynamic status and urine output were representative of the clinical situation, as were the histological changes seen in the myocardium.

Haemodynamic Changes Post Brain Death

In total, thirty three animals underwent brain death with haemodynamic measurements at baseline, two hours and four hours post brain death. Unlike Shivalkar's study, these animals reliably survived four hours for the transplant studies, and six hours in the remaining studies. This allows a clearer impression of the characteristic changes that occur, and are shown in table 44.

Heart rate does not change post brain death remaining at 120 bpm (±3) at four hours compared to 117 bpm (±3). Mean arterial pressure significantly falls to 69 mmHg (±3) from 97 mmHg (±3) whilst left atrial pressure increases from 6.7 mmHg (±0.3) to 8.5 mmHg (±0.8) (figure 72). Cardiac output is maintained at 1658 ml/min (±101) with no significant change to baseline. Systemic vascular resistance significantly decreases post brain death probably due to denervation of the arterioles; at baseline it is 5249 dyne.sec.cm^{-5} (±311) and at four hours drops to 3528 dyne.sec.cm^{-5} (±201) (figure 73). The left ventricular pre load independent recruitable stroke work decreases by 20% to 78.8% (±4.3) of baseline.

In summary left ventricular function is impaired post brain death but the injury is masked by the fall in afterload, the systemic vascular resistance. The pre load increases as shown by the rise in left atrial pressure and systemic pressure falls, due to the decrease of left ventricular contractility and systemic vascular resistance. These factors combine to maintain the cardiac output.

Table 44.

Haemodynamic profile post brain deatn. *±1sem.* (*n*=33).

Mean values for the thirty three animals in the thesis that were made brain dead, with complete data at baseline, two hours and four hours post instigation of brain death. (* denotes p<0.05)

Abbreviations;

SVR; systemic vascular resistance

PVR; pulmonary vascular resistance

LV; left ventricle

RV; right ventricle

PRSW; pre load independent recruitable stroke work

HAEMODYNAMIC PROFILE POST BRAIN DEATH
(N = 33)

		BASELINE		2 HOURS		4 HOURS	
Heart rate	*bpm*	117	*3*	125	*3*	120	*3*
Systolic pressure	*mmHg*	124	*3*	*95	*3*	*93	*3*
Diastolic pressure	*mmHg*	83	*3*	*63	*3*	*57	*3*
Mean arterial pressure	*mmHg*	97	*3*	*73	*3*	*69	*3*
Left atrial pressure	*mmHg*	6.7	*0.3*	7.6	*0.5*	8.5	*0.8*
Pulmonary artery press.	*mmHg*	12.8	*0.4*	13.0	*0.4*	*14.6	*0.5*
Cardiac output	*ml/min*	1568	*68*	1547	*72*	1658	*101*
SVR	*dyn.s.cm-5*	5249	*311*	*4056	*238*	*3528	*201*
Total power	*mW*	67.2	*3.9*	61.3	*3.8*	73.1	*6.2*
Meanpower	*mW*	45.2	*2.6*	45.6	*3.1*	55.1	*4.9*
% mean pwr.		67.4	*1.0*	*73.9	*1.2*	*74.9	*1.1*
Oscillatory pwr.	*mW*	22.0	*1.5*	15.6	*1.0*	18.0	*1.7*
% oscillatory pwr.		32.6	*1.0*	*26.1	*1.2*	*25.1	*1.1*
Input Resist.	*dyn.s.cm-5*	686	*29*	722	*41*	738	*41*
PVR	*dyn.s.cm-5*	326	*23*	297	*26*	313	*28*
Characteristic Imp.	*dyn.s.cm-5*	178	*8*	163	*8*	167	*9*
Transpulm. efficiency		2.54	*0.07*	2.36	*0.07*	2.62	*0.09*
LV PRSW	*erg.103*	76.2	*3.5*	*58.5	*2.1*	*56.5	*2.5*
RV PRSW	*erg.103*	23.6	*1.5*	*14.7	*1.3*	*15.6	*1.2*
% LV PRSW		100.0%	*0.0%*	*79.6%	*3.4%*	*78.8%	*4.3%*
% RV PRSW		100.0%	*0.0%*	*65.6%	*5.1%*	*70.6%	*4.9%*
Change of LV X-int	*ml*	0.0	*0.0*	15.6	*2.4*	12.0	*2.2*
Change of RV X-int	*ml*	0.0	*0.0*	8.4	*1.4*	9.3	*1.8*
Total urine	*ml*	0	*0*	431	*38*	1227	*74*
Cumulative IV fluid	*ml*	0	*0*	1527	*114*	2819	*165*
Haematocrit	*%*	35.5	*0.7*	*45.2	*1.1*	*28.1	*1.0*
Weight	*kg*	26.0	*0.3*				

Figure 72. (Above)

Haemodynamic profile post brain death;

Mean systemic arterial pressure and mean left atrial pressure.

±1sem. (*n*=33).

Mean peripheral arterial pressure significantly falls post brain death ($p<0.05$).
Left atrial pressure is depicted ten times actual value (solid line); the increase post brain death does not achieve significance.

Figure 73. (Bottom)

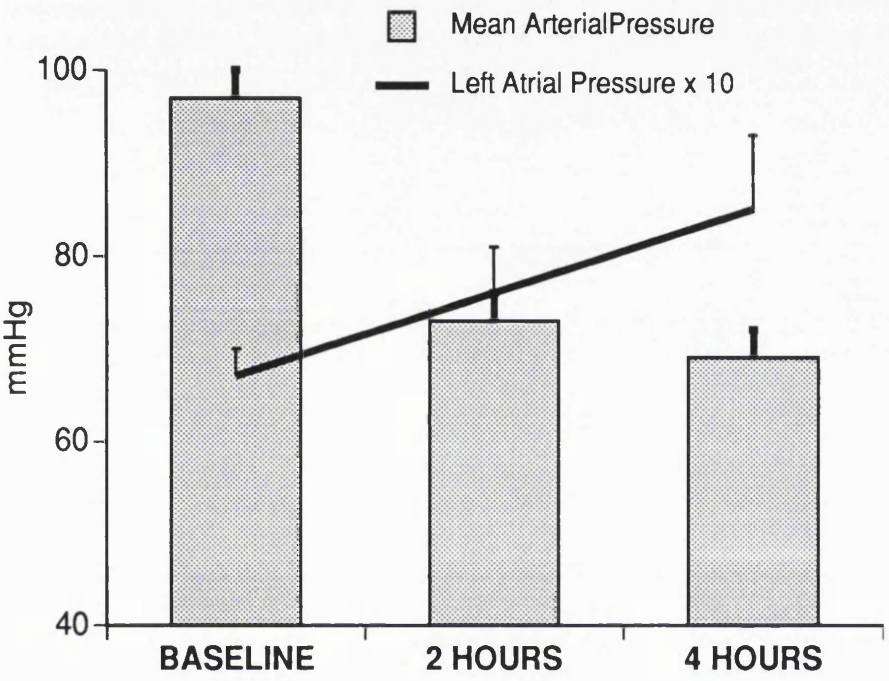
Haemodynamic profile post brain death;

Cardiac output and systemic vascular resistance. *±1sem.* (*n*=33).

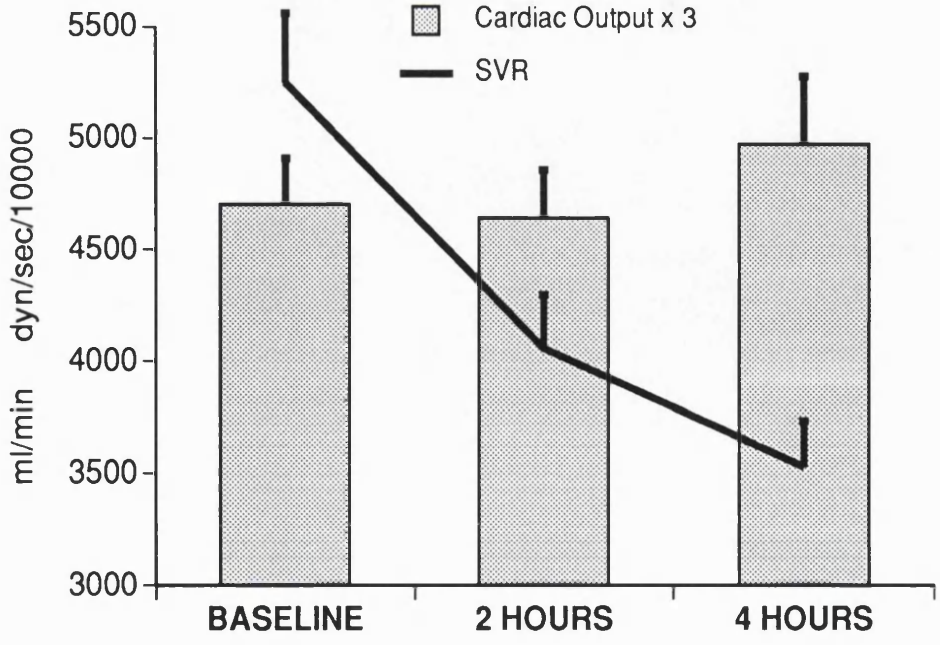
Cardiac output is depicted three times actual value (grey bars); there is no significant change in cardiac output.
Systemic vascular resistance significantly falls post brain death, probably due to denervation of the arterioles ($p<0.05$).

HAEMODYNAMIC PROFILE POST BRAIN DEATH

SYSTEMIC ARTERIAL AND LEFT ATRIAL PRESSURE



CARDIAC OUTPUT AND SVR



The mean pulmonary artery pressure rises significantly to 14.6 mmHg (±0.5) from 12.8 mmHg (±0.4). The pulmonary impedance profile does not change; input resistance 686 dyne.sec.cm^{-5} (±29) vs 738 dyne.sec.cm^{-5} (±41), pulmonary vascular resistance 326 dyne.sec.cm^{-5} (±23) vs 313 dyne.sec.cm^{-5} (±28) and characteristic impedance 178 dyne.sec.cm^{-5} (±8) vs 167 dyne.sec.cm^{-5} (±9) at baseline and four hours post brain death respectively.

Total hydraulic power from the right ventricle shows no significant change from baseline; 73.1 mW (±6.2) vs 67.2 mW (±6.2), nor is there any significant change in actual values for mean and oscillatory power. Figure 75 depicts the significant proportional change in the components of total power. Percentage oscillatory decreases post brain death from 32.6% (±1.0) to 25.1% (±1.1) while mean power increases from 67.4% to 74.9%. Despite less power being wasted in the pulmonary vasculature the transpulmonary efficiency remains unchanged at 2.62 J/L (±0.09).

Overall the pulmonary circulation is maintained in a relatively steady state compared to the systemic circulation. The injury to right ventricular systolic function is shown in the significant reduction of the pre load independent recruitable stroke work. At two hours this injury is more pronounced than for the left ventricle (65.6% (±5.1) vs 79.6% (±3.4)), but at four hours there is no significant difference between left and right ventricles (70.6% (±4.9) vs 78.8% (±4.3)) (figure 74). Despite this injury the pulmonary circulation is maintained, and the defect is exposed only when pulmonary impedance was acutely increased (chapter 9). Right ventricular function has been shown not to be essential for maintenance of satisfactory haemodynamics in prior studies (Santamore et al, 1976), and the results of this brain death group supports those conclusions. The right ventricle has a vital role only when the pulmonary impedance increases either in acute mitral incompetence, chronic left ventricular failure or post transplantation.

Figure 74. (Above)

Haemodynamic profile post brain death;

Pre load independent recruitable stroke work; left and right ventricles.

±1sem. (*n*=33).

The PRSW decreases significantly in left and right ventricles post brain death ($p<0.001$). At two hours right ventricular impairment is more severe, but at four hours there is no difference between left and right ventricles.

Figure 75. (Bottom)

Haemodynamic profile post brain death;

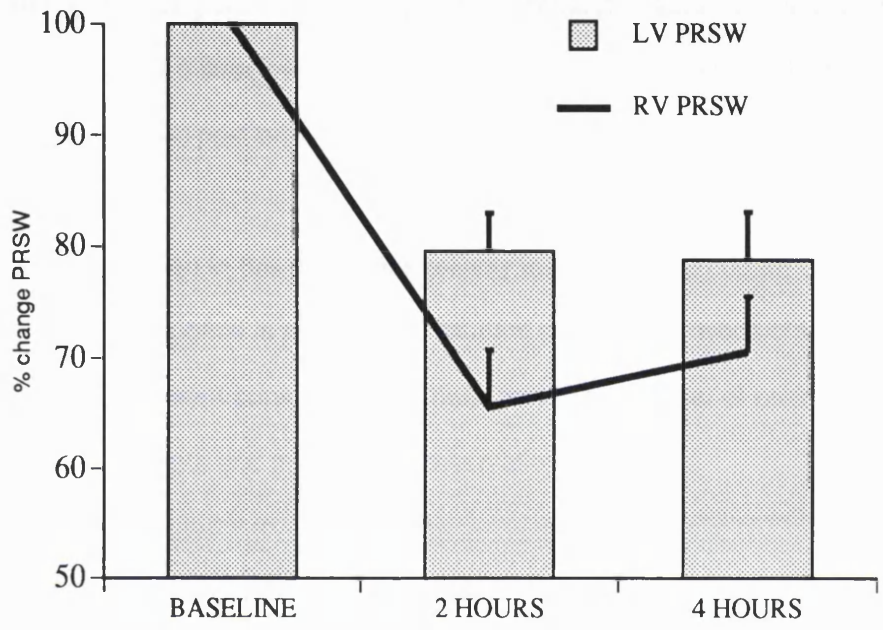
Right ventricular hydraulic power analysis . *±1sem.* (*n*=33).

Total power does not alter significantly post brain death.

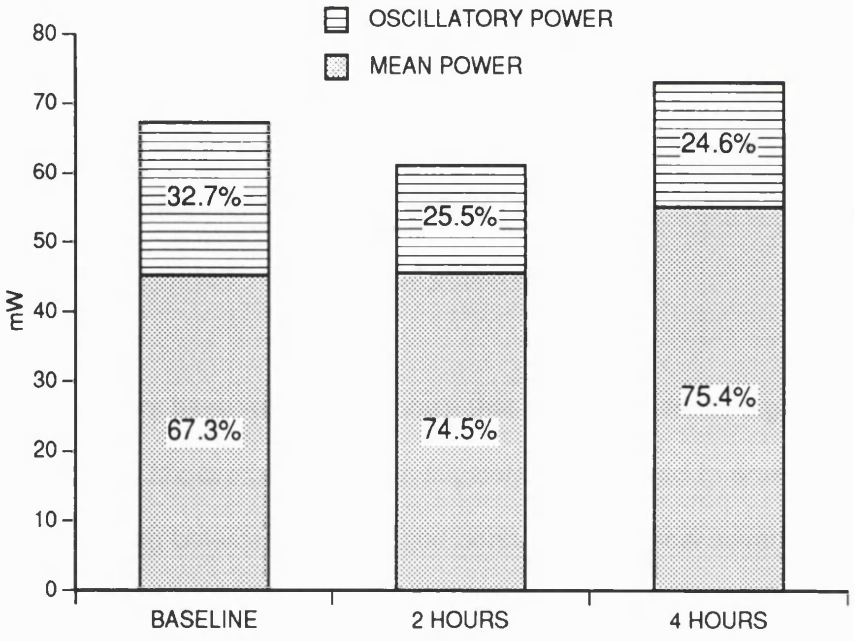
The percentage contribution of mean and oscillatory power significantly changed post brain death ($p<0.05$).

HAEMODYNAMIC PROFILE POST BRAIN DEATH

PRE LOAD INDEPENDENT RECRUITABLE STROKE WORK; LEFT AND RIGHT VENTRICLES



HYDRAULIC POWER ANALYSIS



The increase in haematocrit at fifteen minutes is significant ($p<0.0001$) becoming 45.2% (±1.1) from 35.5% (±0.7) and is almost certainly attributable to increased hydrostatic pressure during the hyperdynamic response. At four hours post brain death this has significantly decreased to 28.1% (±1.0) due in part to haemodilution by the 2.8 litres (±0.2) of intravenous infusion that was required to maintain ventricular filling pressure and systemic arterial pressure. On average this produced a positive fluid balance of 1.6 litres over four hours, with the polyuria resultant from diabetes insipidus making total urine excreted post brain death 1227 ml (±74).

Intracranial balloon inflation in this thesis has reliably instigated brain death and brain stem death. Although the Cushing response is severe, the resultant changes in myocardial, haemodynamic and hormonal function have been consistent, and thus provided a model of brain death to investigate the interaction of brain death and myocardial preservation.

Myocardial Preservation; General Considerations

Human orthotopic cardiac transplantation has been one of the pinnacles of medical achievement. It combines many discoveries from different areas of scientific research. In particular it unites therapeutic advances in immunosuppression with the biomechanical and biochemical knowledge to reliably perform cardiopulmonary bypass. Furthermore, unlike other solid organ transplantation, cardiac transplantation is dependent on immediate adequate donor organ function, as prolonged mechanical support predisposes to increased morbidity and mortality. The cardiac transplant surgeon therefore has paramount interest in optimal preservation, and it is they who have sought to improve the quality and duration of myocardial preservation.

Heart transplant centers have repeatedly shown that prolonged donor organ preservation beyond four hours significantly impairs outcome. Investigators have strived to prolong this maximum time, to allow tissue matching between recipient and donor; a theoretical advantage so as to decrease the chronic immune response and hence decrease the rate and incidence of coronary occlusive disease. Indeed the early research heightened expectations; notable contributions were from

Watson who reliably preserved canine hearts for twenty four hours, and in the primate Wicomb preserved baboon hearts for a similar period (Watson, 1977)(Wicomb et al, 1982). These results in animal models could not be applied to the clinical situation, and many researchers using different solutions, in differing combinations and differing temperatures, failed to make further progress.

In essence prolonged cardiac preservation relies on the cardiac myocyte maintaining cellular integrity, both physically and biochemically. Functional haemodynamic testing of hearts post preservation is a gross analysis to demonstrate cellular protection. The knowledge that cerebral events cause profound cardiac and haemodynamic alterations has been known since clinical cardiac transplantation began. Indeed, DePasquale wrote an editorial in 1969 for the American Heart Journal questioning the 'normality' of hearts from 'heart-beating' cadavers (DePasquale and Burch, 1969). Brain death may disrupt the myocyte to impair its preservation characteristics; whether this is resultant from the catecholamine surge or hormonal depletion requires further investigation.

Despite this insight by DePasquale, research continued in animal models using hearts from anaesthetised donors. The influences of brain death on myocyte function were not considered and the discrepancy of attainable preservation times persisted between animal research and clinical practice. Wicomb was the first to investigate this phenomenon; having worked successfully with 24 hour preservation followed by orthotopic transplantation in the baboon and also assessed brain death in the baboon caused by intracranial balloon inflation, he transferred his focus to the swine model. He also switched to carotid ligation to cause brain death and used the isolated heart preparation to assess function (Wicomb et al, 1986b). He showed brain death to impair left ventricular function, and that preservation reversed the anaerobic metabolism that had developed post brain death.

Six years later Galinanes used Wicomb's ideas and used the rat model; he concluded that brain death impaired left ventricular function, but after six hours preservation this had recovered, as

tested on an isolated preparation (Galinanes and Hearse, 1992). Shivalkar also concentrated on left ventricular function in the canine orthotopic transplant model and discovered a significant detrimental effect of rapid intracranial balloon inflation in the donor (Shivalkar et al, 1993). As already discussed this may be resultant of harvesting the donor heart soon after the Cushing response, during a period of haemodynamic and metabolic instability.

Preload independent measures of systolic function for left **and** right ventricles have not been used before to investigate the effect of brain death on myocardial function, nor assess post transplant cardiac function. This was successfully accomplished with reproducible data collection and analysis. Moreover the two way variate analysis of groups A, B, C and D allowed strong statistical comparisons to be made.

This thesis has furthered the understanding of both the direct effects of brain death on myocardial function and its interaction with prolonged myocardial preservation. The injury to the right ventricle due to brain death has previously been unrecognised and the superimposed injury to this ventricle during preservation is most interesting. Right ventricular dysfunction is most pertinent in the early post-implant period of clinical transplantation; that it may be attributable to the donor heart and not to a raised pulmonary vascular resistance in the recipient is a new concept for the transplant surgeon to consider.

Surgical Technique for Implantation

Dr. Shumway may possibly be regarded as the creator of cardiac transplantation. With Dr. Lower he overcame the technical difficulty of surgical implantation and, after the tragedies of the early clinical series, he persisted with thorough research at Stanford to develop techniques to diagnose and monitor rejection. When Borel discovered cyclosporin, Dr. Shumway had already laid the foundations for efficacious cardiac transplantation.

As a result 18,000 transplants have been performed worldwide with a five year survival of 60%

during which time quality of life is usually excellent. The vast majority have been implanted using the technique of Lower and Shumway, with bi-atrial anastomosis at mid-atrial level. Few problems exist with this method but the resultant large distorted atrial cavities do present some problems. Tricuspid and mitral insufficiency is frequent using this technique, as seen on Doppler echocardiography, probably due to geometrical distortion of the atrioventricular rings. Thrombus and emboli have been reported to originate from the atrial suture line and there is the theoretical impairment of atrial conduction and atrial systolic function.

Since Lower and Shumway's landmark paper in 1960 no further animal studies have assessed any different techniques for surgical implantation of the heart. Recently there have been several clinical reports of complete atrio-ventricular implantation, or combinations of the two methods (Kendall et al, 1993)(Yacoub and Banner, 1989) (Dreyfus et al, 1991) (Blanche et al, 1994). The cardiovascular laboratories in the Department of Surgery at Duke University Medical Center provide an ideal opportunity to compare these techniques in an animal model; the development of geometric ventricular volume models, sophisticated data analysis and the ability to apply the technology to analyse atrial systole, combine to create an environment suitable for such studies.

The conservation of sinus rhythm in the complete group was an outstanding finding. This phenomenon persisted in the subsequent twenty four transplants, although four hour preservation predisposed to a brief episode of ventricular pacing. This difference was probably apparent as no inotropes or chronotropic agents were used; had they been used, and the hearts in the standard group had displayed any of the sensitivity to ß-agonists as the group D hearts, then sinus rhythm may have resumed. Load dependent measures of cardiac function showed no significant differences between techniques, but the trend always favoured the complete method of implantation. This finding applied to the analysis of atrial systole and there were some statistical advantages in the complete group, for rate of atrial pressure change and rate of biventricular volume change.

The significant decrease in right ventricular PRSW almost certainly reflects distortion of the right ventricular and septal piezo-electric crystals, deceiving the geometric model that intra-ventricular volume increases significantly post transplant. However this does confirm the clinical impression that the standard technique disturbs cardiac geometry predisposing to valvular incompetence.

This study of complete implantation combines with the clinical studies to conclude this method is technically possible without increasing morbidity, mortality, ischaemic time and cardiopulmonary bypass time - the original motives for Lower and Shumway to describe their technique. Moreover this study has revealed some definite advantages in this technique, with no evidence of any drawbacks. Whether this evidence is strong enough to universally change implantation techniques is debatable; Lower's method works well for the majority of recipients. Complete implantation may only truly benefit those recipients who need as much function from their new heart as possible.

The author has little doubt that if Lower and Shumway had originally described complete atrioventricular transplantation, instead of their standard technique, it would be near impossible to convince surgeons to change to a method of transplantation that so disrupts atrial anatomy.

SUMMARY

The aim of the author was to complete a research project using an animal model that would be directly applicable to patient care. Therefore the work embodied in this thesis has addressed two areas of cardiac transplantation where there is scope to change clinical practise and improve outcome. It has required the use of almost 150 animals, in a laboratory with some of the most advanced methods of haemodynamic measurement, and has reached conclusions that have hitherto been undiscovered.

Fundamental to this project was the ability to assess the pre-load independent recruitable stroke work and hydraulic power of the right ventricle which has not previously been possible in models of orthotopic cardiac transplantation. Although the right ventricle has been identified as a source of

problems since the advent of clinical cardiac transplantation, only now can it be adequately investigated.

The results of over thirty animals, where brain death has been instigated, conclusively demonstrates a consistent injury to the left **and** right ventricles, which in the right ventricle is not apparent using load dependent analysis of function. Only when outflow resistance is increased, in the recipient or with pulmonary artery banding, does the injury reveal itself. In particular, prolonged preservation exaggerates the dysfunction of the right ventricle, which is identical to clinical procedure and clinical practise where right ventricular failure is frequent post implantation.

It has been fortunate to have combined the investigation of brain death with a comparison of surgical techniques to implant the heart. This latter study revealed deficiencies in right ventricular volume analysis post transplant using the standard technique. Therefore complete implantation was used successfully in the remaining studies providing reliable pressure volume relationships in the right ventricle.

The analysis of complete atrioventricular transplantation in this thesis is the first comparative animal study of surgical technique for implantation, and the first study since Lower's work to assess a new surgical technique for animal orthotopic transplantation. The results demonstrate clear advantages by implanting the intact heart, particularly in conserving sinus rhythm. The role of this technique in clinical practise requires further evaluation.

CONCLUSIONS

The following conclusions are the main significant findings of this thesis, encompassing the broader issues that have been investigated.

(1) Inflation of an intracranial balloon has been validated to reliably cause brain stem death in the canine model.

(2) In this model of brain death, left ventricular function is impaired. Moreover, the function of the **right** ventricle is significantly impaired, not previously recognised.

(3) The injury to the right ventricle is clinically inconspicuous, until an acute increase of pulmonary vascular impedance causes significant right ventricular failure.

(4) Right ventricular injury caused by brain death is further significantly increased by four hour preservation prior to transplantation; the extent of this right ventricular injury is such that inotropic support is required to wean the recipient from cardiopulmonary bypass.

(5) The shell subtraction model, to calculate intraventricular volumes for the right ventricle, may be applied to orthotopic transplantation pre and post implantation. Using complete atrioventricular implantation the data remains consistent and reliable post transplant.

(6) In the canine model of orthotopic cardiac transplantation complete atrioventricular transplantation is a superior method to the standard technique, originally described by Lower and Shumway. Conservation of the integrity of left and right atria maintains sinus rhythm post transplant, and significantly preserves certain aspects of atrial systolic function.

Areas for Future Research

The work embodied in this thesis has examined two areas of cardiac transplantation that have, to some extent, been overlooked by previous researchers; the role of brain death in prolonged myocardial preservation and the surgical technique for orthotopic transplantation. For this reason this work leaves many fruitful topics for future work. In particular the discovery that the right ventricle is subject to dysfunction after brain death opens a previously untouched subject in cardiac research.

The author has employed techniques of haemodynamic measurement, developed in the Department of Surgery at Duke Medical Center, to study orthotopic transplantation. These techniques have developed from initial studies of left ventricular function and have been refined to include analysis of right ventricular function. They rely on the application of tested instrumentation and validated mathematical models of ventricular volume, coupled to recent developments in microcomputers that can handle the necessary computations. Haemodynamic studies can now reliably include assessment of right ventricular function which has previously been unexamined.

Therefore this thesis has tended to assess myocyte cellular function by using functional observations of the ventricles. In considering future areas of research it is important to also concentrate on the cellular and molecular level. The following ideas are presented as interventions in chronological order of the brain death / transplant preparation.

(1). What is the role of the catecholamine surge in inflicting ventricular injury, and is it the same for left and right ventricles? Prior work by Novitzky, 1984, has shown sympathectomy prior to induction to brain death to decrease the myocardial injury. This technique could be applied to the canine model, coupled with studies utilising a short acting ß-blocker prior to brain death and high dose catecholamine infusions to induce a Cushing reflex without brain death. Analysis of bi-ventricular function might show the role of the catecholamine surge in causing ventricular impairment.

(2). Does hormone replacement post brain death reduce left and right ventricular injury, and does it improve the haemodynamic status of the donor to maintain adequate perfusion of the donor organs? This area of research is controversial with conflicting evidence of the efficacy of hormone supplementation in organ donors. The validated model of brain death in this thesis showed significant reduction of certain hormones, and could be used to assess the role of, for instance, vasopressin, cortisol, thyroxine and insulin in donor management. These are hormones that have been used in clinical trials and have not been fully assessed in animal models. In combining the findings from catecholamine research and this hormone research, the causes of ventricular dysfunction post brain death may become apparent.

(3). What changes occur in the vascular endothelium post brain death? The detrimental effect of brain death on myocardial preservation might be mediated by changes in the endothelium. A study of coronary artery endothelium pre and post brain death in terms of function and histological changes may provide insight to preservation characteristics. This work may also address the changes to cardiac myocyte phosphates.

(4). What is the influence of renal dose dopamine post brain death on cardiac function pre and post transplantation? It is now established clinical practice to maintain the organ donor on low dose dopamine to maintain satisfactory haemodynamics and urine output. This thesis has shown consistent changes to ß-receptor status post brain death, and markedly increased ß-receptor sensitisation post transplant. Using haemodynamic functional testing and assays of ß-receptor function the influence of dopamine, post brain death, on the heart could reveal significantly altered responses from those seen in this thesis where inotropic agents were avoided.

(5). What is the optimal preservation solution for the heart from a brain dead donor? The results from this thesis combines with previous work utilising brain dead animal donors to show hearts from these animals have unique preservation characteristics. The hearts from anaesthetised animals are much more robust, demonstrated by the numerous hypothermic solutions that have successfully

preserved them for twenty four hours or longer. This latter research needs to be repeated using 'brain dead' hearts and should incorporate studies of right ventricular function.

(6). Why is the right ventricle is more sensitive than the left ventricle to injury with the interaction of brain death and prolonged preservation? The smaller free wall volume of the right ventricle, coupled to the lower pressures it generates, makes it difficult to explain its predisposition to injury post brain death. Work in this area will have to wait until further research reveals the cause and nature of this injury.

(7). Can significant right ventricular function be recognised prior to implantation? Right ventricular dysfunction is a persistent problem in transplant programs causing significant early morbidity and mortality. If the results of this animal model represents clinical events, the diagnosis of significant ventricular injury would be an important preventative measure. If the cause of the injury is discovered, chemical or histological markers might be utilised to gauge the severity of ventricular impairment. Functional testing in the donor, by inflicting an acute rise of pulmonary impedance, is probably unjustified as the significant injury only occurs after prolonged preservation. The eventual outcome is also dependent on the pulmonary vascular resistance in the recipient and careful consideration is required before rejecting a valuable donor heart, based solely an isolated measure of right ventricular function.

(8). Does complete atrioventricular transplantation confer any advantages in clinical transplantation? The clinical trials to date investigating complete atrioventricular implantation have not demonstrated clear advantages over the standard technique. This is understandable, with small numbers of patients and a heterogeneous collection of recipients and donor hearts. Persistence is required to achieve large enough numbers of patients for statistical comparison. Furthermore, functional assessment is difficult as it requires patient co-operation and should be minimally invasive. Total oxygen consumption during exercise may be the best indicator of overall cardiac function, performed at least one year post transplant.

APPENDIX 1.

DRUGS EMPLOYED IN EXPERIMENTS

Amidate - Etomidate. An induction agent of general anaesthesia. In humans suppresses adrenocortical function for up to 8 hours. Manufactured by Abbott Laboratories, North Chicago. IL.. 60064 USA.

Atropine - Atropine sulphate injection, USP. An anticholinergic agent - by inhibition of smooth muscle and glands innervated by postganglionic cholinergic nerves. Manufactured by Elkins-Sinn, Inc., Cherry Hill, NJ. 08004 USA.

Calcium Chloride - 10mg/10ml water, for injection. American Regent Laboratories, Inc. Subsidiary of Pharmaceuticals Inc. Shirley, NY. 11967 USA.

Combiotic - Combiotic. Penicillin and Dihydrostreptomycin in aqueous suspension for veterinary use. A highly effective combination antimicrobial preparation containing procaine penicillin and dihydrostreptomycin the sulphate. Distributed by the Department of Veterinary Medicine, Pfizer Inc., New York 10017 USA.

Cyclosporine - A lipophilic cyclic polypeptide the fungus that acts by impairing the action of interleukin-2 on T-cells in cell mediated immunity. Now the primary drug in immunosuppression in solid organ transplantation. Sandoz Pharmaceuticals Corporation. East Hanover, NJ. 07936 USA.

Diazepam - Diazepam CIV inj USP. Potent benzodiazepine for intravenous or oral use to obtain degrees of CNS suppression. Manufactured by Elkins-Sin, Inc., Cherry Hill, N.J. 08034 USA.

Dopamine - A beta-receptor agonist and dopamine receptor agonist. In low dosage a renal vasodilator but in higher doses a generalised vasoconstrictor. Gensia Laboratories Inc, Irvine, CA. 92718 USA.

Electrode Gel - Chieftain Ultrasound Transmission Gel. A water soluble contact medium for ultrasonic transmission, used primarily in these experiments to provide electrical "coupling" around the Transonic ultrasound flow probe. Distributed by Baxter Healthcare Corporation-Hospital Supply Division. Deerfield, IL 60015 USA.

Fentanyl - Fentanyl citrate injection, USP CII. Potent narcotic analgesic. Manufactured by Elkins-Sin, Inc., Cherry Hill, N.J. 08034 USA.

Frusemide - Equi-phar 5% for injection (for veterinary use only). Powerful loop diuretic and weak pulmonary vasodilator plus venodilator. Distributed by Vedco, Inc. St. Joseph, MO, 64504 USA.

Gentamycin - Gentamycin sulphate injection, USP. A broad spectrum, widely used antibiotic. Manufactured by Elkins-Sinn, Inc., Cherry Hill, N.J. 08034 USA.

Heparin - Heparin sodium injection, USP. A widely used glycosaminoglycan having anticoagulant properties. It inhibits reactions that lead to the clotting of blood and the formation of fibrin clots both in *vitro* and in *vivo*. Manufactured by Elkins-Sinn, Inc., Cherry Hill, N.J. 08034 USA.

Imuran - Azathioprine tablets. An immunosuppressant agent (a thiopurine) that inhibits purine synthesis. This makes it a myelosuppressive agent inhibiting the generation of T cells in allograft rejection. Burroughs Wellcome. Research Triangle Park, NC. 27709 USA.

Ketaset - Ketamine hydrochloride injection, USP. A rapid acting, non-narcotic, non-barbiturate anaesthetic agent. Distributed by Aveco Co., Inc. 800 Fifth St. N.W. Fort Dodge, Iowa 50501.

Lactated Ringer's Solution - Intravenous volume replacement. (273 osmol/L, pH 6.0-7.5, NaCl 600mg/dl, KCl 30mg/dl.) Manufactured by Abbott Laboratories, North Chicago, IL 60064, USA.

Levophed - Levophed Bitartate. A brand of norepinephrine bitartrate injection, USP (Noradrenaline). Functions primarily as a powerful peripheral vasoconstrictor (alpha-adrenergic action) and secondarily as an inotropic stimulator of the heart and dilator of the coronary arteries (beta-adrenergic action). Manufactured by Winthrop Pharmaceuticals. Division of Sterling Drug Inc., New York, N.Y. 10016.

Lidocaine - Lignocaine 100mg/10ml for injection USP. A local anaesthetic and antiarrhythmic agent due to its effect of stabilising membrane potentials. Used for the prevention of acute ventricular tachyarrhythmias. Abbott Laboratories, Chicago, IL. 60064 USA.

Mannitol - 20% Mannitol for injection. Osmotic diuretic agent and free oxygen radical scavenger. American Regent Laboratories, Inc. Subsidiary of Luitpold Pharmaceuticals Inc. Shirley, NY. 11967 USA.

Methylprednisolone - Sodium succinate for injection. Immunosuppression drug and anti-inflammatory agent. Upjohn Co, Kalamazoo, MI. 49001 USA.

Morphine - Morphine Sulphate for injection USP. Narcotic analgesic used intravenously or intramuscularly. Manufactured by Elkins-Sin, Inc., Cherry Hill, N.J. 08034 USA.

Normasol - Normasol R solution, pH 7.4; Normal saline supplemented with sodium bicarbonate to normalise pH. Manufactured by Abbott Laboratories, North Chicago, IL 60064, USA.

Pavulon - Pancuronium bromide is a long acting, non depolarising, neuromuscular blocking agent. Competes with acetylcholine for binding sites on the post-junctional receptor; depolarisation does not occur and paralysis ensues. Gensia Laboratories Inc, Irvine, CA. 92718 USA.

Pentothal - Thiopental sodium for injection, USP. A thiobarbiturate ultra-short acting depressant on the central nervous system which induces hypnosis and anaesthesia, but not analgesia. Manufactured by Abbott Laboratories, North Chicago, IL 60064, USA.

Phenylephrine - 1% for injection USP (adrenaline). Vasopressive agent and cardiac inotrope via ß adrenergic stimulation. Manufactured by Elkins-Sinn, Inc., Cherry Hill, N.J. 08034 USA.

Potassium for injection - potassium chloride. American Regent Laboratories, Inc. Subsidiary of Luitpold Pharmaceuticals Inc. Shirley, NY. 11967 USA.

Promace - Acepromazine maleate injection. A potent neuroleptic agent with a low toxicity, and rapid action. Manufactured by Ayerst Laboratories, Inc., New York, NY 10017. Distributed by Aveco Co., Inc. 800 Fifth St. N.W. Fort Dodge, Iowa 50501.

Sodium Bicarbonate - 8.4% sodium bicarbonate for injection. Used intravenously in slow bolus injection for correction of acid - base deficits caused by metabolic acidosis. Manufactured by Abbott Laboratories, North Chicago, IL 60064, USA.

Succinylcholine - Quelcin; succinylcholine chloride injection. A short acting, depolarising, neuromuscular blocking agent that mimics the action of acetyl choline. Manufactured by Abbott Laboratories, North Chicago, IL 60064, USA.

APPENDIX 2.

COST OF THESIS.

Salaries:		
Research Fellow 1	$ 35,000	
Research Fellow 2	$ 33,000	
Research Specialist	$ 26,000	
Engineer / Scientist (part-time)	$ 3,000	
Senior Research Analyst (part-time)	$ 5,000	
		$ 102,000
Animals:		
145 dogs @ $350 each	$ 50,750	
3 pigs @ $100 each	$ 300	
		$ 51,050
Drugs:		
58 transplants @ $180 each	$ 10,440	
29 single animal expts @ $30 each	$ 870	
		$ 11,310
Consumables per experiment:		
58 transplants @ $300 each	$ 17,400	
29 single animal expts @ $75 each	$ 2,175	
		$ 19,575
Laboratory investigations:		
B-receptor studies	$ 8,500	
Blood gas analysis	$ 2,175	
Histopathology	$ 1,350	
Hormone studies	$ 9,500	
		$ 23,700
Thesis preparation:		
Artwork	$ 400	
Photography	$ 1,380	
Binding	$ 280	
		$ 2,060
Total:		$ 209,695

REFERENCES

Abel FL 1981 Maximum negative dP/dt as an indicator of end systole. American Journal of Physiology 240: H676-679.

Angerman CE, Spes CH, Tammew A 1990 Anatomic characteristics and valvular function of the transplanted heart: transthoracic versus transoesophageal echocardiographic findings. Journal of Heart Transplantation 9: 331-338.

Arts T, Veenstra PC, Reneman RS 1982 Epicardial deformation and left ventricular wall mechanics during ejection in the dog. American Journal of Physiology 243: H379-88.

Attinger EO, Anne A, McDonald DA 1966 Use of Fourier series for the analysis of biological systems. Biophysics Journal 6: 291-304.

Bart KJ, Macon EJ, Humphries AL, Baldwin RJ, Fitch T, Pope RS, Rich MJ, Langford D, Teutsch SM, Blount JH 1981 Increasing the supply of cadaveric kidneys for transplantation. Transplantation 31: 383-387.

Baumgartner WA, Reitz BA, Bieber CP, Oyer PE, Shumway NE, Stinson EB 1978 Current expectations in cardiac transplantation. Journal of Thoracic and Cardiovascular Surgery 75: 525-527.

Belzer FO, Southard JH 1988 Principles of solid-organ preservation by cold storage. Transplatation 45: 673-676.

Bergel DH, Milnor WR 1965 Pulmonary vascular impedance in the dog. Circulation Research 6: 401-415.

Bethencourt DM, Laks H 1981 Importance of edema and compliance changes during 24 hours of preservation of the dog heart. Journal of Thoracic and Cardiovascular Surgery 81: 440-449.

Bhatia SJS, Kirshenbaum JM, Shemin RJ, Cohn LH, Collins JJ 1987 Time course of resolution of pulmonary hypertension and right ventricular remodeling after orthotopic cardiac transplantation. Circulation 76: 819-826.

Billingham ME, Baumgartner WA, Watson DC, Reitz BA, Masek MA, Shumway NE 1980 Distant heart procurement for human transplantation. Ultrastructural studies. Circulation 62: 11-19.

Bizouarn P, Treilhaud M, Portier D, Train M, Michaud M 1994 Right ventricular function early after total or standard orthotopic heart transplantation. Annals of Thoracic Surgery 57: 183-187.

Blackman RB, Tukey JW 1958 The measurement of power spectra from the point of view of communications engineering. Dover Publications, New York.

Blaine EM, Tallman RD, Frolicher D, Jordan MA, Bluth LL, Howie MB 1984 Vasopressin supplementation in a porcine model of brain-dead potential organ donors. Transplantation 38: 459-464.

Blanche C, Czer LSC, Valenza M, Trento A 1994 Alternative technique for orthotopic heart transplantation. Annals of Thoracic Surgery 57: 765-767.

Blumenstock DA 1963 Prolonged survival of orthotopic homotransplants of the heart in animals treated with methotrexate. Journal of Thoracic and Cardiovascular Surgery 46: 616-622.

Bodenham A, Park GR 1989 Care of the multiple organ donor. Intensive Care Medicine 15: 340-346.

Borel J, Feurer C, Gubler HU, Stanhelin H 1976 Biological effects of cyclospiorin A: a new lymphocytic agent. Agents and Actions 6: 468-470.

Burch GE, Meyers R, Abildskov JA 1954 A new electrocardiographic pattern observed in cerebrovascular accidents. Circulation 9: 719-723.

Burt JM, Copeland JG 1986a Myocardial function after preservation for 24 hours. Journal of Thoracic and Cardiovascular Surgery 92: 238-246.

Calne RY, White DJG, Thiru S 1978 Cyclosporin A in patients receiving renal allografts from cadaver donors. Lancet 2: 1323-1327.

Caro CG, McDonald DA 1961 The relation of pulsatile pressure and flow in the pulmonary vascular bed. Journal of Physiology 157: 426-453.

Carrel A, Guthrie CC 1905 The transplantation of veins and organs. American Journal of Medicine 10: 1101-1109.

Cass MH, Brock R 1959 Heart excision and replacement. Guy's Hospital Reports 108: 285-90.

Caturla MC, Masotti M, Brodde OE, Hjemdahl P, Velasquez D, Tura A, Crexells C 1992 Development of Myocardial ß-adrenergic receptor density and adenylate cyclase activity after human heart transplantation. Journal of Heart and Lung Transplantation 11: 1059-1065.

Caves PK, Stinson EB, Billingham ME, Rider AK, Shumway NE 1973 Diagnosis of human cardiac allograft rejection by serial cardiac biopsy. Journal of Thoracic and Cardiovascular Surgery 66: 461-468.

Chapman CB, Mitchell JH 1965 Starling on the heart. Dawsons of Pall Mall, London.

Chappel CI, Rona G, Balazs T, Gaudry R 1959 Comparison of cardiotoxic actions of certain sympathomimetic amines. Canadian Journal of Biochemistry and Physiology 37: 35-42.

Choong YS, Gavin JB 1991 Functional recovery of hearts after cardioplegia and storage in University of Wisconsin and in St. Thomas' Hospital solutions. Journal of Heart and Lung Transplantation 10: 537-546.

Cooper DKC, de Villiers JC, Smith LS, Barnard CN 1982 Medical, legal and administrative aspects of cadaveric organ donation in the Republic of South Africa. South African Medical Journal 62: 933-938.

Cooper DKC, Wicomb WN, Rose AG, Barnard CN 1983 Orthotopic allotransplantation and autotransplantation of the baboon heart following 24-hr storage by a portable hypothermic perfusion system. Cryobiology 20: 385-394.

Copeland JG, Jones M, Spragg R, Stinson EB 1973 In vitro preservation of canine hearts for 24 to 48 hours followed by successful orthotopic transplantation. Annals of Surgery 178: 687-692.

Cournand A 1964 Air and blood. In: Fishman, A Richards, D (eds) Circulation of the blood. Oxford University Press, New York p.3-7.

Cowley AW Jr (ed) 1982 Vasopressin and cardiovascular regulation: cardiovascular physiology 1V, University Park Press: Baltimore.

Cushing H 1901 Concerning a definite regulatory mechanism of the vascular center which controls blood pressure during cerebral compression. Bulletin from Johns Hopkins Hospital 12: 290-2.

Damiano RJ, Follette PL, Cox JL, Lowe JE, Santamore WP 1991 Significant left ventricular contribution to right ventricular systolic function. American Journal of Physiology 261: H1514-1524.

Darby JM, Stein K, Grenvik A, Stuart SA 1989 Approach to management of the heart-beating 'brain dead' organ donor. Journal of American Medical Association 261: 2222-2228.

Davis AO, Lefkowitz RJ 1984 Regulation of B-adrenergic receptors by steroid hormones. Annals of Revised Physiology 46: 119-130.

Demikhov VP 1960 Some essential points of the technique of transplantation of heart and lungs and other organs. In: (eds) Experimental transplantation of vital organs. Medgiz, State Press for Medical Literature, Moscow p.29-48.

DePasquale NP, Burch GE 1969 How normal is the donor heart. American Heart Journal 77: 719-720.

Dogterom J, van Wimersa B, Greidanus TJ, Dewied D 1978 Vasopressin in cerebrospinal fluid and plasma of man, dog, and rat. American Journal of Physiology 234: E463-E467.

Dreyfus G, Jebara V, Mihaileanu S, Carpentier AF 1991 Total orthotopic heart transplantation: an alternative to the standard technique. Annals of Thoracic Surgery 52: 1181-4.

Elbeery JR, Lucke JC, Speier R, Rankin JS, VanTrigt P 1991 Analysis of myocardial function in orthotopic cardiac allografts after prolonged storage in UW lactobionate solution: comparison with standard techniques. Journal of Heart and Lung Transplantation 10: 527-536.

Emery RW, Cork RC, Levinson MM, Riley JE, Copeland J, McAleer J, Copeland JG 1986 The cardiac donor: a six year experience. Annals of Thoracic Surgery 41: 356-362.

Feemster JA, Lillehei RC 1969 Hypothermic-hyperbaric pulsatile perfusion for preservation of the canine heart. Transplantation Proceedings 1: 138-143.

Feneley MP, Elbeery JR, Gaynor JW, Gall SA, Davis JW, Rankin JS 1990 Ellipsoidal shell subtraction model of right ventricular volume. Circulation research 67: 1427-36.

Feneley MP, Gaynor JW, Maier GW, GAll SA, Kisslo JA, Rankin JS 1988 In vivo estimation of left ventricular wall volume in volume-overloaded canine hearts. American Journal of Physiology 255: H1399-H1404.

Feneley MP, Maier GW, Gaynor JW, Gall SA, Davis JW, Rankin JS 1987 An ellipsoidal shell subtraction model for determining right ventricular volume. Journal of American College of Cardiology 9: A214-25.

Feneley MP, Olsen CO, Glower DD, Rankin JS 1989 Effect of acutely increased right ventricular afterload on work output from the left ventricle in conscious dogs. Circulation Research 65: 135-45.

Finkelstein I, Toledo-Preyra LH, Castellanos J 1987 Physiologic and hormonal changes in experimentally induced brain dead dogs. Transplantation Proceedings. 19: 4156-4158.

Fortin TL, Sundaresan PR 1989 Reserpine but not surgical denervation regulates rat renal B-adrenergic receptors. American Journal of Physiology 256: F532-9.

Fowler MB, Bristow MR, Laser JA, Ginsburg R, Scott LB, Schroeder JS 1984 Beta blocker therapy in severe heart failure; improvement related to beta-adrenergic receptor up-regulation? Circulation 70: 112.

Fukushima N, Shirakura R, Nakata S, Kaneko M, Sawa Y, Matsuwaka R, Naka Y, Matsumiya G, Matsuda H 1992 Effects of terminal cardioplegia with leukocyte-depleted blood on heart grafts preserved for 24 hours. Journal of Heart and Lung Transplantation 11: 676-682.

Galinanes M, Hearse DJ 1992 Brain death - induced impaiment of cardiac contractile performance can be reversed by explantation and may not preclude the use of hearts for transplantation. Circulation Research 71: 1213-1219.

Gaskill HV, Sirinek KR, Levine BA 1983 Haemodynamic effects of vasopressin. Archives of Surgery 118: 434-437.

Gharib H, Horn DB 1972 Triiodothyronine radioimmunoassay, a critical evaluation. Mayo Clinical Proceedings 47: 934-937.

Gilbert EM, Eiswirth CC, Mealey PC, Larrabee BS, Herrick CM, Bristow MR 1989 B-Adrenergic supersenstivity of the transplanted human heart is presynaptic in origin. Circulation 79: 344-349.

Glaubiger G, Tsai BS, Lefkowitz RJ 1978 Chronic guanethidine treatment increases cardiac B-adrenergic receptors. Nature 273: 240-242.

Glower DD, Spratt JA, Snow ND, Kabas JS, Davis JW, Olsen CO, Tyson GS, Sabiston DC, Rankin JS 1985 Linearity of the Frank-Starling relationship in the intact heart: the concept of preload recruitable stroke work. Ciculation 71: 994-1009.

Gott JP, Pan-Chih, Dorsey LMA, Cheung EH, Hatcher CR, Guyton RA 1990 Cardioplegia for transplantation: failure of extracellular solution compared with Stanford or UW solution. Annals of Thoracic Surgery 50: 348-354.

Gramm HJ, Meinhold H, Bickel U, Zimmermann J, Hammerstein B, Keller F, Dennhardt R, Voigt K 1992 Acute endocrine failure after brain death? Transplantation 54: 851-857.

Greenberg JJ, Edmonds LH Jr 1961 Effect of myocardial ischaemia at varying temperatures on left ventricular function and tissue oxygen tension. Journal of Thoracic and Cardiovascular Surgery 42: 84-90.

Greenshoot JH, Reichenbach DD 1969 Cardiac injury and subarachnoid haemmorrhage: a clinical and physiological correlation. Journal of Neurosurgery 30: 521-531.

Guerraty A, Alivizatos P, Warner M, Hess M, Allen L, Lower RR 1981 Successful orthotopic canine heart transplantation after 24 hours of in vitro preservation. Journal of Thoracic and Cardiovascular Surgery 82: 531-537.

Haas M, Glick SM 1978 Plasma vasopressin response to surgery. Archives of Surgery 113: 597-599.

Hairston P 1965 Heart transplantation: past, present and future. Journal of Thoracic and Cardiovascular Surgery 50: 1-3.

Hales S 1733 Statical essays: Containing haemastaticks. In: Library of New York, A o M (eds) History of Medicine Series. Hafner Publishing, New York

Hamill RW, Woolf PD, McDonald JV, Lee LA, Kelly M 1987 Catecholamines predict outcome in traumatic brain injury. Annals of Neurology 21: 438-43.

Hamilton WF, Richards DW 1964 The output of the heart. In: Fishman, A Richards, D (eds) Circulation of the blood. Oxford University Press, New York p.71-125.

Hamm DP, Everson CT, Freedman BM, Pellom GL, Christian C, Wechsler AS 1984 The passive right ventricular volume - dimension relationship in the isolated canine heart. Surgical forum 35: 266-267.

Hammon JH Jr, Smith PK, Hopkins RA, McHale PA, Anderson RW 1977 The effect of elevated left atrial pressure on pulmonary haemodynamics in the awake dog. Circulation 56: 163.

Hammon JH Jr, Smith PK, McHale PA, Van Benthhuysen KM, Anderson RW 1981 Analysis of pulsatile pulmonary artery blood flow in the unanaesthetized dog. Journal of Applied Physiology 50: 805-813.

Hartman J, Koerner J, Lancaster L, Gorczynski R 1985 In vivo calibration of a transit-time ultrasound system for measuring ascending aorta volume flow. The Pharmacologist 27: 217-223.

Harvey W 1628 Exercitatio anatomica de motu cordis et sanguinis in animalibus (translated from Latin by CD Leake). Thomas, Springfield.

Hiatt HH, Lowis S 1957 Diabetes insipidus following head injury. Archives of Internal Medicine 100: 143-146.

Imperial ES, Levy MN, Zieske H Jr 1961 Outflow resistance as an independant determinant of cardiac performance. Circulation Research 9: 1148-56.

Jeevanandam V, Auteri JS, Sanchez JA, Hsu D, Marboe C, Smith CR, Rose EA 1992a Cardiac transplantation after prolonged graft preservation with the University of Wisconsin solution. Journal of Thoracic and Cardiovascular Surgery 104: 224-228.

Jeevanandam V, Barr ML, Auteri JS, Sanchez JA, Fong J, Schenkel FA, Marboe CC, Michler RE, Smith CR, Rose EA 1992b University of Wisconsin solution versus crystalloid cardioplegia for human donor heart transplantation. A randomized blinded prospective clinical trial. Journal of Thoracic and Cardiovascular Surgery 103: 194-199.

Jenkins AM 1976 Causes of failure to harvest cadaver kidneys for transplantation. British Medical Journal 1: 816-817.

Johnson RE, Dorsey LM, Moye SJ, Hatcher CR, Guyton RA 1982 Cardioplegic infusion. The safe limits of pressure and temperature. Journal of Thoracic and Cardiovascular Surgery 83: 813-823.

Judson JF 1979 The eighth day of creation. Simon & Shuster, New York.

Karch SB, Billingham ME 1986 Myocardial contraction bands revisited. Human Pathology 17: 9-13.

Kaye MP, McDonald RH, Randall WC 1961 Systolic hypertension and subendocardial haemorrhages produced by electrical stimulation of the stellate ganglion. Circulation Research 9: 1164-1170.

Kendall SW, Ciulli F, Biocina B, Mullins PA, Schofield P, Wells FC, Wallwork J, Large SR 1993 Atrioventricular orthotopic heart transplantation: A prospective randomised clinical trial in 60 consecutive patients. Transplantation Proceedings 25: 1172-1173.

Kilts C 1983 High performance liquid chromatography with electrochemical detection for quantification of plasma catecholamines. Journal of Neuroscience Methods 11: 257-273.

Kirklin JK, Naftel DC, Kirklin JW, Blackstone EH, White-Williams C, Bourge RC 1988 Pulmonary vascular resistance and the risk of heart transplantation. Journal of heart transplantation 7: 331-335.

Kolin A, Norris JW 1984 Myocardial damage from acute cerebral lesions. Stroke 15: 990-993.

Kosek JC, Hurley EJ, Lower RR 1968 Histopathology of orthotopic canine cardiac homografts. Laboratory Investigation 19: 97-104.

Koskelo P, Punsar S, Sipila V 1964 Subendocardial haemmorrhage and E.C.G. changes in intracranial bleeding. British Medical Journal 1: 1479-1480.

Krukencamp IB, Silverman NA, Pridjian A, Levitsky S 1987 Correlation between the linearized Frank-Starling relationship and myocardial energetics in the ejecting heart. Journal of Thoracic and Cardiovascular Surgery 93: 728-740.

Lamb H 1898 On the velocity of sound in a tube as effected by the elasticity of the walls. Manchester Reports 42: 1-16.

Lanza RP, Cooper DKC, Barnard CN 1984 Lack of efficacy of high-dose verapamil in preventing brain damage in baboons and pigs after prolonged partial cerebral ischaemia. American Journal of Emergency Medicine 2: 481-485.

Leestma JE, Hughes JR, Diamond ER 1984 Temporal correlates in brain death. EEG and clinical relationships to the respirator brain. Archives of Neurology 41: 147-152.

Limas C, Limas C 1984 Rapid recovery of cardiac B-adrenergic receptors after isoproterenol-induced "down"-regulation. Circulation Research 55: 524-531.

Lower RR, Dong E Jr, Glazener FS 1966 Electrocardiograms of dogs with heart homografts. Circulation 33: 455-460.

Lower RR, Dong E Jr, Shumway NE 1965 Long-term survival of cardiac homografts. Surgery 58: 110-116.

Lower RR, Shumway NE 1960 Studies on orthotopic transplantation of the canine heart. Surgical Forum 11: 18-22.

Mahler F, Covell JW, Ross J Jr 1975 Systolic pressure-diameter relations in the normal conscious dog. Cardiovascular Research 9: 447-452.

Mann FC, Priestly JR, Markowitz J, Yates WM 1933 Transplantation of the intact mammalian heart. Archives of Surgery 26: 219-225.

Masson F, Thicoipe M, Latapie MJ, Maurette P 1990 Thyroid function in brain dead donors. Transplant International 3: 226-233.

McDonald DA 1955 The relation of pulsatile pressure to flow in arteries. Journal of Physiology 127: 533-552.

McDonald DA 1974 Blood Flow in Arteries. Williams & Wilkins, Baltimore, Maryland.

McLaughlin RF, Tyler WS, Canada RO 1961 Subgross pulmonary and cardiac anatomy in various mammals. American Journal of Anatomy 108: 149-165.

Melvin JE, Xinjun HE, Baum BJ 1988 Sympathetic denervation fails to produce beta adrenergic supersensitivity in adult rat parotid gland. Journal of Pharmacological and Experimental Therapy 246: 935-40.

Meyers CH, D'Amico TA, Peterseim DS, Jayawant AM, Steenbergen C, Sabiston DC, Van Trigt P 1993 Effects of triiodothyronine and vasopressin on cardiac function and myocardial blood flow after brain death. Journal of Heart and Lung Transplantation 12: 68-80.

Milliken JC, Billingsley AM, Laks H 1989 Modified reperfusate after long-term preservation of the heart. Annals of Thoracic Surgery 47: 725-728.

Milnor WR 1972a Pulmonary haemodynamics. In: Bergel, D (eds) Cardiovascular Fluid Dynamics. Academic Press, London p.299-366.

Milnor WR 1972b Pulsatile blood flow. New England Journal of Medicine 287: 27-34.

Milnor WR 1989 Vascular Impedance. Williams & Wilkins, Baltomore, Maryland.

Milnor WR, Bergel DH, Bargaimer JD 1966 Hydraulic power associated with pulmonary blood flow and its relation to heart rate. Circulation Research 19: 467-480.

Morishita Y, Saigenji H, Higashi T, Umebayashi Y, Taira A, Goto M 1985 Function of the transplanted canine heart after prolonged preservation by simple immersion. Heart and Vessels 1: 220-224.

Morris JJ III, Pellom GL, Hamm DP, Everson CT, Wechsler AS 1986 Dynamic right ventricular function: relation to chamber volume during the cardiac cycle. Journal of Thoracic and Cardiovascular Surgery 91: 879-887.

Moseley JI, Molinari GF, Walker E 1976 Respirator brain. Report of a survey and review of current concepts. Archives of Pathological Laboratory Medicine 100: 61-64.

Mukherjee A, Bush LR, McCoy KE, Duke RJ, Hagler H, Buja LM, Willerson JT 1982 Relationship between B-adrenergic receptor numbers and physiological responses during experimental canine myocardial ischaemia. Circulation Research 50: 735-741.

Neter J, Wasserman W, Kutner MH 1985 Applied linear statistical models. Homewood, Illinois.

Nishimura N, Sugi T 1984 Circulatory support with sympathetic amines in brain death. Resuscitation 12: 25-30.

Novitzky D 1992 Heart transplantation, euthyroid sick syndrome, and triiodothyronine replacement. Journal of Heart and Lung Transplantation 11: S196-198.

Novitzky D, Cooper DKC, Reichart B 1987a Hemodynamic and metabolic responses to hormonal therapy in brain-dead potential organ donors. Transplantation 43: 852-854.

Novitzky D, Wicomb WN, Cooper DKC, Rose AG, Fraser C, Barnard CN 1984 Electrocardiographic, hemodynamic and endocrine changes occurring during experimental brain death in the chacma baboon. Heart Transplantation 4: 63-69.

Novitzky D, Wicomb WN, Cooper DKC, Rose AG, Reichart B 1986 Prevention of myocardial injury during brain death by total cardiac sympathectomy in the chacma baboon. Annals of Thoracic Surgery 41: 520-524.

Novitzky D, Wicomb WN, Cooper DKC, Tjaalgard MA 1987b Improved cardiac function following hormonal therapy in brain dead pigs: relevance to organ donation. Cryobiology 24: 1-10.

Okouchi Y, Shimizu K, Yamaguchi A, Kamada N 1990 Effectiveness of modified University of Wisconsin solution for heart preservation as assessed in heterotopic rat transplant model. Journal of Thoracic and Cardiovascular Surgery 99: 1104-1108.

Olsen CO, Rankin JS, Arentzen CE, Anderson RW 1981 The deformational characteristics of the left ventricle in the conscious dog. Circulation Research 49: 843-855.

Olsen CO, Tyson GS, Maier GW, Spratt JA, Davis JW, Rankin JS 1983 Dynamic ventricular interaction in the conscious dog. Circulation Research 52: 85-104.

Powner DJ, Hendrich A, Nyhuis A, Strate R 1992 Changes in serum catecholamine levels in patients who are brain dead. The Journal of Heart and Lung Transplantation 11: 1046-1053.

Proctor E, Matthews G, Archibald J 1971 Acute orthotopic transplantation of hearts stored for 72 hours. Thorax 26: 99-102.

Qayami AK, Jamieson WRE, Rosado LJ, Lyster DM, Schulzer M, McConville B, Gillespie KD, Hudson MPJ 1991 Comparison of functional and metabolic assessments in preservation techniques for heart transplantation. Journal of Investigative Surgery 4: 93-102.

Raff H, Findling JW 1989 A new immunoradiometric assay for corticotrophin evaluated in normal subjects and patients with Cushing syndrome. Clinical Chemistry 34: 596-600.

Randell TT, Hockerstedt KAV 1992 Triiodothyronine treatment in brain-dead multiorgan donors- a controlled study. Transplantation 54: 736-738.

Rankin JS, Arentzen CE, McHale PA, Ling D, Anderson RW 1977 Viscoelastic properties of the diastolic left ventricle in the conscious dog. Circulation Research 41: 37-45.

Rankin JS, Arentzen CE, Ring WS 1980 The diastolic mechanical properties of the intact left ventricle. Federal Proceedings 39: 141.

Rankin JS, McHale PA, Arentzen CE, Ling D, Greenfield JC Jr, Anderson RW 1976 The three dimensional dynamic geometry of the left ventricle in the conscious dog. Circulation Research 39: 304-313.

Rapaport F, Anaise D 1991 Organ donation - 1990. Transplantation Proceedings 23: 899.

Reemsta K, Williamson WE Jr, Iglesias F, Pena E, Sayegh SF, Creech O Jr 1962 Studies in homologous canine heart transplantation: prolongation with a folic acid antagonist. Surgery 52: 127-132.

Reitz BA, Brody WR, Hickey PR, Michaeiis LL 1974 Protection of the heart for 24 hours with intracellular (high K+) solution and hypothermia. Surgical Forum 25: 149.

Rose AG, Novitzky D, Cooper DKC 1988 Myocardial and pulmonary histopathological changes post brain death. Transplantation Proceedings 20: 29-32.

Rosenfeldt FL, Cobb FR, Bache RJ, Sabiston DC Jr 1979 Effect of increases in afterload before and after coronary occlusion in awake dogs. Cardiovascular Research 13: 392-403.

Ross J Jr, Braunwald E 1964 The study of left ventricular function in man by increasing resistance to ventricular ejection with angiotensin. Circulation 29: 739-43.

Rushmer RF, Franklin DL, Ellis RM 1956 Left ventricular dimensions recorded by sonocardiometry. Circulation Research 4: 684-689.

Sagawa K (ed) 1967 Analysis of the ventricular pumping capacity as a function of input and output pressure loads, W.B Saunders Co.: Philadelphia.

Sakagoshi N, Matsuda H, Watanebe Y in press Alterations in the B-adrenergic receptor systemafter hypothermic ischemia in hearts with pre-ischemic B-receptor desensitization Journal of Surgical Research

Sakagoshi N, Shirakura R, Nakano S, Taniguchi K, Miyamoto Y, Matsuda H 1992 Serial changes in myocardial ß-adrenergic receptor after experimental brain death in dogs. The Journal of Heart and Lung Transplantation 11: 1054-1058.

Salomon Y, Londos C, Rodbell M 1974 A highly sensitive adenylate cyclase assay. Annals of Biochemistry 58: 541-548.

Santamore WP, Carey R, Goodrich D, Bove AA 1981 Measurement of left and right ventricular volume from implanted radiopaque markers. American Journal of Physiology 240: H896-900.

Santamore WP, Lynch PR, Heckman JL, Bove AA, Meier GD 1976 Left ventricular effects on right ventricular developed pressure. Journal of Applied Physiology 41: 925-930.

Sarnoff SJ, Mitchell JH 1961 The regulation of the performance of the heart. American Journal of Medicine 30: 747.

Schwartz I, Bird S, Lotz Z, Innes CR, Hickman R 1993 The influence of thyroid hormone replacement in a porcine brain death model. Transplantation 55: 474-476.

Schwiep F, Cassidy SS, Ramanathan M, Johnson RL 1988 Rapid in-vivo determinations of instantaneous right ventricular pressure and volume in dogs. American Journal of Physiology 254:

Shirakura R, Hirose H, Matsuda H, Nakano S, Nakata S, Ohtani M, Kawaguti A, Miyagawa S, Takami H, Naka Y, Kawashima Y 1989 Resuscitation and preservation of agonally arrested hearts for transplantation: a study of 24 hour stored canine hearts. Transplantation Proceedings 21: 1347-1349.

Shivalkar B, Van Loon J, Wieland W, Tjandra-Maga TB, Borgers M, Plets C, Flameng W 1993 Variable effects of explosive or gradual increase of intracranial pressure on myocardial structure and function. Circulation 87: 230-239.

Shuster S 1960 The electrocardiogram in subarachnoid haemmorrhage. British Heart Journal 22: 316-320.

Smart FW, Mobley RA, Holter VL, Sekela ME, Grinstead WC, Frazier OH, Van Buren CT, Young JB 1991 Variability of organ utilisation criteria exacerbates the national shortage of heart donors. Journal of American College of Cardiology 17: 273A.

Soifer BE, Gelb AW 1989 The multiple organ donor: identification and management. Annals of Internal Medicine 110: 814-823.

Solis E, Tago M, Kaye MP 1985 Cardiac function following prolonged preservation and orthotopic transplantation. Heart Transplantation 4: 357-61.

Sonnenblick EH, Downing SE 1963 Afterload as a primary determinant of ventricular performance. American Journal of Physiology 204: 604-11.

Spray TL, Watson DC, Roberts WC 1977 Morphology of canine hearts after 24 hours' preservation and orthotopic transplantation. Journal of Thoracic and Cardiovascular Surgery 73: 880-886.

Squire J 1981 The structural basis of muscular contraction. Plenum Press, New York.

Stevenson LW, Dadourian BJ, Child JS, Clark SH, Laks H 1987 Mitral regurgitation after heart transplantation. American Journal of Cardiology 60: 119-122.

Suga K, Sagawa K 1974 Instantaneous pressure-volume relationships and their ratio in the in the excised, supported canine left ventricle. Circulation Research 35: 117-127.

Sunamori M, Amano J, Miyamoto H, Shirai T, Suzuki A 1993 Relative advantages of non-depolarzing solution to depolarizing University of Wisconsin solution in donor heart preservation. Transplantation Proceedings 25: 1613-1617.

Swanson DK, Dufek JH, DR Kahn 1979 Myocardial preservation for transplantation. Transplantation Proceedings 11: 1478-1479.

Swanson DK, Myerowitz DP 1983 Effect of reperfusion temperature and pressure on the functional and metabolic recovery of preserved hearts. Journal of Thoracic and Cardiovascular Surgery 86: 242-251.

Swanson DK, Pasaoglu I, Berkoff HA, Southard JA, Hegge JO 1988 Improved heart preservation with UW preservation solution. Journal of Heart Transplantation 7: 456-467.

Tajimi T, Widmann TF, Martsuzaki M, Peterson KL 1984 Differing effects of angiotensin II and phenylephrine on end-systolic pressure-volume relationship in conscious dogs. Journal of American College of Cardiology 3: 523-27.

Tian G, Smith KE, Biro GP, Butler KW, Haas N, Scott J, Anderson R, Deslauriers R 1991 A comparison of UW cold storage solution and St. Thomas' solution II: A 31 P NMR and functional study of isolated porcine hearts. The Journal of Heart and Lung Transplantation 10: 975-985.

Todd GL, Baroldi G, Pieper GM, Clayton FC, Elliot RS 1985 Experimentally catecholamine-induced myocardial necrosis. Morphology, quantification and regional distribution of acute contraction band lesions. Journal of Molecular and Cellular Cardiology 17: 317-338.

Toledo-Pereyra LH, Chee M, Lillehei RC, Jara FM 1979 Forty-eight hours hypothermic pulsatile perfusion of canine hearts before transplantation. Cryobiology 16: 343-347.

VanTrigt P, Olsen CO, Pellom GL 1980 Do acute hemodynamic interventions alter diastolic mechanics of the left ventricle? Circulation 62: 778.

Wandall HH 1970 A review of cardiac transplantation in man. Scandinavian Journal of Thoracic and Cardiovascular Surgery 4: 5-7.

Warbasse JR, Braunwald E, Aygen MM 1963 Starling's law of the heart. VII. Ventricular function in closed-chest unaesthetised dogs. American Journal of Physiology 204: 439-445.

Watson DC 1977 Consistent survival after prolonged donor heart preservation. Transplantation Proceedings 9: 297-9.

Watson DC, Baumgartner WA, Ranay AA, Oyer PE, Stinson EB, Shumway NE 1979 Distant heart procurement for transplantation. Surgery 86: 56-61.

Webb WR, Howard HS, Neely WA 1959 Practical method of homologous cardiac transplantation. Journal of Thoracic and Cardiovascular Surgery 37: 361-6.

Weber KT, Janicki RS, Reeves RC, Hefner LL, Reeves TJ 1974 Determinants of stroke volume in the isolated canine heart. Journal of Applied Physiology 37: 742-747.

Weng Z, Nicolosi AC, Detwiler PW, Hsu DT, Schierman SW, Goldstein AH, Spotnitz HM 1992 Effects of crystalloid, blood, and University of Wisconsin perfusates on weight, water content, and left ventricular compliance in an oedema-prone, isolated porcine heart model. Journal of Thoracic and Cardiovascular Surgery. 103: 504-513.

Wheeldon DR, Potter CDO, Dunning JJ, Gray S, Oduro A, Wallwork J, Large SR 1992 Haemodynamic correction in multiorgan donation. Lancet 339: 1175-1179.

Wicomb W, Cooper DKC, Hassoulas J, Rose AG, Barnard CN 1982 Orthotopic transplantation of the baboon heart after 20 to 24 hours' preservation by continuous hypothermic perfusion with an oxygenated hyperosmolar solution. Journal of Thoracic and Cardiovascular Surgery 83: 1333-140.

Wicomb WN, Cooper DKC, Lanza RP, Novitzky D, Isaacs S 1986b The effects of brain death and 24 hours' storage by hypothermic perfusion on donor heart function in the pig. Journal of Thoracic and Cardiovascular Surgery 91: 896-909.

Wieshammer S, Mendler N, Schmid D 1979 Regional myocardial flow and metabolism in canine heart transplants after preservation for 24 hours. Journal of Thoracic and Cardiovascular Surgery 27: 248-255.

Wilcken DEL, Charlier AA, Hoffman JIE, Guz A 1964 Effects of alterations in aortic impedance on the performance of the ventricles. Circulation Research 14: 282-89.

Williams FA 1948 A history of the heart and circulation. WB Saunders, Philadelphia.

Witzig K 1914 Uber erzwungene wellenbewegungen zaher, inkompressibler flussigkeiten in elastischen rohren. Inaugural Dissitation, University of Bern.

Womersley JR 1955a Method for the calculation of velocity, rate of flow and viscous drag in arteries when the pressure gradient is known. Journal of Physiology 127: 553-563.

Womersley JR 1955b, Oscillatory motion of a viscous fluid in a thin walled elastic tube. Phil Mag: 199-221.

Woolf PD, Hamill RW, Lee LA, Cox C, McDonald JV 1987 The predictive value of catecholamines in assessing outcome in traumatic brain injury. Journal of Neurosurgery 66: 875-82.

Yacoub MH, Banner NA 1989 Recent developments in heart and heart-lung transplantation. In: Morris, P Tilney, N (eds) Transplantation Reviews. Saunders, WB, Philadelphia p.1-29.

Yeh T, Hanan SA, Johnson DE, Rebeyka IM, Abd-Elfattah AS, Lee KF, Wechsler AS 1990 Superior myocardial preservation with modified UW solution after prolonged ischaemia in the rat heart. Annals of Thoracic Surgery 49: 932-9.

Yellin EL, Hori M, Yoran C 1986 Left ventricular relaxation in the filling and non filling intact heart. American Journal of Physiology 250: H620-627.

Yusef S, Theodoropoulos S, Mathias CJ, Dhalla N, Wittes J, Mitchell A, Yacoub M 1987 Increased sensitivity of the denervated transplanted human heart to isoprenaline both before and after B-adrenergic blockade. Circulation 75: 696-704.