

THE DEVELOPMENT OF A SYSTEM OF 24 HOURS PRESERVATION  
OF THE HEART FOR TRANSPLANTATION

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BY

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# THE DEVELOPMENT OF A SYSTEM OF 24 HOURS PRESERVATION OF THE HEART FOR TRANSPLANTATION.

WICOMB, Winston Neville, PhD. University of Cape Town, 1983.

The aim of the study presented here was to preserve functional viability of hearts stored for 24 hours. Preservation of the hearts was obtained by using cardioplegic arrest followed by continuous perfusion at 6 - 8°C with a hyperosmolar fluid, through which 95% oxygen and 5% carbon dioxide were bubbled to maintain the pH at 7.2 - 7.4.

Viability was assessed biochemically and functionally. The biochemical studies involved measurement of tissue high energy phosphates, glycogen stores and metabolic products released into the perfusate (LDH, lactate). The effect of preservation on suborganelles was also evaluated and these included the mitochondria, lysosomes and sarcoplasmic reticulum.

The functional studies were both ex vivo and in vivo. The ex vivo functional evaluation system was developed especially for the assessment of large hearts (pig) but was based on the classical Langendorff rat heart perfusion model. Ex vivo functional evaluation was used as a screening test and allowed rapid assessment of heart function. The in vivo test of functional viability was orthotopic and autotransplantation followed by cardiac catheterization.

From the information obtained from these tests adjustments were made to the perfusate by modifying chemical combinations and adding appropriate drugs. Eight perfusates were evaluated. The perfusion solution of Krebs and Henseleit was the first perfusate in this series and proved to be totally ineffective; high energy phosphate stores became depleted and the release of LDH was excessive. Addition of the amino acid taurine

to the perfusate rapidly decreased the release of LDH. The addition of ATP and insulin to the perfusate and an increase in the magnesium and potassium concentration improved myocardial function, though significant oedema formation was still evident in all hearts. The use of large, osmotically active molecules was shown to be ineffective in preventing fluid accumulation and/or improving consequent myocardial function. Mitochondrial oxidative phosphorylation was normal in these hearts, though myocardial function remained less than optimum. This depressed function was thought to be a result of increased cytosolic calcium concentration, probably related to impairment of the mechanism by which calcium is pumped out of the myocardium (against a large electrochemical gradient) and/or damage to the membrane barrier that normally prevents entry of calcium into the cell. Orthotopic transplantation confirmed that these hearts had been poorly preserved.

A hypothesis then evolved, namely that the combined effect of raised perfusate sodium levels and progressive oedema formation protected the myocardium from the effects of calcium redistribution in the myoplasm. The success of the perfusate subsequently developed was based on this hypothesis. During hypothermia essential ionic pump activity is inhibited and calcium penetrates membrane barriers activating contractile proteins. Enhanced resting myocardial tension results, which in turn inhibits excessive oedema formation during this ischaemic phase. This effect also occurs during warm ischaemia when the myocardium is unperfused. If the intracellular calcium concentration was controlled by decreasing the perfusate calcium, by increasing the extracellular sodium concentration, and adding small osmotically active molecules, then the myocardium remained viable. The probable sequence of events was as follows.

Some of the small osmotically active molecules do not enter the extra vascular compartments immediately, although sodium does enter the cell



due to failure of the sodium-potassium pump. Evidence of failure of the sodium calcium exchange mechanism during hypothermia has not been reported and this mechanism may initially operate to reduce calcium entry into the cells. Should this mechanism fail as time progresses, the small molecules would be protective by slowly accumulating fluid and diluting the tissue calcium concentrations. (Small osmotically active molecules gradually enter the myocardial tissue during hypothermic perfusion). Intracellular or extracellular fluid accumulation would have similar effects as any dilution of the total calcium would be protective. Evidence for this hypothesis was provided by the gradual rate of oedema formation in these well preserved hearts. (By comparison hearts preserved in the same solution by simple storage in ice gained no weight during the period of preservation and showed poor haemodynamic performance and frequent rhythm disturbances.

The successful results obtained from modification of the perfusate led to the development of a portable method of storing donor hearts for 24 hours. This unit enabled hearts to be harvested at distant centres, transported over long distances, and facilitated the logistics of transplantation by allowing time for tissue typing, preparation of the recipient, and convenient timing of the operative procedure. Pig hearts preserved for 24 hours by this portable hypothermic perfusion system and subsequent functionally tested, showed haemodynamic parameters indistinguishable from freshly excised hearts. Orthotopic and autotransplantation of baboon hearts, preserved by an identical technique, was followed by consistent survival of the recipient baboon until rejection. Four human hearts were similarly preserved by continuous perfusion for periods of ischaemia ranging from 7 to 17 hours. After heterotopic transplantation three of these hearts required inotropic support initially, but there were no rhythm or conduction disorders, suggesting

the absence of irreversible ischaemic injuries. The fourth heart supported the circulation without difficulty. One heart was acutely rejected, the three remaining hearts which survived through rejection episodes showed good or adequate haemodynamic performance at cardiac catheterization 5,4 and 3 months respectively after transplantation.

## PREFACE

This thesis describes a series of investigations into the problems that hamper the progress of myocardial preservation for transplantation in man.

Six positive aspects of cardiac preservation have emerged from this study:-

- (1) A clear fluid hyperosmolar solution was formulated that adequately preserved viability of pig and baboon hearts.
- (2) A pneumatically powered portable preservation unit was designed which successfully preserved pig and baboon hearts when assessed by either functional testing or orthotopic transplantation.
- (3) A method of in vitro testing of hearts was developed that correlated with results from orthotopic transplantation.
- (4) A technique of cardiac autotransplantation in baboons was perfected.
- (5) The high release of lysosomal acid phosphatase during the period of hypothermic preservation was shown to be non-pathological and was reversible after a period of warm blood perfusion.
- (6) Successful preservation of human hearts for periods longer than 4 hours, not previously achieved, was obtained.

The preservation solution and the portable preservation unit that emerged from this experimental study were thoroughly investigated before clinical application. During the development of this perfusate the author had numerous consultations and discussions with colleagues and senior members of neighbouring departments (see acknowledgements).

Some of the work presented in chapters 1, 10 and 11 has been published and further work in chapters 11 and 12 has subsequently been submitted for publication. The titles and references of these articles are listed below.

- (1) Ex vivo functional evaluation of pig hearts subjected to 24 hours preservation by hypothermic perfusion. S. Afr. Med. J. 60 (1981) pg.245.
- (2) Orthotopic transplantation of the baboon heart after 20-24 hours preservation by continuous hypothermic perfusion with an oxygenated hyperosmolar solution. J. Thorac. Cardiovasc. Surg. 83 (1982) pg. 133.
- (3) Twenty four hour preservation of the pig heart by a portable hypothermic perfusion system. Transplantation 34 (1982) pg. 246.
- (4) Orthotopic allotransplantation and autotransplantation of the baboon heart following 24 hours storage by a portable hypothermic perfusion system. Accepted for publication in Cryobiology.
- (5) Cardiac transplantation following storage of the donor heart by a portable hypothermic perfusion system. Accepted for publication in Ann. Thorac. Surg.
- (6) The use of acid phosphatase as an indicator of lysosomal membrane changes during hypothermic preservation. Submitted for publication in Basic Research in Cardiology, 1983
- (7) Storage of the donor heart by a portable hypothermic perfusion system: experimental development and clinical experience. Heart Transplantation 2, 1983, pg. 104.

ABBREVIATIONS

|        |                                         |
|--------|-----------------------------------------|
| ADP    | Adenosine diphosphate                   |
| AMP    | Adenosine monophosphate                 |
| AoP    | Aortic pressure                         |
| ATP    | Adenosine triphosphate                  |
| BT     | Biochemical tests                       |
| CF     | Coronary flow                           |
| CK     | Creatine phosphokinase                  |
| cm     | Centimetre                              |
| CO     | Cardiac output                          |
| CP     | Creatine phosphate                      |
| CVR    | Coronary vascular resistance            |
| EDTA   | Ethylenediamine tetraacetic acid        |
| EGTA   | Ethyleneglycol tetraacetic acid         |
| FT     | Functional testing                      |
| g      | Gravity                                 |
| gm     | Gram                                    |
| G6-P   | Glucose 6 - phosphate                   |
| G6-PDH | Glucose 6 - phosphate dehydrogenase     |
| h      | Hour                                    |
| HCl    | Hydrochloric acid                       |
| HK     | Hexokinase                              |
| i.m.   | Intramuscular                           |
| i.v.   | Intravenous                             |
| IVC    | Inferior vena cava                      |
| kg     | Kilogram                                |
| l      | Litre                                   |
| LDH    | Lactate dehydrogenase                   |
| LV     | Left ventricle                          |
| LVP    | Left ventricular pressure               |
| LVEDP  | Left ventricular end-diastolic pressure |

|                  |                                                        |
|------------------|--------------------------------------------------------|
| M                | Molar                                                  |
| meq              | Milliequivalents                                       |
| min              | Minute                                                 |
| ml               | Millilitres                                            |
| mM               | Millimolar                                             |
| mmHg             | Millimetres of mercury                                 |
| mmol             | Millimole                                              |
| mol              | Mole                                                   |
| $\mu$ mol        | Micromole                                              |
| mOsm             | Milliosmoles                                           |
| mRNA             | Messenger RNA                                          |
| mV               | Millivolts                                             |
| n                | Number of observations                                 |
| NAD <sup>+</sup> | Nicotinamide adenine dinucleotide (oxidised)           |
| NADH             | Nicotinamide adenine dinucleotide (reduced)            |
| NADP             | Nicotinamide adenine dinucleotide phosphate (oxidised) |
| nm               | Nanometres                                             |
| p                | Probability                                            |
| pg               | Page                                                   |
| P/O              | Phosphate : oxygen ratio                               |
| QO <sub>2</sub>  | Rate of oxygen uptake                                  |
| RCI              | Respiratory control index                              |
| S.E.M.           | Standard error of the mean                             |
| SR               | Sarcoplasmic reticulum                                 |
| SRV              | Sarcoplasmic reticulum vesicles                        |
| SV               | Stroke volume                                          |
| SVC              | Superior vena cava                                     |
| TRIS             | Tris-(hydroxymethyl) aminomethane                      |
| wt               | Weight                                                 |

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## CHAPTER 1

### 1. INTRODUCTION

This dissertation describes a procedure to achieve 24 hour preservation of the heart. From a review of the literature on methods of maintaining myocardial viability, it appeared that hypothermia would contribute greatly to myocardial protection, because of its metabolic lowering effect (McQuiston 1950, Blair 1960, Bigelow et al 1954).

The literature on kidney preservation indicated that preservation by continuous perfusion has some theoretical advantages over simple cold storage for periods of preservation longer than 24 hours (Freier, 1979) (Belzer 1980). Kidneys subjected to warm ischaemia usually recover after a period of hypothermic perfusion, whereas recovery was poor after storage in ice (Collins and Halasz 1979). Continuous perfusion provides oxygen and nutrients to the myocardium and prevents the accumulation of toxic metabolic end products and fluctuations in pH. Spray et al (1977) have also shown that hypothermically perfused hearts functioned much better than stored hearts preserved under identical conditions. From this information the preservation method of choice appeared to be a combination of hypothermia and continuous perfusion.

#### 1.1 CARDIOPLEGIA

A fundamental aspect in an early stage of cardiac preservation is cardioplegia, which involves the arrest of the in situ heart (Buckberg 1979). The concept of cardioplegia was first introduced by Hooker in 1929 as a method of producing immediate electromechanical arrest, preserving energy stores that would have been expended by anaerobic metabolism. From the standpoint of clinical application Bigelow et al (1954) believed that the

combination of hypothermia and potassium was a risk of two dangerous procedures. Potassium arrest was first used by Melrose and colleagues (1955) using concentrations from 245 to 980 millequivalents of potassium per litre. At that time potassium and cold were not used together. The vital experiment of combining cardioplegia with hypothermia was performed by Cross (1957) who showed that a cold heart withstood ischaemia better than a warm heart, and on reperfusion, the heart recovered more quickly. The use of potassium citrate in cardioplegic solutions was abandoned when Helmsworth (1959) published a paper which reported that myocardial necrosis was always found in the hearts of dogs after potassium citrate arrest. Interest in cardioplegia disappeared due to other priorities in cardiac surgery which expanded rapidly.

In the early seventies a new interest in cardioplegia was stimulated with the Kirsch et al (1972) Bretschneider et al (1975) and later Hearse (1977) solutions. In the Bretschneider solutions the cardioplegic effect was brought about by high procaine hydrochloride levels and also the absence of calcium substituted for by magnesium chloride. The Kirsch solution only had procaine hydrochloride as the active cardioplegic agents and also excluded calcium from the solution. The Hearse solution was characterised by a more complex formulation deviating only slightly from the composition of extra-cellular fluid (Hearse 1977), with potassium, magnesium and procaine as the active cardioplegic agents. More recently Buckberg et al (1978) evaluated hypothermic potassium cardioplegia using cold blood as the vehicle, which he found to be advantageous in comparison to other techniques for myocardial preservation (Lake 1978); Follette 1978).

Apart from periodic asanguinous coronary flushing (Iyengar et al 1972) cardiac arrest and myocardial protection can be obtained by coronary perfusion with blood (Taber et al 1967) local hypothermia (Enright et al 1970) and various pharmacological agents (Webb et al 1966); Davies et al 1968; and Hearse et al 1975). From this review of the literature it was clear that the areas of myocardial protection required further clarification. Most of the published information on cardioplegic solutions showed adequate preservation when tested under the author's experimental conditions. My solution of choice was a modification of that used by Hearse et al (1977) which resembled the extracellular fluid composition but with the addition of procaine hydrochloride and verapamil hydrochloride. Verapamil is a calcium slow channel inhibitor (Singh et al 1978; Opie 1980). It was included in the cardioplegic solution with the aim of preventing any enhanced myocardial tension that may develop due to  $Ca^{++}$  during the period of arrest. The developed tension may progress to the irreversible stone heart condition (Cooley et al 1972) rendering longer periods of preservation by alternative methods futile. The solution was of relatively simple composition and biochemically stable between 37°C to 0°C.

#### 1.1.1 CARDIAC PRESERVATION: A BRIEF REVIEW

It was at the start of the eighteenth century that the concept of in vitro organ preservation (restoration of irritability in dying muscle) was first introduced by the French physiologist Le Gallois (Humphries et al 1974). The first idea of hypothermia in preservation stems from the survival of insects after freezing and the recovery of fish after having been embedded in ice. The pioneering work was done by John Hunter (1766) who froze two carp in river water in an attempt to keep them alive in the frozen state.

The induced hypothermia was a method used to lower the metabolism of the body as a whole and to reduce the dangers of hypoxia. Soon after Hunter's work, Currie (1798) of Liverpool published observations on the effects on man of immersion in cold water, inducing generalised hypothermia, in the treatment of fevers and alcoholic intoxication (Leigh and Atkinson 1968). From 1828 attempts at preservation of most organs were made and by 1868, Ludwig and Schmidt (1868) had constructed a perfusion apparatus being little more elaborate than a syringe.

Fifty years then lapsed before a new interest developed in organ preservation. Perfusion and preservation of organs remained a matter of extreme complexity. Isolated organ perfusion was first used by Carrel and Lindbergh (1935) and in 1938 they published "The Culture of Organs" in which various perfusates were used to sustain at normothermia a variety of isolated organs for days and even weeks (Carrel and Lindbergh 1938). They also concluded that the removal of an organ from the in vivo state initiated a cascade of drastic events and that preservation of an organ would only be improved if these events were understood and the organ made able to withstand the insult of an altered environment. Baker (1956) used human foetuses whose hearts were removed one to two hours after caesarian section, which he perfused retrogradely using an oxygenated Tyrode's solution at 29°C. There were no functional measurements taken but the hearts were able to resume some contractions.

With the use of refrigeration, preservation of other tissues had also progressed such as corneas (Altman 1890), red blood cells (Rouse and Turner 1916) and spermatozoa (Polge et al 1949).

By the early fifties kidneys were being hypothermically preserved with heparinized whole blood with little success (Bogardus and Schlosser 1956), and Telander (1964) diluted blood with a balanced salt solution without any further improvement.

Webb and Howard (1957) attempted the first preservation study on the refrigerated heart, which was isolated, cooled and preserved by perfusion. Successful preservation was limited to eight hours after which time viability rapidly declined. Survival of animals receiving orthotopically placed donor hearts preserved for 24 hours has rarely been for more than a matter of hours. Kondo and his colleagues (1965) reported five days survival in one of 29 puppies after donor heart preservation by hyperbaric oxygenation at 3 - 4 atmospheres. Hyperbaric oxygenation and hypothermic perfusion together have been employed with the survival of one out of 6 animals for 8 days (Feemster and Lillehei 1969). The studies of Proctor and Parker (1972) suggested that hearts preserved by hypothermic perfusion for as long as 72 hours retained viability, but this group did not attempt to attain long term survival after orthotopic transplantation, (transplantation of an organ into its normal in vivo position) discontinuing assessment of the heart after  $1\frac{1}{2}$  to 2 hours function.

Using a preservation system based on that of Proctor, Copeland et al (1973) reported 4 out of 30 dogs surviving longer than 4 days to rejection. Hearts suspended, but not perfused or oxygenated, in a hypothermic, intracellular high potassium solution for up to 26 hours have been successfully transplanted, though only 4 out of 20 survived rejection, the longest survivor being five and a half days (Reitz et al 1974). Watson (1977) achieved survival after orthotopic transplantation of

hypothermically perfused hearts but sacrificed animals after only 24 hours. All hearts were supported by a dopamine infusion and ventilated for the entire post-operative period; some required left ventricular pacing. This study showed, however, that hearts preserved by continuous oxygenated, low pressure, perfusion suffered less extensive histological damage than hearts stored in the same solution but not perfused (Spray et al 1977).

In one study aimed primarily at resuscitation of anoxic hearts, Angell et al (1968) resuscitated and stored anoxic dog hearts by anastomosing the heart heterotopically in the neck of an intermediate host, thus maintaining perfusion over one to three days. The hearts were then orthotopically transplanted into recipient dogs, where 3 hearts which had not suffered anoxic damage supported the circulation of the host, though length of survival was not reported. One heart which had suffered reversible anoxic damage and which was subsequently transplanted after 3 days intermediate host perfusion, did support a recipient animal for 30 days. Although it is difficult to see a homologous intermediate host storage system being used in man, xenogeneic (heterologous with respect to tissue grafts, when donor and recipient belong to widely separated species) storage using an immunosuppressed animal might be a potential answer to short term storage of human cadaver organs (Dupree et al 1969). The number of experimental animals, therefore, which have survived rejection following orthotopic transplantation supported entirely by hearts which had been stored from 20 - 24 hours remain very small. However, recent studies by Guerraty (1981) have shown that dog hearts were successfully preserved for 24 hours when using low pressure, non pulsatile hypothermic perfusion. He preserved

15 hearts in this way; after orthotopic transplantation all the grafts were able to support the circulation, with the exception of two animals dying of cardiac failure after 1 and 5 days. To date this is the only report of a reliable and reproducible method of 24 hour in vitro perfusion of the isolated canine heart.

#### 1.1.2. PRESERVATION UNIT

Hearts have been protected clinically in ice cold cardioplegic solutions for periods of up to 4 hours and a limited period of 5 to 6 hours is anticipated (English et al 1980; Lower et al 1978). For prolonged myocardial protection (24 hours) continuous hypothermic perfusion was my chosen method of preservation. Other methods of preservation (supercooling, hyperbaric oxygenation, normothermic perfusion) was fully reviewed elsewhere (Cooper 1976). The arrested heart was attached to a greatly modified preservation unit based on that originally described by Proctor and Parker (1968). Clinical application demanded further modification to the unit such that it was uncomplicated in design, compact, functionally independent and totally transportable.

#### 1.1.3. THE PERFUSATE

The original solution used in this study was the perfusion solution of Krebs and Henseleit, which was a very well established perfusate used for preserving viability of in vitro rat hearts at normothermia (Opie 1965; Neely et al 1967). The perfusate was developed by determining the effect of combinations of various constituents on the myocardium during 24 hours perfusion. This effect was determined biochemically and improvement made by readjustment of the perfusate. (If beneficial, constituents retained or increased; if detrimental, constituents removed or reduced). The perfusate was easily prepared and sterilised by



filtration with no need for antibiotics. The solution was also stable when subjected to large fluctuations in pH and temperature. Every chemical in the perfusate was essential, and the absence of any one ingredient rendered the solution inadequate in maintaining viability.

#### 1.1.4. EVALUATION OF MYOCARDIAL VIABILITY DURING PERFUSION STORAGE

A test of functional viability of a preserved organ is essential if the preservation method is to become a clinical reality (Maginn et al 1968; Calman et al 1973; Cooper 1976). Tests suggested by others for the determination of myocardial viability were used in this study; oedema formation (Proctor and Jones 1973), coronary flow and coronary resistance (Proctor and Parker 1968). During the development of the perfusate used in the present study, the above criteria were not consistent as a good coronary flow or low coronary resistance did not necessarily imply a viable myocardium. These tests were unreliable and a more extensive assessment was desirable in order to determine the degree of myocardial viability.

A viability test is imperative if successful preservation remains uncertain. Alternatively, it seemed reasonable to believe that preserved organs which appeared biochemically sound after repeated assessment throughout preservation would function satisfactorily. In this report a heart preservation solution was developed that worked routinely and the use of a viability test after preservation was no longer essential, provided no errors in the perfusate composition occurred. The biochemical tests in the development of the perfusion were intermittently performed during the preservation period to detect any likely change that was to occur. These tests included studies of mitochondria, lysosomes and sarcoplasmic reticulum. Other



determinations included high energy phosphates, glycogen, lactate, lactate dehydrogenase, oxygen uptake, glucose uptake and coronary flow.

#### 1.1.5. METABOLISM AND HYPOTHERMIA

One of the most common causes of cell death under normothermic conditions is hypoxia. The pathophysiology of hypoxic death appears to centre on decreased energy production. The metabolism of glucose in the presence of oxygen provides most of this energy. Approximately 45% of the energy is successfully captured in high energy bonds available for later use in the cell (White et al 1964). Under hypothermic conditions these metabolic pathways are altered and may lead to loss of viability. Our current understanding of the biochemical and physiological changes induced by hypothermia is still relatively minimal. One well-documented change is an alteration in cell volume. The maintenance of cellular volume is a continuously active energy-consuming process which is dependent on the active extrusion of sodium at the cell membrane (Leaf 1959). The lack of energy soon causes the sodium pump to fail and as a consequence, sodium and water move into the cell and the cell begins to swell. This correlates with the microscopic swelling which occurs in ischaemic isolated tissue and which is reversible in its early stages (Baker 1956); and also with the increase in the weight found in hypoxic tissues in vitro (Enerson 1966). The cellular swelling has been implicated as a possible cause of breaks sometimes seen in the cell membranes (Ashford and Burdette 1965). This explains the cytoplasmic leakage of potassium (Flink et al 1950) proteins (King et al 1959) and enzymes (Reese 1962). Another well-documented alteration in cells at low temperatures is their solidification of the membranes because of their content of saturated fats (Raison and Lyons 1971). Since many key enzymes are membrane-

associated, the phase transition in the fluidity of the membrane modifies the activity of these enzymes. The generation of energy (ATP) is highly dependent upon membrane bound enzymes in the mitochondria that are dramatically affected by low temperatures. Thus, even though low temperature decreased the rate of ATP utilization, it also decreased ATP synthesis. The problem is that the normal regulatory mechanisms are no longer capable of controlling cell metabolism because of the differential effects of hypothermia on enzyme activity.

The primary physiological basis for the use of hypothermia is its metabolic lowering effect (McQuiston 1950; Bigelow et al 1954; Blair 1960). The fall in oxygen uptake by tissue is said to be exponential with the fall in core temperature (Adolph 1950; Spurr et al 1954). Extrapolation of an exponential curve (from data obtained up to 20°C) shows that oxygen uptake ceases at 10°C (Spurr et al 1954). Actual studies at this level revealed a continuous uptake of between 4% and 11% (Cavallero et al 1962). The van't Hoff Arrhenius rule (the rate of a chemical reaction is doubled for each 10°C rise in temperature) applies only within certain limits. The greatest fall occurs between 37°C and 30°C, below this level the decrease may well be exponential to 20°C; thereafter the decline levels out. Data derived from several sources of animal studies under controlled conditions (Adolph 1950; Bigelow 1954; Cavellero 1962) show the oxygen requirements to be reduced as follows :-

| DEGREES | PERCENTAGE |
|---------|------------|
| 37°     | 100%       |
| 32°     | 60% - 70%  |
| 30°     | 50% - 55%  |
| 28°     | 40%        |
| 25°     | 20% - 25%  |
| 10°     | 10%        |
| 5°      | 5%         |

Further metabolic lowering can be induced by the addition of the so-called metabolic inhibitors such as magnesium sulphate (Fadali and Soloff 1973), chlorpromazine (Noble 1979); by reducing metabolism at 5°C to 0.6% causing 99.4% inhibition of oxygen uptake (see section 10.5.1). The total metabolic state of an organ is the sum of the anaerobic and aerobic pathways. Fisher et al (1956) demonstrated that after a six hour period of hypothermia some oxidative mechanisms were interfered with and that there was no direct relationship between oxygen uptake and the glycogen levels in the liver. The glucose tolerance curve was also flattened below 28°C (Wynn 1964) suggesting an almost complete block of exogenous glucose utilization. In this study, glucose levels were measured in an isolated case to see whether glucose uptake was similar under our conditions of preservation. Our findings agreed with previously reported studies.

#### 1.1.6. EX VIVO FUNCTIONAL EVALUATION

The isolated working rat heart perfusion preparation has been used for the study of cardiac haemodynamic performance (Morgan et al 1965; Neely et al 1967; Chain et al 1969). We have modified the system to allow for evaluation of hearts from larger animals. The preserved hearts were connected to a parabi-  
otic blood circulating system using a perfusor pig for re-oxygenation of the blood. The unit provided a simple method for functional evaluation of the preserved heart. Hearts that failed during functional evaluation also failed to support the circulation after orthotopic transplantation. Successfully preserved and functionally evaluated hearts survived orthotopic transplantation.

#### 1.1.7. ORTHOTOPIC TRANSPLANTATION

The ultimate test for myocardial viability of the preserved heart is orthotopic transplantation, though few have assessed the efficacy of their preservation systems by this procedure (Pitzele et al 1968; Pausescu et al 1978). The number of animals which survived to rejection following orthotopic transplantation supported by hearts which had been stored for 24 hours remains very small. Kondo et al 1965 reported 5 day survival in 1 of 29 puppies after donor heart preservation by hyperbaric oxygenation at 3 - 4 atmospheres. Copeland et al 1973 reported 4 of 30 dogs

surviving longer than 4 days to rejection. Watson 1977 achieved survival after orthotopic transplantation of hypothermically perfused hearts, but sacrificed surviving animals after only 24 hours. All animals were supported by a dopamine infusion and ventilated for the entire post-operative period; some required left ventricular pacing. Animals surviving orthotopic transplantation have both haemodynamic function and microscopic appearance affected by rejection.

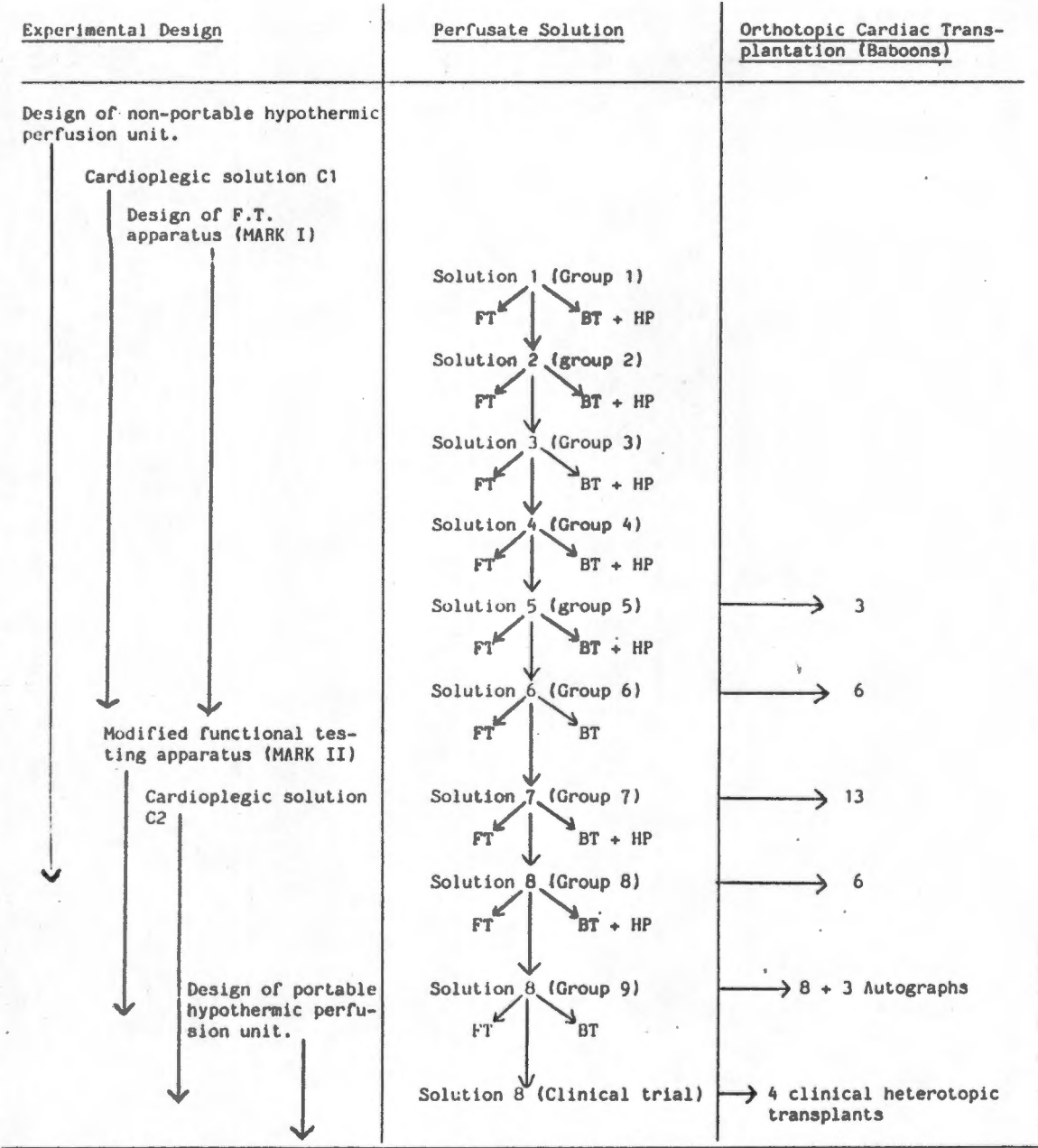
#### 1.1.8. AUTOGRAFTS

To determine the long-term effect of perfusion preservation on an organ, and to avoid the intervention of rejection, animals were subjected to 24 hours cardiopulmonary bypass while their own hearts were preserved for the same period. Thereafter their own hearts were orthotopically re-implanted. This method was abandoned as liver, kidney and brain damage always occurred due to the long bypass period. With continuous failure of the above procedure, we developed a new experimental approach. In this method a baboon's heart was removed for preservation while his own circulation was maintained by an orthotopic cardiac allograft. At the end of this period the baboon's own heart, preserved ex vivo for 24 hours, was replaced. The condition of these hearts was a true reflection of the preservation effect. Any microscopic, biochemical or functional change would be a consequence of inadequate preservation.

#### 1.1.9. CLINICAL EXPERIENCE

Obtaining consistent success in both biochemical and functional studies, as well as orthotopic transplantation and autotransplantation, the programme was extended to clinical transplantation. Conditions were somewhat different, for donors were brain dead and recipients were terminally ill. At this stage a compact portable preservation unit was completed in which four human hearts were preserved for 13, 8, 17 and 7 hours respectively.

FLOW DIAGRAM OF EXPERIMENTAL PROCEDURES



In all experiments pig hearts were used except where otherwise stated.  
Abbreviation:  
FT = functional testing  
BT = biochemical tests  
HP = histopathology

Table 2

## CHAPTER 2

### 2.1. EXPERIMENTAL PROCEDURES

The heart is unique as a transplanted organ in that it must be capable of full function immediately after its insertion into the recipient. The need for a test of functional viability is, therefore, essential if short term storage of the heart is to become a clinical reality. The ultimate measure of viability of the preserved heart is its capacity to support the circulation after orthotopic transplantation, although ideally the viability should be known throughout the period of storage and certainly at the time of transplantation.

Similarly, orthotopic transplantation as a means of testing all cardioplegic agents and storage perfusates is logistically impossible. Preservation of organs is an empirical and largely passive procedure in which a great deal of effort is expended to maintain the status quo of the tissue by arresting the damage incurred before preservation starts and in minimising any further damage resulting from preservation.

Katz (1980) has pointed out that "The large number of individual ingredients of cardioplegic solutions in various concentrations, volumes, temperatures, pressures and rates of injection, and in all their possible combinations according to the effect of each on the heart by all the possible means and models of looking at structures, compositions and functions, require something in the order of  $10^6$  experiments". Orthotopic transplantation is, therefore, impossible as a routine method of evaluation of cardioplegic agents, perfusates and perfusion systems, since the choice of perfusions is similarly almost unlimited.



Ideally any such test of organ viability should be simple, rapid and reproducible. The search for such a test has explored two main routes (Cooper 1976). The first of these is simple, single measurement or observation that confirms that tissues under study are not irreversibly damaged; this may take the form of e.g.,

a visual assay of a myocardial enzyme system, a histochemical change, or the monitoring of a fundamental metabolic event such as anaerobic glycolysis or lactic acid production.

Experience gained during the course of this study suggested that no single test was efficient to predict organ viability and as a result a series of tests were performed. The type of test used to detect the areas of damage in myocardium was largely empirically based, although some information was obtained from preceding biochemical and functional studies.

The aim of the work described in this thesis was to develop a successful preservation system. The major problem was the development of a solution which would maintain myocardial viability for periods of up to 24 hours.

Studies relating to 8 solutions are presented in detail, and the results of various preliminary screening tests are summarised in Appendix 4.

Whenever changes in the methodology were introduced, new sets of control values were determined. For example, initial mitochondrial studies were first carried out in the absence of BSA, whereas in chapter 10 BSA was included in the isolation procedure, yielding improved results. At this stage new control values were determined.

The original functional testing apparatus was also modified during the course of the experimental work (chapter 9) in order to subject the hearts to greater stress in a less rigid and more physiological environment; with this alteration a new set of control values was determined.

#### 2.1.1.1. PHYSIOLOGICAL CHANGES DURING HYPOTHERMIC PERFUSION

1. The non pulsatile coronary flow was obtained by a timed collection of fluid from the coronary sinus. The rate was measured at the start and after 24 hours of preservation. Coronary flow in ml/min/gm initial heart weight was used as an index of function.

2. Coronary vascular resistance (CVR) was calculated as follows:-

$$\text{CVR} = \frac{\text{Coronary perfusion pressure (cm H}_2\text{O)} \times \text{initial heart weight (gm)}}{\text{Coronary flow (ml/min)}}$$

3. Oxygen tension was measured with a Clarke's oxygen electrode and myocardial oxygen uptake was calculated using the following formula:

$$\begin{aligned} \text{O}_2 \text{ consumption } (\mu \text{ mole/h/gm}) = & \frac{\text{arterial - venous O}_2 \text{ tension (mm Hg)}}{22.4 \text{ (ml/mole)}} \\ & \times \frac{\text{solubility of O}_2 \text{ at 4}^\circ\text{C (ml/ml H}_2\text{O)}}{22.4 \text{ (ml/mole)}} \\ & \times \frac{\text{Coronary flow (ml/h)}}{\text{dry weight of heart (gm)}} \end{aligned}$$

The oxygen tension was measured at the start and at the end of the 24 hour preservation period.

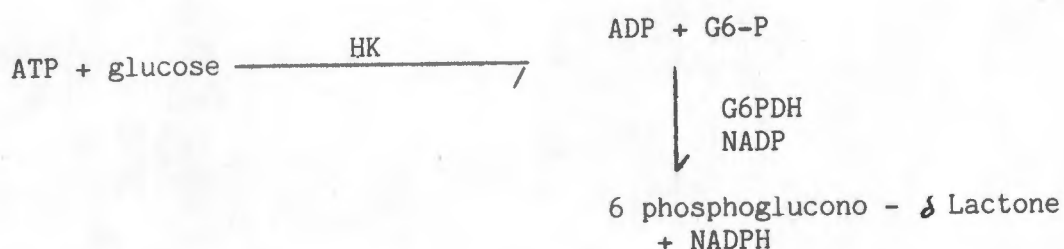
4. Myocardial fluid gain or loss was calculated by subtracting the initial heart weight from the weight at the end of the preservation period and the result was expressed as a percentage increase or decrease.

## 2.1.2. BIOCHEMICAL OBSERVATIONS OBTAINED DURING THE PERIOD OF HYPOTHERMIC PERFUSION

### 1. ADENOSINE TRIPHOSPHATE (ATP) AND PHOSPHOCREATINE (CP)

The assay mixture (2.8ml) contained 200 mM Tris HCl (pH 7.5). 20mM  $MgCl_2$ , 100mM glucose, 1.3mM NADP and G6PDH (5mg/ml). Routinely 0.2ml tissue extract was included. The reaction was started by the addition of hexokinase (3mg/ml) and followed spectrophotometrically at 340 nm. ATP concentration was calculated using an extinction coefficient of  $6.22 \times 10^{-6} M^{-1} cm^{-1}$ .

In order to assay CP 10mM ADP was added to the assay mixture and further change in absorption at 340 nm was noted. Background, endogenous ATP contaminants were determined by inclusion of water blanks in place of tissue extracts and these were subtracted. These values were always less than 2%. Internal standards were routinely included in accordance with Lamprechts and Trautschold (1963). The ATP assay was based on the following reaction :



The function of NADPH was measured spectrophotometrically at 340 nm and the glucose concentration calculated using an extinction coefficient of  $6.22 \times 10^{-6} M^{-1} cm^{-1}$ . The CP assay was based on the following reaction :



### 2.1.3. LACTATE DEHYDROGENASE (LDH) (EC 1.1.1.27)

The perfusate LDH concentration was determined using the Boehringer Mannheim biochemical test - combination kit number 124893. The test principle :



The formation of  $\text{NAD}^+$  was monitored at 340 nm and LDH concentrations were estimated using an extinction coefficient of  $6.22 \times 10^{-6} \text{M}^{-1} \text{cm}^{-1}$ . Samples were taken from the coronary sinus at 4 hourly intervals.

### 2.1.5. LACTATE

The perfusate lactate concentration was determined by the use of the Boehringer Mannheim test - combination kit number 124842. The test principle :



The formation of NADH was monitored at 340 nm and lactate activity was estimated using an extinction coefficient of  $6.22 \times 10^{-6} \text{M}^{-1} \text{cm}^{-1}$ . Samples were taken from the coronary sinus after 1 hour and 12 hours hypothermic perfusion and at the end of the hypothermic preservation period.

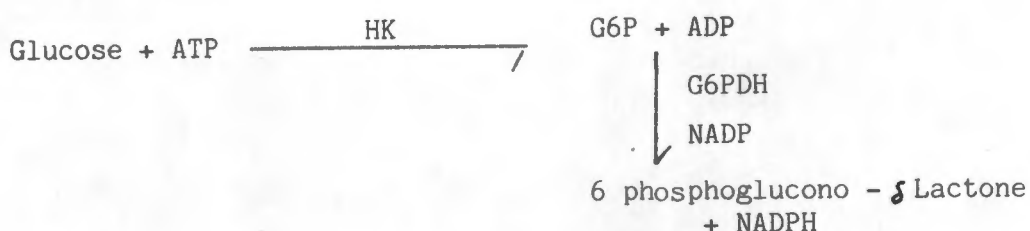
### 2.1.6. GLUCOSE UPTAKE

Perfusate glucose concentrations were enzymatically determined (Hohorst 1963). The test principle was the same as that of glycogen. Perfusate samples were taken at the beginning and at the end of the preservation period and the difference between these two concentrations were recorded as myocardial glucose uptake.

### 2.1.7. DETERMINATION OF GLYCOGEN CONCENTRATION

(Hohorst 1963). The tissue was digested with 40% KOH at 95°C and the glycogen precipitated by the addition of cold ethanol. The precipitated glycogen was acid hydrolysed (2M HCl at 96°C), The extract neutralised and the glucose assayed enzymatically.

Assay principle :



The formation of NADPH was measured spectrophotometrically at 340 nm and the glucose concentration calculated using an extinction coefficient of  $6.22 \times 10^{-6} \text{ M}^{-1} \text{ cm}^{-1}$ .

The biochemical assessments were determined during various stages of preservation :

1. Before and immediately after the period of cardioplegic arrest.
2. During the period of 24 hour hypothermic preservation.
3. After 1 hour normothermic ex vivo functional evaluation.

Biopsies from hearts in stage 1 were treated as controls as they were not subjected to prolonged preservation periods.

### 2.1.8. ISOLATION OF MITOCHONDRIA

The mitochondria were isolated from 2 groups of hearts :

1. Freshly arrested hearts (control group).
2. Hearts preserved for 24 hours and then reperfused with blood for 1 hour.

The method of isolation differed slightly from the procedure described by Sordahl and Schwartz (1971) by omitting the 0.5% bovine serum albumin (section 5.4.2). In more recent studies

bovine serum albumin (sigma fraction V) was included in the medium (section 10.3.4.).

5 grams of tissue were biopsied from the apex of the left ventricle, cooled to 4°C, finely diced, placed in medium containing 0.18 M KCl and 10mM EDTA, pH 7.2 - 7.4 (8 volumes medium per gram of tissue) and then homogenised for 10 seconds with a polytron type PT 10 - 35 tissue processor at a rheostat setting of 2. All remaining procedures were conducted at 0 - 4°C. The suspension was further homogenised using a teflon and a dounce homogeniser to ensure complete cellular disruption. Mitochondria were separated by differential centrifugation and washed twice in the isolation medium (Sordahl AND Schwartz 1971). After the final centrifugation of 1200 g for 10 minutes the pellet was suspended in 2 ml isolation medium.

#### ASSAY PROCEDURE

Oxygen consumption was measured by the method of Chance and Williams (1956) using a Clarke oxygen electrode with the vessel temperature controlled at 30°C. The assay medium (3 ml) consists of 10mM glutamate and 10mM  $K_2HPO_4$  in 0.25 M sucrose - 10mM tris - HCl pH 7.4. The reaction was initiated by the addition of 0.2ml pig heart mitochondria (10-20 mg/ml protein ).

After an initial downward deflection the mitochondria reached a steady state of stage 4 respiration (figure 2.0). Addition of 1.68  $\mu$ moles of ADP resulted in state 3 respiration. Respiratory rates were calculated in nano atoms of oxygen used per mg of protein per minute in state 3 ( $QO_2$ ).

The respiratory control index (RCI) (the ratio of the state 3 to the state 4 rate) and the ADP:O ratio (the amount of ADP phosphorylated per molecule of oxygen consumed was calculated (figure 2.0). Mitochondrial protein concentrations were determined by the procedure of Lowry et al (1951) using bovine serum albumin (sigma) as a standard. In the freshly arrested unpreserved control group, the mitochondrial  $QO_2$  was also determined at 23°C and 8°C respectively.

#### 2.1.9. LYSOSOMAL ISOLATION

The lysosomes were isolated from 3 groups of hearts :

1. Control (freshly excised immediately after cardioplegic arrest).
2. Hypothermically preserved for 24 hours.
3. Hypothermically preserved for 24 hours followed by 1 hour warm blood reperfusion.

The entire isolation procedure was carried out at 4°C. The hearts were placed on ice and 5 grams of tissue accurately dissected from the apex before being placed in isolation medium consisting of 0.01M EDTA and 0.18M KCl at pH 7.4 Wildenthal (1975). Intermittent homogenisation (4 x 4 seconds) of the tissue was carried out using a polytron PT 10-35. The homogenate was suspended in 40 ml isolation medium, gently homogenised using a teflon dounce homogeniser and centrifuged at 1200 g for 10 minutes. The resulting pellet was kept on ice and the supernatant filtered through a cheese cloth, and again centrifuged at 20 000g for 30 minutes. The supernatant was stored on ice and the pellet washed a further 2 times by centrifugation at 20 000g for 3 minutes. The final pellet was dissolved in 2ml of isolation buffer. The resulting fractions, supernatant, particulate and nuclear were then stressed in the detergent Triton X-100 (1%) to determine their latent activities.

In addition the particulate fractions were also subjected to milder treatment by stressing in Triton X-100 (0.1%).

#### ASSAY PROCEDURE

Lysosomal acid phosphatase activities were determined by the formation of p-nitrophenol at 405 nm from the substrate p-nitrophenol phosphate. The acid phosphatase activity in the supernatant, particulate and nuclear fractions of freshly preserved hearts were compared with those in the corresponding fractions of freshly isolated control hearts.

#### 2.1.10 ISOLATION OF SARCOPLASMIC RETICULUM VESICLES (SRV) ELETR AND INESI 1972

The sarcoplasmic reticulum (SRV) from 3 groups of hearts were isolated:

1. Control (freshly excised immediately after cardioplegic arrest).
2. Hypothermically preserved for 24 hours.
3. Hypothermically preserved for 24 hours followed by 1 hour warm

blood perfusion. 60 gms ventricular tissue were diced in cubes of about 2 cms each, washed and cooled in 0.1mM EDTA pH 7.4 and homogenised in 300ml of medium 1:10mM histidine (pH 7.4), 10% sucrose and 0.1 mM EDTA using a Waring blender. Homogenisation was carried out every 5 minutes for 15 bursts of 12 seconds, maintaining the pH at 7.4 after each homogenisation with 5% NaOH. The homogenate was then centrifuged at 15 000g for 20 minutes and the pellet discarded. The supernatant was filtered through glass wool previously washed in medium 1. The filtered suspension was centrifuged at 40 000g for 90 minutes and the sediment resuspended in 55ml of medium 2:10mM histidine, (pH 7.4) and 0.6M KCl and incubated for 40 minutes at 2 - 3°C, resulting in the solubilisation of actomyosin. The suspension was recentrifuged at 15 000g



for 20 minutes and the pellet discarded. The supernatant was again centrifuged at 78 000g for 60 minutes and the final sediment resuspended in 1 ml imidazole pH 7.4, 0.25M sucrose. The protein content of the final pellet was determined using the folin Lowry method with bovine serum albumin(*sigma*) as a standard (Lowry et al 1951).

#### ASSAY OF CALCIUM TRANSPORT (MILLIPORE FILTRATION TECHNIQUE)

The method is essentially that described by Martonosi and Ferretos (1964), in which the amount of radioactive calcium transported into the SRV is measured by the radio activity remaining on a millipore filter. The inclusion of oxalate, a freely permeable anion ensures that the transported calcium is precipitated within the vesicular lumen as insoluble calcium oxalate. The assay mixture (0.5ml) contained 20 mM histidine pH 6.85, 50mM KCl, 5 mM  $MgCl_2$ , 0.5 mM EGTA, 0.5 mM  $^{45}CaCl_2$  (3000cpm/nmol, free calcium ion concentration = 11.2  $\mu M$ ), 5mM potassium oxalate and 10 - 50  $\mu g$  SRV protein. The reaction at 25°C was initiated by addition of ATP and terminated at various time intervals by filtering aliquots (0.2ml) through a millipore filter (type HA 0.5  $\mu$  pore size) and washing with 15 ml water. The reaction was considered to be terminated as soon as washing commenced. The filter discs were dried, placed in 10ml of instagel (Pacard) and assayed for radioactivity. The amount of calcium transported per milligram protein, calculated from the radioactivity remaining on the filter was plotted as a function of time (figure 2.1) and found to be linear for at least one minute. The calcium uptake velocity (nmol calcium/min/mg) was calculated from the slope of the curve. Controls containing no ATP (figure 2.1) were assayed for each preparations and were always  $\leq 1\%$ .

### 2.1.11 ORTHOTOPIC TRANSPLANTATION

#### COLD CARDIOPLEGIC ARREST AND DONOR HEART EXCISION

Baboons weighing 10 to 20 kg were premedicated with ketamine hydrochloride 100 mg i.m. The baboon was given pancuronium 2 mg i.v., atropine 0.3 mg i.v., morphine 7.5 mg i.v., intubated endotracheally and ventilated with oxygen (4ℓ/min) and nitrous oxide (6ℓ/min). Cephalothin sodium 1gm i.v., and methylprednisolone 125 mg i.v. The heart was not excised until blood gases and acid-base balance were within normal limits.

Through a median sternotomy the azygos vein was doubly ligated and divided. Heparin (1000 units/kg i.v.) was given. The superior vena cava (SVC) was then doubly ligated cephalad to the azygos vein and divided. The brachiocephalic artery was ligated distally and cannulated for subsequent cardioplegic infusion, the tip of the cannula lying in the arch of the aorta.

The aorta was cross-clamped distal to the cannula, followed by immediate division of the inferior vena cava (IVC) and one or more pulmonary veins, and 500 mℓs of cardioplegic solution (Table 2.1) at 4°C was infused, the infusion pressure not being allowed to rise above 100 mmHg. Cessation of heart beat occurred within 10 to 15 seconds if both right and left sides of the heart were adequately decompressed; the infusion of 500 mℓ solution was completed within 1 to 3 minutes by which time myocardial temperature had fallen to approximately 17°C.

Excision of the heart was then completed by division of the pulmonary veins, the pulmonary artery at its bifurcation, the aorta proximal to the brachiocephalic artery, and the

mediastinal tissue posterior to the heart. The heart was then serially immersed for one minute in each of two bowls containing cold 4°C normal saline or cardioplegic solution causing the myocardial temperature to fall to approximately 12°C.

The heart was then weighed and transferred to the perfusion apparatus. The time interval between aortic cross-clamping and the initiation of perfusion under refrigeration conditions was approximately 10 - 12 minutes.

#### 2.1.12 TECHNIQUE OF ORTHOTOPIC TRANSPLANTATION

Baboons weighing 10 to 20 kg and matched for AB blood group with donor baboons, were premedicated, anaesthetised and ventilated as were the donors.

Double doses of pancuronium, atrophine and morphine were given and these doses were repeated after opening the chest. Cephalothin sodium 1 gm i.v. was given and repeated eight hourly for three days. Methylprednisolone 25 mg i.v. was given and was repeated at intervals during the operative and early post-operative period until a total of 125 mg had been administered (or 10 mg/kg/body weight in those baboons to be immunosuppressed).

After the recipient heart had been excised, the donor heart was removed from the overnight perfusion apparatus. The donor heart was weighed. At 20 minute intervals throughout the period of insertion, saline at 4°C was syringed into the pericardium to cover the heart to maintain a low myocardial temperature. Myocardial temperature rose to approximately 20°C during the period of insertion.

It has been our policy to continue pump-oxygenator support for 60 minutes following the release of the aortic clamp. Spontaneous coordinated contractions or, rarely, ventricular fibrillation requiring electrical defibrillation occurred. Before discontinuation of cardiopulmonary bypass, an isoproterenol hydrochloride infusion (1 microgram/minute) was set up, and if urine output was low, frusemide 3 - 5 mg i.v. administered.

#### 2.1.13 POST-OPERATIVE MANAGEMENT

The baboon was weaned off ventricular support over the next four hours, isoproterenol support was maintained electively for one to two hours and then slowly reduced. On a few occasions it has been necessary to add a dopamine hydrochloride infusion (100 micrograms/minute) to maintain an adequate pressure during the immediate post-operative period; this was discontinued as soon as possible. Once the baboon was haemodynamically stable without inotropic support, maintaining satisfactory blood gases, breathing spontaneously on air, it was extubated and returned to its cage.

An immunosuppressive regime (to 12 of the 22 baboons) consisting of azathioprine 2.5 mg/kg/day and a decreasing dose of methylprednisolone (until by the fourth post-operative day the baboon was receiving a maintenance of 2 mg/kg/day) was given by orogastric tube passed under ketamine sedation. No attempt was made to treat acute rejection episodes (suggested by diminishing QRS voltage on ECG) by increased immunosuppressive therapy.

### 2.2.1 CARDIOPLEGIA

#### COMPOSITION OF THE CARDIOPLEGIC PERFUSATE

There is an obvious parallel between the short-term cardiac arrest during open-heart surgery and long-term 24 hours preservation of hearts for subsequent transplantation. In each case, functional integrity must be preserved as well as possible, during a period of imposed reduction of metabolic activity. A brief historical review of methods of myocardial protection and the type of solution chosen for this study was introduced in section 1.1. The determination of an ideal cardioplegic agent reaches far beyond the scope of this study and the choice of an existing effective solution was, therefore, compulsory. Small changes were, however, made with the intention of preventing cardioplegic induced injury from extending into the hypothermic perfusion period. The composition of the cardioplegic agents used during the stages of development of the perfusates are shown in table 2.1. The solution entitled C1 was used to arrest the fresh control hearts and hearts preserved in groups 1 - 6. The solution entitled C2 was used for the arrest of groups 7, 8 and 9. It was hoped that the solution C2 would provide the myocardium with most of its natural ionic constituents (Hearse et al 1975). The effects of potassium (section 5.1.1), magnesium (section 5.1.2) and procaine (section 5.1.4) and the importance of calcium (section 10.5.1) on cardiac metabolism are discussed elsewhere. Although absent from the final perfusate, insulin was still present in the cardioplegic solution and was considered essential for the following reasons. During cardioplegic arrest myocardial activity was converted from an active to a passive stage, during which time, insulin was thought essential to facilitate the use of substrate until contractile motion ceased

and to possibly maintain glycogen stores. In combination with hypothermia the cardioplegic effect was produced by potassium, (Wright et al 1978; Gharagozloo et al 1979; Lucas et al 1980) magnesium (Hearse et al 1978) procaine (Fey et al 1977) and verapamil (Nicholson et al 1978). Calcium may not be essential in cardioplegic solutions used during open-heart surgery because it is supplied to the myocardium by non-coronary collateral flow (Buckberg 1979). On the other hand, in the isolated heart used in cardiac transplantation, the non-coronary collateral flow is absent and calcium is then a pre-requisite in cardioplegic solutions as its absence results in a so-called calcium paradox (Zimmerman et al 1967; Jynge et al 1977; Yates 1975) and may even lead to the rare extreme form of ischaemic damage called "stone heart" (Cooley et al 1972).

During the development of the perfusate certain hearts in various groups developed enhanced resting myocardial tension, becoming hard to the touch and sometimes resembling the condition of myocardial contracture. Some of these features occurred immediately after the initial cardiac arrest and in most cases at the end of the period of preservation. The exact cause of this phenomenon was not identified but it was thought to be related to changes in myocardial calcium distribution. To help protect against some induced injury during the cardioplegic period, the slow channel calcium inhibitor verapamil (calcium antagonist) was included in the new cardioplegic solution. This new solution was used to arrest hearts in groups 7, 8, 9, and human hearts preserved for clinical transplantation. According to Poole-Wilson (1981), verapamil acts by inhibiting only the slow channel calcium flow of myocardial smooth muscle and pacemaker cells. Thus, a conduction system-induced negative inotropic effect

occurs, reducing the work of the heart at the onset of ischaemia, delaying and later protecting the rate of ATP consumption (Singh et al 1978; Poole-Wilson 1981). Verapamil protects against toxic doses of isoprenaline and also has vasodilator properties (Singh et al 1978); Opie 1980).

## RESULTS

These observations were made during the development of the various perfusates with the aim of preventing any possibility of irreversible damage occurring during or after the period of cardioplegic arrest.

### 2.2.2. ATP, CP AND GLYCOGEN

Changes in tissue levels of ATP, CP and glycogen are shown in table 2.0. After cardioplegic arrest these values all remained within the normal range. Therefore, no depletion occurred during arrest.

### 2.2.3. LDH

The release of LDH during the period of cardioplegic arrest (using C1) is shown in figure 2.3. As the blood was washed from the coronary vessels, the LDH level rapidly decreased. The myocardium did not release significant levels of LDH during this period.

### 2.2.4. TEMPERATURE CHANGES DURING CARDIOPLEGIA

The effect of cardioplegic infusion and topical cooling on myocardial temperature is shown in figure 2.4. An initial rapid decrease in temperature (25°C) occurred, followed by a more gradual decrease.



## 2.2.5. DISCUSSION AND CONCLUSIONS

Most cardioplegic solutions offer some degree of protection to the myocardium during ischaemia but, on rare occasions, hearts respond unusually to cardioplegia resulting in ischaemic contracture "stone hearts". In this dissertation 264 pig hearts were cardioplegically arrested, of which 5 developed enhanced resting myocardial tension. Subsequent preservation of these 5 hearts followed by functional testing resulted in no cardiac output at all under the testing conditions. The purpose of this brief study was to choose or modify an existing cardioplegic solution such that the above changes would not occur. The results of measurement of the tissue high energy phosphates and glycogen suggest that no injury had been sustained by the myocardium during cardioplegic arrest. The pattern of release of LDH similarly indicated no injury. The cardioplegic solution C2 was also used to arrest baboon (N=30) hearts for subsequent valve replacement. The solution was infused into the myocardium every 20 minutes. After cessation of cardiopulmonary bypass these hearts regained sinus rhythm and only rarely required inotropic support. Mean pressure ranged between 80 - 120 mmHg and no problems were ever encountered; all the animals were returned to their cages within 4 - 10 hours after cardiopulmonary bypass.

In this study the actual cause of injury to the myocardium during cardioplegia was not identified, but a significant feature was the rapid decline in myocardial temperature (25°C) within the first minute of infusion of cardioplegic solution (figure 2.4).

The initial rate of temperature change was induced by cardioplegic infusion and topical cooling was 0.4°C/second, 14 fold greater than the threshold rate of injury of 0.03°C/second (Lovelock 1954). The mean rate of cooling during the second and third minute of



cardioplegia was  $0.038^{\circ}\text{C}/\text{second}$  and  $0.052^{\circ}\text{C}/\text{second}$  respectively (figure 2.4). Smith (1954) has shown that many types of living cells suffer damage on rapid cooling, though slow cooling or prolonged exposure to low temperature may be harmless. Changes in a physical environment producing this type of damage in sensitive cells are described by the general term "thermal shock" (Lovelock 1955). Lovelock (1955) describes the nature of the damage to be mechanical failure of the cell membrane to cope with the stress of differential temperature gradients. He attributes the extent of damage to the temperature of the cells and the interval of the temperature through which it is cooled. In these experiments using both bull spermatozoa and red blood cells, he showed a change in temperature from  $30^{\circ}\text{C}$  to  $0^{\circ}\text{C}$  to destroy 90% of the cells, provided the rate of cooling was greater than  $0.03^{\circ}\text{C}/\text{seconds}$ . The membrane also hardens as the temperature falls due to solidification of the lipid components increasing the tension and predisposing it to fracture (Raison and Lyons 1970). This phase transition in the fluidity of the membrane also affects membrane-associated enzyme activity. Nucleated cells have also been shown to be destroyed by reduction in temperature without freezing (Ashwood-Smith and Forrant 1980). The effect of cooling on the myocardium in this respect is unclear but it is reasonable to assume that a drastic change in myocardial temperature would affect membrane structural components, although a relative qualitative difference between bull spermatozoa, red blood cells and myocardial cells exist.

It was hoped that this cardioplegic solution would protect against vast temperature changes and calcium overload. Protection against temperature induced injury was provided by the procaine hydrochloride (Buckberg 1979) and the elevated magnesium

sulphate concentration (Holland 1964). Injury by calcium overload was presumably prevented by incorporating the slow channel inhibitor verapamil into the perfusate. The dosage of verapamil was calculated not to lead to myocardial depression.

### 2.3.1. THE HYPOTHERMIC PERFUSION APPARATUS

The non-pulsatile perfusion apparatus (figure 2.2) was based on that originally described by Proctor and Parker (1968) and subsequently used by Copeland et al (1973). In this apparatus the heart was perfused at 8 to 10 cm H<sub>2</sub>O pressure for 20 to 24 hours under refrigerated conditions (6°C to 8°C). The perfusate was a clear fluid hyperosmolar solution to which 95% O<sub>2</sub> and 5% CO<sub>2</sub> were continuously bubbled to maintain the perfusate pH between 7.2 and 7.4. Myocardial temperature stabilized after 1 - 2 hours at approximately 8°C. The non-pulsatile perfusion apparatus consisted of a cylindrical perspex 4 litre chamber in which the heart was suspended by the aorta, through which it was perfused. The two perfusion circuits consisted of sterile polyvinyl tubing and two peristaltic roller pumps. One circuit incorporated a 0.8 Micron filter delivering perfusate from the lower reservoir to the aorta, the second circuit decompressed the right ventricle by aspirating coronary venous return from it and returning it to the lower reservoir into which the gas mixture was bubbled. This system was primed with 2 litres of perfusate whose pH was measured at 30 minute intervals and adjusted by increasing or decreasing the gas mixture flow. All perfusate entering the right ventricle was coronary venous return, coronary flow could therefore be readily collected and measured. At the beginning and end of the perfusion period, coronary flow and coronary arterial venous O<sub>2</sub>

uptake were measured, coronary resistance was calculated.

After the period of perfusion the heart was either subjected to further biochemical analysis or weighed and functionally evaluated.

#### 2.4.1. FUNCTIONAL TESTING APPARATUS

Various techniques of ex vivo functional testing have been used in the evaluation of cardioplegic solutions and isolated heart function (Neely et al 1967; Pitzele et al 1968; Levitsky et al 1970).

Absolute confidence in these systems as a method of functional assessment is lacking, and orthotopic transplantation is the ultimate determinant of viability. The latter is, however, a difficult, time-consuming and expensive procedure. For the results of ex vivo testing to be meaningful, any evaluation must be correlated with and confirmed by orthotopic transplantation. This study describes a simple method for the functional evaluation of the heart which we believe provides an accurate prediction of the function of the heart if that heart were to be orthotopically transplanted. This system is thus suitable for quantitative screening of various storage techniques without the need for orthotopic transplantation. This system is shown in figure 2.5.

After the 24 hour preservation period the hearts were functionally evaluated. In groups 1 to 6 the apparatus was unmodified whereas in groups 7 to 9 an aortic balloon and a non-linear resistance were included in the apparatus (figure 2.5) (See Appendix 2). Parameters determined during functional testing therefore differed quantitatively between groups 1 to 6 and groups 7 to 9.

Parameters determined during the hypothermic preservation period were not influenced by the change to the functional testing apparatus.

Oxygenated blood matched for group was supplied by a perfusor pig, to which deoxygenated blood was returned (ventilation of this animal was monitored by repeated determination of arterial  $PO_2$

pCO<sub>2</sub> and pH). Initially the aorta was retrogradely perfused as a Langendorff system supplying blood to the coronary arteries of the isolated heart. This allowed a period of recovery, usually continued for 5 - 15 minutes. When the myocardial temperature registered 37°C, the heart was defibrillated and the model converted into the working mode. Blood was then directed into the left atrium of the isolated heart, converting the role of the heart from a passive to an active one by challenging the left ventricle with blood from the left atrial reservoir. If myocardial preservation had been good, the left ventricle pumped blood (through the aortic balloon and non-linear resistance of the modified apparatus) to a height of a 100cm H<sub>2</sub>O after which the blood was returned to the left atrial reservoir. Preload and afterload were adjustable but usually kept constant at 11cm H<sub>2</sub>O and 80mmHg respectively during the period of testing. All hearts were tested for one hour during which time haemodynamic parameters were recorded. Cardiac output measurements were made twice during the one hour period. The pressure determinations were recorded at the same time. Coronary flow, stroke volume and oxygen uptake were measured at the end of the one hour period. The hearts were then weighed and biopsied for biochemical and histological examinations.

In reality a functional measurement that can be related to the precise number of myocardial cells destroyed or damaged within the heart at a given period of time would be ideal. This measure presently does not exist, so the classical method of Neely et al (1967) (described below) was used for myocardial assessment. Use was not made of the intra ventricular balloon which had frequently been made to assess heart function in larger animals (Pitzele et al 1968; Levitsky et al 1970); this method involves removal of the mitral valve leaflets allowing the insertion of a soft latex

balloon into the left ventricular cavity. The mitral valve ring is then closed using a purse string suture and the balloon inflated with fluid. The fluid was unejectable and the model was considered unphysiological.

#### 2.4.2. FUNCTIONAL EVALUATION OF FRESHLY ISOLATED (CONTROL) AND EXPERIMENTAL HEARTS

##### METHODS

Healthy pigs, weighing 15 - 25 kgs were used as heart donors. After overnight starvation and premedication with ketamine hydrochloride (5mg/kg) the animal was anaesthetised with intravenous (i.v.) thiopentone sodium (2.5 mg/kg) and morphine (1.5 mg/kg). Alcuronium chloride (0.5 mg/kg i.v.) was used as a muscle relaxant. Endotracheal intubation was carried out and ventilation maintained with oxygen (3L/min and nitrous oxide (6L/min). Ventilation was monitored by repeated determination of arterial  $pO_2$ ,  $pCO_2$  and pH.

#### 2.4.3. COLD CARDIOPLEGIC ARREST AND EXCISION OF THE HEART

A median sternotomy was performed. Heparin (1000 units/kg i.v.) was given. The branchiocephalic artery was ligated distally and cannulated proximally, the tip of the cannula lying in the aorta. The superior vena cava, azygos vein, and both right pulmonary veins were each doubly ligated and divided. The aorta was cross-clamped distal to the origin of the branchiocephalic artery. The inferior vena cava (after clamping at the diaphragm) and both left pulmonary veins were divided, followed immediately by the infusion of 500 ml of cold 4°C cardioplegic solution (table 2.1) through the branchiocephalic artery cannula, the infusion pressure not being allowed to rise above 100 mmHg. The heart was also bathed in 500 ml of normal saline at 4°C. Arrest of the heart occurred within 15 - 20 seconds after the beginning of infusion (which was completed within 1 - 3

minutes) by which time myocardial temperature had fallen to approximately 10 - 15°C.

Excision of the heart was then complete, with the heart immersed in cold 4°C normal saline for 1 minute. While immersed the bridge of tissue between the orifices of the left superior and inferior pulmonary veins was excised and a continuous suture placed around this opening, for subsequent ligation around the left atrial cannula of the testing apparatus. The heart was then weighed and either transferred to the functional testing apparatus or subjected to 24 hours hypothermic perfusion preservation. The total time from initiation of infusion of cardioplegic solution taking approximately 10 - 14 minutes.

#### 2.4.4. PARAMETERS DETERMINED DURING EX VIVO FUNCTIONAL EVALUATION

The system is shown in figure 2.5. The following parameters were either measured or recorded.

1. Cardiac output (CO) was obtained by a timed collection of aortic output plus coronary venous return (CF).
2. Stroke volume (SV) was calculated using the formula  $CO = HR \times SV$  where HR = heart rate.
3. Pressure determinations were recorded using a Statham P23H transducer: Left ventricular pressure (LVP), left ventricular end-diastolic pressure (LVEDP) and aortic pressure (AOP).
4. Oxygen tension was measured with an oxygen electrode from which myocardial oxygen uptake was calculated by the following formula:

$$\text{Oxygen consumption mmol/h/gm} = \frac{\text{Arterial-venous oxygen tension (mm Hg)}}{760 \text{ (mm Hg)}} \times \frac{\text{Solubility of oxygen at } 37^{\circ}\text{C (ml/ml H}_2\text{O)}}{22.4 \text{ (ml/mole)}}$$

X

$$\frac{\text{Coronary flow (ml/h)}}{\text{Dry weight of heart (gm)}}$$

#### 2.4.5. CONTROL HEARTS

The functional testing apparatus was used to test control hearts and hearts preserved in groups 1 - 6. Thereafter the apparatus was modified incorporating an aortic balloon and a non-linear peripheral resistance. This modified version was used to test hearts in groups 7 - 9. The advantages and proposed dynamics of the new system are discussed in appendix 2.



TISSUE ADENOSINE TRIPHOSPHATE, CREATINE PHOSPHATE AND GLYCOGEN LEVELS AT THE  
BEGINNING AND AT THE END OF CARDIOPLEGIC INFUSION

|  | C1<br>n = 8                                                                                                                                |                                         | C2<br>n = 8                             |                                           |
|--|--------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|-----------------------------------------|-------------------------------------------|
|  | At the beginning<br>of Cardioplegia                                                                                                        | At the end<br>of cardioplegia           | At the beginning<br>of cardioplegia     | At the end<br>of cardioplegia             |
|  | ATP ( $\mu\text{mol/gm/wet wt}$ )<br>CP ( $\mu\text{ mol/gm/wet wt}$ )<br>Glycogen ( $\mu\text{ mol/gm/wet wt}$ )<br>(Glucose equivalents) | 3.61 (0.29)<br>5.02 (0.87)<br>30 (2.91) | 3.98 (0.35)<br>5.98 (0.89)<br>34 (3.22) | 4.06 (0.26)<br>6.76 (0.66)<br>29.2 (1.71) |

C = cardioplegic solution

CONSTITUTION OF THE CARDIOPLEGIC AGENTS

|                                          | C1               |       | C2               |       |
|------------------------------------------|------------------|-------|------------------|-------|
|                                          | g/L              | (mM)  | g/L              | (mM)  |
| 1. NaCl                                  | 6.54             | 111.9 | 6.00             | 102.0 |
| 2. NaHCO <sub>3</sub>                    | 2.10             | 25.0  | 0.38             | 4.0   |
| 3. KCL                                   | 0.82             | 11.0  | 0.75             | 10.0  |
| 4. KH <sub>2</sub> PO <sub>4</sub>       | 0.16             | 1.2   | -                | -     |
| 5. CaCl <sub>2</sub> · 2H <sub>2</sub> O | 0.15             | 1.1   | 0.15             | 1.1   |
| 6. MgSO <sub>4</sub> · 7H <sub>2</sub> O | 0.29             | 1.2   | 3.50             | 14.0  |
| 7. Procaine hydrochloride                | 0.27             | 1.0   | 0.27             | 1.0   |
| 8. Insulin                               | 40 units/L       |       | 20 units/L       |       |
| 9. Glucose                               | 2.00             | 11.1  | -                | -     |
| 10. Dextrose                             | -                | -     | 50               | 278   |
| 11. Verapamil hydrochloride              | -                | -     | 1.5mg            | 3.1µM |
| 12. Osmolality                           | 300 milliosmoles |       | 320 milliosmoles |       |
| 13. pH at 4°C                            | 7.4              |       | 7.4              |       |

C = Cardioplegic solution

TABLE 2.1

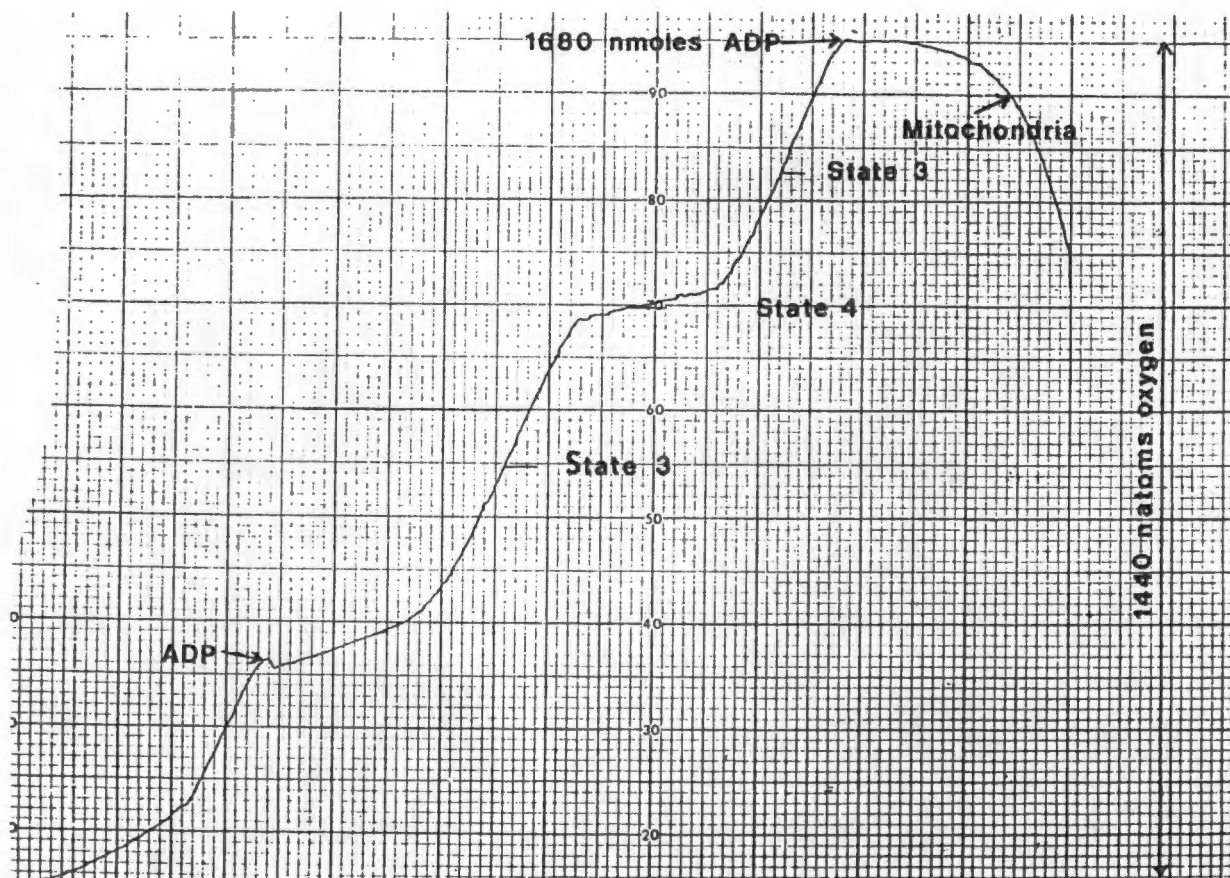


Fig. 2.0

A recording of in vitro mitochondrial oxygen uptake using a Clarke's oxygen electrode.

State 4 represents a steady state of respiration.

Addition of ADP results in state 3 respiration in which ADP is phosphorylated forming ATP.

Once all the ADP has been phosphorylated, the mitochondria resort to state 4 respiration again.

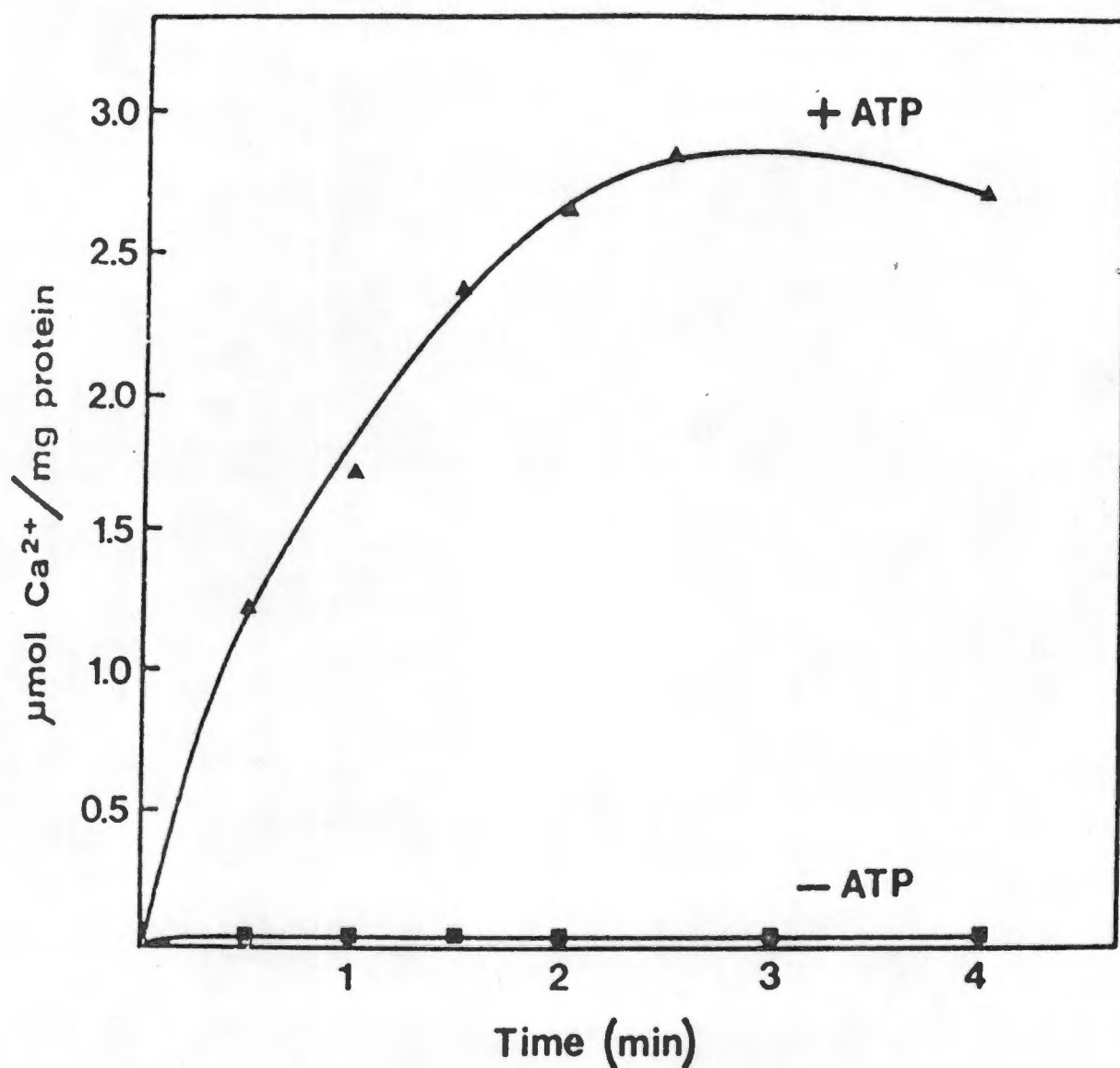


Fig. 2.1.

Kinetics of  $\text{Ca}^{++}$  transport by SR vesicles.  $\text{Ca}^{++}$  uptake was measured in the standard assay mixture containing 10 ng/ml SR protein. The reaction at 25°C was initiated by addition of ATP (5mM) and terminated by filtering aliquots through a millipore filter at the various time intervals indicated. After washing, the  $^{45}\text{Ca}^{++}$  remaining on the dried filters was assayed in a scintillation counter.

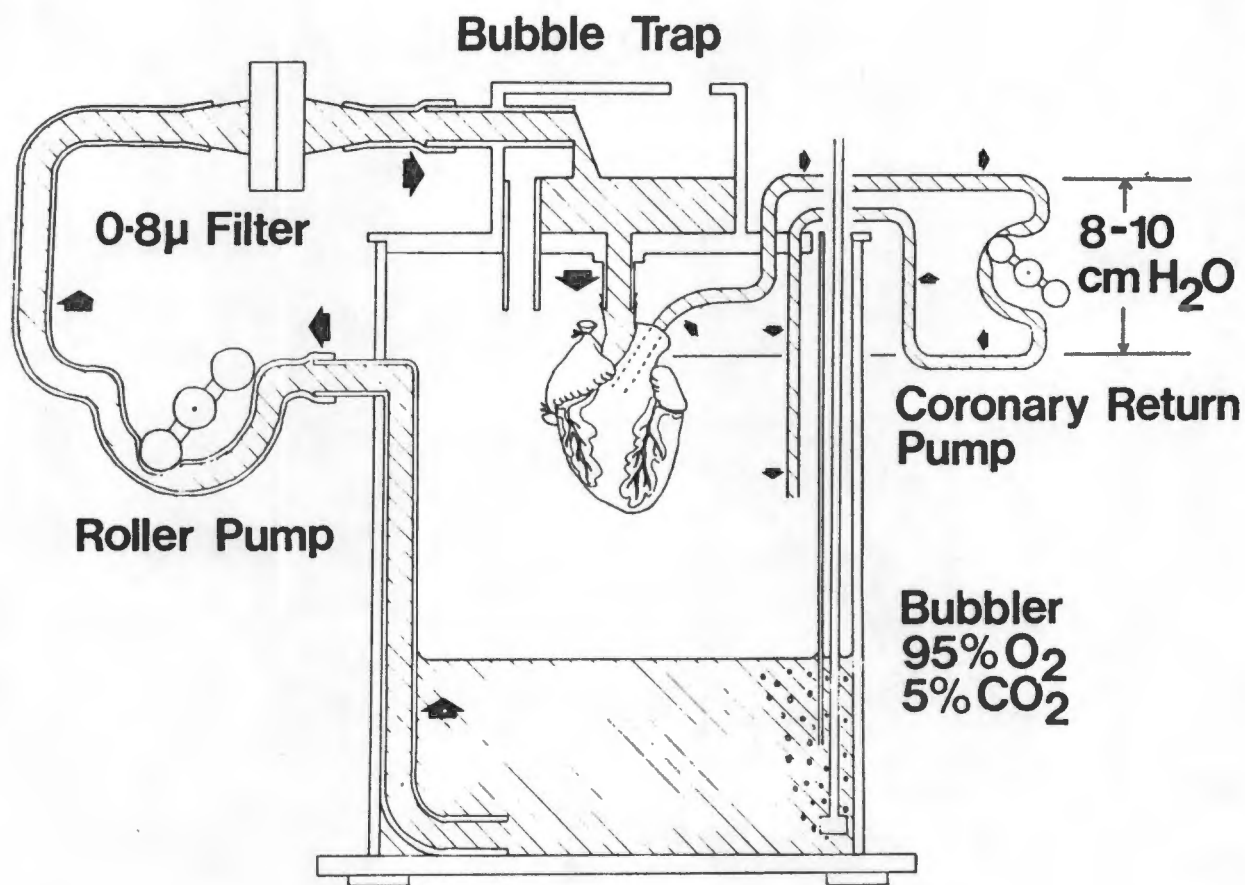


Fig. 2.2.

Diagram of the laboratory non-portable hypothermic perfusion unit.

Perfusate is pumped from the lower reservoir through a 0.8 micron filter into the upper reservoir (bubble trap), from which the aorta is retrogradely perfused. Coronary effusate is pumped from the right ventricle back into the lower resêvoir.

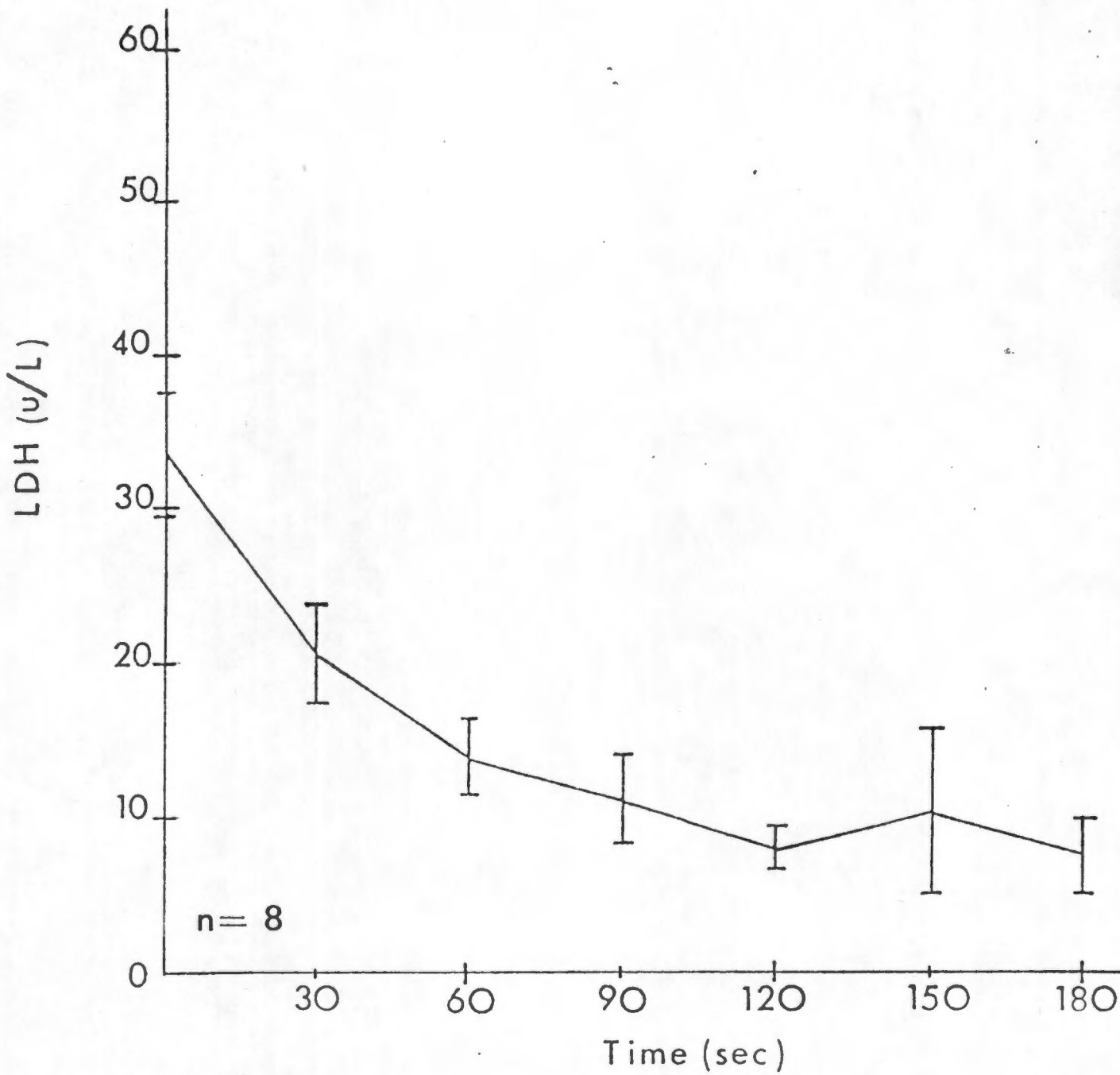


Figure 2.3

The effect of 4°C cardioplegic infusion on myocardial lactate dehydrogenase (LDH) release, taken over a period of 3 minutes. Infusion of cardioplegic solution began at time zero seconds and continued throughout the 3 minutes. Bars represent S.E.M.

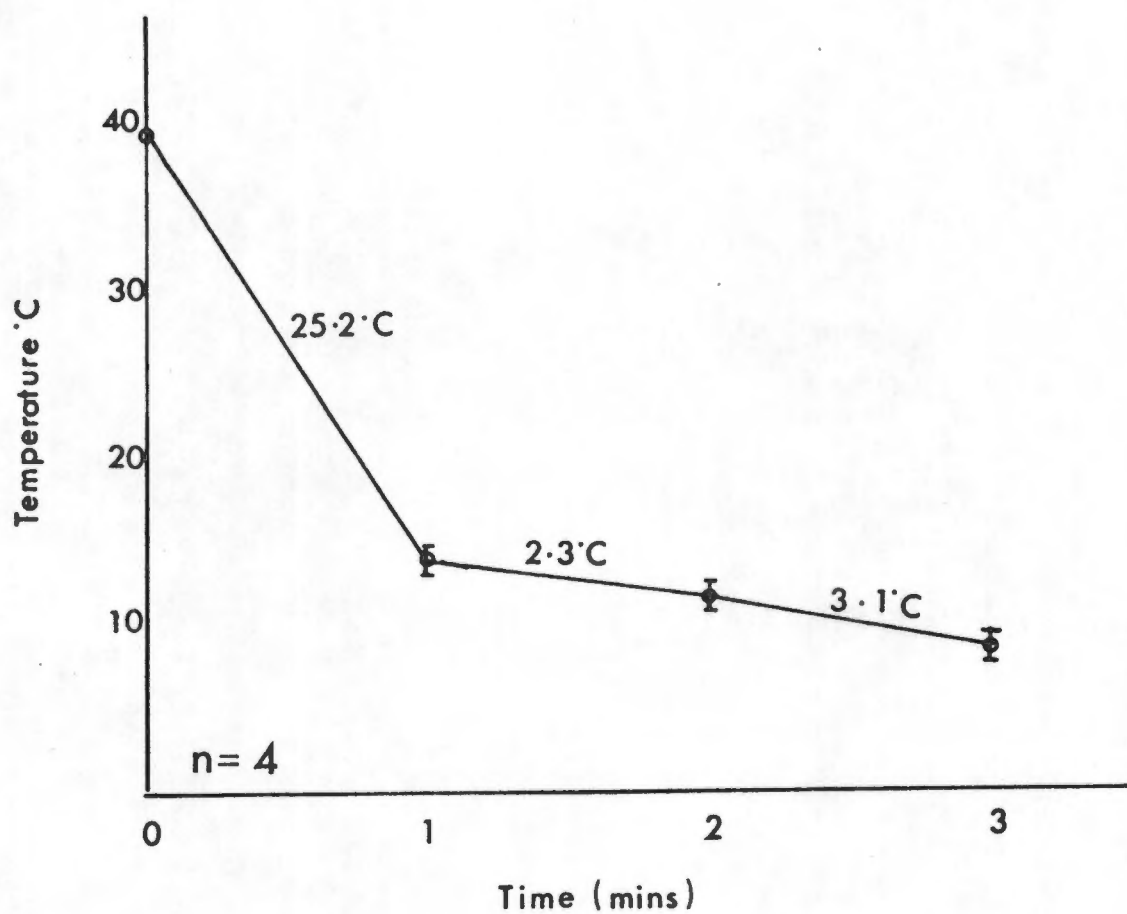


Fig. 2.4.

Myocardial septal temperature during the period of 4°C cardioplegic infusion. Bar represents S.E.M.

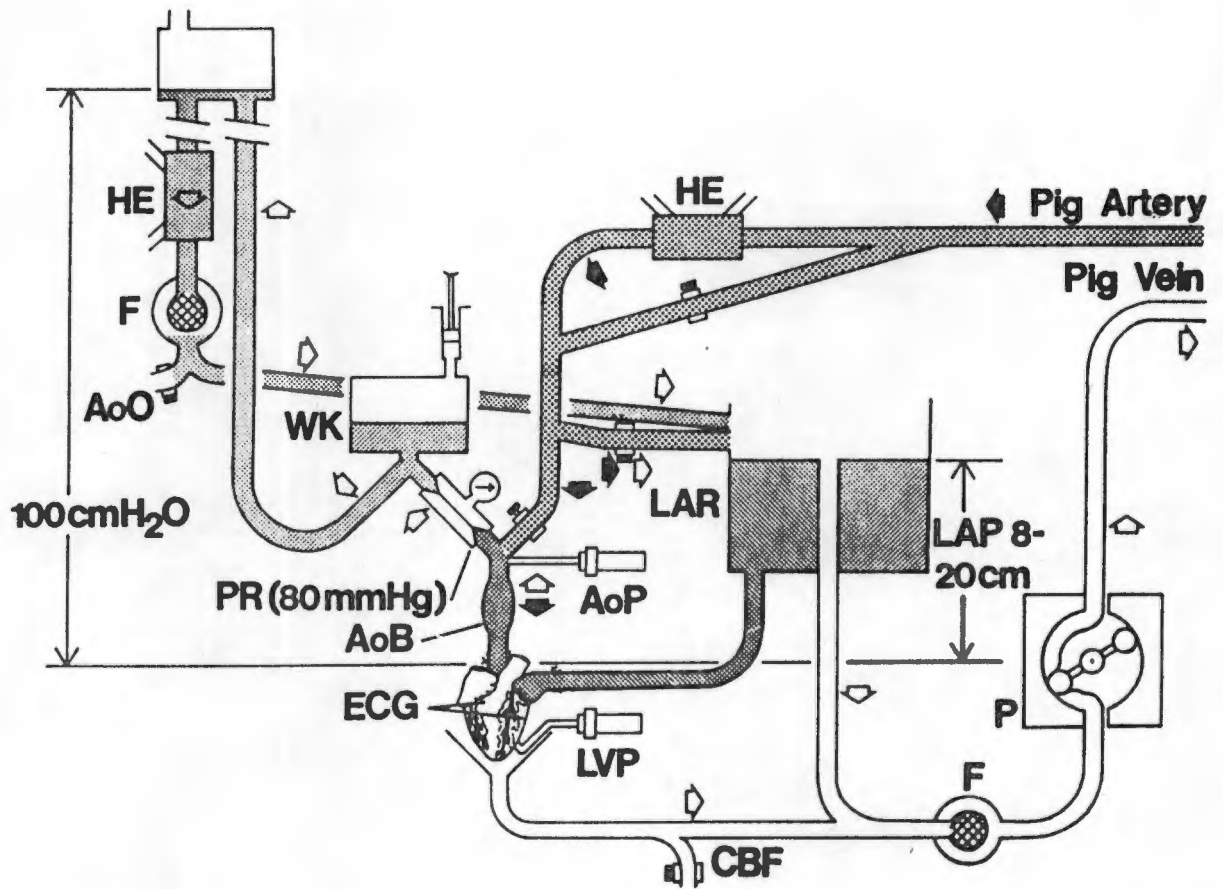


Fig. 2.5.

Functional testing apparatus. HE = heat exchanger, WK = windkessel, PR = periforal resistance, AOB = aortic ballon, LAR = left atrial resêvoir, LVP = left ventricular pressure, CBF = coronary blood flow, LAP = left atrial pressure, F = filter, P = pump.

Oxygenated blood is supplied to the circuit from an anaesthetized perfusor pig (black arrows). Blood flows from the left atrial resêvoir to the left atrium at a fixed pressure of 11cm H<sub>2</sub>O. The blood is then pumped through the circuit by the heart as illustrated by the white arrows. CBF returns to the perfusor pig for re-oxygenation.

For functional determinations see text.



## CHAPTER 3

### GROUP 1

#### 3 PRESERVATION CONDITIONS

There are an infinitenumber of chemical combinations and concentrations possible for the preparation of an ideal perfusate (Katz 1980) and consequently no record of a perfect solution has been reported. At the beginning of this investigation my knowledge about preservation was limited and the simple, well studied Krebs and Henseleit solution was chosen as the initial perfusate (table 3.1). This perfusate was originally used by Neely et al (1967) to preserve functional viability of rat hearts at normothermia. All preserved hearts in the present study were hypothermically perfused for 24 hours. The preservation system is fully described elsewhere (Chapter 2). The parameters used for the estimation of viability after 24 hours preservation are tabulated in tables 3.2 and 3.3.

##### 3.1.1. ADENOSINE TRIPHOSPHATE (ATP) AND CREATINE PHOSPHATE (CP)

Determination of tissue ATP and CP prior to preservation and during the 24 hour preservation period are shown in figures 3.1 and 3.2 respectively. The ATP gradually decreased to less than 50% of the initial value and the CP had a more rapid decline within the first 3 hours of preservation.

##### 3.1.2. LACTATE DEHYDROGENASE (LDH)

Changes in the release of LDH into the preservation medium were monitored for 20 hours during the preservation period and the results are shown in figure 3.3. A large increase in perfusate LDH levels occurred after 9 hours of preservation reaching abnormally high values after 20 hours.

### 3.1.3. CREATINE KINASE (CK)

The release of CK into the perfusing medium was monitored for a 20 hour preservation period and the results are shown in figure

3.4. The perfusate concentration steadily increased throughout the period of preservation.

### 3.2.1. OBSERVATIONS DURING THE PERIOD OF HYPOTHERMIC PERFUSION

Coronary flow at the beginning of the perfusion period was 0.77ml/min/gm and became steadily less over the 24 hours; in five of the ten hearts no coronary flow could be recorded after 24 hours, and in the remaining five hearts the mean flow was 0.045ml/min/gm.

### MICROSCOPY

Histological examination showed interstitial oedema but no other significant changes.

### 3.3.1. OBSERVATIONS DURING THE PERIOD OF FUNCTIONAL TESTING

These hearts required repeated episodes of defibrillation, with many rhythm disturbances throughout the period of functional testing. The hearts gained 21% of their initial weight (table 3.4) after 24 hours preservation and gained an additional 2% after 1 hour warm blood reperfusion. The functional response was always poor. Contractions were weak and no cardiac output could be measured in six of the ten hearts tested (table 3.5). The mean coronary flow of these hearts was low when compared with control values (Table 3.6). With such poor functional response no further parameters were determined. At the end of the testing period the hearts felt hard to the touch.

### 3.4.1. DISCUSSION AND CONCLUSIONS

These results are characteristic of organ ischaemia (Trump et al), 1976) in which a progressive failure to maintain energy metabolism was manifested as a decline in ATP and CP (Calman 1974; Collins et al 1977). These hearts were hard to the touch which suggested that they had undergone ischaemic contracture of the myocardium.

(Cooley et al 1972; Katz and Tada 1977). The condition resembled rigor mortis in skeletal muscle; the left ventricle was firm and contracted, and this systolic arrest was irreversible. The resultant cellular derangements included acidosis (Vogt and Farber 1968), swelling, ionic exchange resulting in the release of destructive lysosomal enzymes (Malbica 1971; Wilkinson and Robinson 1974). A more dramatic result was the increased release of LDH and CK after 12 hours preservation (figures 3.3 and 3.4).

A high release of LDH was also found in poorly preserved kidneys (Johnson 1972). At this stage it was felt that a further gain in heart weight after reperfusion was related to poor preservation. It was well recognised that maintenance of a normal cell volume was an energy requiring process which used a major proportion in order to balance the osmotic effect of the Donnan equilibrium produced by the protein anions (Willis 1972). Thus, when the sodium potassium pump was inhibited by cold or the use of metabolic inhibitors, cell swelling was a characteristic response. Thores et al (1972) further suggested that this effect not only changes intracellular organelles, but also amplified and prolonged the ischaemic injury by interfering with restoration of blood flow to the organ. Belzer et al (1972) have shown vascular endothelium to be particularly sensitive to ATPase depression which was directly related to the no-flow effect.

The no-flow phenomenon was demonstrated in this study in which five hearts had no measurable coronary flow.

COMPOSITION OF PERFUSION SOLUTIONS USED TO PRESERVE  
HEARTS IN 9 DIFFERENT GROUPS

| Constitution of<br>perfusates (mM)   | 1     |
|--------------------------------------|-------|
| NaCl                                 | 118.5 |
| NaHCO <sub>3</sub>                   | 25    |
| KCl                                  | 4.75  |
| KH <sub>2</sub> PO <sub>4</sub>      | 1.20  |
| MgSO <sub>4</sub> 7H <sub>2</sub> O  | 1.20  |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O | 2.5   |
| MgCl <sub>2</sub>                    | -     |
| Glucose                              | 11    |
| Taurine                              | -     |
| ATP                                  | -     |
| Insulin                              | -     |
| Procaine HCl                         | -     |
| Dextran 40                           | -     |
| Chlorpromazine                       | -     |
| Phenoxybenzamine                     | -     |
| Glycerol                             | -     |
| Sucrose                              | -     |
| Osmolality (mOsm/L)                  | 277   |

TABLE 3-1

|                                       | GROUPS |   |   |   |   |   |   |   |   |
|---------------------------------------|--------|---|---|---|---|---|---|---|---|
|                                       | 1      | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| ATP                                   | +      | - | - | - | + | - | + | + | - |
| CP                                    | +      | - | - | - | + | - | + | + | - |
| LDH                                   | +      | + | - | - | - | - | + | + | - |
| CPK                                   | +      | - | - | - | - | - | - | - | - |
| Glycogen                              | -      | - | - | - | + | + | - | + | - |
| Lactate                               | -      | - | - | - | + | + | - | + | - |
| Wt gain                               | +      | + | + | + | + | + | + | + | + |
| Acid phosphatase                      | -      | - | - | - | - | - | - | + | - |
| Sarcoplasmic Reticulum<br>Ca++ uptake | -      | - | - | - | - | - | - | + | - |
| Oxygen uptake                         | -      | - | - | - | - | + | + | + | + |
| Glucose uptake                        | -      | - | - | - | + | - | - | - | - |
| Coronary flow                         | +      | - | - | - | - | + | + | + | + |
| Coronary resistance                   | -      | - | - | - | - | + | + | + | + |

The relevant information (results and statistical details) is tabulated in the respective chapters. This table is an over-view of all the biochemical and physiological determinations carried out during the period of hypothermic perfusion. ( + = tests performed; - = no tests performed).

TABLE 3.2

|                                                   | G R O U P S |   |   |   |   |   |   |   |   |
|---------------------------------------------------|-------------|---|---|---|---|---|---|---|---|
|                                                   | 1           | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Acid phosphatase                                  | -           | - | - | - | + | - | - | + | - |
| ATP                                               | -           | - | - | - | - | + | + | + | - |
| CP                                                | -           | - | - | - | - | + | + | + | - |
| Glycogen                                          | -           | - | - | - | + | - | - | + | - |
| % wt loss                                         | -           | - | - | - | + | - | - | + | - |
| Oxidative phosphorylation                         | -           | - | + | - | - | + | + | + | - |
| Oxygen uptake                                     | -           | - | - | - | - | + | + | + | + |
| Sarcoplasmic reticulum<br>Ca <sup>++</sup> uptake | -           | - | - | - | - | - | - | + | - |
| CO                                                | +           | + | + | + | + | + | + | + | + |
| CF                                                | +           | - | + | + | + | + | + | + | + |
| SV                                                | -           | - | - | - | - | + | + | + | + |
| LVP                                               | -           | - | - | - | - | + | + | + | + |
| LVEDP                                             | -           | - | - | - | - | + | + | + | + |
| AOP                                               | -           | - | - | - | - | + | + | + | + |
| Histology (light)                                 | +           | + | + | + | + | - | + | + | - |
| Electron microscopy                               | -           | - | + | + | + | - | - | - | - |

The relevant information (results and statistical details) is tabulated in the respective chapters. This table is an over-view of all the biochemical and functional determinations carried out during the period of functional testings. (+ = tests performed; - = no tests performed).

TABLE 3:3

CHANGES IN HEART WEIGHT DURING 24 HOUR PERIOD OF  
HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

|                                                                                    |     |
|------------------------------------------------------------------------------------|-----|
| GROUPS (Perfusates)                                                                | 1   |
| Mean % wt gain                                                                     | 21  |
| SEM                                                                                | 1.9 |
| Statistical comparison:<br>Experimental groups vs<br>preceding experimental groups | -   |
| n                                                                                  | 10  |

TABLE 3.4.



CARDIAC OUTPUT DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUPS                                                                            | Control | 1           |
|-----------------------------------------------------------------------------------|---------|-------------|
| CO(ml/min/100 g)                                                                  | 3259    | 690<br>n =4 |
| SEM                                                                               | 202     | 135         |
| Statistical comparison:<br>Control vs experimental group                          | -       | <0.001      |
| Statistical comparison:<br>experimental groups vs preceding<br>experimental group | -       | -           |
| n                                                                                 | 12      | 10          |

TABLE 3.5.

CORONARY FLOW DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUPS                                                                           | Control | 1      |
|----------------------------------------------------------------------------------|---------|--------|
| CF<br>(ml/min/100g)                                                              | 328     | 122    |
| SEM                                                                              | 30.2    | 27.0   |
| Statistical comparison:<br>Control vs<br>Experimental group                      | -       | < .001 |
| Statistical comparison:<br>Experimental group vs<br>Preceding experimental group | -       | -      |
| n                                                                                | 12      | 10     |

TABLE 3.6.

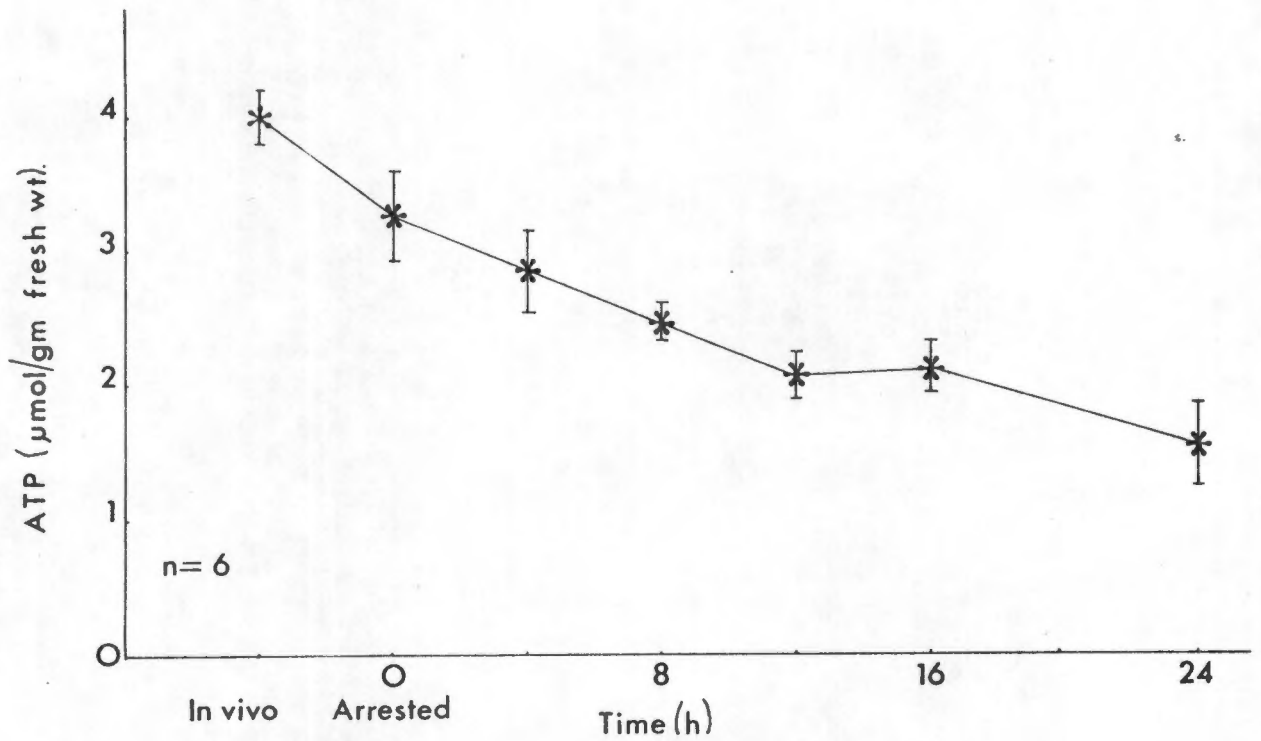


Fig. 3.1.

Myocardial biopsies for the determination of tissue adenosine triphosphate (ATP) levels before cardioplegia (in vivo) after cardioplegia (arrested) and during hypothermic perfusion taken over a period of 24 hours. Bars represent S.E.M.

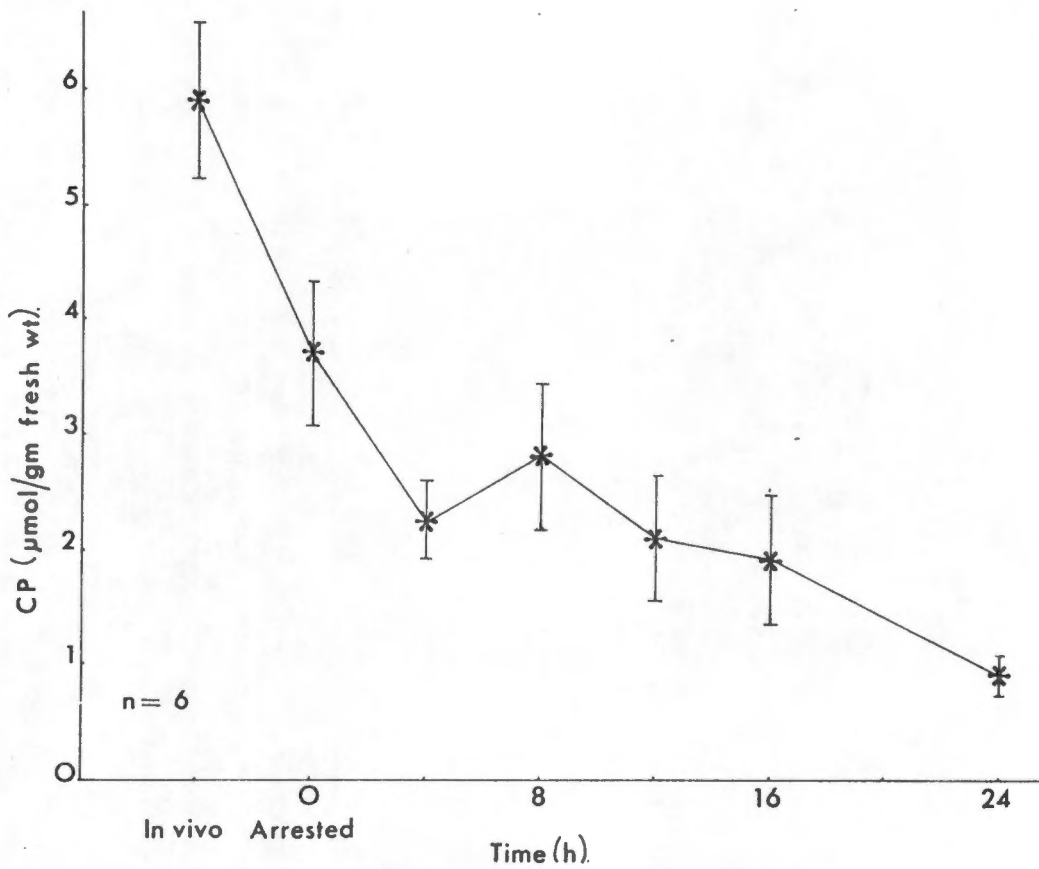


Fig. 3.2.

Myocardial biopsies for the determination of tissue creatine phosphate (CP) levels before cardioplegia (in vivo) after cardioplegia (arrested) and during hypothermic perfusion, taken over a period of 24 hours.

Bars represent S.E.M.

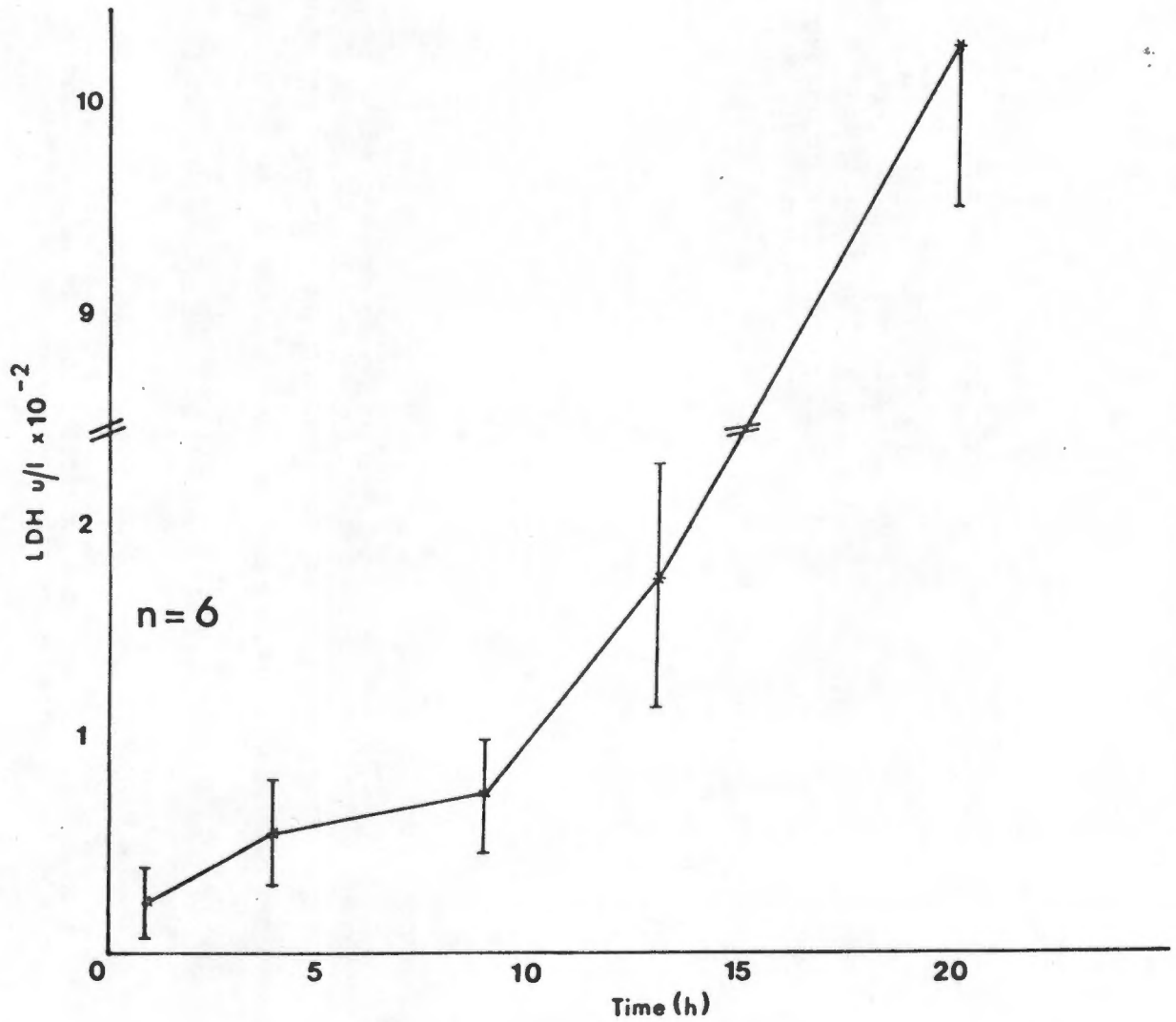


Fig. 3.3.

Perfusate lactate dehydrogenase (LDH) concentrations determined during the period of hypothermic perfusion. Bars represent S.E.M.

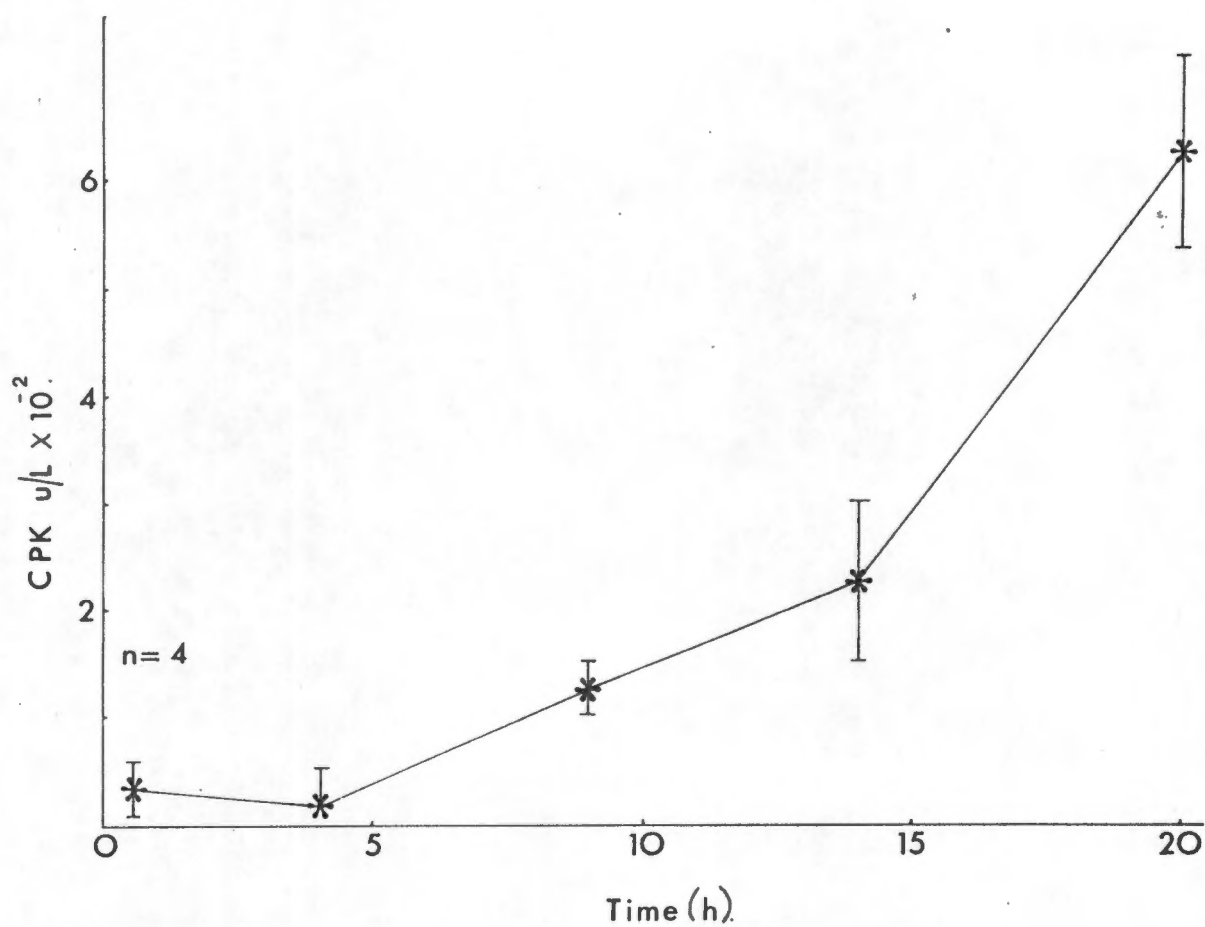


Fig. 3.4.

Perfusate creatine kinase (CK) concentrations determined during the period of hypothermic perfusion. Bars represent S.E.M.

## CHAPTER 4

### GROUP 2

#### 4 MODIFICATIONS TO THE PERFUSATE

The only alteration to this perfusate included the addition of the amino acid taurine (appendix 4) (table 4.1). This amino acid is found in the mammalian body concentrated in many tissues particularly heart muscle, where it constitutes about 50% of the free amino acid pool (Jacobsen and Smith, 1968). Taurine also plays a role in ion movements in the heart (Guidotti et al, 1971), and prevents the loss of potassium, leading to stabilization of the membrane potential (Chazov et al, 1974). Its absorption into the myocardium is rapid (moving against a concentration gradient) where it is converted into isethionic acid, which helps maintain the normal electrical rhythm of the heart (Chazov et al, 1974) and has been shown to reverse digoxin and adrenaline, - induced arrhythmias (Read and Welty, 1963). This zwitterion also has a protective effect on ATPase activities with an even stronger preservation effect on sarcoplasmic reticulum, calcium binding and transport (Huxtable and Bressler, 1973). With these numerous advantages at normothermia it was felt worthwhile to study the effect of this amino acid in hearts preserved by hypothermic perfusion for twenty-four hours. The parameters studied during hypothermic perfusion (table 3.2) and functional testing (table 3.3) are tabulated.

### RESULTS

#### 4.1. OBSERVATIONS DURING THE PERIOD OF HYPOTHERMIC PERFUSION

The myocardial texture was firm, unlike the soft texture after cardioplegic arrest alone. A mean weight gain of 23% occurred (table 4.2).

The release of LDH into the perfusate is shown in figure 4.1. The upper curve demonstrates an uncontrolled release of LDH in the absence of taurine (Krebs and Henseleit solution) whereas the lower curve was determined in the presence of taurine.

#### 4.1.1. MICROSCOPY

Histological examinations showed only interstitial oedema and no other significant features.

#### 4.2.1. OBSERVATIONS DURING THE PERIOD OF FUNCTIONAL TESTING

All the hearts required electrical defibrillation and were able to overcome the afterload of the system (table 4.3) and rhythm disturbances were virtually absent. Although contractions were not vigorous, the heart still managed to maintain a consistent CO throughout the period of evaluation (table 4.3). These hearts gained weight (2%) after functional evaluation.

#### 4.3.1. DISCUSSION AND CONCLUSIONS

Three notable advantageous effects of adding taurine were observed in this study:

1. The release of LDH was effectively decreased. (The effect of taurine on the release of LDH was maintained throughout the period of preservation).
2. All the hearts were able to overcome the afterload of the system.
3. The hearts had regular rhythm.

Although preservation had improved, heart function was far from satisfactory when compared with function of the control group (table 4.3). The perfusate obviously required improvements. It was hoped to conserve the beneficial effect of taurine in the presence of other perfusate modifications.



COMPOSITION OF PERFUSION SOLUTIONS USED TO PRESERVE  
HEARTS IN 9 DIFFERENT GROUPS

| Constitution of perfusates (mM)      | 1     | 2     |
|--------------------------------------|-------|-------|
| NaCl                                 | 118.5 | 118.5 |
| NaHCO <sub>3</sub>                   | 25    | 25    |
| KCl                                  | 4.75  | 4.75  |
| KH <sub>2</sub> PO <sub>4</sub>      | 1.20  | 1.20  |
| MgSO <sub>4</sub> 7H <sub>2</sub> O  | 1.20  | 1.20  |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O | 2.5   | 2.5   |
| MgCl <sub>2</sub>                    | -     | -     |
| Glucose                              | 11    | 11    |
| Taurine                              | -     | 4     |
| ATP                                  | -     | -     |
| Insulin                              | -     | -     |
| Procaine HCl                         | -     | -     |
| Dextran 40                           | -     | -     |
| Chlorpromazine                       | -     | -     |
| Phenoxybenzamine                     | -     | -     |
| Glycerol                             | -     | -     |
| Sucrose                              | -     | -     |
| Osmolality (mOsm/L)                  | 277   | 280   |

TABLE 4.1.

CHANGES IN HEART WEIGHT DURING 24 HOUR PERIOD OF  
HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUPS (Perfusates)                                                                | 1   | 2   |
|------------------------------------------------------------------------------------|-----|-----|
| Mean % wt gain                                                                     | 21  | 23  |
| SEM                                                                                | 1.9 | 2.4 |
| Statistical comparison:<br>Experimental groups vs<br>preceding experimental groups | -   | NS  |
| n                                                                                  | 10  | 5   |

TABLE 4.2.

CARDIAC OUTPUT DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUPS                                                                            | Control | 1         | 2      |
|-----------------------------------------------------------------------------------|---------|-----------|--------|
| CO(ml/min/100 g)                                                                  | 3259    | 690<br>=4 | 631    |
| SEM                                                                               | 202     | 135       | 178    |
| Statistical comparison:<br>Control vs experimental group                          | -       | <0.001    | <0.001 |
| Statistical comparison:<br>experimental groups vs preceding<br>experimental group | -       | -         | <0.05  |
| n                                                                                 | 12      | 10        | 5      |

TABLE 4.3.

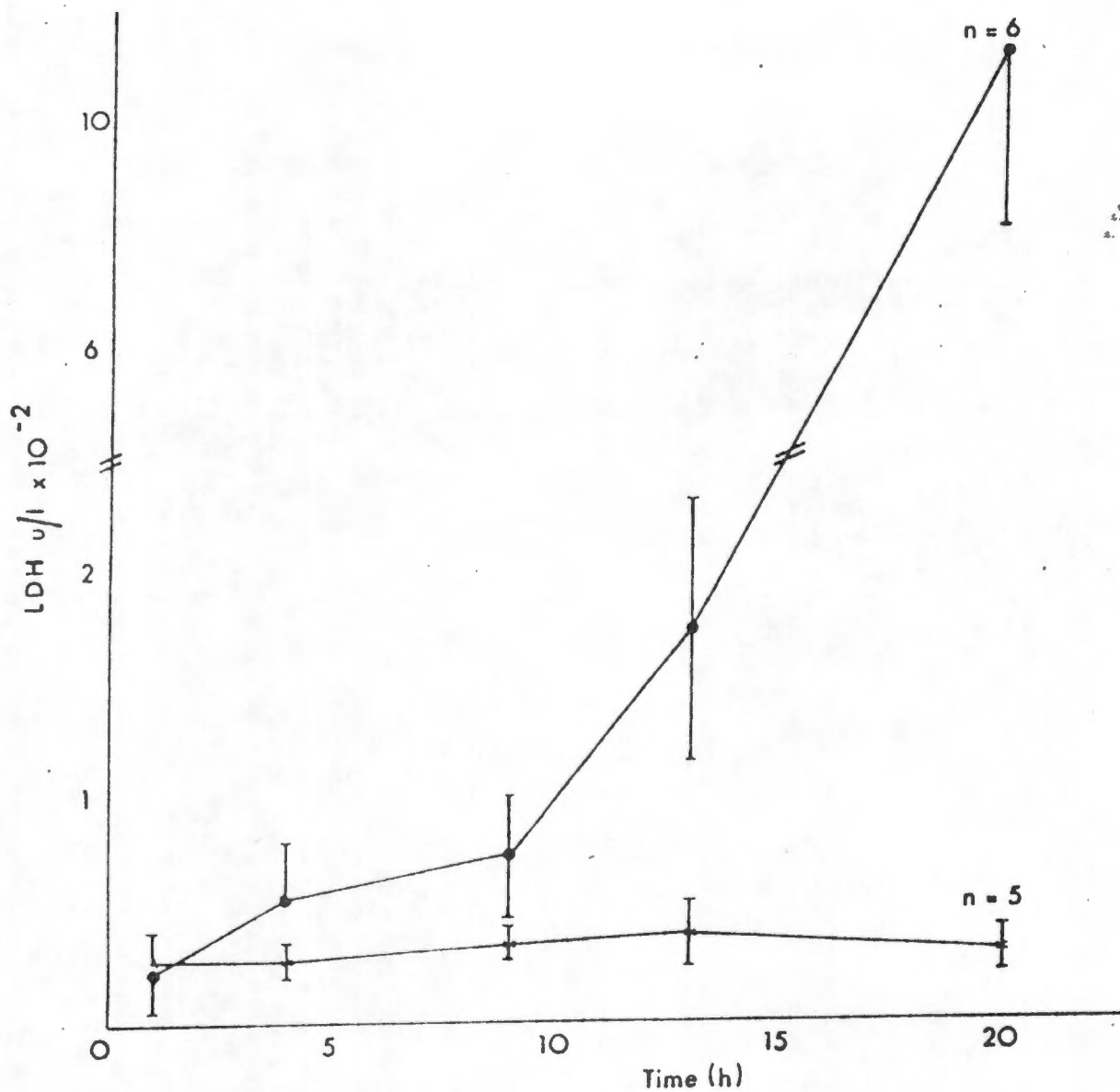


Fig. 4.1

Perfusate lactate dehydrogenase (LDH) concentrations determined during the period of hypothermic perfusion: (●—●) Krebs and Henseleit solution, (▲—▲) Krebs and Henseleit solution plus taurine.

Bars represent S.E.M.

## CHAPTER 5

### GROUP 3

#### 5. MODIFICATIONS TO THE PERFUSATE

This perfusate was a modified version of that used by Hearse et al (1976) (table 5.1). The solution of Hearse was an extracellular based fluid used to perfuse rat hearts that had been subjected to 30 minutes of normothermic ischaemia. These hearts recovered 93% of their pre-ischaemic function. (The use of intracellular perfusion solutions was first suggested by Keeler et al (1966) and later used by Collins et al (1969). These techniques which produced successful renal preservation have proved incapable of similar success with the heart (Copeland, 1973).

The perfusate constituted the following ingredients based on the evidence put forward by Hearse et al (1976). Evidence supporting the effectiveness of some of the constituents are mentioned below. Group 3 solution changes included raised potassium and magnesium concentrations and the addition of insulin ATP and Procaine HCl (table 5.2).

##### 5.1.1. POTASSIUM

The potassium concentration was increased to prevent the loss of intracellular potassium and to block the fast sodium channels by voltage inactivation (partial depolarization of the cells) (Keeler et al, 1966; Reitz et al 1974; Hearse et al 1976). A vasoconstrictive effect of potassium in concentrations greater than 12meq/l was detected in kidney preservation by Scott et al (1959) and Levowitz et al (1969). In heart muscle this has not been previously demonstrated but tables 9.5 (coronary flow) and 9.7 (coronary

vascular resistance) clearly show that at the end of the preservation period the elevated potassium concentration of 16mM in group 6 was associated with significantly lower coronary flow and higher coronary vascular resistance when compared with group 7 (10.9mM) where a lower potassium concentration was used. Again with reference to renal preservation Schwartz et al (1980) has demonstrated hypotensive effects by using potassium concentrations greater than 8 meq/L. Johnson (1972) on the other hand found that little loss or leakage of potassium occurred when potassium perfusates of 4 - 6 meq/L were used.

On the other hand, both Keeler et al (1966) in kidneys and Copeland et al (1973) in hearts described the rapid loss of intracellular cations during cold perfusion. To prevent the loss of potassium Martin et al (1970), demonstrated that the constitution of an intracellular perfusate led to improved function. To create a balance between these effects a potassium concentration of 16mM was chosen.

#### 5.1.2 MAGNESIUM

The primary use of magnesium is as a metabolic inhibitor, being superior to fluoride and adenosine (Webb et al 1966). Its inhibitory effect was demonstrated by Nakae et al (1967) who also showed tolerance to normothermic ischaemia. Magnesium is known to be essential as a catalyst in many enzyme systems involved in anaerobic glycolysis in the pentose monophosphate shunt, and in phosphate metabolism (Aikawa, 1963). It is essential for the function of the hexokinases, co-enzyme A, phosphatases, transphosphorylases, some of the peptidases, the

conversion of alphaketoglutaric acid to succinic acid (Aikawa 1963) and in the function of myosin and actomysin (Mommaerts and Seraidarian 1947). In addition, for ATP to be operable it must form a complex of magnesium in a 1:1 ratio (White et al 1973). Magnesium is also essential to provide structural integrity for ATP (Welt 1964). Other advantages of magnesium include: reduced membrane permeability to potassium, stabilising the membrane potential (Holland 1964); delayed oedema formation (magnesium is poorly diffusable) (Belzer and Southard 1980); increased perfusate magnesium concentrations result in higher adenine nucleotide content (Calman et al 1973).

#### 5.1.3. INSULIN

Was added to the perfusate with the intention of maintaining myocardial glycogen stores by increasing the uptake of glucose into the cells. Calman et al (1973) demonstrated this effect in perfused hearts finding a significant increase in survival. Contrary to these findings, Copeland et al (1973) found that insulin was not necessary in preservation solutions.

#### 5.1.4 PROCAINE HYDROCHLORIDE

The importance of the local anaesthetic procaine hydrochloride in myocardial protection was described by Holscher (1967). The inclusion of 1mM procaine in this perfusate was based on its cardioplegic effect, maintaining polarization by blocking efflux and its anti-dysrhythmic properties (Holscher 1967; Kirsch et al 1972; Hearse et al 1976; Hearse et al 1981). Various doses of procaine have been used by others ranging from 1mM - 11mM (Kirsch et al 1972; Bretschneider et al 1975; Hearse et al 1976), but an ideal dose was only established by Hearse et al (1981), at which time, our studies had already been

completed. Other beneficial effects of procaine include; membrane stabilization, prolonging the duration of asystole (Buckberg 1979); and vasodilatation (Freier 1979). Watkins et al (1971) demonstrated that procaine was detrimental in renal preservation as indicated by elevated serum creatinine levels. This possibility resulted from its degradation to para-amino benzoic acid (Watkins et al 1971).

#### 5.1.5 PHOSPHORYLATED COMPOUNDS

Phosphorylated compounds, (purine nucleotides) do not cross the intact cell membrane to any great extent (Fleit et al 1975 ; Harms and Stirling 1977), but ATP has been demonstrated extracellularly in the plasma under extreme physiological conditions (Forrester 1972). The increase in loss of purine nucleotides during ischaemia (Collins et al 1977), can hardly be caused by a diffusion of purine nucleotides from the cell, but must be due to a loss of diffusible non-phosphorylated purine nucleosides.

Contrary to these findings Chaudry and Gould (1970) demonstrated that Adenosine Triphosphate was in fact able to cross the muscle cell membrane. Hearts perfused in a solution rich in ATP protects the myocardium against ischaemic injury (Hearse et al 1976).

Whether these high energy phosphates enter the cell or act on the cell membrane remains unknown. Calman (1974) similarly demonstrated the beneficial effect of perfusates containing ATP. Experimenting with ATP, ADP and AMP, he showed that only ATP significantly increased the storage time. All determinations made during the hypothermic perfusion period (table 3.2) and during the normothermic ex vivo function evaluation period (table 3.3) are tabulated.



### 5.2.1. BIOCHEMICAL DETERMINATIONS

Energy required by living cells is conserved in useful form, mainly that of ATP. This energy is used in powering cellular functions. The mitochondrion is a major contributor to cellular ATP pools, and its integrity during preservation is, therefore, essential for the viability of the organ. The process of oxidative phosphorylation, which includes the oxidation of pyruvate by the tricarboxylic acid cycle, followed by the phosphorylation of ADP by the electron transport chain, is carried out entirely within the mitochondrion. Details about the structure and function of the mitochondrion can be found in Appendix 1. Isolation procedures and calculations are listed under materials and methods. Mitochondria isolated from fresh, unpreserved hearts (control) were compared with hearts that had been preserved for 24 hours and functionally evaluated for one hour.

## RESULTS

### 5.3.1. OBSERVATIONS DURING THE PERIOD OF HYPOTHERMIC PERFUSION

#### % CHANGE IN WEIGHT

A comparison of the results using Group 3 solution with those of previous groups show that a significant increase in weight occurred (table 5.3).

### 5.4.1 OBSERVATIONS DURING THE PERIOD OF FUNCTIONAL TESTING

Cardiac output and coronary flow are presented in tables 5.4 and 5.5 respectively. The mean cardiac output of Group 3 was less than half of the control groups ( $P < 0.001$ ) but significantly higher than Group 2 ( $P < 0.05$ ). The percentage weight gain, presumably due to fluid accumulation by Group 3 hearts, was at least partly reversible upon perfusion with blood, as there was

a mean weight loss of 13% after 1 hour's functional testing (table 5.6). Eleven hearts were evaluated of which one did not function at all; another was just able to overcome the afterload of the system. The remaining hearts had a remarkable functional improvement compared with Group 2 (table 5.4).

#### 5.4.2 MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION

These results are shown in table 5.7 in which the mitochondrial function of a fresh control group was compared with hearts preserved for 24 hours and functionally evaluated for 1 hour. The three parameters, P/O ratio (the amount of ADP phosphorylated, per amount of oxygen consumed), RCI (measure of the tightness of coupling of the mitochondria) and  $QO_2$  (the activity of the enzymatic protein) were determined at the end of 1 hour functional testing and used to compare mitochondrial functional integrity between the two groups.

#### 5.4.3. MICROSCOPY

Histological examinations showed interstitial oedema and perinuclear vacuolation with no other significant features.

#### 5.4.4. ELECTRON MICROSCOPY

The myofibres appeared generally poorly preserved. This included loss and disruption of myofilaments, increased numbers of mitochondria and dilatation of the T-tubule system.

#### 5.5.1. DISCUSSION AND CONCLUSIONS

The combination of hypothermia plus perfusion preserved functional viability of the mitochondria in this study (table 5.7) and functional viability of the entire organ was improved compared to previous solutions but not normal (table 5.4).

An exact explanation for the improved preservation effect of this solution was not possible as the perfusate was extensively modified (table 5.2).

The major contributors to functional improvement were presumably the procaine hydrochloride and magnesium chloride. In vitro mitochondrial functional studies showed that functional integrity was preserved in ischaemic tubular cells well after the onset of irreversibility (Vogt and Farber 1968; Reimer and Jennings 1971). Trump et al (1976) provided an explanation for this phenomenon. They stated that ischaemic or normal mitochondria isolated in the laboratory were placed in a medium resembling the normal physiological intracellular environment. During the isolation procedure, the "weaker" mitochondria were more prone to destruction, leaving only the intact mitochondria in the final fraction. These were capable of normal metabolic activity. Conversely, in vivo mitochondria were subjected to an adverse environment that recovered very gradually from the ischaemic insult, because of the delay in the removal of toxic metabolites. Light microscopy and ultrastructural studies provided some of the initial evidence of tissue damage. The ultrastructural changes noted by others in similar circumstances were the development of oedema (Ganote et al 1976), the disappearance of glycogen particles, (Bernelle - Zezzera and Gaja 1964; Grana et al 1968) and mitochondrial swelling (Whalen et al 1974).

Hearts subjected to hypothermic perfusion gradually accumulated fluid (Kleinzeller 1960; Whittenbury 1965; Copeland et al 1973; Swanson et al 1979). Proctor and Jones (1973) suggested that this fluid accumulation has no pathological implications and differs from fluid accumulation in ischaemically injured organs.

They termed the condition "water of equilibration". Conversely, a badly preserved heart may develop oedema and become hard to the touch and not lose weight on reperfusion (see section 3.3.1.). It was felt that Group 3 hearts preserved in this study gained some degree of "water of equilibration" as 13% of it was lost after 1 hour reperfusion. This non-pathological state could also account for the normal mitochondrial respiration.

A proposed pathological effect of fluid accumulation may be as follows: a perfusate lacking in oncotic pressure may result in a differential uptake of fluid, with a most severe effect taking place in the vascular endothelial cells with which it is in direct contact. These cells then failed to function normally after warm blood reperfusion, with impaired "blood tissue" exchange of substrates leading to ionic imbalances, vasoconstriction and accumulation of toxic metabolites.

SOLUTION USED FOR MYOCARDIAL PROTECTION  
DURING ISCHAEMIA. (HEARSE ET AL 1976).

|                                      | <u>mm</u> |
|--------------------------------------|-----------|
| NaCl                                 | 92.9      |
| NaHCO <sub>3</sub>                   | 25        |
| K Cl                                 | 10.8      |
| KH <sub>2</sub> PO <sub>4</sub>      | 1.2       |
| MgSO <sub>4</sub> .7H <sub>2</sub> O | 1.2       |
| CaCl <sub>2</sub> .2H <sub>2</sub> O | 2.5       |
| MgCl <sub>2</sub>                    | 14.8      |
| Glucose                              | 11.0      |
| ATP                                  | 10.0      |
| Procaine HCl                         | 1.0       |
| Creatine Phosphate                   | 10.0      |

TABLE 5.1

COMPOSITION OF PERFUSION SOLUTIONS USED TO PRESERVE  
HEARTS IN 3 DIFFERENT GROUPS

| Constitution of perfusates (mM)      | 1     | 2     | 3     |
|--------------------------------------|-------|-------|-------|
| NaCl                                 | 118.5 | 118.5 | 92.90 |
| NaHCO <sub>3</sub>                   | 25    | 25    | 25    |
| KCl                                  | 4.75  | 4.75  | 16    |
| KH <sub>2</sub> PO <sub>4</sub>      | 1.20  | 1.20  | 1.20  |
| MgSO <sub>4</sub> 7H <sub>2</sub> O  | 1.20  | 1.20  | 1.20  |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O | 2.5   | 2.5   | 2.25  |
| MgCl <sub>2</sub>                    | -     | -     | 14.8  |
| Glucose                              | 11    | 11    | 11    |
| Taurine                              | -     | 4     | 4     |
| ATP                                  | -     | -     | 10    |
| Insulin                              | -     | -     | 40u/L |
| Procaine HCl                         | -     | -     | 1     |
| Dextran 40                           | -     | -     | -     |
| Chlorpromazine                       | -     | -     | -     |
| Phenoxybenzamine                     | -     | -     | -     |
| Glycerol                             | -     | -     | -     |
| Sucrose                              | -     | -     | -     |
| Osmolality (mOsm/L)                  | 277   | 280   | 295   |

TABLE 5.2.

CHANGES IN HEART WEIGHT DURING 24 HOUR PERIOD OF  
HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUPS (Perfusates)                                                                | 1   | 2   | 3      |
|------------------------------------------------------------------------------------|-----|-----|--------|
| Mean % wt gain                                                                     | 21  | 23  | 33.8   |
| SEM                                                                                | 1.9 | 2.4 | 2.8    |
| Statistical comparison:<br>Experimental groups vs<br>preceding experimental groups | -   | NS  | < 0.05 |
| n                                                                                  | 10  | 5   | 11     |

TABLE 5.3.

CARDIAC OUTPUT DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUPS                                                                            | Control | 1         | 2      | 3      |
|-----------------------------------------------------------------------------------|---------|-----------|--------|--------|
| CO(ml/min/100 g)                                                                  | 3259    | 690<br>=4 | 631    | 1486   |
| SEM                                                                               | 202     | 135       | 178    | 222    |
| Statistical comparison:<br>Control vs experimental group                          | -       | <0.001    | <0.001 | <0.001 |
| Statistical comparison:<br>experimental groups vs preceding<br>experimental group | -       | -         | <0.05  | <0.05  |
| n                                                                                 | 12      | 10        | 5      | 11     |

TABLE 5.4.



CORONARY FLOW DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUPS                                                                           | Control | 1      | 3      |
|----------------------------------------------------------------------------------|---------|--------|--------|
| CF<br>(mL/min/100g)                                                              | 328     | 122    | 222    |
| SEM                                                                              | 30.2    | 27.0   | 18.3   |
| Statistical comparison:<br>Control vs<br>Experimental group                      | -       | < .001 | < 0.01 |
| Statistical comparison:<br>Experimental group vs<br>Preceding experimental group | -       | -      | -      |
| n                                                                                | 12      | 10     | 10     |

TABLE 5.5.

CHANGES IN HEART WEIGHT FOLLOWING ONE HOUR EX VIVO FUNCTIONAL TESTING

|                                                                                   |      |
|-----------------------------------------------------------------------------------|------|
| GROUPS<br>(Perfusates)                                                            | 3    |
| % Wt<br>Loss                                                                      | 13   |
| SEM                                                                               | 2.00 |
| Statistical Comparison:<br>Experimental groups vs<br>preceding experimental group | -    |
| n                                                                                 | 11   |

TABLE 5.6.

MITOCHONDRIAL ADP:O RATIO, QO<sub>2</sub> AND RCI VALUES IN FRESHLY ISOLATED CONTROL  
HEARTS AND HEARTS REPERFUSED FOR ONE HOUR AT NORMOTHERMIA

|  | CONTROL                                          | GROUP 3                                          |
|--|--------------------------------------------------|--------------------------------------------------|
|  | 2.75 (0.18)<br><br>219 (40.4)<br><br>3.52 (0.32) | 2.78 (0.28)<br><br>160 (32.0)<br><br>2.90 (0.44) |

Figures in brackets denote SEM

TABLE 5.7

## CHAPTER 6

### GROUP 4

#### 6. MODIFICATION TO THE PERFUSATE

Two changes were made to the perfusate: ATP was omitted and 4g/L of dextran 40 was added. The change increased the osmolarity of the perfusate to 315mOsm/L (table 6.1).

ATP was excluded from the perfusate for three reasons:

1. Nucleotides do not cross cell membranes (only under extreme physiological conditions).
2. Mitochondrial function was comparatively normal (see section 5.4.2).
3. The extreme cost of ATP.

In chapter 5 it was proposed that a differential fluid-accumulating gradient developed across the membrane of endothelial cells. As these cells were in direct contact with the perfusate which has no oncotic pressure, they were subject to more pronounced deleterious effects, such as fluid accumulation, and ionic imbalance, whereas areas situated deeper in the endocardium were less affected. (Limas et al 1977).

Other workers in the field of renal preservation have also shown a progressive perfusion damage to the endothelial cells of the vascular system (Hill et al 1976); Cerra et al 1977; Limas et al 1977) leading to metabolic death of these cells (Belzer et al 1978) even though a good renal blood flow continued.

Attempts at controlling the differential failure of cell volume regulation have been made by adding to the perfusate osmotically active, impermeant molecules such as various sugars or large molecular weight anions (Belzer et al 1978). These large molecules were effective for short periods but progressive swelling did occur over a period of 24 hours. Copeland et al (1973) used various combinations for maintenance of oncotic pressure and graft function failed at perfusate levels of 40g/L and concentration of 6g/L was not necessary for successful preservation. Pegg (1977) also showed that dextran was very efficient in preventing over-hydration in perfused kidneys. On the basis of these findings, dextran was included in the perfusate in the hope of protecting myocardial tissues against oedema formation, especially in the endothelial cells. All determinations made during the hypothermic perfusion period (table 3.2) and during normothermic ex vivo functional evaluation period (table 3.3) are tabulated.

## RESULTS

### 6.1.1. OBSERVATIONS DURING THE PERIOD OF HYPOTHERMIC PERFUSION

The predicted effect of "less oedema formation occurring" was not seen and the tissue fluid content increased rather than decreased (table 6.2). This increase was not of statistical significance.

### 6.2.1 OBSERVATIONS DURING THE PERIOD OF FUNCTIONAL TESTING

After approximately 10 minutes of passive warm blood reperfusion, vigorous ventricular fibrillation occurred. All 18 reperfused hearts required electrical defibrillation followed by the resumption of sinus rhythm with good contractile motion. When challenging the L V of these hearts with blood, only 12 managed to successfully overcome the afterload of the system. The mean

cardiac output (table 6.3) and coronary flow increased though only the latter being significant by comparison with Group 3 (table 6.4). Haemodynamic performance was inferior to that in the control group (table 6.3). The hearts demonstrated a 17% loss of weight during reperfusion (table 6.5). Haemodynamic function did not change significantly throughout this period.

#### 6.2.2. MICROSCOPY

The myofibres appeared well preserved although some areas showed myofibrillar degeneration. There were also areas of scanty haemorrhage and very severe interstitial oedema. Small arteries and veins appeared structurally normal although devoid of blood. Few intact capillaries were seen, the majority being disrupted.

#### 6.2.3. ELECTRON MICROSCOPY

The myofilaments were disrupted suggesting severe damage. Other changes included dilatation of the T-tubules system and the mitochondria had increased in number.

#### 6.3.1. DISCUSSION AND CONCLUSIONS

These hearts did not show much haemodynamic improvement, but some salient observations are worth discussing. Firstly there was a significant increase in coronary flow compared with group 3, suggesting either dilatation of the coronary vessels or else improved preservation of the endothelial cells (as well as other cells). The dextran may have had some effect on protecting the cells of closest proximity (endothelial cells) against overdistension and osmotic shock (Hardy 1971). The expected effect of dextran did not seem to materialize (weight gain and endothelial protection) as histological examination showed unexpected features of disrupted capillaries and small bloodless vessels

suggesting that the dextran had been ineffective against osmotic forces. Instead of reducing the effect of fluid retention, the mean % weight gained by the preserved hearts increased (table 6.2). The mean heart weight decreased after reperfusion although this decrease was not significantly different when compared with group 3 (table 6.5).

These observations gave little guidance towards further modification to the perfusate.

Certain fundamental questions remained unanswered:

Was oedema formation really destructive? Did prolonged hypothermia cause damage? Was lysosomal injury responsible for poor myocardial performance following preservation?

COMPOSITION OF PERFUSION SOLUTIONS USED TO PRESERVE  
HEARTS IN 9 DIFFERENT GROUPS

| Constitution of perfusates (mM)      | 1     | 2     | 3     | 4     |
|--------------------------------------|-------|-------|-------|-------|
| NaCl                                 | 118.5 | 118.5 | 92.90 | 92.90 |
| NaHCO <sub>3</sub>                   | 25    | 25    | 25    | 25    |
| KCl                                  | 4.75  | 4.75  | 16    | 16    |
| KH <sub>2</sub> PO <sub>4</sub>      | 1.20  | 1.20  | 1.20  | 1.20  |
| MgSO <sub>4</sub> 7H <sub>2</sub> O  | 1.20  | 1.20  | 1.20  | 1.20  |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O | 2.5   | 2.5   | 2.25  | 2.25  |
| MgCl <sub>2</sub>                    | -     | -     | 14.8  | 14.8  |
| Glucose                              | 11    | 11    | 11    | 11    |
| Taurine                              | -     | 4     | 4     | 4     |
| ATP                                  | -     | -     | 10    | -     |
| Insulin                              | -     | -     | 40u/L | 40u/L |
| Procaine HCl                         | -     | -     | 1     | 1     |
| Dextran 40                           | -     | -     | -     | 4g/L  |
| Chlorpromazine                       | -     | -     | -     | -     |
| Phenoxybenzamine                     | -     | -     | -     | -     |
| Glycerol                             | -     | -     | -     | -     |
| Sucrose                              | -     | -     | -     | -     |
| Osmolality (mOsM/L)                  | 277   | 280   | 295   | 315   |

TABLE 6.1.



CHANGES IN HEART WEIGHT DURING 24 HOUR PERIOD OF  
HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUPS (Perfusates)                                                                | 1   | 2   | 3      | 4    |
|------------------------------------------------------------------------------------|-----|-----|--------|------|
| Mean % wt gain                                                                     | 21  | 23  | 33.8   | 41.4 |
| SEM                                                                                | 1.9 | 2.4 | 2.8    | 4.3  |
| Statistical comparison:<br>Experimental groups vs<br>preceding experimental groups | -   | NS  | < 0.05 | NS   |
| n                                                                                  | 10  | 5   | 11     | 18   |

TABLE 6.2.

CARDIAC OUTPUT DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUPS                                                                            | Control | 1         | 2      | 3      | 4      |
|-----------------------------------------------------------------------------------|---------|-----------|--------|--------|--------|
| CO(ml/min/100 g)                                                                  | 3259    | 690<br>=4 | 631    | 1486   | 1825   |
| SEM                                                                               | 202     | 135       | 178    | 222    | 325    |
| Statistical comparison:<br>Control vs experimental group                          | -       | <0.001    | <0.001 | <0.001 | <0.001 |
| Statistical comparison:<br>experimental groups vs preceding<br>experimental group | -       | -         | <0.05  | <0.05  | NS     |
| n                                                                                 | 12      | 10        | 5      | 11     | 18     |

TABLE 6.3.

CORONARY FLOW DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUPS                                                                           | Control | 1      | 3      | 4       |
|----------------------------------------------------------------------------------|---------|--------|--------|---------|
| CF<br>(ml/min/100g)                                                              | 328     | 122    | 222    | 291     |
| SEM                                                                              | 30.2    | 27.0   | 18.3   | 14.7    |
| Statistical comparison:<br>Control vs<br>Experimental group                      | -       | < .001 | < 0.01 | NS      |
| Statistical comparison:<br>Experimental group vs<br>Preceding experimental group | -       | -      | -      | < 0.001 |
| n                                                                                | 12      | 10     | 10     | 12      |

TABLE 6.4.

CHANGES IN HEART WEIGHT FOLLOWING ONE HOUR EX VIVO FUNCTIONAL TESTING

| GROUPS<br>(Perfusates)                                                            | 3    | 4    |
|-----------------------------------------------------------------------------------|------|------|
| % Wt<br>Loss                                                                      | 13   | 17   |
| SEM                                                                               | 2.00 | 5.00 |
| Statistical Comparison:<br>Experimental groups vs<br>preceding experimental group | -    | NS   |
| n                                                                                 | 11   | 18   |

TABLE 6.5.

## CHAPTER 7

### GROUP 5

#### 7. MODIFICATION TO THE PERFUSATE

With little function improvement in group 4 hearts, the study was repeated with the emphasis on biochemical observations. The only change introduced into the perfusate was the lowering of the dextran 40 concentration to 2gm/L (table 7.1). If this change in dextran concentration showed little effect in terms of biochemical and functional viability, its subsequent use would be discontinued.

The aim of the study was to locate injured areas within the myocardium associated with poor preservation. The tests used in this investigation are listed in tables 3.2 and 3.3.

#### 7.1 BIOCHEMICAL DETERMINATION

The effects of 24 hour preservation on the in situ release of acid phosphatase activity was compared in pig hearts freshly excised (control) and in hearts preserved for 24 hours and then perfused for one hour (group 5). Acid phosphatase is an enzyme occurring in many tissues and species and is not entirely confined to the lysosome. (Wildenthal 1975). It catalyses the hydrolysis of phosphoric monoesters; p-nitrophenol phosphate was used as a substrate in this investigation. The assay mixture included 15mM tartrate as a phosphatase inhibitor, although phosphoprotein phosphatase and glucose 6 phosphatase were not sensitive to the inhibitory effect (Wildenthal 1975). Various fractions were differentially isolated, (supernatant, particulate, nuclear); but only the particulate fractions were of relevance when interpreting the results as other free contaminating enzymes were absent.

### 7.1.1. LYSOSOMES

As ischaemic myocytes become irreversibly injured, they sustain specific structural lesions which are not reversible upon the resumption of coronary flow. (Jennings and Ganote 1974; Kloner et al 1974; Jennings 1976). The basic features are discussed in sections 5.5.1; these include : dilated mitochondria, glycogen depletion and breakdown of sarcolemmal integrity, and lysosomal rupture (Decker et al 1977; Decker and Wildenthal 1978; (a) Decker and Wildenthal 1978 (b)

Many of these subcellular alterations that accompany ischaemia involve structural damage in organellar membranes and it has been hypothesised that intracellular release of normally latent lysosomal degradative enzymes during ischaemia contribute to this membrane damage and accelerate the progression of injury to necrosis (van Lancker and Holtzer 1959). This hypothesis has been supported by observations that the time course of ischaemic-induced lysosomal disruption is closely linked to the development of ultrastructural signs of ischaemic necrosis in normal hearts (Decker et al 1977; Decker and Wildenthal 1978 (b) ).

The release of acid phosphatase was used as an index of lysosomal integrity with a high release implying consequent or subsequent myocardial injury. These results were hoped to correlate with functional studies and other parameters such as biochemical tests and ultrastructural determinations. Details about the isolation and assay procedures of lysosomes are listed under materials and methods section 2.1.9.

## RESULTS

### 7.2.1. OBSERVATIONS DURING THE PERIOD OF HYPOTHERMIC PERFUSION

These hearts remained soft to the touch during the entire period of preservation with little noticed change in coronary flow.

(Coronary flow was crudely determined by counting the droplets seeping from the coronary sinus). The increased mean percentage weight change was not significantly higher than in group 4 hearts despite the reduction in the perfusate dextran concentration (table 7.2).

### 7.2.2. GLYCOGEN

Changes in the content of tissue glycogen are shown in figure 7.1. Myocardial biopsies were taken from the in situ organ, immediately after arrest and intermittently throughout the period of hypothermic preservation.

### 7.2.3 LACTATE

Perfusate lactate concentrations were determined after one hour, 12 hour and 24 hour hypothermic preservation (figure 7.2). The mean perfusate lactate concentration remained relatively unchanged during the preservation period.

### 7.2.4. GLUCOSE

The results of perfusate glucose concentrations are shown in table 7.5. These levels are very low showing the combined inhibitory effect of the perfusate and hypothermia on glucose uptake.

### 7.3.1. OBSERVATIONS DURING THE PERIOD OF FUNCTIONAL TESTING

The pattern of recovery was similar to group 4 hearts, namely initial ventricular fibrillation, being followed by sinus rhythm with good contractions. Twenty-four hearts were functionally evaluated of which four had weak contractions, were hard to the touch and unable to overcome the afterload of the system. The mean cardiac outputs of groups 4 and 5 were similar, with both differing significantly from that of the control group (table 7.6). The increase in coronary flow in these hearts was comparable to the control group ( $P > 0.1$ ) (table 7.7). The hearts demonstrated a 19% loss of weight (table 7.8) during reperfusion and haemodynamic function did not significantly change during this period (table 7.6).

### 7.3.2. ATP AND CP

The results obtained from 8 hearts are shown in table 7.3 (ATP) and table 7.4 (CP). Both values fall within the range of control hearts.

### 7.3.3. GLYCOGEN

Myocardial glycogen levels gradually decreased during the period of preservation. After a period of 1 hour warm blood reperfusion, the tissue glycogen levels dramatically increased with an overshoot significantly exceeding control values (figure 7.1).

### 7.3.4. LYSOSOMES

Pig hearts were divided into two groups from which myocardial lysosomes were differentially isolated.

1. Freshly excised (control).
2. Hypothermically perfused for 24 hours and then reperfed with blood at normothermia for 1 hour (group 5).



The final isolate consisted of three fractions, namely, supernatant, particulate and nuclear. These fractions were not stressed in the detergent Triton X-100. The free acid phosphatase activities in each fraction of both the control and group 5 hearts are shown in figure 7.3. A predominant feature was the increased activity in the group 5 hearts, with the greater increase in free activity occurring in the particulate fraction, although the total free activity of this fraction was the lowest (figure 7.3).

#### 7.3.5. MICROSCOPY

The myofibres and the nuclei appeared generally well preserved although some fibres had lost their cross striations with moderate damage. Interstitial oedema and small foci of mucus were present as well as areas of contraction banding; the latter may have been artefactual.

#### 7.3.6. ELECTRON MICROSCOPY

Changes include the degeneration of myofilaments, increased number of mitochondria and dilatation of the T-tubule system. The glycogen content appeared to be normal.

#### 7.4.1 ORTHOTOPIC TRANSPLANTATION

The results of three experiments are shown in table 7.9. All three animals were weaned off cardiopulmonary bypass and supported by a continuous infusion of dopamine. These hearts were only responsive to dopamine for a mean period of 9.5 hours after which time graft failure occurred (table 7.9).

### 7.5.1. DISCUSSION AND CONCLUSIONS

An interpretation of the results is difficult. However, by comparing biochemical and functional data from unpreserved (control) hearts with that of preserved (group 5) hearts, valuable information was obtained about cardiac metabolism under the hypothermic conditions of this study.

The primary physiological basis for the use of hypothermia is its metabolic lowering effect (McQuiston 1950; Bigelow et al 1954; Blair 1960) but various other functions and pathways are also altered which may lead under certain conditions to loss of viability from such phenomena as suppression of the sodium potassium pump leading to intracellular electrolyte derangements and loss of cell volume regulation (Martin et al 1972; Belzer and Southard 1980).

The decrease in myocardial glycogen concentrations during the period of preservation was an indication of anaerobic metabolism (Swanson et al 1979; Newsholme and Crabtree 1981) to maintain basal metabolic activity and structural integrity. If anaerobic metabolism was in progress, tissue lactate concentrations were expected to increase with subsequent release into the perfusate. Increased lactate levels were not, however, recovered in the perfusate, probably due to impaired membrane exchange systems. The low uptake of glucose under these preservation conditions (ranging between 1 and 22  $\mu\text{gm/gm wet wt/h}$  (table 7.5) was probably insufficient to replenish the glycogen stores. Thus, glycogen depletion appears to be a result of impaired glucose uptake rather than anaerobic glycolysis. The normal ATP and CP tissue levels were probably maintained by glycogenolysis; These levels can be maintained for a long period of time in ischaemic myocardium (Jennings 1976).

Another possible effect responsible for the loss of energy was calcium enhanced resting myocardial tension. If a passive flux of ions did occur due to hypothermically induced pump failure, myoplasmic calcium concentrations would increase due to calcium redistribution (entry from the extracellular spaces and also loss from the sarcoplasmic reticulum) without any change in total myocardial calcium concentrations. This effect would in turn activate a number of energy consuming reactions, notably those of the cardiac contractile protein and of the mitochondria (Fleckenstein et al 1974; Katz and Tada 1972).

Glycogen breakdown may then be enhanced to maintain the tissue high energy phosphates as a decline in intracellular ATP leads to the formation of rigor bonds between the thick and thin filaments (Weber and Murray 1973). Evidence for this phenomenon came from information about the change in texture (stiffness) of some of the hearts evaluated in this group.

Another important finding was the effect of preservation on the release of lysosomal acid phosphatase. The results clearly show (figure 7.3) (particulate fraction) an increased free acid phosphatase activity in the preserved hearts, suggesting ischaemic damage had occurred. This may imply that other proteolytic lysosomal enzymes may also be released contributing to membrane damage and accelerating the progression of injury to necrosis. This effect was also observed in unpreserved ischaemic tissue in which the proportion of lysosomal enzymes recovered in the residual particulate fraction was reduced (Van Lancker and Holtzer 1959; De Duve and Beaufay 1959).

In this study an attempt was made to locate areas in the myocardium that had been poorly preserved. Some destructive features were identified; decreased tissue glycogen during preservation, increased lysosomal acid phosphatase release, and oedema formation. Other changes remain purely speculative such as the distribution of calcium during hypothermic preservation and the effect of lysosomal acid phosphatase in the myoplasm.

This injury was subsequently confirmed by failure of graft function after orthotopic transplantation of preserved hearts.

My further approach to solving this problem was to find chemicals suitable as metabolic inhibitors and others as membrane stabilizers to prevent (or reduce) the loss of endogenous substrate and to protect cellular and lysosomal membranes against irreversible injury.

COMPOSITION OF PERFUSION SOLUTIONS USED TO PRESERVE  
HEARTS IN 5 DIFFERENT GROUPS

| Constitution of perfusates (mM)      | 1     | 2     | 3     | 4     | 5     |
|--------------------------------------|-------|-------|-------|-------|-------|
| NaCl                                 | 118.5 | 118.5 | 92.90 | 92.90 | 92.90 |
| NaHCO <sub>3</sub>                   | 25    | 25    | 25    | 25    | 25    |
| KCl                                  | 4.75  | 4.75  | 16    | 16    | 16    |
| KH <sub>2</sub> PO <sub>4</sub>      | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  |
| MgSO <sub>4</sub> 7H <sub>2</sub> O  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O | 2.5   | 2.5   | 2.25  | 2.25  | 2.25  |
| MgCl <sub>2</sub>                    | -     | -     | 14.8  | 14.8  | 14.8  |
| Glucose                              | 11    | 11    | 11    | 11    | 11    |
| Taurine                              | -     | 4     | 4     | 4     | 4     |
| ATP                                  | -     | -     | 10    | -     | -     |
| Insulin                              | -     | -     | 40u/L | 40u/L | 40u/L |
| Procaine HCl                         | -     | -     | 1     | 1     | 1     |
| Dextran 40                           | -     | -     | -     | 4g/L  | 2g/L  |
| Chlorpromazine                       | -     | -     | -     | -     | -     |
| Phenoxylbenzamine                    | -     | -     | -     | -     | -     |
| Glycerol                             | -     | -     | -     | -     | -     |
| Sucrose                              | -     | -     | -     | -     | -     |
| Osmolality (mOsm/L)                  | 277   | 280   | 295   | 315   | 302   |

TABLE 7.1.

CHANGES IN HEART WEIGHT DURING 24 HOUR PERIOD OF  
HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUPS (Perfusates)                                                                | 1   | 2   | 3      | 4    | 5    |
|------------------------------------------------------------------------------------|-----|-----|--------|------|------|
| Mean % wt gain                                                                     | 21  | 23  | 33.8   | 41.4 | 45.4 |
| SEM                                                                                | 1.9 | 2.4 | 2.8    | 4.3  | 2.5  |
| Statistical comparison:<br>Experimental groups vs<br>preceding experimental groups | -   | NS  | < 0.05 | NS   | NS   |
| n                                                                                  | 10  | 5   | 11     | 18   | 23   |

TABLE 7.2.

TISSUE ADENOSINE TRIPHOSPHATE (ATP) CONCENTRATIONS FOLLOWING  
24 HOURS HYPOTHERMIC PRESERVATION USING DIFFERENT PERFUSATES

| GROUPS (Perfusates)                                         | Control<br>(Unperfused) | 1       | 5     |
|-------------------------------------------------------------|-------------------------|---------|-------|
| ATP<br>( $\mu\text{mol/gm wet wt}$ )                        | 3.725                   | 1.551   | 3.500 |
| SEM                                                         | 0.253                   | 0.264   | 0.430 |
| Statistical comparison:<br>Control vs<br>Experimental Group | -                       | < 0.001 | NS    |
| n                                                           | 19                      | 8       | 8     |

TABLE 7.3.

TISSUE CREATINE PHOSPHATE (CP) CONCENTRATIONS FOLLOWING  
24 HOURS HYPOTHERMIC PRESERVATION USING DIFFERENT PERFUSATES

| GROUPS                                                            | Control<br>(unpreserved) | 1       | 5     |
|-------------------------------------------------------------------|--------------------------|---------|-------|
| CP<br>( $\mu\text{mol/g}$ wet wt)                                 | 6.611                    | 1.064   | 5.513 |
| SEM                                                               | 0.720                    | 0.215   | 1.288 |
| Statistical comparison:<br>Control group vs<br>experimental group | -                        | < 0.001 | NS    |
| n                                                                 | 7                        | 8       | 8     |

TABLE 7.4.



| Perfusate glucose concentration |            | Glucose uptake (mg/24h) | Glucose uptake ( $\mu$ gm/gm wet wt/h) |
|---------------------------------|------------|-------------------------|----------------------------------------|
| Initial (mM)                    | Final (mM) |                         |                                        |
| 10.44                           | 10.18      | 47                      | 13                                     |
| 9.18                            | 9.14       | 7                       | 2                                      |
| 9.60                            | 9.38       | 40                      | 10                                     |
| 9.58                            | 9.56       | 4                       | 1                                      |
| 9.92                            | 9.48       | 79                      | 21                                     |
| 9.00                            | 8.90       | 18                      | 7                                      |
| 9.11                            | 8.71       | 72                      | 22                                     |
| 9.40                            | 9.10       | 54                      | 17                                     |
| 9.50                            | 9.30       | 36                      | 13                                     |

Glucose uptake at the beginning and end of the period of 24 hours hypothermic perfusion

TABLE 7.5

CARDIAC OUTPUT DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUPS                                                                            | Control | 1         | 2      | 3      | 4      | 5      |
|-----------------------------------------------------------------------------------|---------|-----------|--------|--------|--------|--------|
| CO(ml/min/100 g)                                                                  | 3259    | 690<br>=4 | 631    | 1486   | 1825   | 1701   |
| SEM                                                                               | 202     | 135       | 178    | 222    | 325    | 328    |
| Statistical comparison:<br>Control vs experimental group                          | -       | <0.001    | <0.001 | <0.001 | <0.001 | <0.001 |
| Statistical comparison:<br>experimental groups vs preceding<br>experimental group | -       | -         | <0.05  | <0.05  | NS     | NS     |
| n                                                                                 | 12      | 10        | 5      | 11     | 18     | 23     |

TABLE 7.6.

CORONARY FLOW DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUPS                                                                           | Control | 1      | 3      | 4       | 5    |
|----------------------------------------------------------------------------------|---------|--------|--------|---------|------|
| CF<br>(mL/min/100g)                                                              | 328     | 122    | 222    | 291     | 242  |
| SEM                                                                              | 30.2    | 27.0   | 18.3   | 14.7    | 25.9 |
| Statistical comparison:<br>Control vs<br>Experimental group                      | -       | < .001 | < 0.01 | NS      | NS   |
| Statistical comparison:<br>Experimental group vs<br>Preceding experimental group | -       | -      | -      | < 0.001 | NS   |
| n                                                                                | 12      | 10     | 10     | 12      | 12   |

TABLE 7.7.

CHANGES IN HEART WEIGHT FOLLOWING ONE HOUR EX VIVO FUNCTIONAL TESTING

| GROUPS<br>(Perfusates)                                                            | 3    | 4    | 5    |
|-----------------------------------------------------------------------------------|------|------|------|
| % Wt<br>Loss                                                                      | 13   | 17   | 19.1 |
| SEM                                                                               | 2.00 | 5.00 | 4.33 |
| Statistical Comparison:<br>Experimental groups vs<br>preceding experimental group | -    | NS   | NS   |
| n                                                                                 | 11   | 18   | 23   |

TABLE 7.8.

RESULTS OF ORTHOTOPIC TRANSPLANTATION FOLLOWING 24 HOUR

STORAGE OF THE DONOR HEART

| Baboon (Group 5) | Survival (days) | Cause of death  | Myocardial Histology |
|------------------|-----------------|-----------------|----------------------|
| 1                | .29 ( 7.0 h)    | Cardiac Failure | No sample taken      |
| 2                | .44 (10.6 h)    | Cardiac Failure | No sample taken      |
| 3                | .46 (11.0 h)    | Cardiac Failure | No sample taken      |

TABLE 7.9

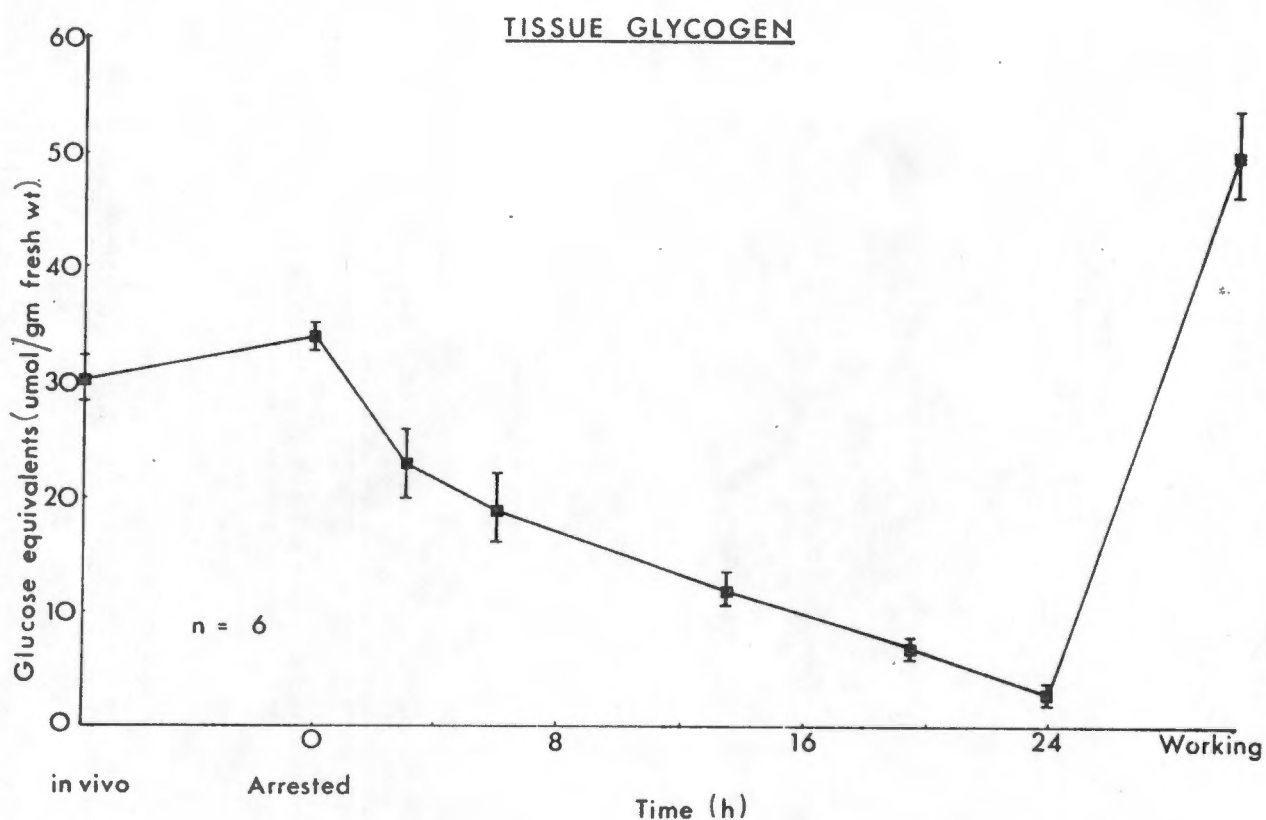


Figure 7.1.

Myocardial tissue glycogen levels (expressed as glucose equivalents) before cardioplegia (in vivo), after cardioplegia (arrested) and during the hypothermic perfusion period. After one hour warm blood reperfusion biopsies for glycogen content were again taken from the working heart (working). Bars represent S.E.M.

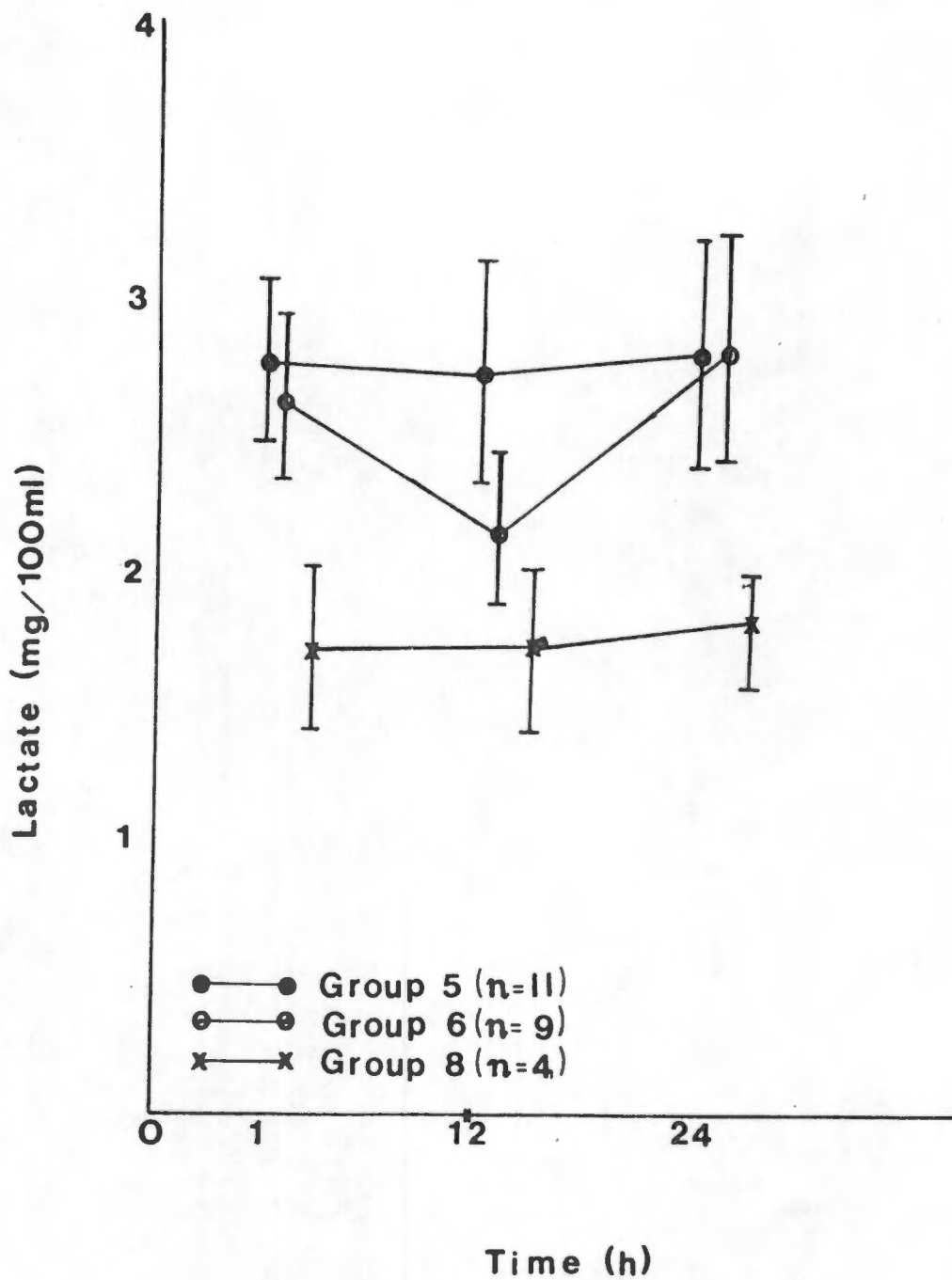


Fig. 7.2.

Perfusate lactate concentrations during the period of hypothermic perfusion. Bars represent S.E.M.

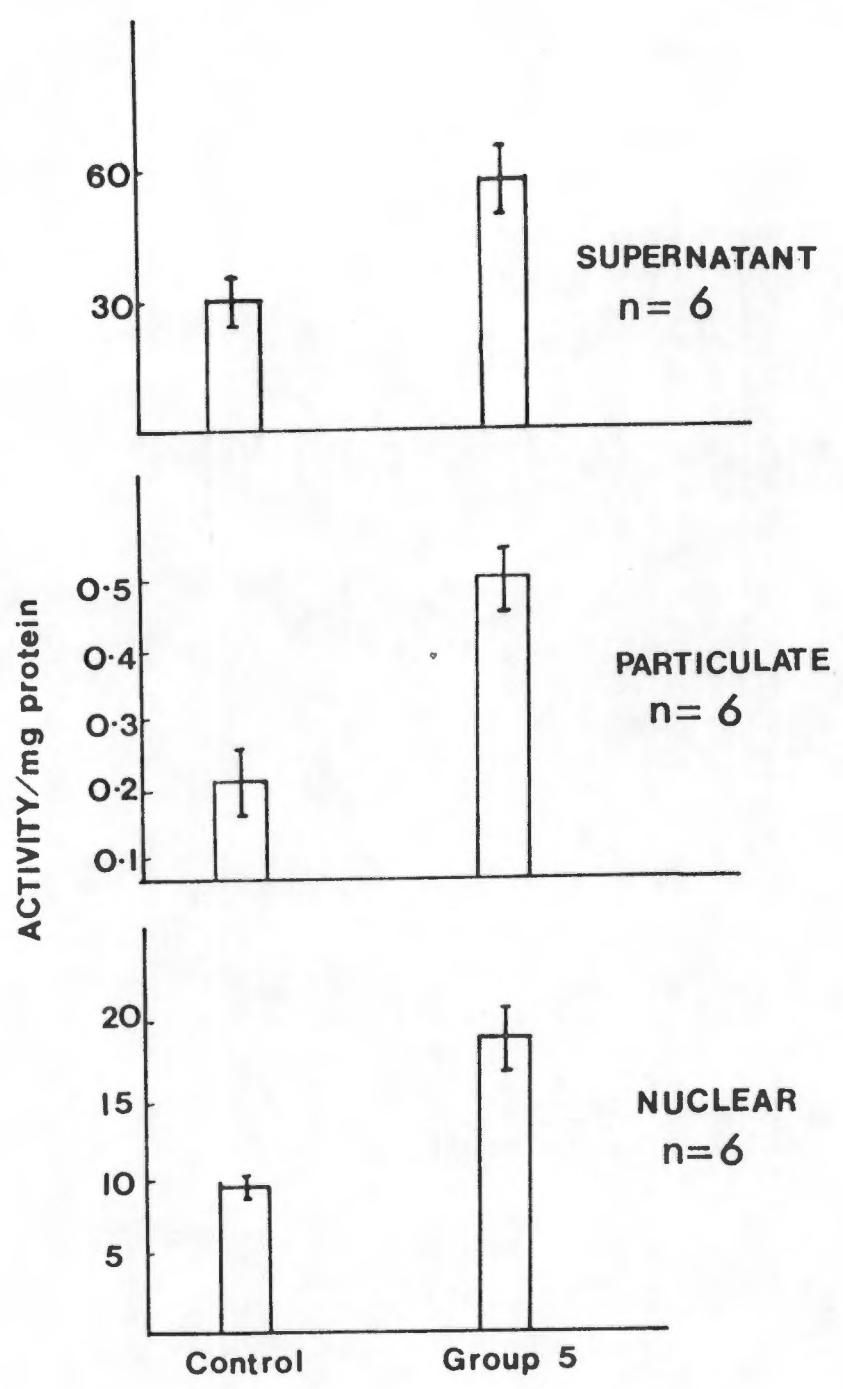


Fig. 7.3.

A comparison between free lysosomal acid phosphatase activities of a control and an experimental group 5. Each group consists of three differentially isolated fractions, supernatant, particulate and nuclear with each fraction differing significantly from its related control value.



## CHAPTER 8

### GROUP 6

#### 8. MODIFICATIONS TO THE PERFUSATE

This solution was modified with the aim of preventing some of the injury observed in group 5 hearts. As Dextran 40 did not contribute towards the prevention of fluid accumulation its further use was discontinued. In a screening test in which two pig hearts were perfused with a 5% albumin solution, no functional improvement occurred and the use of large molecular weight osmotic compounds was stopped. Similar observations were reported by Belzer et al (1978). Throughout the use of the previous five solutions some hearts functioned poorly although all were subjected to identical conditions with the exception of the change in the perfusate. Poor function was thus thought to be due to enhanced resting myocardial tension in the myocardium brought about by possibly overloading the calcium transport system by the available  $\text{Ca}^{++}$  in the perfusate. In a screening test used to assess the affect of a calcium free perfusate, ischaemic contracture resulted (two hearts tested) thus calcium was included at a concentration of 1.1mM in this perfusate which resembled the free calcium concentration perfusing normal myocardium in vivo. Further screening details are tabulated in appendix 4.

The use of high concentrations of a single drug may produce toxic side effects, therefore, several drugs known to be membrane stabilizers were added in lower concentrations.

##### 8.1.1. CHLORPROMAZINE

The need for more effective membrane stabilisation was demonstrated in the preceding chapter, which showed an increased release of free lysosomal acid phosphatase.

A chemical of suitable choice was chlorpromazine, which like other phenothiazines has a general inhibitory effect on the leakage of lysosomal enzymes (Guth et al 1963), cellular metabolism, (Dawkins et al 1959) and mitochondrial swelling (Judah 1960). Phenothiazines also conserve purine nucleotides by inhibiting the membrane-bound 5 nucleotidase (Lokkegaard et al 1979), prevents catecholamine depletion by inhibiting adenylyl cyclase (Noble 1979), and may have the additional function of alpha adrenergic blockade preventing vasospasm (Lokkegaard 1979). Its effectiveness during hypothermia was demonstrated by Laborit and Huguenard (1951) who used the combination of chlorpromazine and hypothermia to induce so-called "artificial hibernation". In red blood cells chlorpromazine causes expansion of the membrane and protects them against exposure to hypertonic saline and rapid cooling (Seeman et al 1969; Kwant and van Steveninck 1969).

Phenothiazines have also been demonstrated to aid in the maintenance of normal membrane permeability (Guttman and Friedman 1963) and in the prevention of electrolyte shifts and cellular swellings. Eyal et al (1965) reported that preservation of hearts was improved by the addition of chlorpromazine, and Turner et al (1970) confirmed its value by successfully storing kidneys for 24 hours using hypothermia and chlorpromazine.

#### 8.1.2. PHENOXYBENZAMINE

Phenoxybenzamine was chosen as one of the perfusate constituents for its vasodilation properties and its lysosomal membrane stabilising effect (Rangel et al 1969; Fonkalsrud et al 1970). Investigations by Fonkalsrud et al (1969) also showed that phenoxybenzamine protected mitochondria from osmotic induced swelling. Calman and Bell (1972), however, reported that it decomposed rapidly in dilute physiological solutions.

Other effects of phenoxybenzamine include; inhibition of catecholamine uptake and degradation (Smith 1973) as well as inhibition of serotonin and histamine activity resulting in vasodilation (Rangel et al 1969). The composition of the perfusate is shown in table 8.1. All determinations made during the hypothermic perfusion (table 3.2) and during the normothermic ex vivo functional evaluation period (table 3.3) are tabulated.

## RESULTS

### 8.2.1. OBSERVATIONS DURING THE PERIOD OF HYPOTHERMIC PRESERVATION

The appearance and "feel" of these hearts remained relatively unchanged at the end of the preservation period, but for the 47% increase in heart weight (table 8.2). The myocardial oxygen uptake was determined at the beginning and end of the preservation period with no significant change occurring between these two intervals (table 8.3).

There was a significant decrease in the coronary flow at the end of the preservation period (table 8.4) and the coronary resistance increased significantly (table 8.5).

### 8.2.2. GLYCOGEN

The control and experimental tissue glycogen levels are shown in figure 8.1. The level was significantly higher than glycogen values obtained in group 5 hearts (see section 7.2.3). This estimation was only made at the end of the period of hypothermic preservation.

### 8.2.3. LACTATE

The perfusate lactate concentration was determined after 1, 12 and 24 hours hypothermic perfusion and is shown in figure 7.2. The mean lactate level did not change significantly during the perfusion period.

### 8.3.1. OBSERVATIONS DURING THE PERIOD OF FUNCTIONAL TESTING

These hearts all required defibrillation and then maintained sinus rhythm with forceful contractions. In this group 16 hearts were tested of which 4 were in a contracted state and failed to relax properly during diastole. Haemodynamic measurements and calculations are listed in tables 8.6 (cardiac output) 8.7 (coronary flow) and 8.8 (pressure determinations) with a significant increase in CO compared with group 5 (table 8.6).

Myocardial oxygen uptake (table 8.9) did not change significantly from control values. The percentage weight loss is shown in table 8.9.1 (14.8%).

### 8.3.2. ATP and CP

The ATP and CP levels are shown in tables 8.9.2 and 8.9.3. Only the ATP value differed significantly from the control group.

### 8.4.1. ORTHOTOPIC TRANSPLANTATION

The results of 6 experiments are shown in table 8.9.4. Two animals could not be weaned off cardiopulmonary bypass, and three eventually failed to support the circulatory load while receiving a continuous dopamine infusion and one animal survived 1.5 days. The myocardium was examined histologically in 5 cases (table 8.9.4). The injury to the myocardium varied. The animals surviving less than one day showed features of ischaemic damage, notably myofibrillar degeneration (contraction band necrosis and loss of cross-striation) and also more severe damage of focal coagulative necrosis and myocytolysis.

### 8.5.1 DISCUSSION AND CONCLUSIONS

A definite biochemical and functional improvement was observed in hearts preserved in this group. The coronary flow decreased and the coronary vascular resistance increased significantly but this did not affect oxygen uptake significantly (table 8.3). The change in coronary flow and coronary vascular resistance was thought to be due to tissue oedema formation and possibly a vasoconstrictive effect brought about by the relatively high perfusate potassium concentration. The tissue glycogen (figure 8.1) levels were also maintained and no change in the release of lactate occurred during hypothermic perfusion (figure 7.2).

Functionally, these hearts were much improved and a significant increase in CO occurred when compared with any of the hearts preserved in groups 1 - 5, although the CO was significantly less when compared with the control groups. The tissue ATP levels were significantly lower than the control group.

Although the perfusate calcium concentration was reduced, myocardial tone was observed in 4 hearts and cytosolic calcium concentrations were still thought to be the cause of the failure observed in these hearts. These observations were considered when constituents of the next perfusate were selected. Orthotopic transplantation, however, of baboon hearts similarly preserved proved to be unsuccessful (table 8.9.4). These hearts were unable to support the circulatory load and required support by a dopamine infusion. Eventually these hearts were unresponsive to cardiotonic support (dopamine, isoprenaline, adrenaline) and complete failure followed.

The lack of correlation in failure rates between hearts tested on the functional testing apparatus and hearts tested by orthotopic transplantation led to modification of the functional testing apparatus. The aim of the modification was to subject the heart to a more physiological state than previously evaluated hearts (appendix 2). It was hoped that this change would lead to a more effective assessment of heart function during the one hour period of functional testing. Hearts which functioned well would undergo the ultimate test of orthotopic transplantation. A new group of freshly-arrested hearts were similarly tested on this modified functional testing apparatus to provide new control observations for comparison.

COMPOSITION OF PERFUSION SOLUTIONS USED TO PRESERVE  
HEARTS IN 9 DIFFERENT GROUPS

| Constitution of perfusates (mM)      | 1     | 2     | 3     | 4     | 5     | 6       |
|--------------------------------------|-------|-------|-------|-------|-------|---------|
| Nac1                                 | 118.5 | 118.5 | 92.90 | 92.90 | 92.90 | 92.90   |
| NaHCO <sub>3</sub>                   | 25    | 25    | 25    | 25    | 25    | 25      |
| KCl                                  | 4.75  | 4.75  | 16    | 16    | 16    | 16      |
| KH <sub>2</sub> PO <sub>4</sub>      | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20    |
| MgSO <sub>4</sub> 7H <sub>2</sub> O  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20    |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O | 2.5   | 2.5   | 2.25  | 2.25  | 2.25  | 1.1     |
| MgCl <sub>2</sub>                    | -     | -     | 14.8  | 14.8  | 14.8  | 14.8    |
| Glucose                              | 11    | 11    | 11    | 11    | 11    | 11      |
| Taurine                              | -     | 4     | 4     | 4     | 4     | 4       |
| ATP                                  | -     | -     | 10    | -     | -     | -       |
| Insulin                              | -     | -     | 40u/L | 40u/L | 40u/L | 40u/L   |
| Procaine HCl                         | -     | -     | 1     | 1     | 1     | 1       |
| Dextran 40                           | -     | -     | -     | 4g/L  | 2g/L  | -       |
| Chlorpromazine                       | -     | -     | -     | -     | -     | .005g/L |
| Phenoxybenzamine                     | -     | -     | -     | -     | -     | .01g/L  |
| Glycerol                             | -     | -     | -     | -     | -     | -       |
| Sucrose                              | -     | -     | -     | -     | -     | -       |
| Osmolality (mosM/L)                  | 277   | 280   | 295   | 315   | 302   | 321     |

TABLE 8.1.

CHANGES IN HEART WEIGHT DURING 24 HOUR PERIOD OF  
HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUPS (Perfusates)                                                                | 1   | 2   | 3      | 4    | 5    | 6    |
|------------------------------------------------------------------------------------|-----|-----|--------|------|------|------|
| Mean % wt gain                                                                     | 21  | 23  | 33.8   | 41.4 | 45.4 | 47.3 |
| SEM                                                                                | 1.9 | 2.4 | 2.8    | 4.3  | 2.5  | 3.28 |
| Statistical comparison:<br>Experimental groups vs<br>preceding experimental groups | -   | NS  | < 0.05 | NS   | NS   | NS   |
| n                                                                                  | 10  | 5   | 11     | 18   | 23   | 16   |

TABLE 8.2.



CORONARY ARTERIO-VEINOUS OXYGEN UPTAKE AT THE BEGINNING AND AT THE END  
OF THE PERIOD OF HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP<br>Perfusates | O <sub>2</sub> uptake<br>at the beginning<br>of preservation<br>mmol/h/gm<br>(X10 <sup>-3</sup> ) | O <sub>2</sub> uptake<br>at the end of<br>preservation<br>mmol/h/gm<br>(X10 <sup>-3</sup> ) | Statistical comparison:<br>Beginning vs end<br>of perfusion | n  |
|---------------------|---------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|-------------------------------------------------------------|----|
| 6                   | 3.9<br>(0.62)                                                                                     | 4.2<br>(0.56)                                                                               | NS                                                          | 21 |

TABLE 8.3.

CORONARY FLOW AT THE BEGINNING AND END OF 24 HOUR PERIOD  
OF HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP<br>Perfusates | Coronary flow<br>at beginning<br>of preservation<br>ml/min/gm<br>(X10 <sup>-2</sup> ) | Coronary flow<br>at end of<br>preservation<br>ml/min/gm<br>(X10 <sup>-2</sup> ) | Statistical comparison:<br>Beginning vs end of<br>preservation | n  |
|---------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------|----|
| 6                   | 15.74<br>(0.70)                                                                       | 6.77<br>(0.47)                                                                  | < 0.001                                                        | 21 |

TABLE 8.4.

CORONARY VASCULAR RESISTANCE AT THE BEGINNING AND END OF 24 HOUR  
PERIOD OF HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP<br>(Perfusates) | Coronary vascular resistance<br>cm H <sub>2</sub> O/gm/min/ml |                               | Statistical comparison:<br>Beginning vs end<br>of preservation | n  |
|-----------------------|---------------------------------------------------------------|-------------------------------|----------------------------------------------------------------|----|
|                       | At the beginning<br>of preservation                           | At the end of<br>preservation |                                                                |    |
| 6                     | 53<br>(2.2)                                                   | 210<br>(21.3)                 | < 0.001                                                        | 21 |

TABLE 8.5.

CARDIAC OUTPUT DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUPS                                                                            | Control | 1         | 2      | 3      | 4      | 5      | 6      |
|-----------------------------------------------------------------------------------|---------|-----------|--------|--------|--------|--------|--------|
| CO(ml/min/100 g)                                                                  | 3259    | 690<br>=4 | 631    | 1486   | 1825   | 1701   | 2572   |
| SEM                                                                               | 202     | 135       | 178    | 222    | 325    | 328    | 141    |
| Statistical comparison:<br>Control vs experimental group                          | -       | <0.001    | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Statistical comparison:<br>experimental groups vs preceding<br>experimental group | -       | -         | <0.05  | <0.05  | NS     | NS     | <0.05  |
| n                                                                                 | 12      | 10        | 5      | 11     | 18     | 23     | 16     |

TABLE 8.6.

CORONARY FLOW DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUPS                                                                           | Control | 1      | 3      | 4       | 5    | 6      |
|----------------------------------------------------------------------------------|---------|--------|--------|---------|------|--------|
| CF<br>(mL/min/100g)                                                              | 328     | 122    | 222    | 291     | 242  | 270    |
| SEM                                                                              | 30.2    | 27.0   | 18.3   | 14.7    | 25.9 | 27.0   |
| Statistical comparison:<br>Control vs<br>Experimental group                      | -       | < .001 | < 0.01 | NS      | NS   | NS     |
| Statistical comparison:<br>Experimental group vs<br>Preceding experimental group | -       | -      | -      | < 0.001 | NS   | < 0.02 |
| n                                                                                | 12      | 10     | 10     | 12      | 12   | 12     |

TABLE 8.7.

HAEMODYNAMIC PERFORMANCE DURING FUNCTIONAL TESTING FOLLOWING 24 HOURS

HYPOTHERMIC PRESERVATION USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                   | Control      | 6            |
|----------------------------------------------------------------------|--------------|--------------|
| LVP (mm Hg)                                                          | 131<br>(4.4) | 90<br>(3.6)  |
| LVEDP (mm Hg)                                                        | 5.3<br>(1.0) | 7.5<br>(1.3) |
| AOP (mm Hg)                                                          | 114<br>(2.8) | 83<br>(4.3)  |
| Statistical comparison:<br>LVP<br>Control vs Experimental<br>group   | -            | 0.001        |
| Statistical comparison:<br>LVEDP<br>Control vs experimental<br>group | -            | NS -         |
| Statistical comparison:<br>AOP<br>Control vs experimental<br>group   | -            | 0.001        |
| n                                                                    | 9            | 16           |

FIGURES IN BRACKETS DENOTE SEM

TABLE 8.8.

OXYGEN UPTAKE DURING THE PERIOD OF FUNCTIONAL TESTING FOLLOWING  
24 HOURS HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                            | Control | 6    |
|-------------------------------------------------------------------------------|---------|------|
| O <sub>2</sub> uptake during functional testing mmol/h/g (X10 <sup>-1</sup> ) | 6.87    | 7.16 |
| SEM                                                                           | 1.08    | 1.37 |
| Statistical comparison: Control vs Experimental group                         | -       | NS   |
| Statistical comparison: Experimental group vs preceding experimental group    | -       | -    |
| n                                                                             | 9       | 16   |

TABLE 8.9.

CHANGES IN HEART WEIGHT FOLLOWING ONE HOUR EX VIVO FUNCTIONAL TESTING

| GROUPS<br>(Perfusates)                                                            | 3    | 4    | 5    | 6    |
|-----------------------------------------------------------------------------------|------|------|------|------|
| % Wt<br>Loss                                                                      | 13   | 17   | 19.1 | 14.8 |
| SEM                                                                               | 2.00 | 5.00 | 4.33 | 1.61 |
| Statistical Comparison:<br>Experimental groups vs<br>preceding experimental group | -    | NS   | NS   | NS   |
| n                                                                                 | 11   | 18   | 23   | 16   |

TABLE 8.9.1.



TISSUE ADENOSINE TRIPHOSPHATE (ATP) CONCENTRATIONS FOLLOWING  
24 HOURS HYPOTHERMIC PRESERVATION USING DIFFERENT PERFUSATES

| GROUPS (Perfusates)                                         | Control<br>(Unperfused) | 1         | 5     | 6        |
|-------------------------------------------------------------|-------------------------|-----------|-------|----------|
| ATP<br>( $\mu\text{mol/gm wet wt}$ )                        | 3.725                   | 1.551     | 3.500 | 2.601    |
| SEM                                                         | 0.253                   | 0.264     | 0.430 | 0.235    |
| Statistical comparison:<br>Control vs<br>Experimental Group | -                       | $< 0.001$ | NS    | $< 0.05$ |
| n                                                           | 19                      | 8         | 8     | 18       |

TABLE 8.9.2.

TISSUE CREATINE PHOSPHATE (CP) CONCENTRATIONS FOLLOWING  
24 HOURS HYPOTHERMIC PRESERVATION USING DIFFERENT PERFUSATES

| GROUPS                                                            | Control<br>(unpreserved) | 1       | 5     | 6     |
|-------------------------------------------------------------------|--------------------------|---------|-------|-------|
| CP<br>( $\mu\text{mol/g}$ wet wt)                                 | 6.611                    | 1.064   | 5.513 | 5.764 |
| SEM                                                               | 0.720                    | 0.215   | 1.288 | 0.964 |
| Statistical comparison:<br>Control group vs<br>experimental group | -                        | < 0.001 | NS    | NS    |
| n                                                                 | 7                        | 8       | 8     | 18    |

TABLE 8.9.3.

RESULTS OF ORTHOTOPIC TRANSPLANTATION FOLLOWING  
24 HOUR STORAGE OF THE DONOR HEART

| Baboon (Group 6) | Survival (days) | Cause of Death     | Myocardial Histology                               |
|------------------|-----------------|--------------------|----------------------------------------------------|
| 1                | 0               | Myocardial failure | No sample taken                                    |
| 2                | 0.25 (6.0 h)    | Myocardial failure | Myofibrillar degeneration                          |
| 3                | 0.71(17.0 h)    | Myocardial failure | Subendocardial haemorrhage/myocytolysis            |
| 4                | 0               | Myocardial failure | Myofibrillar degeneration/interstitial haemorrhage |
| 5                | 1.5             | Myocardial failure | Coagulative necrosis                               |
| 6                | 0.79(19.0 h)    | Myocardial failure | Subendocardial haemorrhage                         |

TABLE 8.9.4

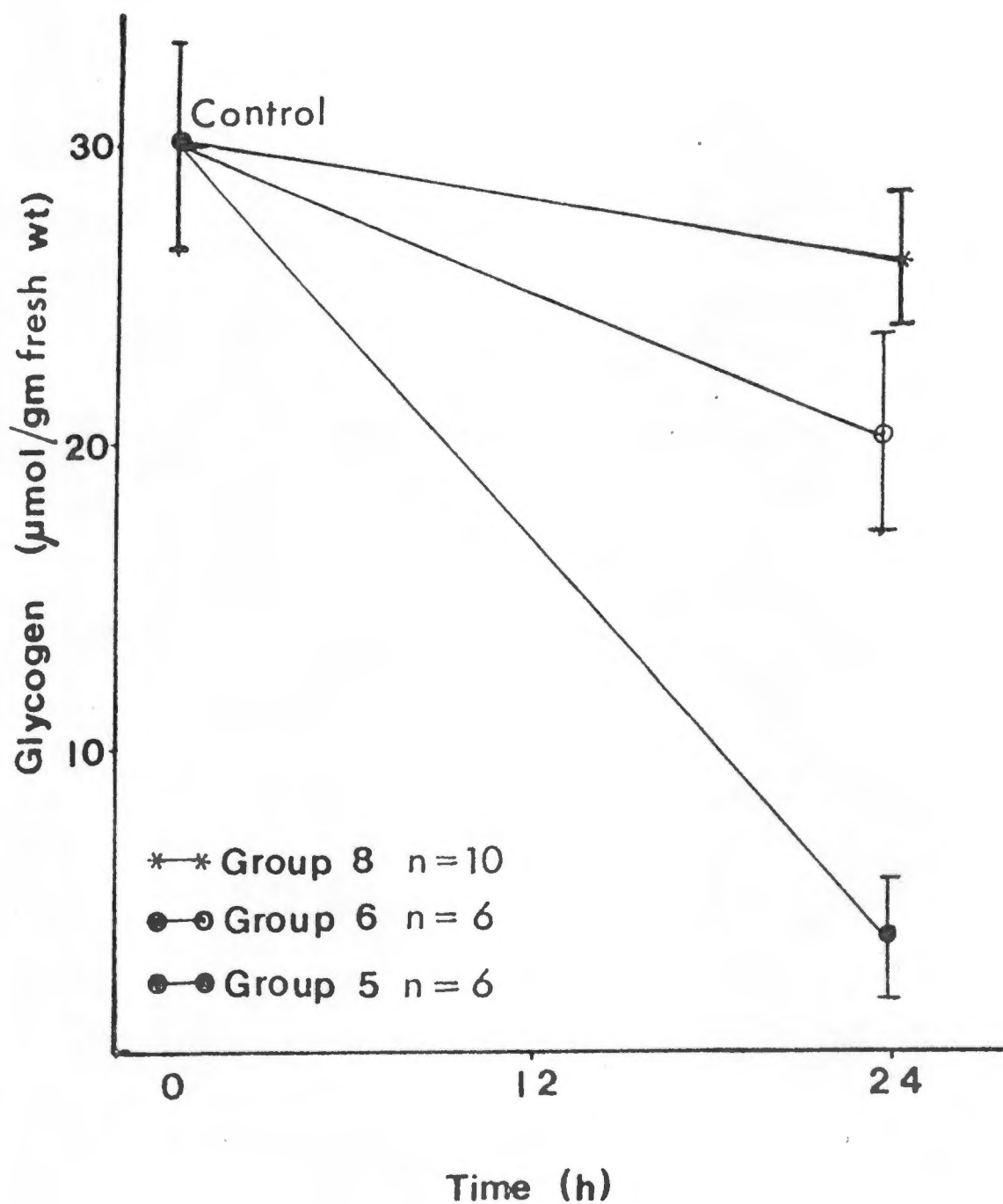


Fig. 8.1.

A comparison of tissue glycogen (glucose equivalents) levels between a control group (freshly excised hearts) and three experimental groups subjected to 24 hours of hypothermic preservation. Bars represent S.E.M.

## CHAPTER 9

### GROUP 7

#### 9. MODIFICATIONS TO THE PERFUSATE

Suggestions towards further modifications to the perfusate came from the results in chapter 8 concerning changes in coronary flow and coronary resistance during hypothermic preservation, and advice from Pegg (Personal communication 1980) stating that the prevention of tissue oedema was essential for graft viability during preservation (table 9.0).

The problem of oedema formation was still unresolved, although (Lee et al 1980) produced evidence that oedema formation did not reduce flow through an organ nor was it critical for the maintenance of organ viability (Copeland et al 1973). Having shown that hearts that lost weight (groups 3 to 7) (table 9.1) perform better than hearts that gain weight (groups 1 and 2) (Sections 3.3.1 and 4.2.1) after functional testing, a general hypothesis was stated as follows : Oedema formation was not critical for organ viability if it was reversible after reperfusion with warm blood.

##### 9.1.1. GLYCEROL

Large molecular weight molecules (12% albumin) in the perfusate did not protect against oedema formation (section 8.0) as progressive organ swelling occurred over a period of 24 hours (Belzer et al 1978). A review of the literature revealed that oedema formation was unavoidable unless extremely unphysiological concentrations of osmotic agents were used (Copeland et al 1973; Belzer et al 1978).

As a result an alternative approach was used. This involved the use of a small molecule (glycerol) (appendix 4) effective against oedema formation at 37°C (Gonzalez et al 1977). This small molecule would easily diffuse into the cell and presumably leave the cell without much difficulty on reperfusion with warm blood. Oedema formation was considered beneficial as toxic end products may be diluted. The role of glycerol in the perfusate was two fold, namely to slow down the initial process of oedema formation (Gonzalez et al 1977) and to impart some cryoprotective effects on the myocardium. Ice formation was unlikely to occur at 4 - 10°C although nucleated cells can be destroyed simply by reduction in temperature without freezing (Meynell 1958; Knight et al 1975). Cryoprotective agents (glycerol) may also be important in preventing damage by sudden temperature change; it has been shown that both rapid and slow cooling can be harmful, depending on the type of tissue that is being cooled (Ashwood - Smith and Forrant 1980). Raison and Lyons (1971) have also shown that cryoprotective agents protect against mitochondrial damage at temperatures below 20°C. Glycerol is non-toxic (Meyer et al 1971) and in both liver and kidney it sequesters most of the tissue ATP as glycerophosphate (Burch et al 1970) which may be used in the glycerophosphate shuttle transferring electrons from cytoplasmic NADH to the mitochondrial electron transport chain preparing the cells for oxidative metabolism at the onset of warm blood reperfusion (Lehninger 1972).

#### 9.1.2. POTASSIUM

The decrease in coronary flow and the increase in coronary resistance was thought to be related to the perfusate potassium concentration. From observations obtained following renal preservation potassium concentration in the perfusate in excess of 12 meq/l results in vasoconstriction, (Levowitz et al 1969; Scott et al 1959). The perfusate concentration was consequently reduced to 10.9mM and the idea of intracellular solutions suggested by Martin et al (1970) was rejected.

#### 9.1.3. SODIUM

The perfusate sodium concentration was raised because of the passive access of sodium into the myocardial cells under hypothermic conditions. The sodium presumably influenced calcium transport by a  $\text{Na}^+ - \text{Ca}^{++}$  exchanged mechanism in which the downhill entry of sodium into the myocardial cell was coupled to the uphill transport of calcium from the cytosol to the extracellular space (Reuter 1974). Evidence for the energy dependency of the exchange mechanism in cardiac muscle was provided by Jundt and Reuter (1976).

## RESULTS

### 9.2.1. OBSERVATIONS DURING THE PERIOD OF HYPOTHERMIC PRESERVATION

All hearts increased in weight (42%) during the period of perfusion due to oedema formation and this increase did not differ significantly from group 6 hearts (table 9.2). Initial mean coronary flow was significantly greater in group 6 hearts than group 7 (table 9.3). The coronary flow at the end of the perfusion period decreased significantly (table 9.4) from the initial determination but was significantly higher than group 6 hearts at the end of perfusion (table 9.5). Initial mean coronary vascular resistance was significantly greater in group 7 than group 6 (table 9.6). The coronary resistance pattern at the end of perfusion was now reversed as group 7 was now significantly lower than group 6 (table 9.7). the coronary vascular resistance at the beginning and end of perfusion differed significantly (table 9.8). There was a consistent pattern in coronary arteriovenous oxygen uptake in both groups with no significant difference occurring at the beginning and end of perfusion (table 9.9) despite the changes in coronary flow and coronary resistance between these two intervals.

### 9.2.2. ATP and CP

The results of ATP and CP tissue levels are shown in figure 9.1. The tissue high energy phosphate levels remained relatively normal compared with in vivo values but decreased towards the end of the preservation period.

### 9.2.3. LDH

The changes in release of LDH during the hypothermic perfusion period is shown in figure 9.2. The release of LDH slowly increased with time but increased insignificantly after 20 hours.



#### 9.3.1. OBSERVATIONS DURING THE PERIOD OF FUNCTIONAL TESTING

These heart all required defibrillation. 8 Hearts were functionally tested and all were able to overcome the afterload of the system. There were no rhythm disturbances and contractions were forceful. The haemodynamic measurements and calculations (SV) are shown in tables 9.9.1 (cardiac output), 9.9.2 (coronary flow), 9.9.3 (stroke volume) and 9.9.4 (pressure determinations). The CO LVP and SV differed significantly from the control group. Myocardial oxygen uptake (table 9.9.5) did not change significantly from control values and these results were similar to those of group 6. The percentage weight loss did not differ significantly from group 6 (9.74%) (table 9.1).

#### 9.3.2. ATP AND CP

The ATP and CP levels are shown in tables 9.9.6 and 9.9.7 and both these values are significantly lower than the control values.

#### 9.4.1. ORTHOTOPIC TRANSPLANTATION

#### 9.4.2. OBSERVATIONS DURING THE PERIOD OF HYPOTHERMIC PERFUSION

All the hearts gained weight (32%) at the end of the period of preservation, with little change taking place in coronary vascular resistance although the coronary flow decreased significantly (table 9.9.8).

#### 9.4.3. RESULTS OF ORTHOTOPIC TRANSPLANTATION

Thirteen experiments had been performed of which six animals survived to rejection, two could not be weaned from cardiopulmonary bypass, one death occurred after a rapid infusion of sodium bicarbonate (unexplained) (table 9.9.9).

The remaining animals died of myocardial failure. Several hearts required inotropic support to ensure satisfactory functioning immediately after discontinuation of cardio-pulmonary bypass when mean arterial pressures of only 45 - 55 mm Hg were obtained. Immediately before returning the baboons to the cage (6 - 12 hours after discontinuation of cardio-pulmonary bypass) mean arterial pressure had risen to 90-100mm Hg with mean central venous pressures of 1 - 5 cm H<sub>2</sub>O without inotropic support. In all animals surviving longer than 48 hours the ECG was normal; occasional flattening or inversion of the T-wave in the left chest leads has been noted, although we have found this to be a not uncommon finding in healthy baboons.

Good haemodynamic functioning has been confirmed at cardiac catheterisation in these immunosuppressed animals (table 9.9.9.1); virtually normal pressures were obtained. The slight pressure gradient between right ventricle and distal pulmonary artery was accounted for by some narrowing occurring at the pulmonary artery suture line at necropsy.

The myocardium was examined histologically in most cases (table 9.9.9). All the preserved hearts had some interstitial oedema, but no features of major ischaemic injury. The baboon sacrificed on the 10th day showed minor features of ischaemic damage notably myofibrillar degeneration (contraction band necrosis and loss of cross-striation). Those baboons which survived until rejection showed features of severe acute rejection with mononuclear cell infiltration, oedema and myofibrillar damage.

Only in those baboons in which myocardial function was obviously poor throughout the post-operative period from inadequate preservation have there been microscopic features of severe ischaemic injury to the myocardium. Some showed focal myofibrillar degeneration, early myocytolysis, plus focal coagulative necrosis. Interstitial haemorrhage was also present in areas.

These findings suggested a reperfusion type of myofibre necrosis; the heart failed within 48 hours.

#### 9.5.1. DISCUSSION AND CONCLUSIONS

Although there was once again a further improvement in most myocardial function, some parameters suggested that preservation was not perfect, such as the steady increase in release of LDH (figure 9.2) and the significant decrease in LVP, CO and SV during functional testing when compared with control values.

The inclusion of glycerol in the perfusate did not result in a decreased percentage weight gain nor in a greater percentage weight loss after reperfusion although an advantage of glycerol in the perfusate was observed. The coronary flow at the beginning of preservation was higher in group 6 than 7, but at the end of preservation the situation was reversed with a higher coronary flow in group 7. A similar improvement was observed in coronary vascular resistance, which was lower in group 7 at the end of preservation. This effect may be due to the decreased potassium concentration. The important observation was that all the hearts were able to cope with the afterload of the modified functional testing apparatus without any failure occurring.

Although the haemodynamic performance was not as good as control hearts, the criterion for a trial by orthotopic transplantation was satisfied. The results of orthotopic transplantation largely confirmed the effectiveness of this perfusate and 4 hearts were unable to support the circulation after orthotopic transplantation. Thus the perfusate still needed some modification.

the tissue ATP levels were maintained at a relatively normal concentration during the period of preservation (figure 9.1) whereas the CP level was very low at the end of the period of preservation. This decrease suggested that the myocardium was unable to make full use of available substrates and slowly depleted reserves instead. A similar problem was encountered earlier when glycogen levels were reduced. In an attempt to prevent glycogen depletion, a metabolic inhibitory approach was pursued as opposed to a metabolic stimulatory effect in which substrate utilisation was enhanced. The presence of insulin in the perfusate was queried. The decrease in tissue CP levels was evidence of endogenous metabolic activity, during which time a gradual accumulation of metabolic waste products occurred. These waste products presumable stimulated other metabolic pathways to assist in the eliminatory process, with the latter using additional energy resulting in further waste production. It was further speculated that the myocardium could be protected from injury induced by this futile cycle by reducing the effective rate of metabolic waste production.

This could be brought about by inducing a gradual accumulation of fluid within the myocardium. The accumulated fluid would dilute the endogenous toxic waste products; dilute the extracellular calcium concentration preventing possible enhanced resting myocardial tension (calcium may passively diffuse from the sarcoplasmic reticulum and the interstitial spaces through phospholipid moieties in the cell membrane into the cells under hypothermic conditions) and maintain a relatively constant endogenous acid-base balance.

It was on these assumptions that the composition of the next perfusate was based. By a careful selection of osmotically active molecules the above effect was envisaged; however it was essential that these small molecules would be able to rapidly diffuse back into the vascular compartment following reperfusion with blood.

COMPOSITION OF PERFUSION SOLUTIONS USED TO PRESERVE  
HEARTS IN 9 DIFFERENT GROUPS

| Constitution of perfusates (mM)      | 1     | 2     | 3     | 4     | 5     | 6       | 7       |
|--------------------------------------|-------|-------|-------|-------|-------|---------|---------|
| NaCl                                 | 118.5 | 118.5 | 92.90 | 92.90 | 92.90 | 92.90   | 115.7   |
| NaHCO <sub>3</sub>                   | 25    | 25    | 25    | 25    | 25    | 25      | 25      |
| KCl                                  | 4.75  | 4.75  | 16    | 16    | 16    | 16      | 10.9    |
| KH <sub>2</sub> PO <sub>4</sub>      | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20    | 1.20    |
| MgSO <sub>4</sub> 7H <sub>2</sub> O  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20    | 1.20    |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O | 2.5   | 2.5   | 2.25  | 2.25  | 2.25  | 1.1     | 1.1     |
| MgCl <sub>2</sub>                    | -     | -     | 14.8  | 14.8  | 14.8  | 14.8    | 14.8    |
| Glucose                              | 11    | 11    | 11    | 11    | 11    | 11      | 11      |
| Taurine                              | -     | 4     | 4     | 4     | 4     | 4       | 4       |
| ATP                                  | -     | -     | 10    | -     | -     | -       | -       |
| Insulin                              | -     | -     | 40u/L | 40u/L | 40u/L | 40u/L   | 40u/L   |
| Procaine HCl                         | -     | -     | 1     | 1     | 1     | 1       | 1       |
| Dextran 40                           | -     | -     | -     | 4g/L  | 2g/L  | -       | -       |
| Chlorpromazine                       | -     | -     | -     | -     | -     | .005g/L | .005g/L |
| Phenoxybenzamine                     | -     | -     | -     | -     | -     | .01g/L  | .01g/L  |
| Glycerol                             | -     | -     | -     | -     | -     | -       | 136     |
| Sucrose                              | -     | -     | -     | -     | -     | -       | -       |
| Osmolality (mOsM/L)                  | 277   | 280   | 295   | 315   | 302   | 321     | 330     |

TABLE 9.0.

CHANGES IN HEART WEIGHT FOLLOWING ONE HOUR EX VIVO FUNCTIONAL TESTING

| GROUPS<br>(Perfusates)                                                            | 3    | 4    | 5    | 6    | 7    |
|-----------------------------------------------------------------------------------|------|------|------|------|------|
| % Wt<br>Loss                                                                      | 13   | 17   | 19.1 | 14.8 | 9.74 |
| SEM                                                                               | 2.00 | 5.00 | 4.33 | 1.61 | 2.65 |
| Statistical Comparison:<br>Experimental groups vs<br>preceding experimental group | -    | NS   | NS   | NS   | NS   |
| n                                                                                 | 11   | 18   | 23   | 16   | 17   |

TABLE 9.1.

CHANGES IN HEART WEIGHT DURING 24 HOUR PERIOD OF  
HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUPS (Perfusates)                                                                | 1   | 2   | 3      | 4    | 5    | 6    | 7    |
|------------------------------------------------------------------------------------|-----|-----|--------|------|------|------|------|
| Mean % wt gain                                                                     | 21  | 23  | 33.8   | 41.4 | 45.4 | 47.3 | 42.0 |
| SEM                                                                                | 1.9 | 2.4 | 2.8    | 4.3  | 2.5  | 3.28 | 2.56 |
| Statistical comparison:<br>Experimental groups vs<br>preceding experimental groups | -   | NS  | < 0.05 | NS   | NS   | NS   | NS   |
| n                                                                                  | 10  | 5   | 11     | 18   | 23   | 16   | 17   |

TABLE 9.2.



COMPARISON OF CORONARY FLOWS AT THE BEGINNING OF 24 HOUR PERIOD OF  
HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES.

| GROUP (Perfusates)                                                               | 6    | 7      |
|----------------------------------------------------------------------------------|------|--------|
| Coronary flow at the beginning of preservation<br>ml/min/gm ( $\times 10^{-2}$ ) | 15.4 | 13.4   |
| SEM                                                                              | 0.70 | 0.80   |
| Statistical comparison:<br>Experimental group vs<br>preceding experimental group | -    | < 0.05 |
| n                                                                                | 16   | 8      |

TABLE 9.3.

CORONARY FLOW AT THE BEGINNING AND END OF 24 HOUR PERIOD  
OF HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP<br>Perfusates | Coronary flow<br>at beginning<br>of preservation<br>ml/min/gm<br>(X10 <sup>-2</sup> ) | Coronary flow<br>at end of<br>preservation<br>ml/min/gm<br>(X10 <sup>-2</sup> ) | Statistical comparison:<br>Beginning vs end of<br>preservation | n  |
|---------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------|----|
| 6                   | 15.74<br>(0.70)                                                                       | 6.77<br>(0.47)                                                                  | < 0.001                                                        | 21 |
| 7                   | 13.40<br>(0.81)                                                                       | 8.77<br>(0.57)                                                                  | < 0.001                                                        | 9  |

TABLE 9.4.

COMPARISON OF CORONARY FLOWS AT THE END OF 24 HOUR PERIOD OF HYPOTHERMIC  
PERFUSION USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                         | 6    | 7      |
|----------------------------------------------------------------------------|------|--------|
| Coronary flow at the end of preservation ml/min/gm ( $\times 10^{-2}$ )    | 6.77 | 8.77   |
| SEM                                                                        | 0.47 | 0.57   |
| Statistical comparison: Experimental group vs preceding experimental group | -    | < 0.02 |
| n                                                                          | 16   | 8      |

TABLE 9.5.

CORONARY VASCULAR RESISTANCE AT THE BEGINNING OF 24 HOUR  
PERIOD OF HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                                           | 6   | 7      |
|----------------------------------------------------------------------------------------------|-----|--------|
| Coronary vascular resistance<br>At beginning of preservation<br>cmH <sub>2</sub> O/gm/min/ml | 53  | 65     |
| SEM                                                                                          | 2.2 | 4.5    |
| Statistical comparison:<br>Experimental group vs<br>preceding experimental group             | -   | < 0.02 |
| n                                                                                            | 16  | 8      |

TABLE 9.6.

CORONARY VASCULAR RESISTANCE AT THE END OF 24 HOUR PERIOD OF HYPOTHERMIC

PERFUSION USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                                     | 6    | 7       |
|----------------------------------------------------------------------------------------|------|---------|
| Coronary vascular resistance<br>At end of preservation<br>cmH <sub>2</sub> O/gm/min/ml | 210  | 127     |
| SEM                                                                                    | 21.3 | 6.7     |
| Statistical comparison:<br>Experimental group vs<br>preceding experimental group       | -    | < 0.001 |
| n                                                                                      | 16   | 8       |

TABLE 9.7.

CORONARY VASCULAR RESISTANCE AT THE BEGINNING AND END OF 24 HOUR  
PERIOD OF HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP<br>(Perfusates) | Coronary vascular resistance<br>cm H <sub>2</sub> O/gm/min/ml |                               | Statistical comparison:<br>Beginning vs end<br>of preservation | n  |
|-----------------------|---------------------------------------------------------------|-------------------------------|----------------------------------------------------------------|----|
|                       | At the beginning<br>of preservation                           | At the end of<br>preservation |                                                                |    |
| 6                     | 53<br>(2.2)                                                   | 210<br>(21.3)                 | < 0.001                                                        | 21 |
| 7                     | 65<br>(4.5)                                                   | 127<br>( 6.7)                 | < 0.001                                                        | 9  |

TABLE 9.8.

CORONARY ARTERIO-VEINOUS OXYGEN UPTAKE AT THE BEGINNING AND AT THE END  
OF THE PERIOD OF HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP<br>(Perfusates) | O <sub>2</sub> uptake<br>at the beginning<br>of preservation<br>mmol/h/gm<br>(X10 <sup>-3</sup> ) | O <sub>2</sub> uptake<br>at the end of<br>preservation<br>mmol/h/gm<br>(X10 <sup>-3</sup> ) | Statistical comparison:<br>Beginning vs end<br>of perfusion | n  |
|-----------------------|---------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|-------------------------------------------------------------|----|
| 6                     | 3.9<br>(0.62)                                                                                     | 4.2<br>(0.56)                                                                               | NS                                                          | 21 |
| 7                     | 4.8<br>(0.60)                                                                                     | 4.9<br>(0.58)                                                                               | NS                                                          | 9  |

TABLE 9.9.

CARDIAC OUTPUT DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                               | Control | 7      |
|----------------------------------------------------------------------------------|---------|--------|
| CO (mℓ/min/100g)                                                                 | 2827    | 1577   |
| SEM                                                                              | 22      | 222    |
| Statistical comparison:<br>Control vs experimental<br>group                      | -       | < 0.01 |
| Statistical comparison:<br>Experimental group vs<br>preceding experimental group | -       | -      |
| n                                                                                | 9       | 8      |

TABLE 9.9.1.



CORONARY FLOW DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                               | Control | 7   |
|----------------------------------------------------------------------------------|---------|-----|
| CF (ml/min/100g)                                                                 | 258     | 295 |
| SEM                                                                              | 33      | 43  |
| Statistical comparison:<br>Control vs experimental<br>group                      | -       | NS  |
| Statistical comparison:<br>Experimental group vs<br>preceding experimental group | -       | -   |
| n                                                                                | 9       | 8   |

TABLE 9.9.2.

STROKE VOLUME DURING EX VIVO FUNCTIONAL TESTING FOLLOWING 24 HOURS

STORAGE USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                               | Control | 7    |
|----------------------------------------------------------------------------------|---------|------|
| SV (mL/100g)                                                                     | 17.0    | 10.7 |
| SEM                                                                              | 1.27    | 1.54 |
| Statistical comparison:<br>Control vs experimental<br>group                      | -       | 0.01 |
| Statistical comparison:<br>Experimental group vs<br>preceding experimental group | -       | -    |
| n                                                                                | 9       | 8    |

TABLE 9.9.3.

# HAEMODYNAMIC PERFORMANCE DURING FUNCTIONAL TESTING FOLLOWING 24 HOURS

## HYPOTHERMIC PRESERVATION USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                   | Control      | 6            | 7            |
|----------------------------------------------------------------------|--------------|--------------|--------------|
| LVP (mm Hg)                                                          | 131<br>(4.4) | 90<br>(3.6)  | 130<br>(4.0) |
| LVEDP (mm Hg)                                                        | 5.3<br>(1.0) | 7.5<br>(1.3) | 7.4<br>(1.6) |
| AOP (mm Hg)                                                          | 114<br>(2.8) | 83<br>(4.3)  | 115<br>(3.2) |
| Statistical comparison:<br>LVP<br>Control vs Experimental<br>group   | -            | 0.001        | NS           |
| Statistical comparison:<br>LVEDP<br>Control vs experimental<br>group | -            | NS           | NS           |
| Statistical comparison:<br>AOP<br>Control vs experimental<br>group   | -            | 0.001        | NS           |
| n                                                                    | 9            | 16           | 8            |

FIGURES IN BRACKETS DENOTE SEM

TABLE 9.9.4.

OXYGEN UPTAKE DURING THE PERIOD OF FUNCTIONAL TESTING FOLLOWING  
24 HOURS HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                            | Control | 6    | 7    |
|-------------------------------------------------------------------------------|---------|------|------|
| O <sub>2</sub> uptake during functional testing mmol/h/g (X10 <sup>-1</sup> ) | 6.87    | 7.16 | 8.07 |
| SEM                                                                           | 1.08    | 1.37 | 1.41 |
| Statistical comparison: Control vs Experimental group                         | -       | NS   | NS   |
| Statistical comparison: Experimental group vs preceding experimental group    | -       | -    | NS   |
| n                                                                             | 9       | 16   | 8    |

TABLE 9.9.5.

TISSUE ADENOSINE TRIPHOSPHATE (ATP) CONCENTRATIONS FOLLOWING  
24 HOURS HYPOTHERMIC PRESERVATION USING DIFFERENT PERFUSATES

| GROUPS (Perfusates)                                         | Control<br>(Unperfused) | 1         | 5     | 6        | 7        |
|-------------------------------------------------------------|-------------------------|-----------|-------|----------|----------|
| ATP<br>( $\mu\text{mol/gm wet wt}$ )                        | 3.725                   | 1.551     | 3.500 | 2.601    | 2.614    |
| SEM                                                         | 0.253                   | 0.264     | 0.430 | 0.235    | 0.476    |
| Statistical comparison:<br>Control vs<br>Experimental Group | -                       | $< 0.001$ | NS    | $< 0.05$ | $< 0.05$ |
| n                                                           | 19                      | 8         | 8     | 18       | 8        |

TABLE 9-9.6.

TISSUE CREATINE PHOSPHATE (CP) CONCENTRATIONS FOLLOWING  
24 HOURS HYPOTHERMIC PRESERVATION USING DIFFERENT PERFUSATES

| GROUPS                                                            | Control<br>(unpreserved) | 1         | 5     | 6     | 7        |
|-------------------------------------------------------------------|--------------------------|-----------|-------|-------|----------|
| CP<br>( $\mu\text{mol/g}$ wet wt)                                 | 6.611                    | 1.064     | 5.513 | 5.764 | 2.314    |
| SEM                                                               | 0.720                    | 0.215     | 1.288 | 0.964 | 0.541    |
| Statistical comparison:<br>Control group vs<br>experimental group | -                        | $< 0.001$ | NS    | NS    | $< 0.01$ |
| n                                                                 | 7                        | 8         | 8     | 18    | 8        |

TABLE 9.9.7.

# CHANGES IN MEASURED PARAMETERS DURING THE PERIOD OF CONTINUOUS PERFUSION

| GROUP          | Mean Heart Weight<br>gm   | Mean % Change<br>in Heart weight | Coronary flow<br>(ml/min/gm)<br>( $\times 10^{-2}$ ) | Mean % Change<br>in coronary flow | Coronary Vascular Resistance<br>(cmH <sub>2</sub> O/gm/min/ml) | Mean % Change<br>in Coronary Vascular Resistance |
|----------------|---------------------------|----------------------------------|------------------------------------------------------|-----------------------------------|----------------------------------------------------------------|--------------------------------------------------|
| GROUP 7        | At beginning of perfusion | 97.0 (8.4)                       | 14.0 (1.2)                                           |                                   | 68.4 (9.8)                                                     |                                                  |
|                | At end of perfusion       | 116.4 (5.0)<br>$p < 0.001$       | 12.0 (1.0)<br>$p < 0.01$                             | -11% (4.3%)                       | 76.6 (10.8)<br>NS                                              | +15% (6.3%)                                      |
| GROUP 8        | At beginning of perfusion | 70.5 (7.5)                       | 22.0 (1.5)                                           |                                   | 37.0 (2.6)                                                     |                                                  |
|                | At end of perfusion       | 126.0 (9.1)<br>$p < 0.001$       | 17.0 (1.8)<br>$p < 0.01$                             | -25% (6.0%)                       | 51.1 (6.6)<br>$p < 0.05$                                       | +37% (10.5%)                                     |
| Comparison 7/8 | At beginning of perfusion | $P < 0.001$                      | $p < 0.001$                                          |                                   | 0.01                                                           | -                                                |
|                | At end of perfusion       | NS                               | $p < 0.05$                                           | NS                                | $p < 0.05$                                                     | NS                                               |

Figures in brackets denote standard error.

NS = not statistically significant

TABLE 9.9.8

# RESULTS OF ORTHOTOPIC TRANSPLANTATION FOLLOWING 24 HOUR

## STORAGE OF THE DONOR HEART

| Baboon (Group 7) | Survival (days) | Cause of Death                                                 | Myocardial Histology                             |
|------------------|-----------------|----------------------------------------------------------------|--------------------------------------------------|
| 1                | 6               | Rejection                                                      | Myocytolysis/coagulative necrosis                |
| 2                | 6               | (Rejection) ?                                                  | Myocytolysis                                     |
| 3                | 2.5             | Respiratory failure (Atelectasis)                              | Granular myofibres & indistinct cross striations |
| 4                | 10              | Sacrificed                                                     | Myofibrillar degeneration/early rejection        |
| 5                | 8               | Sacrificed (Gangrene of foot)                                  | No sample taken                                  |
| 6                | 0               | Myocardial failure                                             | Contraction band and disrupted capillaries       |
| 7                | 1               | Myocardial failure                                             | Coagulative necrosis/Interstitial haemorrhage    |
| 8                | 7               | Rejection                                                      | Myocytolysis                                     |
| 9                | 0               | Myocardial failure                                             | No sample taken                                  |
| 10               | 1               | Myocardial failure                                             | No sample taken                                  |
| 11               | 1.42            | Unclear (cardiac arrest following NaHCO <sub>3</sub> infusion) | Well preserved                                   |
| 12               | 26              | Rejection                                                      | Rejection                                        |
| 13               | 29              | Rejection                                                      | Rejection                                        |

TABLE 9.9.9



DATA OBTAINED AT CARDIAC CATHETERISATION 6 - 10 DAYS AFTER  
ORTHOTOPIC TRANSPLANTATION

|                                  | GROUP 7<br>(n = 3) | GROUP 8<br>(n = 2) |
|----------------------------------|--------------------|--------------------|
| Right atrium                     | 11.5 (1.3)         | 6.0 (0)            |
| Right ventricle (systolic)       | 40.0 (4.6)         | 29.0 (0)           |
| Main pulmonary artery (systolic) | 31.7 (1.5)         | 21.0 (2.5)         |
| Pulmonary capillary wedge        | 11.2 (0.6)         | 6.5 (3.0)          |
| Left ventricle (systolic)        | 97.8 (6.3)         | 107 (6.0)          |
| Left ventricular end-diastolic   | 6.0 (2.1)          | 6.0 (2.0)          |
| Aorta (systolic)                 | 91.7 (7.9)         | 103 (7.5)          |
| Cardiac output (L/min) (n = 2)   | 1.4                | 1.62               |

Figures refer to mean pressure in mm Hg  
Figures in brackets denote SEM

TABLE 9.9.9.1

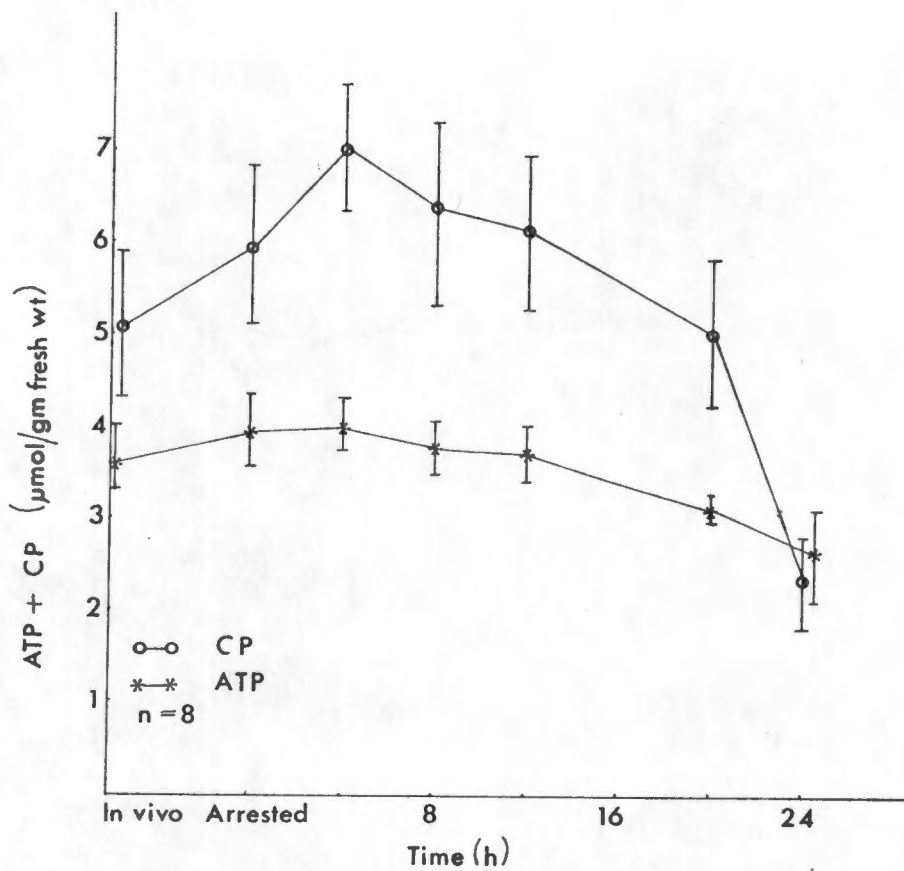


Figure 9.1.

Myocardial tissue adenosine triphosphate (ATP) and creatine phosphate (CP) levels before cardioplegic infusion (in vivo), after cardioplegic infusion (arrested), during hypothermic perfusion.

Bars represent S.E.M.

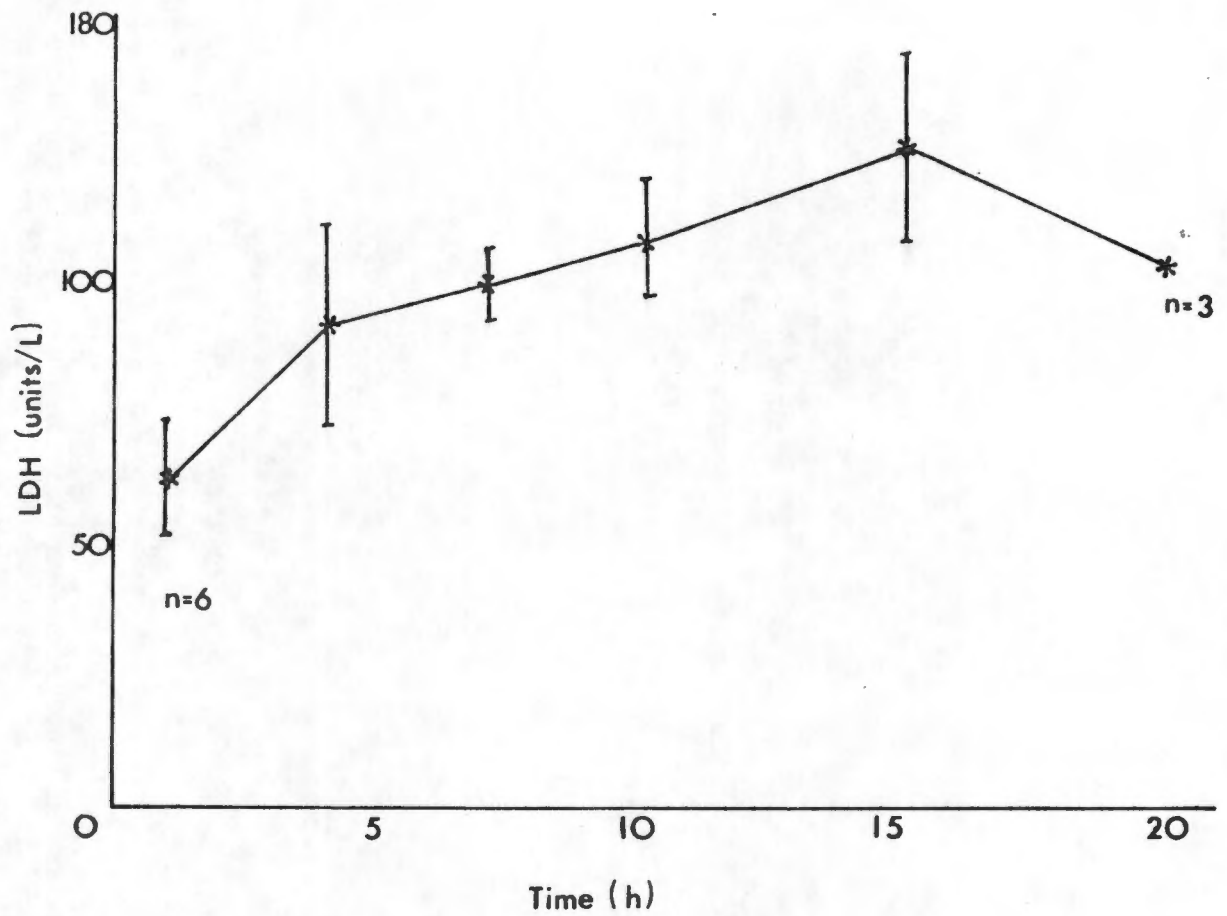


Fig. 9.2.

Perfusate lactate dehydrogenase (LDH) concentrations during the period of hypothermic perfusion. Bars represent S.E.M.

## CHAPTER 10

### GROUP 8

#### 10. MODIFICATIONS TO THE PERFUSATE

The composition of this perfusate (table 10.1) was based on the hypothesis discussed in chapter 9 concerning the effect of oedema formation on preservation. The actual changes or additions to the perfusate were as follows:

##### 10.1.1. INSULIN

The use of insulin was discontinued because its effect on glucose uptake was negligible (table 7.5). Insulin also has other undesirable stimulatory effects on the myocardium, such as activation of pyruvate kinase and pyruvate dehydrogenase, resulting in dephosphorylation of the latter enzyme (Hardie 1981). Pyruvate kinase transfers a phosphate group from phosphoenolpyruvate to ADP generating pyruvate and ATP (Lehninger 1972). The ATP may then be available for unwanted metabolic activity during preservation, whereas the high energy state, phosphoenolpyruvate was preferred until such time that normothermic conditions prevailed. Insulin was also concerned in the regulation of synthesis of membrane-linked transport systems for glucose and other metabolites for enhancing the rate of translation of specific mRNA species at the ribosome (Lehninger 1972). With the desire to inhibit metabolism, insulin was omitted from the perfusate.

#### 10.1.2. POTASSIUM DIHYDROGEN PHOSPHATE

$\text{KH}_2\text{PO}_4$  was used in preference to  $\text{KCl}$ . This substitution decreased the potassium and increased the phosphate concentration to 8mM. The potassium concentration was reduced to ensure that a potassium induced vasoconstriction did not occur (Scott et al 1959; Levowitz et al 1969). The phosphate concentration was increased approximately seven fold for an osmotic effect, and as a substrate for possible phosphorylation reactions in the myocardium.

#### 10.1.3. MAGNESIUM SULPHATE

$\text{MgCl}_2$  was substituted by  $\text{MgSO}_4$ . The effects of magnesium are discussed in detail in chapter 5. Magnesium reduces membrane permeability to potassium and therefore helps to maintain a normal membrane potential (Holland 1964), acts as a catalyst in phosphate metabolism, and is essential to nearly all vital metabolic systems (Aikawa 1963). The substitution of sulphate for chloride was carried out because sulphate anions are more osmotically active (Collins et al 1977).

#### 10.1.4. SUCROSE

Sucrose is effective in helping to prevent cellular swelling (in the presence of sodium rich or sodium poor solutions) (Lambotte et al 1979), although swelling may occur with time (Pegg 1977). Sucrose in aqueous solutions become hydrated and in this form it blocks pores in membranes (Srere 1981) and also protects against freezing damage (Woolgar 1974). With these modifications the osmolarity increased from 330mOsm/L to 385mOsm/L. All determinations made during the hypothermic perfusion period (table 3.2) and during the normothermic ex vivo functional evaluation period (table 3.3) are tabulated.

### RESULTS

#### 10.2.1. OBSERVATIONS DURING THE PERIOD OF HYPOTHERMIC PERFUSION

These hearts appeared grossly enlarged at the end of the period of perfusion although the myocardium was still soft to the touch. Myocardium oxygen uptake at the beginning and the end of the perfusion period did not change significantly (table 10.2) but the coronary flow decreased (table 10.3) and the coronary vascular resistance increased (table 10.4) significantly. When comparing the above parameters of group 7 with group 8, only the coronary vascular resistance at the end of the preservation period differed significantly between the two groups (table 10.5) being less in group 8. The coronary flow at the end of preservation did not differ significantly (table 10.6). The most striking feature after 24 hours preservation was the increase in weight (81%) (table 10.7).

#### 10.2.2. ATP AND CP

Changes in the tissue ATP and CP levels over the 24 hour preservation period are shown in figure 10.1. Very little change in these two high energy phosphates were observed during preservation. At this stage hypothermic perfusion was discontinued by removing the heart from the perfusion apparatus and leaving it on a table at room temperature to progressively rewarm. The reason for this delay was to allow the myocardium to equilibrate ionic distribution and fluid content, diminishing myocardial stiffness when the hearts were functionally evaluated. (This one hour ischaemic period would be inevitable in the clinical situation of transplantation as implantation of the organ would take approximately the same time). A sharp decrease in CP occurred when the hearts were subjected to one hour of re-warming. During this period there was a passive loss of fluid at a rate of 7.6 uL/min/gm wet wt as measured during the first 30 minutes (table 10.8).

#### 10.2.3. GLYCOGEN

The changes in tissue glycogen levels are shown in figure 10.2. The perfusate appeared to preserve the glycogen stores effectively as little change was noticed over this period.

#### 10.2.4. LDH

The release of LDH was monitored intermittently for 20 hours and during this period little change was observed (figure 10.3).

#### 10.2.5. LACTATE

The perfusate lactate levels are shown in figure 7.2. These values did not change significantly during the preservation period.

#### 10.3.1. OBSERVATIONS DURING THE PERIOD OF FUNCTIONAL TESTING

After electrical defibrillation these hearts quickly resumed sinus rhythm with forceful contractions. In this group 14 hearts were evaluated and all were able to overcome the afterload of the system. Haemodynamic performance did not deteriorate during the testing period and no rhythm disturbances were ever noticed. The cardiac output did not differ significantly from the control group, and was significantly higher than group 7 (table 10.9). The coronary flow (table 10.9.1) and stroke volume (table 10.9.2) remained within the range of control values, and the stroke volume was significantly higher than group 7 hearts. The aortic and left ventricular pressures were, however, significantly lower than in the control group (table 10.9.3) and the myocardial oxygen uptake increased significantly when compared with the control and group 7 hearts (table 10.9.4). A mean heart weight loss of 8.3% occurred in the hearts evaluated (table 10.9.5).

#### 10.3.2. ATP AND CP

The ATP and CP tissue levels were determined at the end of the period of functional testing and these results are shown in tables 10.9.6 and 10.9.7. The tissue high energy phosphates were well preserved and did not differ significantly from the control group.



### 10.3.3. VENTRICULAR FUNCTION CURVE

The results are shown in figure 10.4. A similar functional response was observed between control hearts and hearts preserved for 24 hours.

### 10.3.4. MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION

The results are shown in table 10.9.8. The three parameters; P:O ratio and RCI and  $QO_2$  were determined in freshly arrested (control) and hearts preserved for 24 hours and then reperfused for 1 hour with warm blood. In all three cases the control and experimental values of the RCI and  $QO_2$  were somewhat lower than their control counterparts.

### 10.3.5. RELEASE OF LYSOSOMAL ACID PHOSPHATASE

The results are shown in figure 10.5. Three fractions - supernatant, particulate and nuclear - were isolated from 3 groups of hearts. These were : group A (fresh), group B (from hearts subjected to 24 hours hypothermic preservation) group C (from hearts subjected to 24 hours hypothermic preservation and then reperfused for one hour with warm blood). A detailed presentation of the free total acid phosphatase activities and protein concentrations are tabulated in appendix 3. The supernatant, particulate and nuclear, free acid phosphatase activities of group B were significantly higher than the control group A. In group C only the particulate and nuclear free acid phosphatase activities were significantly higher than the control group A. The free acid phosphatase activities of the three groups shown in the supernatant, particulate and nuclear fractions were regarded as basal activities.

(These activities were determined by incubation of each of these fractions in isolation buffer for a period of 15 minutes at 37°C then measuring the total free acid phosphatase activity after centrifugation at 20 000g). The latent activities of the 3 lysosomal fractions were then determined by stressing the fractions in Triton X-100 (1%) (table 10.9.9). The particulate fraction was also mildly stressed in Triton X-100 (0.1%). The free latent activities in group B were all significantly higher than the free latent activities in the control group, whereas only the nuclear fraction of group C was significantly higher than the control group A (table 10.9.9). These results were difficult to interpret.

#### 10.3.6. SARCOPLASMIC RETICULUM

The results are shown in figure 10.6. During the period of hypothermic preservation the calcium uptake activity in the sarcoplasmic reticulum was significantly reduced although this inhibitory effect was reversible after a period of reperfusion with warm blood.

#### 10.4.1. ORTHOTOPIC TRANSPLANTATION

#### 10.4.2. OBSERVATIONS DURING THE PERIOD OF HYPOTHERMIC PERFUSION (TABLE 9.9.8).

Hearts in group 7 had, by chance, an initial greater mean weight than those in group 8. All hearts increased in weight during the period of perfusion due to oedema formation. The mean increase in weight was 32% in hearts in group 7 and 82% in group 8 ( $P < 0.001$ ). Initial mean coronary flow was significantly greater in group 8 than in group 7.

Coronary flow fell or remained unchanged in all hearts during the perfusion period, the mean percentage fall being greater in group 8 hearts, though this difference was not statistically significant. The coronary flow at the end of the perfusion period remained, however, significantly greater in group 8 hearts compared with group 7. Initial mean coronary vascular resistance was significantly greater in group 7 than group 8 and rose during the perfusion period in all cases except one. Coronary vascular resistance at the end of the perfusion period was similarly significantly greater in group 7 hearts, but actual increase in resistance from the beginning to the end of perfusion was not statistically significant in group 7 though it was in group 8. However, the mean % increase in resistance in group 8 hearts was not significantly different from that in group 7 hearts.

#### 10.4.3. RESULTS OF ORTHOTOPIC TRANSPLANTATION

In this group all 6 baboons survived until rejection with a mean survival period of 19.5 days (table 10.9.9.1). Immediately after discontinuation of cardiopulmonary bypass myocardial function was impressively good with mean arterial pressures of 60 - 80 mmHg and inotropic support clearly not being essential. The ECG voltages as determined in the donor animals were 4.7 mV and decreased to 3.9 mV in the recipient after hypothermic preservation though this decrease was not significant ( $P > 0.05$ ) (table 10.9.9.2). Catheterisation of two animals confirmed good haemodynamic function (table 9.9.9.1); virtually normal pressures were obtained. Histological examinations of the myocardium showed features of severe rejection with mononuclear cell infiltration and oedema and myofibre damage (table 10.9.9.1).

#### 10.5.1. DISCUSSION AND CONCLUSIONS

This perfusate was assessed in detail (biochemically and functionally) and found to be largely effective over the period of 24 hours preservation. Myocardial oedema occurred as predicted. Within five hours the myocardium gained 50% of its total fluid and the remaining fluid uptake occurred more gradually during the latter period of preservation (figure 10.7). The development of enhanced resting myocardial tension would theoretically counteract gross oedema formation as myofibres would gradually be shortened, preventing fluid-induced expansion of the myocardium. The theory put forward by Katz and Reuter (1979) (calcium leakage results in resting myocardial tension and the oedema hypothesis of this study (accumulation of fluid dilutes myoplasmic calcium concentrations) were supported by observations depicted in figure 10.7. Hearts that gained the least fluid were hard to the touch and functioned poorly (figure 10.7 - group 1) whereas the most severely oedematous hearts were soft to the touch and functioned exceptionally well (figure 10.7 - group 8). Biochemical studies have also shown that hearts preserved in group 8 were far more viable than hearts preserved in any of the preceding groups. Parallel with this observation was the fact that fluid accumulation in the myocardium was maximal in group 8 and minimal in group 1. The intermediate groups (3 - 7) had a significantly higher fluid accumulation than groups 1 and 2 (table 10.7) and had a superior functional response when compared with the latter groups (table 8.6). From these results it was feasible to conclude that oedema formation was beneficial for myocardial protection during hypothermic perfusion preservation. The reason for maintaining the heart in an unperfused state for 1 hour was to allow ionic redistribution during gradual rewarming and loss of acquired fluid, preventing

possible damage to the myocardium during the initial stages of contractile motion. Despite the loss of fluid during this period, the LVP was notably significantly reduced due to myocardial wall stiffness (table 10.9.3) (Pogatsa et al 1976; Schaff et al 1978). During this passive rewarming period the CP levels (figure 10.1) dropped significantly but this phenomenon was reversible as these values were re-established after a period of 1 hour normothermic blood reperfusion (tables 10.9.6 and 10.9.7).

Oedema formation did not affect coronary flow and coronary vascular resistance significantly because hearts with the highest percentage weight gain had the highest coronary flow (table 10.6) and the lowest coronary vascular resistance (table 10.5) at the end of the period of preservation. These results were contrary to those previously reported in which oedema formation affected flow through an organ (Limas et al 1977; Ames et al 1968; Engelman et al 1975; Krug et al 1966; Gavin et al 1978) and was a manifestation of ischaemic injury (Trump et al 1976; Guerraty 1981; Belzer and Southard 1980; Whalen et al 1974; Willerson et al 1977). It is not implied that myocardial oedema was not a harmful phenomenon as intracellular oedema following myocardial ischaemic injury resulting from loss of cell volume regulation leads to possible sarcolemmal injury (Kloner et al 1974; Whalen et al 1974). However, the oedema formation in the hearts in this study would appear to result from the influx of small, osmotically-active molecules rather than a result of ischaemic injury. Possibly this would also explain why a good correlation between control and experimental results was observed. Oedema formation did not significantly affect the integrity of the mitochondria (table 10.9.8) nor the sarcoplasmic reticulum, (figure 10.6) although the significantly reduced calcium uptake activity during the period of preservation was presumably due to a combined inhibitory effect of the perfusate and hypothermia on the calcium ATPase, rather than a consequence

of oedema formation. The inhibitory effect of hypothermia on mitochondrial respiration is shown in table 10.9.9.3. In the absence of chemical metabolic inhibitors, the rate of mitochondrial oxygen uptake was markedly reduced at low temperatures and this temperature sensitive phenomenon was completely reversible upon rewarming to 30°C. The most important observation was that the mitochondria were still respiring slowly at 8°C although they were uncoupled. As a result no<sup>2</sup> ATP was synthesised by the mitochondria for other metabolic needs. With little lactate production (figure 7.2), it was presumed that minimal energy was supplied by the anaerobic glycolytic pathway. The net energy production in the myocardium was subsequently very low or absent and the lack of adequate energy production was considered responsible for the absence of successful preservation described earlier in this dissertation. By using suitable metabolic inhibitors and reducing myocardial energy demands, successful preservation was achieved. A direct explanation for the increased release of free lysosomal acid phosphatase during preservation was not possible.

An analogous situation occurred in ischaemic tissue resulting in damage to membranes of subcellular organelles (Decker et al 1980). The intracellular enzymes of latent lysosomes was found to be closely linked to the damage which accelerated the progression of injury to necrosis (van Lancker and Holtzer 1959); De Duve and Wattiaux 1965; Decker et al 1980). If necrosis were a consequence of free lysosomal enzyme activity in the myocardium, then poor myocardial integrity would be anticipated. The results of this investigation indicate that a high free acid phosphatase tissue concentration had no permanent pathological implications under hypothermic conditions. The latencies of the three fractions from each group are shown in table 10.9.9. The release of latent activity was induced by stressing each fraction in Triton X-100 (1%).

The latent activity of each fraction in group B was significantly higher than the corresponding fraction in group A (table 10.9.9.). A similar finding was shown when comparing the basal activities between the two groups. These findings implied that continuous hypothermic perfusion (group B) stimulated the release of acid phosphatase and that myocardial lysosomal membranes preserved by this method were more susceptible to injury following treatment with detergent. Alternatively, the perfusion preservation may have increased the activity by converting acid phosphatase from an inhibited, bound form to an uninhibited, unbound form. (Injury induced by the free enzyme during this period was not expected to be severe due to the metabolic inhibitory effect of the perfusate and hypothermia).

In group C the hearts were again reperfused with warm blood, and lysosomal acid phosphatase activities were similarly determined. By comparing the control group A and reperfused group C hearts, the following observations were made : the basal activities of the nuclear and particulate fractions were significantly higher than the control group. Only the latent activity of the nuclear fraction was significantly higher than the control group (table 10.9.9). These significant differences again implied that a higher concentration of free acid phosphatase was present in the myocardial tissues of reperfused hearts. In this group only the nuclear fraction was more susceptible to detergent treatment. At this stage of interpretation of the results it was clear that lysosomes of group B and group C had undergone some change. These results were conflicting with biochemical and functional studies and the problem was further investigated. By dividing the latent activities (Triton X-100 1%) of the particulate fractions of each group by their respective basal activities, their relative latent activities were determined.



To correct for the different preservation conditions the latent activities of the particulate fractions were divided by the basal activity of group A hearts (unpreserved control hearts). These results are shown in figure 10.8. A considerable free enzyme activity was noted in group B and the free activity of group C was reduced to within the normal range of group A. This reduction in activity was either a consequence of or loss of lysosomal acid phosphatase activity during the period of isolation, functional testing period, or possibly the re-uptake of acid phosphatase by myocardial lysosomes (Herzog 1981). The other possibilities were ruled out because loss of acid phosphatase from the particulate fraction would either be recovered in the nuclear or the supernatant fraction. It was unlikely that the enzyme was rendered inactive during the period of reperfusion as no increased activity was noted in the serum of reperfused hearts (group C). Evidence for the phenomenon of internalisation (Herzog 1981) of free acid phosphatase was provided by determining the fragility of the particulate fractions of the three groups by subjecting them to different concentrations of detergent treatment (Triton X-100 1% and 0.1%). The ratio of 1% : 0.1% of the particulate fraction of each group was compared (figure 10.9). The fragility ratios of the three groups were not significantly different from one another, implying that the membrane fragilities were similar when determined under their respective preservation conditions. These results further implied that the high free lysosomal acid phosphatase activity was a consequence of reversible membrane permeability changes rather than irreversible structural damage to the membrane. An explanation for the high free activity in group B was probably due to a physical response of the lysosomal membrane to hypothermia. Additional evidence for the altered membrane permeability theory was obtained from studies of mitochondria of homeothermic animals in which a phase change in the membrane lipid occurred at reduced temperatures. (Raison and Lyons 1971).



In the lysosomes of homeothermic animals such a phase change was envisaged in which the effect of the induced change may result in increased membrane permeability. On rewarming the lysosomal membrane may then re-establish its original structure, exhibiting similar properties to that of freshly isolated lysosomes. The cause of the increased permeability and the biological effects of the free enzymes in the preserved myocardial tissues were not clearly understood and these changes did not influence myocardial function significantly.

The effectiveness of steroids against the release of lysosomal enzymes was first demonstrated by de Duve (1959) and Weismann (1964) who showed that high concentrations of steroids retarded the release of lysosomal enzymes (acid phosphatase and  $\beta$ -glucuronidase). However, on taking into account the high concentrations of steroids required in vitro to achieve this inhibition and extrapolating it to in vivo situations, it was unlikely that lysosomes throughout the myocardium would be coated by such amounts of protective steroids. For this reason the use of steroids was omitted from the perfusate.

In an additional study the metabolic inhibitory effect of hypothermia on the mitochondrial rate of oxygen uptake was determined. The results were intriguing in that most of the metabolic activity (oxygen uptake) was inhibited (greater than 90%) during the first 8°C drop in temperature (table 10.9.9.3). At 8°C the mean rate of oxygen uptake was reduced to 0.4% being inhibited by 99.6%.

The percentage inhibition of oxygen uptake of hearts during hypothermic preservation may be calculated from figures presented in tables 10.2 and 10.9.4. The oxygen uptake of the entire organ was also inhibited by 99%, being similar to results obtained from mitochondrial studies. Although the importance of oxygen utilisation during hypothermic preservation was not assessed, its necessity in preservation solution requires further investigation.

Finally, the efficacy of the preservation system was tested by orthotopic transplantation of baboon hearts. These hearts gained weight from oedema during the period of hypothermic preservation (pig hearts gained 81%) and the coronary flow was significantly higher (table 9.9.8) than the corresponding pig values at the beginning and end of preservation. This implied that in baboons the perfusate had an even more marked effect on the maintenance of an adequate coronary flow and coronary resistance. The preservation effect of the perfusate on baboon hearts was confirmed by orthotopic transplantation. The present study, therefore, constituted a considerable advance on previous solutions in that all the baboons survived to rejection (table 10.9.9.1) with a mean survival period of 19.5 days.

COMPOSITION OF PERFUSION SOLUTIONS USED TO PRESERVE  
HEARTS IN 9 DIFFERENT GROUPS

| Constitution of perfusates (mM)      | 1     | 2     | 3     | 4     | 5     | 6       | 7       | 8       |
|--------------------------------------|-------|-------|-------|-------|-------|---------|---------|---------|
| NaCl                                 | 118.5 | 118.5 | 92.90 | 92.90 | 92.90 | 92.90   | 115.7   | 115.7   |
| NaHCO <sub>3</sub>                   | 25    | 25    | 25    | 25    | 25    | 25      | 25      | 25      |
| KCl                                  | 4.75  | 4.75  | 16    | 16    | 16    | 16      | 10.9    | -       |
| KH <sub>2</sub> PO <sub>4</sub>      | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20    | 1.20    | 8       |
| MgSO <sub>4</sub> 7H <sub>2</sub> O  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20    | 1.20    | 14.4    |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O | 2.5   | 2.5   | 2.25  | 2.25  | 2.25  | 1.1     | 1.1     | 1.1     |
| MgCl <sub>2</sub>                    | -     | -     | 14.8  | 14.8  | 14.8  | 14.8    | 14.8    | -       |
| Glucose                              | 11    | 11    | 11    | 11    | 11    | 11      | 11      | 11      |
| Taurine                              | -     | 4     | 4     | 4     | 4     | 4       | 4       | 4       |
| ATP                                  | -     | -     | 10    | -     | -     | -       | -       | -       |
| Insulin                              | -     | -     | 40u/L | 40u/L | 40u/L | 40u/L   | 40u/L   | -       |
| Procaine HCl                         | -     | -     | 1     | 1     | 1     | 1       | 1       | 1       |
| Dextran 40                           | -     | -     | -     | 4g/L  | 2g/L  | -       | -       | -       |
| Chlorpromazine                       | -     | -     | -     | -     | -     | .005g/L | .005g/L | .005g/L |
| Phenoxybenzamine                     | -     | -     | -     | -     | -     | .01g/L  | .01g/L  | .01g/L  |
| Glycerol                             | -     | -     | -     | -     | -     | -       | 136     | 136     |
| Sucrose                              | -     | -     | -     | -     | -     | -       | -       | 7       |
| Osmolality (mOsm/L)                  | 277   | 280   | 295   | 315   | 302   | 321     | 330     | 385     |

TABLE 10.1.

CORONARY ARTERIO-VEINOUS OXYGEN UPTAKE AT THE BEGINNING AND AT THE END  
OF THE PERIOD OF HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP<br>(Perfusates) | O <sub>2</sub> uptake<br>at the beginning<br>of preservation<br>mmol/h/gm<br>(X10 <sup>-3</sup> ) | O <sub>2</sub> uptake<br>at the end of<br>preservation<br>mmol/h/gm<br>(X10 <sup>-3</sup> ) | Statistical comparison:<br>Beginning vs end<br>of perfusion | n  |
|-----------------------|---------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|-------------------------------------------------------------|----|
| 6                     | 3.9<br>(0.62)                                                                                     | 4.2<br>(0.56)                                                                               | NS                                                          | 21 |
| 7                     | 4.8<br>(0.60)                                                                                     | 4.9<br>(0.58)                                                                               | NS                                                          | 9  |
| 8                     | 5.4<br>(1.50)                                                                                     | 7.1<br>(0.63)                                                                               | NS                                                          | 7  |

TABLE 10.2.

CORONARY FLOW AT THE BEGINNING AND END OF 24 HOUR PERIOD  
OF HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP<br>Perfusates | Coronary flow<br>at beginning<br>of preservation<br>ml/min/gm<br>( $\times 10^{-2}$ ) | Coronary flow<br>at end of<br>preservation<br>ml/min/gm<br>( $\times 10^{-2}$ ) | Statistical comparison:<br>Beginning vs end of<br>preservation | n  |
|---------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------|----|
| 6                   | 15.74<br>(0.70)                                                                       | 6.77<br>(0.47)                                                                  | < 0.001                                                        | 21 |
| 7                   | 13.40<br>(0.81)                                                                       | 8.77<br>(0.57)                                                                  | < 0.001                                                        | 9  |
| 8                   | 15.80<br>(1.80)                                                                       | 9.77<br>(0.13)                                                                  | < .02                                                          | 7  |

TABLE 10.3.

CORONARY VASCULAR RESISTANCE AT THE BEGINNING AND END OF 24 HOUR  
PERIOD OF HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP<br>(Perfusates) | Coronary vascular resistance<br>cm H <sub>2</sub> O/gm/min/ml |                               | Statistical comparison:<br>Beginning vs end<br>of preservation | n  |
|-----------------------|---------------------------------------------------------------|-------------------------------|----------------------------------------------------------------|----|
|                       | At the beginning<br>of preservation                           | At the end of<br>preservation |                                                                |    |
| 6                     | 53<br>(2.2)                                                   | 210<br>(21.3)                 | < 0.001                                                        | 21 |
| 7                     | 65<br>(4.5)                                                   | 127<br>( 6.7)                 | < 0.001                                                        | 9  |
| 8                     | 55<br>(7.3)                                                   | 87<br>( 8.4)                  | < 0.05                                                         | 7  |

TABLE 10.4.

CORONARY VASCULAR RESISTANCE AT THE END OF 24 HOUR PERIOD OF HYPOTHERMIC  
PERFUSION USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                                     | 6    | 7       | 8      |
|----------------------------------------------------------------------------------------|------|---------|--------|
| Coronary vascular resistance<br>At end of preservation<br>cmH <sub>2</sub> O/gm/min/ml | 210  | 127     | 87     |
| SEM                                                                                    | 21.3 | 6.7     | 8.4    |
| Statistical comparison:<br>Experimental group vs<br>preceding experimental group       | -    | < 0.001 | < .001 |
| n                                                                                      | 16   | 8       | 14     |

TABLE 10.5.

COMPARISON OF CORONARY FLOWS AT THE END OF 24 HOUR PERIOD OF HYPOTHERMIC

PERFUSION USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                         | 6    | 7      | 8    |
|----------------------------------------------------------------------------|------|--------|------|
| Coronary flow at the end of preservation ml/min/gm ( $\times 10^{-2}$ )    | 6.77 | 8.77   | 9.77 |
| SEM                                                                        | 0.47 | 0.57   | 0.13 |
| Statistical comparison: Experimental group vs preceding experimental group | -    | < 0.02 | NS   |
| n                                                                          | 16   | 8      | 14   |

TABLE 10.6.



CHANGES IN HEART WEIGHT DURING 24 HOUR PERIOD OF  
HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUPS (Perfusates)                                                                | 1   | 2   | 3      | 4    | 5    | 6    | 7    | 8       |
|------------------------------------------------------------------------------------|-----|-----|--------|------|------|------|------|---------|
| Mean % wt gain                                                                     | 21  | 23  | 33.8   | 41.4 | 45.4 | 47.3 | 42.0 | 81.3    |
| SEM                                                                                | 1.9 | 2.4 | 2.8    | 4.3  | 2.5  | 3.28 | 2.56 | 2.01    |
| Statistical comparison:<br>Experimental groups vs<br>preceding experimental groups | -   | NS  | < 0.05 | NS   | NS   | NS   | NS   | < 0.001 |
| n                                                                                  | 10  | 5   | 11     | 18   | 23   | 16   | 17   | 13      |

TABLE 10.7.

RATE OF FLUID LOSS FROM THE MYOCARDIUM OF HEARTS THAT WERE  
SUBJECTED TO GRADUAL REWARMING FOLLOWING PERFUSION PRESERVATION.

| FLUID LOSS (mℓ) AFTER 30 MINUTES | RATE OF FLUID LOSS (μL/gm/min) |
|----------------------------------|--------------------------------|
| 24                               | 6.5                            |
| 30                               | 9.4                            |
| 29                               | 8.9                            |
| 17                               | 5.6                            |
|                                  | Mean rate<br>of fluid<br>loss  |
|                                  | 7.6 (0.92)                     |

TABLE 10.8

CARDIAC OUTPUT DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                               | Control | 7      | 8      |
|----------------------------------------------------------------------------------|---------|--------|--------|
| CO (ml/min/100g)                                                                 | 2827    | 1577   | 2636   |
| SEM                                                                              | 22      | 222    | 144    |
| Statistical comparison:<br>Control vs experimental<br>group                      | -       | < 0.01 | NS     |
| Statistical comparison:<br>Experimental group vs<br>preceding experimental group | -       | -      | <0.001 |
| n                                                                                | 9       | 8      | 14     |

TABLE 10.9

CORONARY FLOW DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                               | Control | 7   | 8   |
|----------------------------------------------------------------------------------|---------|-----|-----|
| CF (ml/min/100g)                                                                 | 258     | 295 | 314 |
| SEM                                                                              | 33      | 43  | 28  |
| Statistical comparison:<br>Control vs experimental<br>group                      | -       | NS  | NS  |
| Statistical comparison:<br>Experimental group vs<br>preceding experimental group | -       | -   | NS  |
| n                                                                                | 9       | 8   | 14  |

TABLE 10.9.1.

STROKE VOLUME DURING EX VIVO FUNCTIONAL TESTING FOLLOWING 24 HOURS

STORAGE USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                               | Control | 7    | 8    |
|----------------------------------------------------------------------------------|---------|------|------|
| SV (mL/100g)                                                                     | 17.0    | 10.7 | 17.0 |
| SEM                                                                              | 1.27    | 1.54 | 1.10 |
| Statistical comparison:<br>Control vs experimental<br>group                      | -       | 0.01 | NS.  |
| Statistical comparison:<br>Experimental group vs<br>preceding experimental group | -       | -    | 0.01 |
| n                                                                                | 9       | 8    | 14   |

TABLE 10.9.2.

HAEMODYNAMIC PERFORMANCE DURING FUNCTIONAL TESTING FOLLOWING 24 HOURS

HYPOTHERMIC PRESERVATION USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                   | Control      | 6            | 7            | 8            |
|----------------------------------------------------------------------|--------------|--------------|--------------|--------------|
| LVP (mm Hg)                                                          | 131<br>(4.4) | 90<br>(3.6)  | 130<br>(4.0) | 114<br>(5.2) |
| LVEDP (mm Hg)                                                        | 5.3<br>(1.0) | 7.5<br>(1.3) | 7.4<br>(1.6) | 5.3<br>(1.0) |
| AOP (mm Hg)                                                          | 114<br>(2.8) | 83<br>(4.3)  | 115<br>(3.2) | 94<br>(2.5)  |
| Statistical comparison:<br>LVP<br>Control vs Experimental<br>group   | -            | 0.001        | NS           | 0.05         |
| Statistical comparison:<br>LVEDP<br>Control vs experimental<br>group | -            | NS           | NS           | NS           |
| Statistical comparison:<br>AOP<br>Control vs experimental<br>group   | -            | 0.001        | NS           | 0.01         |
| n                                                                    | 9            | 16           | 8            | 14           |

FIGURES IN BRACKETS DENOTE SEM

TABLE 10.9.3.

OXYGEN UPTAKE DURING THE PERIOD OF FUNCTIONAL TESTING FOLLOWING  
24 HOURS HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                            | Control | 6    | 7    | 8       |
|-------------------------------------------------------------------------------|---------|------|------|---------|
| O <sub>2</sub> uptake during functional testing mmol/h/g (X10 <sup>-1</sup> ) | 6.87    | 7.16 | 8.07 | 20.05   |
| SEM                                                                           | 1.08    | 1.37 | 1.41 | 3.28    |
| Statistical comparison: Control vs Experimental group                         | -       | NS   | NS   | < 0.001 |
| Statistical comparison: Experimental group vs preceding experimental group    | -       | -    | NS   | < 0.05  |
| n                                                                             | 9       | 16   | 8    | 14      |

TABLE 10.9.4.

CHANGES IN HEART WEIGHT FOLLOWING ONE HOUR EX VIVO FUNCTIONAL TESTING

| GROUPS<br>(Perfusates)                                                            | 3    | 4    | 5    | 6    | 7    | 8    |
|-----------------------------------------------------------------------------------|------|------|------|------|------|------|
| % Wt<br>Loss                                                                      | 13   | 17   | 19.1 | 14.8 | 9.74 | 8.3  |
| SEM                                                                               | 2.00 | 5.00 | 4.33 | 1.61 | 2.65 | 1.45 |
| Statistical Comparison:<br>Experimental groups vs<br>preceding experimental group | -    | NS   | NS   | NS   | NS   | NS   |
| n                                                                                 | 11   | 18   | 23   | 16   | 17   | 13   |

TABLE 10.9.5.



TISSUE ADENOSINE TRIPHOSPHATE (ATP) CONCENTRATIONS FOLLOWING  
24 HOURS HYPOTHERMIC PRESERVATION USING DIFFERENT PERFUSATES

| GROUPS (Perfusates)                                         | Control<br>(Unperfused) | 1         | 5     | 6        | 7        | 8     |
|-------------------------------------------------------------|-------------------------|-----------|-------|----------|----------|-------|
| ATP<br>( $\mu\text{mol/gm wet wt}$ )                        | 3.725                   | 1.551     | 3.500 | 2.601    | 2.614    | 3.500 |
| SEM                                                         | 0.253                   | 0.264     | 0.430 | 0.235    | 0.476    | 0.201 |
| Statistical comparison:<br>Control vs<br>Experimental Group | -                       | $< 0.001$ | NS    | $< 0.05$ | $< 0.05$ | NS    |
| n                                                           | 19                      | 8         | 8     | 18       | 8        | 7     |

TABLE 10.9.6.

TISSUE CREATINE PHOSPHATE (CP) CONCENTRATIONS FOLLOWING  
24 HOURS HYPOTHERMIC PRESERVATION USING DIFFERENT PERFUSATES

| GROUPS                                                            | Control<br>(unpreserved) | 1       | 5     | 6     | 7      | 8     |
|-------------------------------------------------------------------|--------------------------|---------|-------|-------|--------|-------|
| CP<br>( $\mu\text{mol/g wet wt}$ )                                | 6.611                    | 1.064   | 5.513 | 5.764 | 2.314  | 5.295 |
| SEM                                                               | 0.720                    | 0.215   | 1.288 | 0.964 | 0.541  | 0.520 |
| Statistical comparison:<br>Control group vs<br>experimental group | -                        | < 0.001 | NS    | NS    | < 0.01 | NS    |
| n                                                                 | 7                        | 8       | 8     | 18    | 8      | 7     |

TABLE 10.9.7.

MITOCHONDRIAL ADP:O RATIO, QO<sub>2</sub> AND RCI VALUES IN FRESHLY ISOLATED CONTROL HEARTS  
AND HEARTS REPERFUSED FOR ONE HOUR AT NORMOTHERMIA

|                                                        | Control     | Group 8     | P     |
|--------------------------------------------------------|-------------|-------------|-------|
|                                                        |             |             |       |
| ADP:O ratio                                            | 4.07 (0.26) | 4.64 (0.30) | > 0.1 |
| QO <sub>2</sub> (natoms O <sub>2</sub> /min/mg protein | 301 (42.0)  | 244 (19.0)  | > 0.5 |
| RCI (Respiratory control index)<br>n                   | 20.08<br>8  | 15.83<br>14 | > 0.1 |

Figures in brackets denote SEM

TABLE 10.9.8

| FRACTIONS                                       | GROUP                          |                                |                                |
|-------------------------------------------------|--------------------------------|--------------------------------|--------------------------------|
|                                                 | A                              | B                              | C                              |
|                                                 | Activity/Mg protein<br>(n = 8) | Activity/Mg protein<br>(n = 8) | Activity/Mg protein<br>(n = 8) |
| SUPERNATANT (BASAL ACTIVITY)                    | 57.0 (10.)                     | 298.4 (54.6)<br>< 0.001        | 64.5 (11.3)<br>> 0.1           |
| SUPERNATANT (LATENT ACTIVITY)                   | 59.0 (7.2)                     | 315.0 (36.2)<br>< 0.001        | 70.0 (1.01)<br>> 0.1           |
| NUCLEAR (BASAL ACTIVITY)                        | 7.2 (1.5)                      | 79.0 ( 7.1)<br>< 0.001         | 15.0 (2.1)<br>< 0.02           |
| NUCLEAR +<br>TRITON X-100 (LATENT ACTIVITY)     | 7.2 (0.9)                      | 99.2 ( 6.8)<br>< 0.001         | 20.0 (3.3)<br>< 0.05           |
| PARTICULATE (BASAL ACTIVITY)                    | 0.1 (0.03)                     | 0.44 ( 0.1)<br>< 0.02          | 0.17(0.02)<br>< 0.05           |
| PARTICULATE +<br>TRITON X-100 (LATENT ACTIVITY) | 0.75 (0.06)                    | 4.0 (0.26)<br>< 0.001          | 1.24(0.13)<br>> 0.01           |

A comparison of free lysosomal acid phosphatase activity in unstressed (basal activities) and stressed (latent activities) fractions. Group A lysosomes were isolated from freshly excised hearts, Group B from hearts preserved for 24 hours and Group C from hearts preserved for 24 hours followed by one hour of warm blood reperfusion. Group B showed a marked increase in free acid phosphatase activity whereas Group C returned to almost control values in both unstressed and stressed fractions.

TABLE 10.9.9

RESULTS OF ORTHOTOPIC TRANSPLANTATION FOLLOWING 24 HOUR  
STORAGE OF THE DONOR HEART

| Baboon (Group 8) | Survival (days) | Cause of Death      | Myocardial Histology |
|------------------|-----------------|---------------------|----------------------|
| 1                | 27              | Rejection           | Rejection            |
| 2                | 33              | Rejection (Empyema) | Rejection            |
| 3                | 12              | Rejection           | Rejection            |
| 4                | 8               | Rejection           | Rejection            |
| 5                | 6               | Rejection           | Rejection            |
| 6                | 31              | Rejection           | Rejection            |

TABLE 10.9.9.1

ECG VOLTAGES OF DONOR AND RECIPIENT BABOONS BEFORE AND AFTER A  
PERIOD OF 24 HOURS HYPOTHERMIC PRESERVATION

| Donor voltage (mV) | Recipient voltage (mV) |
|--------------------|------------------------|
| 3.3                | 3.6                    |
| 5.6                | 4.8                    |
| 5.8                | 3.0                    |
| 3.4                | 2.9                    |
| 4.1                | 4.9                    |
| 6.1                | 4.3                    |

TABLE 10.9.9.2

RATE OF OXYGEN CONSUMPTION (NATOMS O<sub>2</sub>/MIN/Mg PROTEIN)  
IN MITOCHONDRIA AT DIFFERENT TEMPERATURES

| 8°C       | 12°C | 23°C | 30°C |
|-----------|------|------|------|
| 2.85      | 5.70 | 7.76 | 281  |
| 2.25      | 6.25 | 9.10 | 465  |
| 1.65      |      |      | 480  |
| 0.90      |      |      | 250  |
| 1.50      |      |      | 555  |
| 0.72      |      |      | 253  |
| 0.35      |      |      | 123  |
| 0.48      |      |      | 249  |
| Mean 1.34 | 5.98 | 8.43 | 332  |
| SEM 0.31  | 0.28 | 0.67 | 53   |

TABLE 10.9.9.3

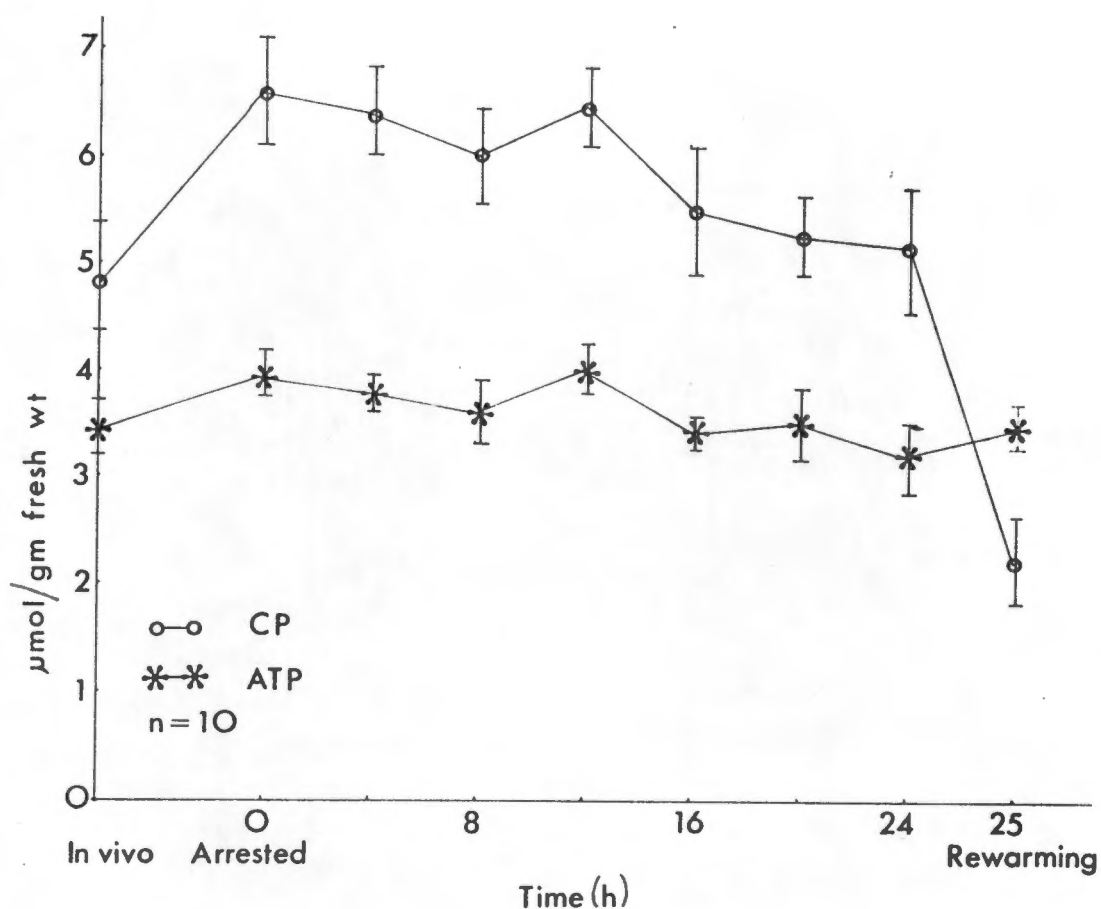


Figure 10.1.

Determination of myocardial tissue adenosine triphosphate (ATP) and creatine phosphate (CP) levels before cardioplegic infusion (in vivo) after cardioplegic infusion (arrested) and during hypothermic perfusion. Thereafter the hearts were removed from the perfusion apparatus and left to rewarm at room temperature for one hour, ATP and CP were again determined (rewarming). Bars represent S.E.M.



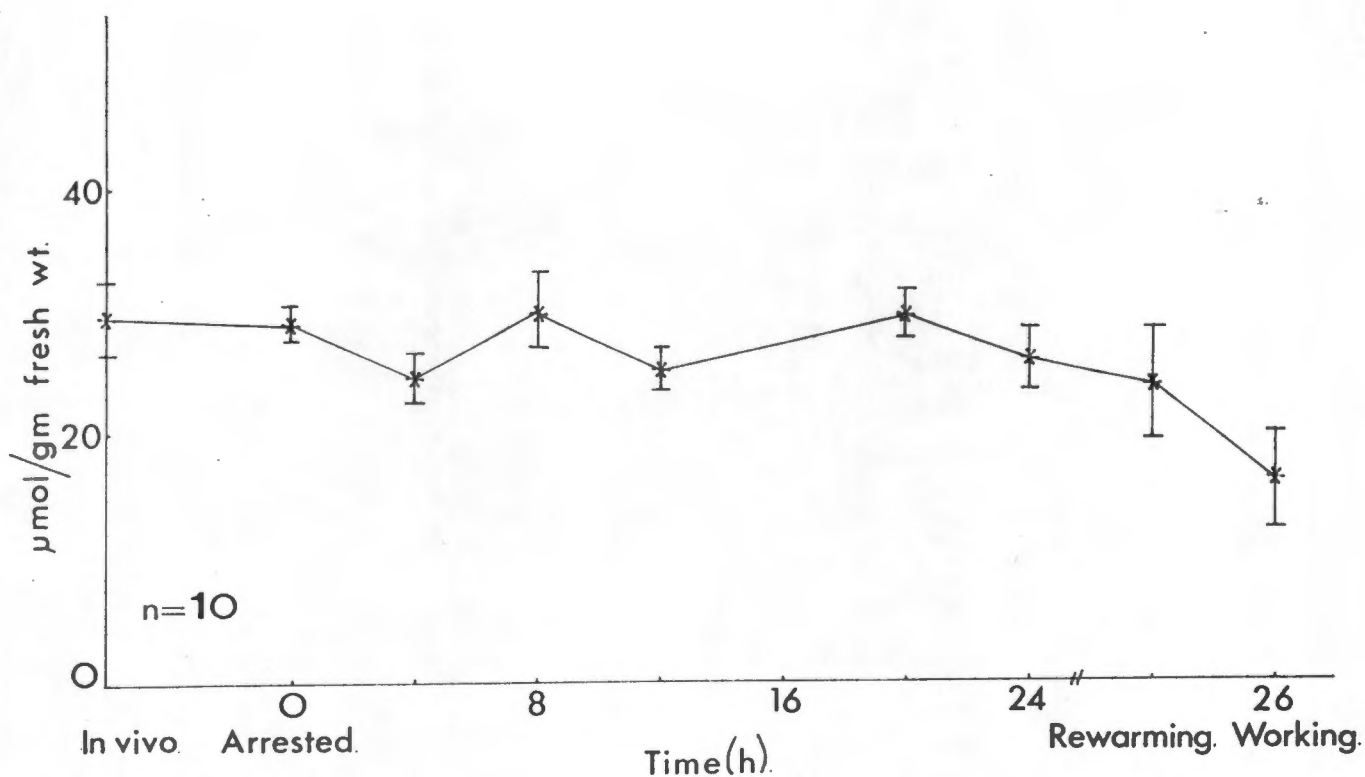


Figure 10.2.

Determination of myocardial tissue glycogen (glucose equivalents) levels before cardioplegic infusion (in vivo) after cardioplegic infusion (arrested) and during hypothermic perfusion. Thereafter the hearts were removed from the perfusion apparatus and left to rewarm at room temperature for one hour and the glycogen level was again determined (rewarming). Following the rewarming period the hearts were reperfused with warm blood and switched to the working mode (working). Bars represent S.E.M.

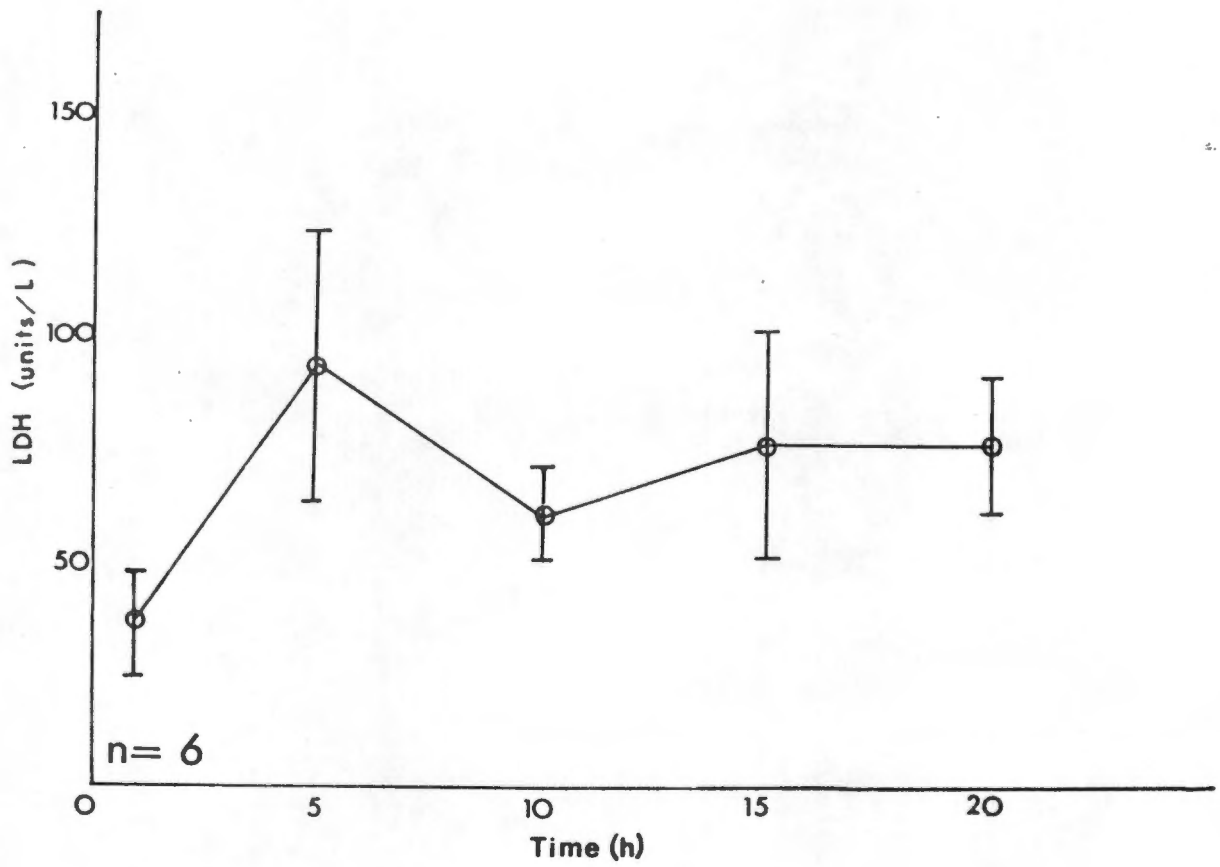


Fig. 10.3.  
Perfusate lactate dehydrogenase (LDH) concentrations during the period of hypothermic perfusion. Bars represent S.E.M.

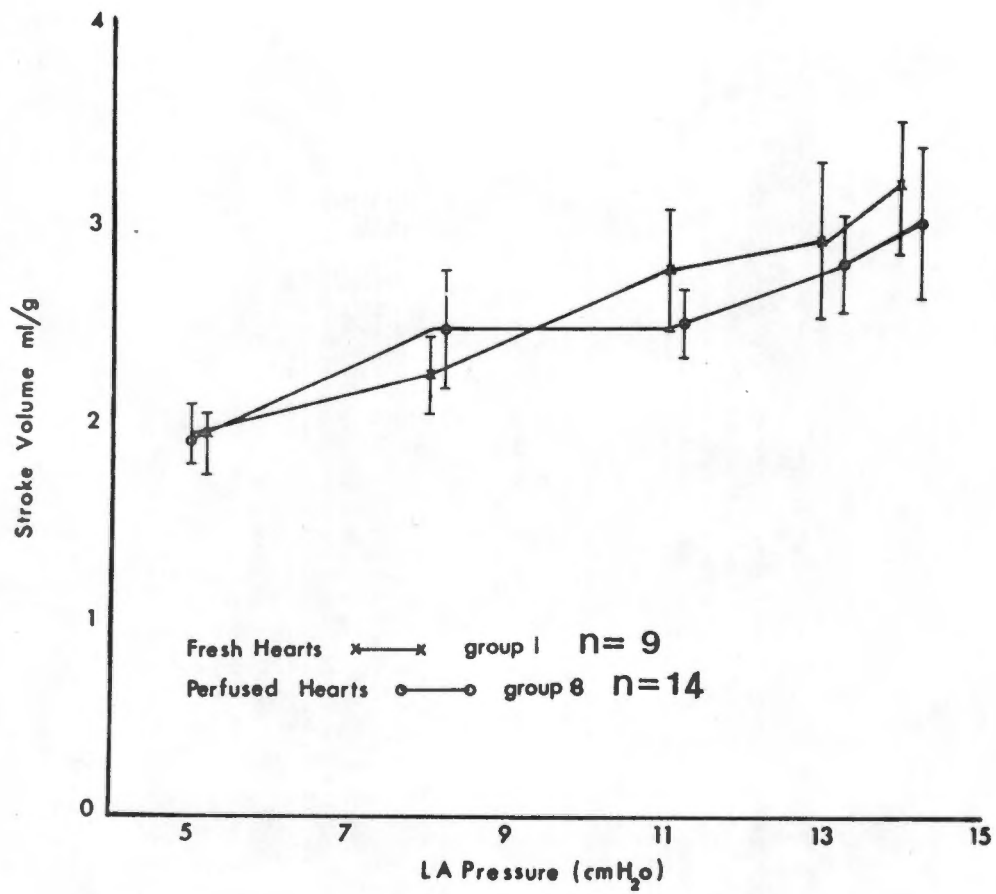


Fig. 10.4.

Ventricular function curves of control hearts ('x----x) and hearts preserved for 24 hours. ('o-----o). Bars represent S.E.M.

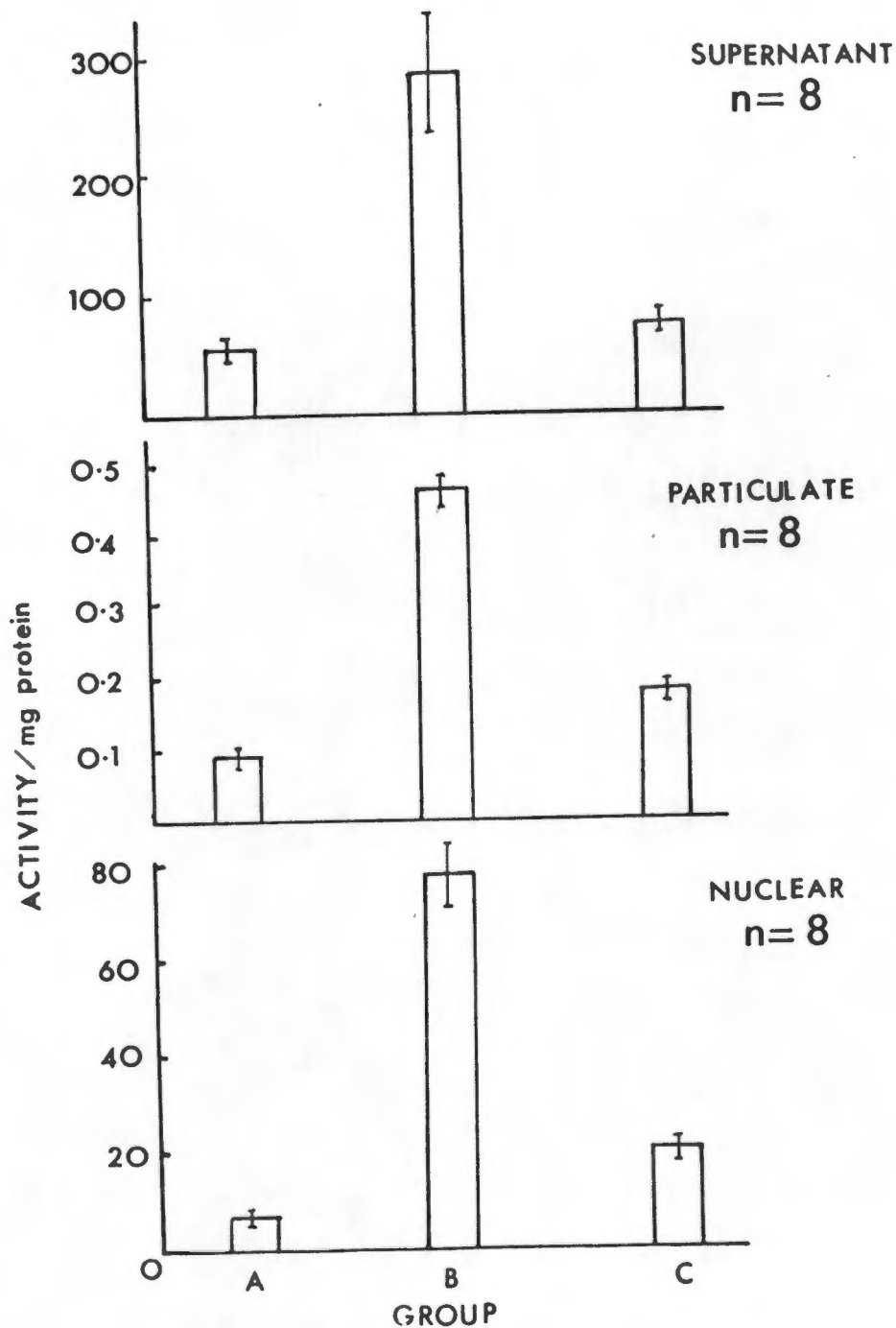


Fig. 10.5.

A comparison of free lysosomal acid phosphatase activities of 3 groups Group A (freshly excised); Group B (24 hour preserved), and Group C (24 hour preserved followed by one hour warm blood reperfusion). Each group consists of three differentially isolated lysosomal fractions; supernatant, particulate and nuclear. Acid phosphatase activities are significantly higher in group B compared with groups A and C. For details see text. Bars represent S.E.M.

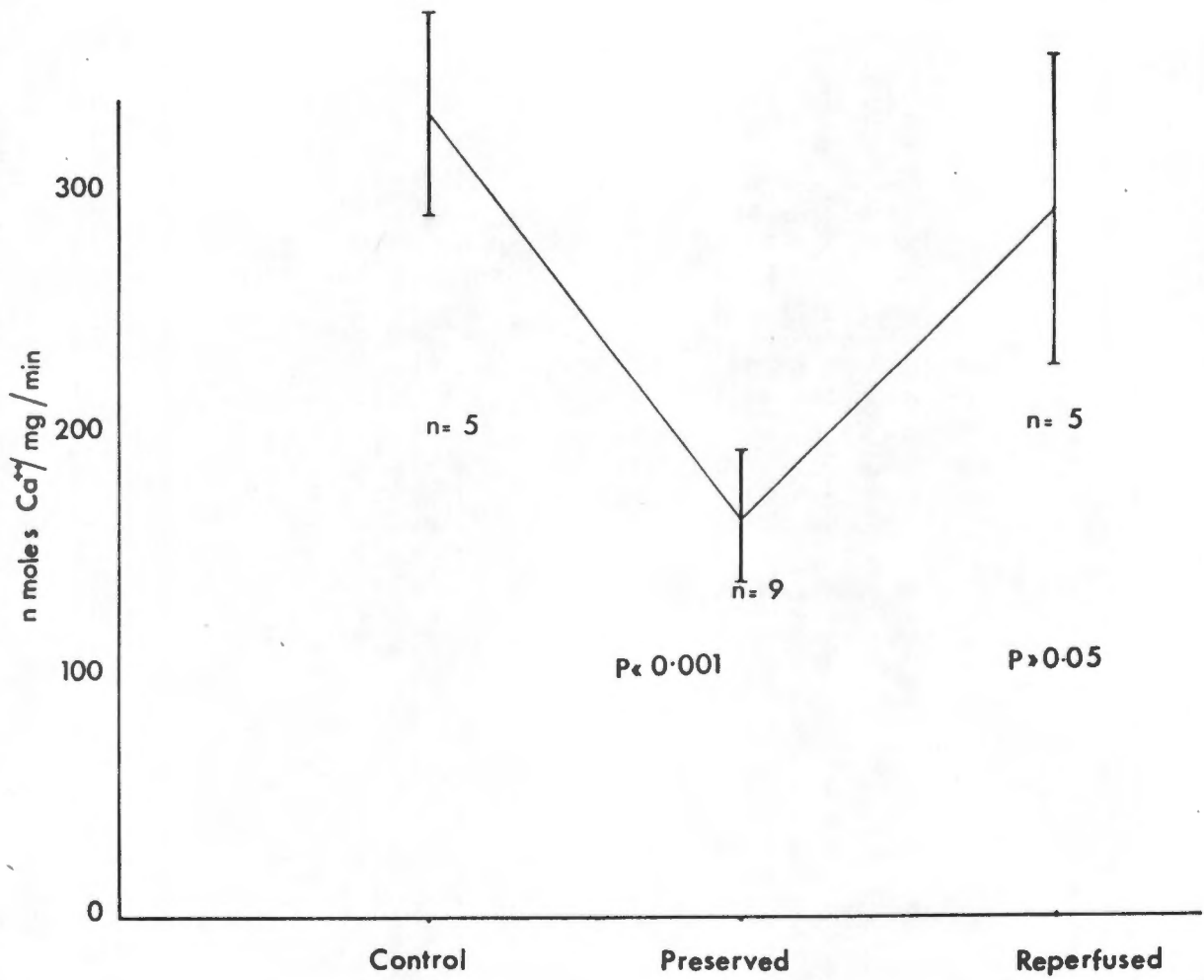


Fig. 10.6.

Sarcoplasmic reticulum calcium uptake of three groups of hearts:

(1) control (2) 24 hour preserved (preserved) and 24 hour preserved followed by one hour of warm blood reperfusion (reperfused). Bars represent S.E.M.

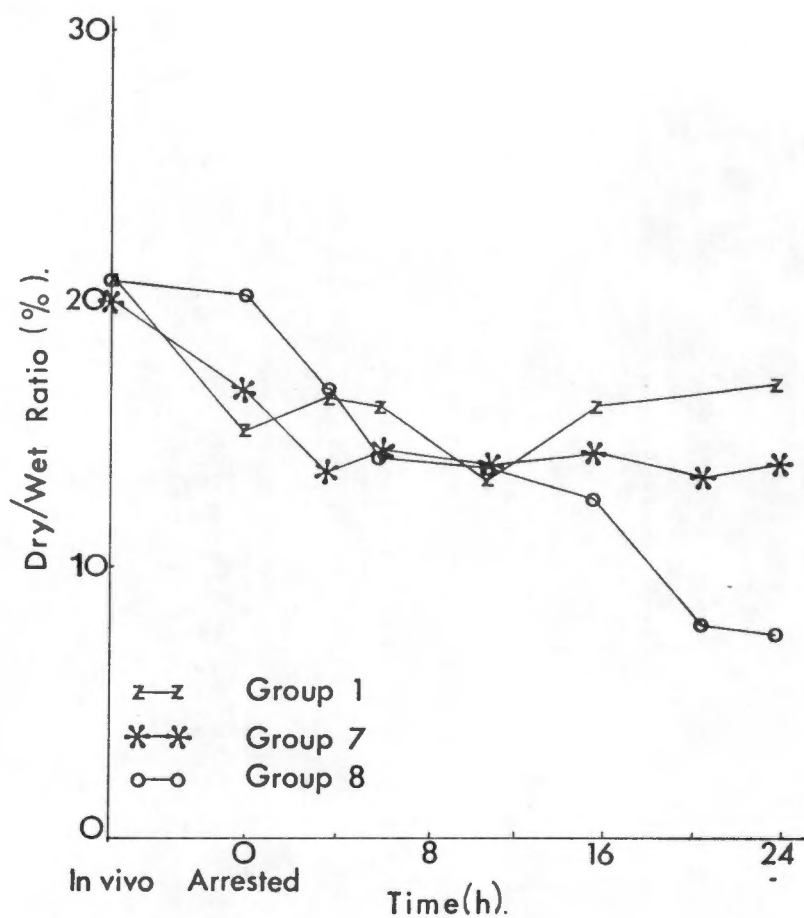


Figure 10.7.

Water gained by three groups of hearts during perfusion, indicated by diminishing dry weight : wet weight ratios. After 24 hours group 8 had the lowest ratio indicating considerable fluid retention relative to group 7 and group 1 which developed the least fluid retention.

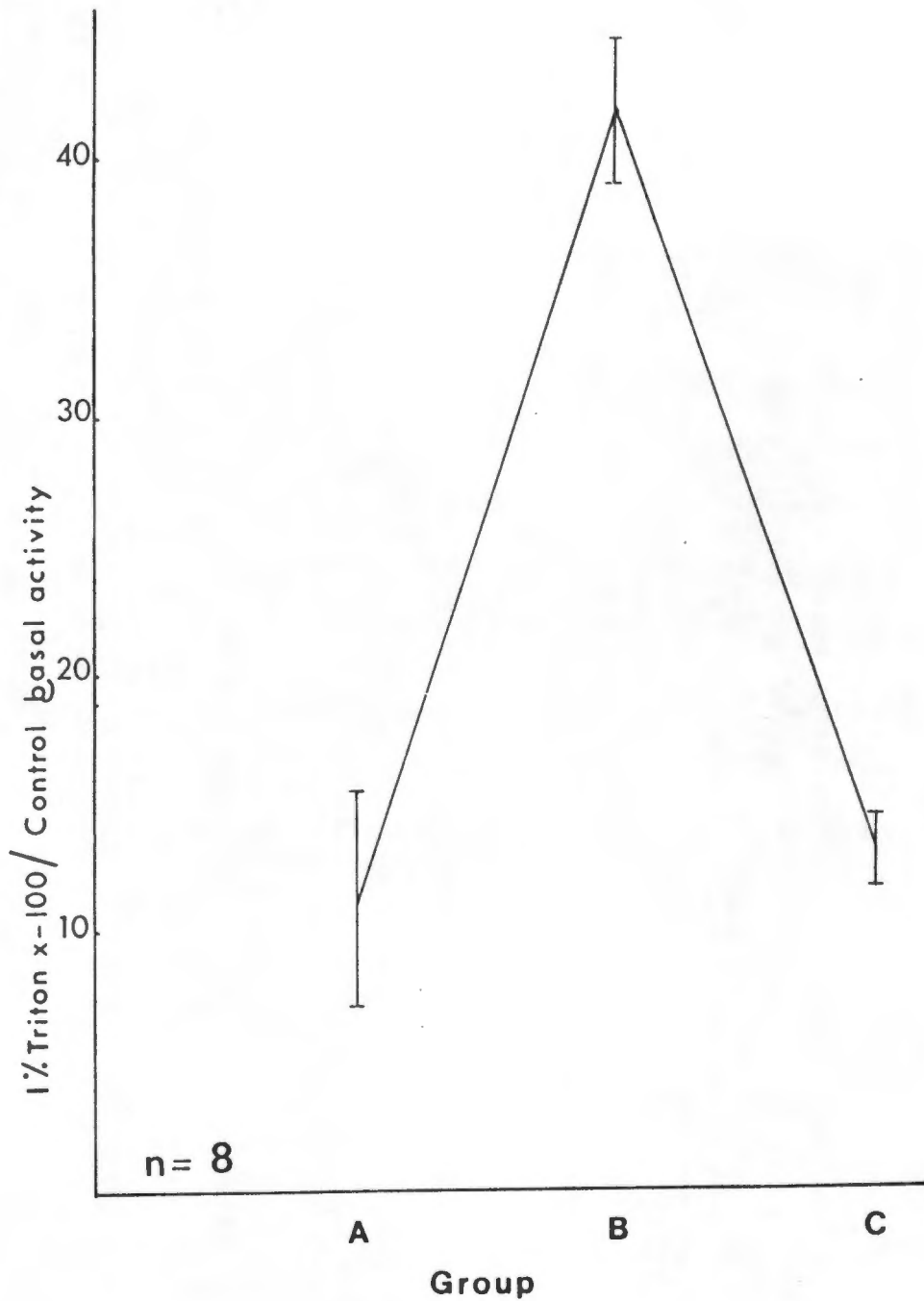


Fig. 10.8.

The latency of the particulate fraction of each of three groups (group A) (freshly isolated lysosomes), (group B) (lysosomes isolated from hearts perfused for 24 hours) and (group C) (lysosomes isolated from hearts perfused 24 hours followed by one hour warm blood reperfusion) was determined by incubation of the particulate fraction in 1% Triton X - 100. The latencies of groups B and C were then corrected by dividing them by the basal activity of group A. Only group B differed significantly from control group A. Bars represent S.E.M.

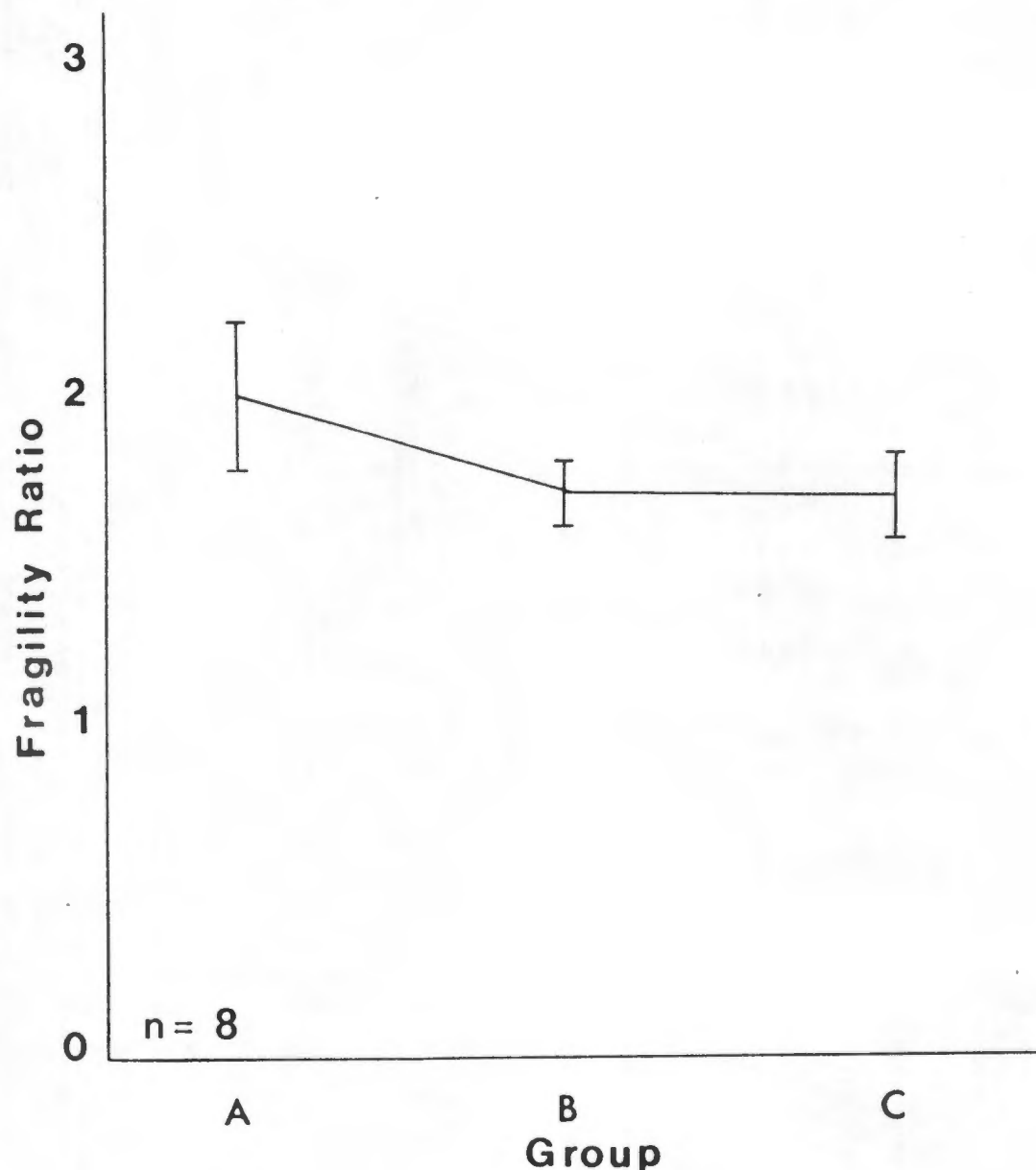


Fig. 10.9.

The fragility ratio was calculated from the particulate fraction of three groups of hearts (group A) freshly isolated lysosomes, (group B) lysosomes isolated from hearts preserved for 24 hours and group C lysosomes isolated from hearts perfused for 24 hours followed by a hour warm blood reperfusion. the particulate fractions of groups A, B and C were stressed in 1% and 0.1% Triton X - 100. The two resulting activities of each group were expressed as ratio (1% Triton X - 100/0. 1% Triton X - 100) referred to as the fragility ratio. Groups A,B and C did not differ significantly, showing that the fragility of the lysosomal membrane did not change during the period of preservation nor during the period of reperfusion. Bars represent S.E.M.



## CHAPTER 11

### GROUP 9

#### 11. THE PORTABLE PRESERVATION SYSTEM

Portable preservation units for the transplantation of livers (Norman 1968) and kidneys (Collins et al 1969) have been designed, but portable units for heart preservation have rarely been reported (Cooper 1975), although hearts have been portably preserved by simple storage on ice for periods up to 4 hours and orthotopically transplanted (Thomas et al 1978). An inadequate supply of donors contributes to the high mortality in potential recipients for whom a donor is not found. Storage of donors hearts for periods of up to 24 hours would enable transplantation over long distances and would therefore increase donor supply.

Following the consistent success with in vitro functional testing of preserved pig hearts and orthotopic transplantation of baboon hearts it was desirable that a portable preservation unit be designed before clinical application of this system. Essential features of the design were simplicity, compactness, independence from any source of power, and absolute sterility. These requirements would minimise complications arising during transit.

For future clinical application the non-portable laboratory preservation unit would not satisfy these requirements. The new system was pneumatically powered and the design was based on that of the airlift principle in which an oxygenated perfusate is recycled between two vertically located reservoirs (figure 11.1) (Dumbleton 1953).

An exact explanation of the dynamics of the system is complex and a simplified version is offered below. By immersing one end of an open-ended pipe into a container with fluid, the water level will stand in the pipe at the same height as in the container.

If air is injected into the foot of the pipe, the state of equilibrium is destroyed as the density of the mixture of gas and fluid is less than that of the fluid alone and fluid therefore rushes into the pipe from outside, pushing the aerated column upwards to restore equilibrium. It is desirable that the stream of air be broken up as much as possible in the form of small bubbles to facilitate the displacement of fluid. This is achieved by having a small ejection port preventing the slippage of intermittently produced fluid-air interfaces. The displaced fluid passes from the lower reservoir to the upper reservoir. Once the upper reservoir is full, a fluid-gas mixture drains through the overflow into the lower reservoir. The continuous fluid-gas drainage results in a pressure buildup in the lower reservoir until a state of equilibrium is reached, preventing any further fluid-gas drainage. The upper reservoir fluid height now rises, until the equilibrium is upset and a continuous drainage of only fluid now passes down the overflow. This raised lower reservoir pressure now decreases the bubble size at the ejector port, resulting in a more efficient fluid delivery to the upper reservoir. This improvement in efficiency takes approximately 3 - 5 minutes to be effective and the gas delivery can then be decreased, conserving the compressed gas store, yet providing sufficient perfusate to the myocardium.

The volume of fluid delivered also depends on the lower reservoir fluid depth, the ejector diameter, and the rate of the gas flow. The maximum rate of fluid delivery is 150ml/min with a vertical lift of 20 - 25cm. An in-line 20 micron filter is also fitted between the upper and lower reservoirs, filtering any contaminant macromolecule that might interrupt flow through the coronary vasculature (figure 11.1). The glass unit is then placed into a stainless steel insulated box containing ice, which cools the perfusate to approximately 4 - 10°C.

The gas mixture from the pressurised gas cylinder has four essential functions:

1. circulates the fluid
2. oxygenates the medium
3. maintains the acid-base balance between pH 7.0 - 7.8
4. maintains sterility by creating a positive pressure inside the glass unit ensuring the unidirectional outward gas flow.

The advantages of the system are as follows:

- i) no electrical pumping device is required
- ii) there are no moving parts
- iii) the fluid delivery pipe can be bent at any angle with little decrease in fluid delivery.

## RESULTS

The efficiency of the portable preservation system was confirmed by three methods.

- (a) Ex vivo functional evaluation of pig hearts
- (b) orthotopic transplantation of baboon hearts
- (c) autotransplantation of baboon hearts

The pig and baboon hearts were arrested using cardioplegic solution C<sub>2</sub> (table 2.1) followed by 24 hours perfusion preservation in solution 8 or 9 (table 11.1).

#### 11.1.1. OBSERVATIONS DURING THE PERIOD OF HYPOTHERMIC PERFUSION

These hearts were always very soft after the preservation period yet markedly oedematous (table 11.2). The 20 micron filters were never occluded and allowed continuous cycling of a small number of red blood cells which were still intact though very shrunken when examined under phase contrast microscopy. After 24 hours the perfusate itself was opaque due to unfiltered red blood cells and other circulatory metabolites. The modified filtration system did not appear to have a deleterious effect on subsequent function.

The perfusate  $pO_2$  when measured at 30°C was high and varied between 1000 and 2000 mm Hg due to the increased partial pressure of oxygen in the lower reservoir. Myocardial oxygen uptake both at the beginning and end of preservation did not change significantly (table 11.2.1). Myocardial temperature remained low throughout the period of preservation with an initial temperature of 10°C dropping steadily to 4°C shortly before the start of hypothermic perfusion. A similar decrease in temperature occurred in the non-portable preservation unit. The decrease in coronary flow (table 11.3) and the increase in coronary resistance (table 11.4) as noted in previous sections, did not reach statistical significance on this occasion over the period of preservation.

A comparison of coronary flow at the beginning of preservation between groups 8 and 9 did not differ significantly (table 11.6) nor did the coronary flow at the end of preservation (table 11.7). Perfusate samples taken after the period of preservation for bacterial culture were all found to be sterile.

#### 11.2.1. OBSERVATIONS DURING THE PERIOD OF FUNCTIONAL EVALUATION

The pattern of recovery was similar to group 8 hearts, namely, initial vascular fibrillation requiring defibrillation, being followed by sinus rhythm, with good contractions. After one hour of reperfusion with blood, a mean weight loss of 7.5% occurred (table 11.5). There was a slow but steady improvement in performance over the course of one hour testing. Haemodynamic observations during the "active" phase are listed in tables 11.8 (stroke volume), 11.9 (cardiac output), 11.9.1 (coronary flow) and 11.9.2 (pressure determinations). Only the left ventricular pressure was significantly different from control values. Rhythm disorders were not seen in these hearts. Oxygen uptake was significantly higher in both groups 8 and 9 when compared with the control group (table 11.9.2.1). Histological examinations revealed interstitial oedema, but the myofibres and nuclei appeared well preserved. Myofibrillar degeneration and scanty haemorrhage were present on occasions. No subendocardial haemorrhage or coagulative necrosis were observed.

#### 11.3.1. ORTHOTOPIC TRANSPLANTATION

The method of orthotopic transplantation is described in section 2.1.12.

Eleven baboon hearts were transplanted after preservation of which three were electively killed after 2 days.

### 11.3.2. OBSERVATIONS DURING THE PERIOD OF HYPOTHERMIC PERFUSION

These hearts were markedly oedematous, gaining 80.3% weight. The initial coronary flow was  $21.15 \times 10^{-2}$  ml/min/gm and fell to  $14.49 \times 10^{-2}$  ml/min/gm at the end of the perfusion period ( $P < 0.0001$ ). Coronary resistance rose from an initial 42 cm H<sub>2</sub>O/gm/min/ml to 50 cm H<sub>2</sub>O/gm/min/ml at the end of perfusion ( $P < 0.001$ ).

### 11.3.3. RESULTS OF ORTHOTOPIC TRANSPLANTATION

After approximately 10 - 15 minutes of rewarming following removal of the aortic cross-clamp, vigorous ventricular fibrillation had occurred, spontaneously reverting to sinus rhythm requiring no defibrillation. The animals were weaned off cardiopulmonary bypass with minimum inotropic support. The hearts of the three electively sacrificed animals gained a mean weight of 66.3% after preservation. After orthotopic transplantation (2 days) these hearts lost 47.3% of their gained weight. This was remarkable as the oedema formation was at least in part reversible. The remaining eight animals survived until rejection with a mean survival period of 27 days (table 11.9.3). Good haemodynamic function has been confirmed at cardiac catheterisation in six animals (table 11.9.4); virtually normal pressures were again obtained. Narrowing at the pulmonary suture lines confirmed at necropsy caused a slight gradient between right ventricle and distal pulmonary artery.

Histological examination showed features of severe acute rejection (table 11.9.3).

#### 11.4.1. AUTOTRANSPLANTATION

Three experiments were performed. Outbred chacma baboons weighing 10, 12 and 15 kg were used.

##### Stage 1

The methods of pre-medication, anaesthesia and ventilation are described in section 2.1.11.

#### 11.4.2. COLD CARDIOPLEGIC ARREST AND HEART EXCISION

A median sternotomy was performed. Total cardiopulmonary bypass was initiated after cannulation of the SVC, IVC and distal ascending aorta. Cooling to 28°C was carried out. A left ventricular vent was inserted at the apex. A needle attached by sterile tubing to a 500ml bag containing cardioplegic agent at 4°C (table 2.1) was inserted into the root of the aorta. The distal ascending aorta proximal to the aortic cannula was cross-clamped and 500ml cardioplegic agent infused; an incision was made in the right atrium and coronary sinus return sucked to waste. The pericardial cavity was filled with normal saline at 4°C to externally cool the heart. The heart was then excised, the line of excision being carried into the SVC and IVC, taking care to include the region of the sinus node with the heart; the aorta and pulmonary artery was divided approximately 3 cm distal to their respective valves. The heart was then weighed and transferred to the perfusion apparatus. An orthotopic transplant was then performed using a heart excised from a donor baboon matched for AB blood group. The techniques of donor heart excision and orthotopic transplantation were basically as described in section 2.1.12.

Cardiopulmonary bypass was then discontinued and, once donor heart function was deemed satisfactory, the chest was closed and the baboon returned to its cage. No immunosuppressive therapy was administered.

### Stage 2

On the following day, when the excised heart had undergone continuous hypothermic perfusion for 20-24 hours, the baboon was again premedicated, anaesthetised and ventilated as described before. The sternotomy was reopened and cardiopulmonary bypass instituted as before. The ascending aorta was cross-clamped and the transplanted heart excised. The animal's own heart was then removed from the perfusion apparatus, weighed and replaced in its original position.

Post-operative management was as in section 2.1.13.

## 11.4.3. RESULTS OF AUTOTRANSPLANTATION

Three autografts were performed (table 11.9.5).

### Case 1

The first baboon made an excellent recovery and was extremely active and obviously well until he died suddenly on the 31st post-operative day. During the first week after operation he developed some oedema of the scrotum for which he was given Frusemide 5 mg i.m. on three occasions; the oedema did not recur. Necropsy revealed that a false aneurysm situated between the ascending aorta and sternum, and communicating with the aorta through a pinhole at the aortic suture line, had ruptured into the right pleural cavity, exsanguinating the animal. Unfortunately, post-operative cardiac catheterisation had not yet been performed.



Histological examination by light microscopy of the myocardium showed interstitial oedema and scanty myocytolysis only; the small blood vessels were well preserved.

#### Case 2

This baboon made an excellent recovery and remains well and active five months after autotransplantation. This animal also developed swelling of the lower abdomen and scrotum within the first few days after operation and required furosemide 5 mm i.m. on four occasions; again, the oedema did not recur. Cardiac catheterisation has been performed on two occasions at 1 and 2 months (table 11.9.6) and the data obtained differed insignificantly from that obtained from the same baboon pre-operatively, except with regard to a small right ventricular-pulmonary artery gradient and an unexplained increase in pulmonary capillary wedge pressure; left ventricular end-diastolic pressure, however, remains normal.

#### Case 3

The third animal developed a dysentery from a severe enterocolitis which progressed to a purulent peritonitis in the early post-operative period and, despite therapy, death occurred on the 7th day. Though sick throughout this period, making it difficult to assess his haemodynamic state reliably, cardiac function appeared satisfactory. There was no dependent oedema. Cardiac catheterisation was not performed. Necropsy confirmed a grossly distended colon and purulent peritonitis.

### 11.5.1. DISCUSSION AND CONCLUSIONS

The modifications to the preservation unit did not affect myocardial function after a period of 24 hours hypothermic preservation both in pig and baboon hearts. In the former animal this was confirmed by functional evaluation and in the latter by orthotopic transplantation. Under these experimental conditions myocardial function following preservation did not appear to be influenced by relatively wide fluctuation in pH (7.0 to 7.8) and  $pO_2$  (1000 - 2000 mm Hg) nor by the lower degree of filtration. The hearts remained sterile during the preservation period and associated infections did not occur in the surviving baboons.

To confirm the superiority of perfusion preservation over simple storage in ice, a group of 10 pig hearts were stored simply in solution 8 in ice. The mean percentage weight gain during the period of storage was 6.8% but by the end of 60 minutes reperfusion with blood and functional testing, there was a further increase of 72.9%.

These hearts, on rewarming, quickly resumed spontaneous sinus rhythm and, in the main, did not require defibrillation.

Whilst still being passively perfused, initial contractions were forcible, but in all cases, deterioration occurred over the first ten minute period, during which, the hearts were expected to support a work load. Haemodynamic measurements, made after switching to the working mode, are listed in table 11.9.7.

Cardiac function showed only little recovery after the initial deterioration. Rhythm disorders were common in this group. Microscopy showed interstitial oedema with capillary disruption in areas. It would appear that these stored hearts had sustained damaged cell membranes, allowing an accumulation of intracellular fluid during the period of reperfusion; as this occurred, myocardial function deteriorated.

Even the preservatives of the major constituents of the cardioplegic solution and the perfusate would appear to be important. At one stage in the development of this system, we unwittingly used procaine hydrochloride made up in 0.5% phenol; this resulted in inferior cardiac output when compared with hearts in group 9 in which the procaine hydrochloride was made up into 0.1% chlorocresol. The haemodynamic performances of these two groups of hearts on testing are shown in table 11. 9.8; it should be stressed that the only difference in the two groups was the preservative used with regard to the procaine in both cardioplegic solution and the perfusate. The "phenol" hearts, however, gained weight (mean 1.8%) during the 1 hour period of reperfusion of functional testing, in contrast to the "cresol" group where there was a mean weight loss of 7.5%.

We are unable to give a definite explanation for the mechanism by which 0.5% phenol altered the effect of the cardioplegic agent and the perfusate, though its damaging effect on tissue is well known. It may be that the phenol in the cardioplegic and perfusate damaged the protein of the cell membrane, so that on reperfusion integrity was lost, allowing fluid to enter the cells.

Any weight gain during reperfusion, even 1 or 2%, appeared to be a sign of inadequate preservation.

Orthotopic transplantation of 8 baboon hearts similarly preserved in the 0.5% "phenol" solution resulted in myocardial failure and none of the animals were able to support the circulatory load following discontinuation of cardiopulmonary bypass.

The final experiment of autotransplantation confirmed successful preservation. In these experiments the long-term effect of perfusion preservation on an organ was determined by avoiding the intervention of rejection. The performance of these hearts was a true reflection of the preservation.

With confidence in the portable system (confirmed above) the project of preservation of human hearts for heterotopic transplantation was embarked upon.

COMPOSITION OF PERFUSION SOLUTIONS USED TO PRESERVE

HEARTS IN 9 DIFFERENT GROUPS

| Constitution of perfusates (mM)      | 1     | 2     | 3     | 4     | 5     | 6       | 7       | 8       | 9<br>Portable Unit |
|--------------------------------------|-------|-------|-------|-------|-------|---------|---------|---------|--------------------|
| NaCl                                 | 118.5 | 118.5 | 92.90 | 92.90 | 92.90 | 92.90   | 115.7   | 115.7   | 115.7              |
| NaHCO <sub>3</sub>                   | 25    | 25    | 25    | 25    | 25    | 25      | 25      | 25      | 25                 |
| KCl                                  | 4.75  | 4.75  | 16    | 16    | 16    | 16      | 10.9    | -       | -                  |
| KH <sub>2</sub> PO <sub>4</sub>      | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20    | 1.20    | 8       | 8                  |
| MgSO <sub>4</sub> 7H <sub>2</sub> O  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20  | 1.20    | 1.20    | 14.4    | 14.4               |
| CaCl <sub>2</sub> ·2H <sub>2</sub> O | 2.5   | 2.5   | 2.25  | 2.25  | 2.25  | 1.1     | 1.1     | 1.1     | 1.1                |
| MgCl <sub>2</sub>                    | -     | -     | 14.8  | 14.8  | 14.8  | 14.8    | 14.8    | -       | -                  |
| Glucose                              | 11    | 11    | 11    | 11    | 11    | 11      | 11      | 11      | 11                 |
| Taurine                              | -     | 4     | 4     | 4     | 4     | 4       | 4       | 4       | 4                  |
| ATP                                  | -     | -     | 10    | -     | -     | -       | -       | -       | -                  |
| Insulin                              | -     | -     | 40u/L | 40u/L | 40u/L | 40u/L   | 40u/L   | -       | -                  |
| Procaine HCl                         | -     | -     | 1     | 1     | 1     | 1       | 1       | 1       | 1                  |
| Dextran 40                           | -     | -     | -     | 4g/L  | 2g/L  | -       | -       | -       | -                  |
| Chlorpromazine                       | -     | -     | -     | -     | -     | .005g/L | .005g/L | .005g/L | .005g/L            |
| Phenoxybenzamine                     | -     | -     | -     | -     | -     | .01g/L  | .01g/L  | .01g/L  | .01g/L             |
| Glycerol                             | -     | -     | -     | -     | -     | -       | 136     | 136     | 136                |
| Sucrose                              | -     | -     | -     | -     | -     | -       | -       | 7       | 7                  |
| Osmolality (mOsm/L)                  | 277   | 280   | 295   | 315   | 302   | 321     | 330     | 385     | 385                |

TABLE 11.1

CHANGES IN HEART WEIGHT DURING 24 HOUR PERIOD OF  
HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUPS (Perfusates)                                                                | 1   | 2   | 3      | 4    | 5    | 6    | 7    | 8       | 9    |
|------------------------------------------------------------------------------------|-----|-----|--------|------|------|------|------|---------|------|
| Mean % wt gain                                                                     | 21  | 23  | 33.8   | 41.4 | 45.4 | 47.3 | 42.0 | 81.3    | 79.2 |
| SEM                                                                                | 1.9 | 2.4 | 2.8    | 4.3  | 2.5  | 3.28 | 2.56 | 2.01    | 6.28 |
| Statistical comparison:<br>Experimental groups vs<br>preceding experimental groups | -   | NS  | < 0.05 | NS   | NS   | NS   | NS   | < 0.001 | NS   |
| n                                                                                  | 10  | 5   | 11     | 18   | 23   | 16   | 17   | 13      | 8    |

TABLE 11.2.

CORONARY ARTERIO-VEINOUS OXYGEN UPTAKE AT THE BEGINNING AND AT THE END  
OF THE PERIOD OF HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP<br>(Perfusates) | O <sub>2</sub> uptake<br>at the beginning<br>of preservation<br>mmol/h/gm<br>(X10 <sup>-3</sup> ) | O <sub>2</sub> uptake<br>at the end of<br>preservation<br>mmol/h/gm<br>(X10 <sup>-3</sup> ) | Statistical comparison:<br>Beginning vs end<br>of perfusion | n  |
|-----------------------|---------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|-------------------------------------------------------------|----|
| 6                     | 3.9<br>(0.62)                                                                                     | 4.2<br>(0.56)                                                                               | NS                                                          | 21 |
| 7                     | 4.8<br>(0.60)                                                                                     | 4.9<br>(0.58)                                                                               | NS                                                          | 9  |
| 8                     | 5.4<br>(1.50)                                                                                     | 7.1<br>(0.63)                                                                               | NS                                                          | 7  |
| 9                     | 4.3<br>(0.85)                                                                                     | 5.6<br>(0.80)                                                                               | NS                                                          | 8  |

FIGURES IN BRACKETS DENOTE SEM

TABLE 11.2.1.

CORONARY FLOW AT THE BEGINNING AND END OF 24 HOUR PERIOD  
OF HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP<br>Perfusates | Coronary flow<br>at beginning<br>of preservation<br>ml/min/gm<br>(X10 <sup>-2</sup> ) | Coronary flow<br>at end of<br>preservation<br>ml/min/gm<br>(X10 <sup>-2</sup> ) | Statistical comparison:<br>Beginning vs end of<br>preservation | n  |
|---------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------|----|
| 6                   | 15.74<br>(0.70)                                                                       | 6.77<br>(0.47)                                                                  | < 0.001                                                        | 21 |
| 7                   | 13.40<br>(0.81)                                                                       | 8.77<br>(0.57)                                                                  | < 0.001                                                        | 9  |
| 8                   | 15.80<br>(1.80)                                                                       | 9.77<br>(0.13)                                                                  | < .02                                                          | 7  |
| 9                   | 13.56<br>(1.62)                                                                       | 9.18<br>(0.75)                                                                  | NS                                                             | 8  |

FIGURES IN BRACKETS DENOTE SEM

TABLE 11.3.



CORONARY VASCULAR RESISTANCE AT THE BEGINNING AND END OF 24 HOUR  
PERIOD OF HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP<br>(Perfusates) | Coronary vascular resistance<br>cm H <sub>2</sub> O/gm/min/ml |                               | Statistical comparison:<br>Beginning vs end<br>of preservation | n  |
|-----------------------|---------------------------------------------------------------|-------------------------------|----------------------------------------------------------------|----|
|                       | At the beginning<br>of preservation                           | At the end of<br>preservation |                                                                |    |
| 6                     | 53<br>(2.2)                                                   | 210<br>(21.3)                 | < 0.001                                                        | 21 |
| 7                     | 65<br>(4.5)                                                   | 127<br>( 6.7)                 | < 0.001                                                        | 9  |
| 8                     | 55<br>(7.3)                                                   | 87<br>( 8.4)                  | < 0.05                                                         | 7  |
| 9                     | 74<br>(20.9)                                                  | 85<br>( 7.1)                  | NS                                                             | 8  |

FIGURES IN BRACKETS DENOTE SEM

TABLE 11.4.

CHANGES IN HEART WEIGHT FOLLOWING ONE HOUR EX VIVO FUNCTIONAL TESTING

| GROUPS<br>(Perfusates)                                                            | 3    | 4    | 5    | 6    | 7    | 8    | 9    |
|-----------------------------------------------------------------------------------|------|------|------|------|------|------|------|
| % Wt<br>Loss                                                                      | 13   | 17   | 19.1 | 14.8 | 9.74 | 8.3  | 7.5  |
| SEM                                                                               | 2.00 | 5.00 | 4.33 | 1.61 | 2.65 | 1.45 | 1.40 |
| Statistical Comparison:<br>Experimental groups vs<br>preceding experimental group | -    | NS   | NS   | NS   | NS   | NS   | NS   |
| n                                                                                 | 11   | 18   | 23   | 16   | 17   | 13   | 8    |

TABLE 11.5.

COMPARISON OF CORONARY FLOWS AT THE BEGINNING OF 24 HOUR PERIOD OF  
HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES.

| GROUP (Perfusates)                                                                  | 6    | 7      | 8    | 9     |
|-------------------------------------------------------------------------------------|------|--------|------|-------|
| Coronary flow at the<br>beginning of preservation<br>ml/min/gm ( $\times 10^{-2}$ ) | 15.4 | 13.4   | 15.8 | 13.56 |
| SEM                                                                                 | 0.70 | 0.80   | 1.80 | 1.62  |
| Statistical comparison:<br>Experimental group vs<br>preceding experimental group    | -    | < 0.05 | NS   | NS    |
| n                                                                                   | 16   | 8      | 14   | 8     |

TABLE 11.6.

COMPARISON OF CORONARY FLOWS AT THE END OF 24 HOUR PERIOD OF HYPOTHERMIC

PERFUSION USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                         | 6    | 7      | 8    | 9    |
|----------------------------------------------------------------------------|------|--------|------|------|
| Coronary flow at the end of preservation ml/min/gm (X10 <sup>-2</sup> )    | 6.77 | 8.77   | 9.77 | 9.81 |
| SEM                                                                        | 0.47 | 0.57   | 0.13 | 0.75 |
| Statistical comparison: Experimental group vs preceding experimental group | -    | < 0.02 | NS   | NS   |
| n                                                                          | 16   | 8      | 14   | 8    |

TABLE 11.7.

STROKE VOLUME DURING EX VIVO FUNCTIONAL TESTING FOLLOWING 24 HOURS

STORAGE USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                               | Control | 7    | 8    | 9    |
|----------------------------------------------------------------------------------|---------|------|------|------|
| SV (mL/100g)                                                                     | 17.0    | 10.7 | 17.0 | 17.3 |
| SEM                                                                              | 1.27    | 1.54 | 1.10 | .74  |
| Statistical comparison:<br>Control vs experimental<br>group                      | -       | 0.01 | NS   | NS   |
| Statistical comparison:<br>Experimental group vs<br>preceding experimental group | -       | -    | 0.01 | NS   |
| n                                                                                | 9       | 8    | 14   | 8    |

TABLE 11.8.

CARDIAC OUTPUT DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                               | Control | 7      | 8       | 9    |
|----------------------------------------------------------------------------------|---------|--------|---------|------|
| CO (mL/min/100g)                                                                 | 2827    | 1577   | 2636    | 2543 |
| SEM                                                                              | 22      | 222    | 144     | 88   |
| Statistical comparison:<br>Control vs experimental<br>group                      | -       | < 0.01 | NS      | NS   |
| Statistical comparison:<br>Experimental group vs<br>preceding experimental group | -       | -      | < 0.001 | NS   |
| n                                                                                | 9       | 8      | 14      | 8    |

TABLE 11.9

CORONARY FLOW DURING EX VIVO FUNCTIONAL TESTING FOLLOWING  
24 HOURS STORAGE USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                               | Control | 7   | 8   | 9    |
|----------------------------------------------------------------------------------|---------|-----|-----|------|
| CF (ml/min/100g)                                                                 | 258     | 295 | 314 | 362  |
| SEM                                                                              | 33      | 43  | 28  | 31   |
| Statistical comparison:<br>Control vs experimental<br>group                      | -       | NS  | NS  | 0.05 |
| Statistical comparison:<br>Experimental group vs<br>preceding experimental group | -       | -   | NS  | NS   |
| n                                                                                | 9       | 8   | 14  | 8    |

TABLE 11.9.1.

# HAEMODYNAMIC PERFORMANCE DURING FUNCTIONAL TESTING FOLLOWING 24 HOURS

## HYPOTHERMIC PRESERVATION USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                   | Control      | 6            | 7            | 8            | 9            |
|----------------------------------------------------------------------|--------------|--------------|--------------|--------------|--------------|
| LVP (mm Hg)                                                          | 131<br>(4.4) | 90<br>(3.6)  | 130<br>(4.0) | 114<br>(5.2) | 114<br>(3.1) |
| LVEDP (mm Hg)                                                        | 5.3<br>(1.0) | 7.5<br>(1.3) | 7.4<br>(1.6) | 5.3<br>(1.0) | 4.8<br>(1.5) |
| AOP (mm Hg)                                                          | 114<br>(2.8) | 83<br>(4.3)  | 115<br>(3.2) | 94<br>(2.5)  | -            |
| Statistical comparison:<br>LVP<br>Control vs Experimental<br>group   | -            | 0.001        | NS           | 0.05         | 0.05         |
| Statistical comparison:<br>LVEDP<br>Control vs experimental<br>group | -            | NS           | NS           | NS           | NS           |
| Statistical comparison:<br>AOP<br>Control vs experimental<br>group   | -            | 0.001        | NS           | 0.01         | -            |
| n                                                                    | 9            | 16           | 8            | 14           | 8            |

FIGURES IN BRACKETS DENOTE SEM

TABLE 11.9.2.



OXYGEN UPTAKE DURING THE PERIOD OF FUNCTIONAL TESTING FOLLOWING  
24 HOURS HYPOTHERMIC PERFUSION USING DIFFERENT PERFUSATES

| GROUP (Perfusates)                                                            | Control | 6    | 7    | 8       | 9      |
|-------------------------------------------------------------------------------|---------|------|------|---------|--------|
| O <sub>2</sub> uptake during functional testing mmol/h/g (X10 <sup>-1</sup> ) | 6.87    | 7.16 | 8.07 | 20.05   | 19.42  |
| SEM                                                                           | 1.08    | 1.37 | 1.41 | 3.28    | 1.76   |
| Statistical comparison: Control vs Experimental group                         | -       | NS   | NS   | < 0.001 | < 0.05 |
| Statistical comparison: Experimental group vs preceding experimental group    | -       | -    | NS   | < 0.05  | NS     |
| n                                                                             | 9       | 16   | 8    | 14      | 8      |

TABLE 11.9.2.1.

RESULTS OF ORTHOTOPIC TRANSPLANTATION FOLLOWING 24 HOUR

STORAGE OF THE DONOR HEART

| Baboon (Group 9) | Survival (days) | Cause of death | Myocardial Histology |
|------------------|-----------------|----------------|----------------------|
| 1                | 49              | Rejection      | Rejection            |
| 2                | 10              | Rejection      | Rejection            |
| 3                | 36              | Rejection      | Rejection            |
| 4                | 13              | Rejection      | Rejection            |
| 5                | 28              | Rejection      | Rejection            |
| 6                | 31              | Rejection      | Rejection            |
| 7                | 33              | Rejection      | Rejection            |
| 8                | 42              | Rejection      | Rejection            |

TABLE 11.9.3

DATA OBTAINED AT CARDIAC CATHETERISATION 6 - 14 DAYS

AFTER ORTHOTOPIC TRANSPLANTATION

|                                  | GROUP 9    |
|----------------------------------|------------|
| Right Atrium                     | 7.3 (1.1)  |
| Right Ventricle (systolic)       | 34.8 (2.9) |
| Main Pulmonary Artery (systolic) | 22.6 (2.1) |
| Pulmonary Capillary Wedge        | 6.5 (1.5)  |
| Left Ventricle (systolic)        | 106 (1.0)  |
| Left Ventricular end-diastolic   | 6 ( 0 )    |
| Aorta (systolic)                 | 96.5 (6.5) |
| Cardiac output (L/min) n = 3     | 3.2 (0.2)  |

Figures refer to mean pressure in mm Hg  
Figures in brackets denote SEM

TABLE 11.9.4

SURVIVAL OF ANIMALS RECEIVING AUTOGRAFTS FOLLOWING 24 HOURS HYPOTHERMIC

PERFUSION

| Baboon No. | Date of Operation | Survival (days) | Cause of Death                   |
|------------|-------------------|-----------------|----------------------------------|
| 138        | 6.8.1980          | 31              | Rupture of false aortic aneurysm |
| 217        | 16.9.1981         | Living 15.6.83  | -                                |
| 340        | 17.11.1981        | 8               | Sacrificed - (Enterocolitis)     |

TABLE 11.9.5

DATA OBTAINED AT CARDIAC CATHETERISATION AFTER AUTOTRANSPLANTATION  
OF A BABOON HEART PRESERVED FOR 24 HOURS BY HYPOTHERMIC PERFUSION.

|                                  | Pre-op | 1 Month<br>post-op | 2 Months<br>post-op |
|----------------------------------|--------|--------------------|---------------------|
| Right Atrium                     | 4      | 7                  | 8                   |
| Right Ventricle (systolic)       | 28     | -                  | 40                  |
| Main Pulmonary artery (systolic) | 28     | 20                 | 20                  |
| Pulmonary capillary wedge        | 3      | 8                  | 12                  |
| Left Ventricle (systolic)        | 110    | 90                 | 100                 |
| Left Ventricle end-diastolic     | 2      | 3                  | 2.5                 |
| Aortic (systolic)                | 110    | 95                 | 105                 |
| Cardiac output (L/min)           | 4.9    | 6.02               | -                   |

Figures refer to mean pressure in mm Hg

TABLE 11.9.6

|                                                    | Control<br>(n = 10) | Ice Stored<br>Group<br>(n=10) | Statistical<br>comparison |
|----------------------------------------------------|---------------------|-------------------------------|---------------------------|
| CARDIAC OUTPUT (l/min)                             | 2.8 (0.22)          | 1.4 (0.16)                    | (P< 0.001)                |
| CORONARY FLOW (ml/min)                             | 258 (32.50)         | 326 (45.60)                   | (NS)                      |
| STROKE VOLUME (ml/beat)                            | 17.4(1.27)          | 12.7 (1.34)                   | (P<0.05)                  |
| LEFT VENTRICULAR PRESSURE (mm Hg)                  | 131 (4.42)          | 102 (3.86)                    | (P<0.001)                 |
| LEFT VENTRICULAR END DIASTOLIC<br>PRESSURE (mm Hg) | 5.3 (1.03)          | 5.9 (1.34)                    | (NS)                      |
| MYOCARDIAL OXYGEN UPTAKE (mmol/h/g)                | 0.68(0.11)          | 1.00 (0.23)                   | (NS)                      |

Figures in brackets denote standard error (SEM)

NS = Not statistically significant (P<0.05)

Haemodynamic performance of two groups of hearts preserved for 24 hours by (a) hypothermic perfusion (b) simple ice storage. An identical perfusate (solution 8) was used in both cases.

TABLE 11.9.7

HAEMODYNAMIC PERFORMANCE ON FUNCTIONAL TESTING FOLLOWING HYPOTHERMIC  
PERFUSION USING PERFUSATES WITH DIFFERENT PROCAINE PRESERVATIVES

|                                                       | PROCAINE HCL IN<br>0.1% CHLORO-CRESOL<br>(n=10) |         | PROCAINE HCL IN<br>0.5% PHENOL<br>(n=10) |         | STATISTICAL<br>COMPARISON A:B |
|-------------------------------------------------------|-------------------------------------------------|---------|------------------------------------------|---------|-------------------------------|
| CARDIAC OUTPUT<br>(l/min)                             | 2.5                                             | (0.09)  | 1.6                                      | (0.34)  | $P < 0.05$                    |
| CORONARY FLOW<br>(ml/min)                             | 362                                             | (30.88) | 312                                      | (40.20) | NS                            |
| STROKE VOLUME<br>(ml/beat)                            | 17.3                                            | (0.74)  | 13.8                                     | (2.50)  | NS                            |
| LEFT VENTRICULAR<br>PRESSURE (mm Hg)                  | 114                                             | (3.11)  | 107                                      | (3.62)  | NS                            |
| LEFT VENTRICULAR<br>END-DIASTOLIC<br>PRESSURE (mm Hg) | 4.8                                             | (1.05)  | 8.3                                      | (1.41)  | NS                            |
| MYOCARDIAL OXYGEN<br>UPTAKE (mmol/h/g)                | 1.74                                            | (0.24)  | 1.99                                     | (0.22)  | NS                            |

NS = not statistically significant ( $P > 0.05$ )

Figures in brackets denote standard error (SEM)

TABLE 11.9.8

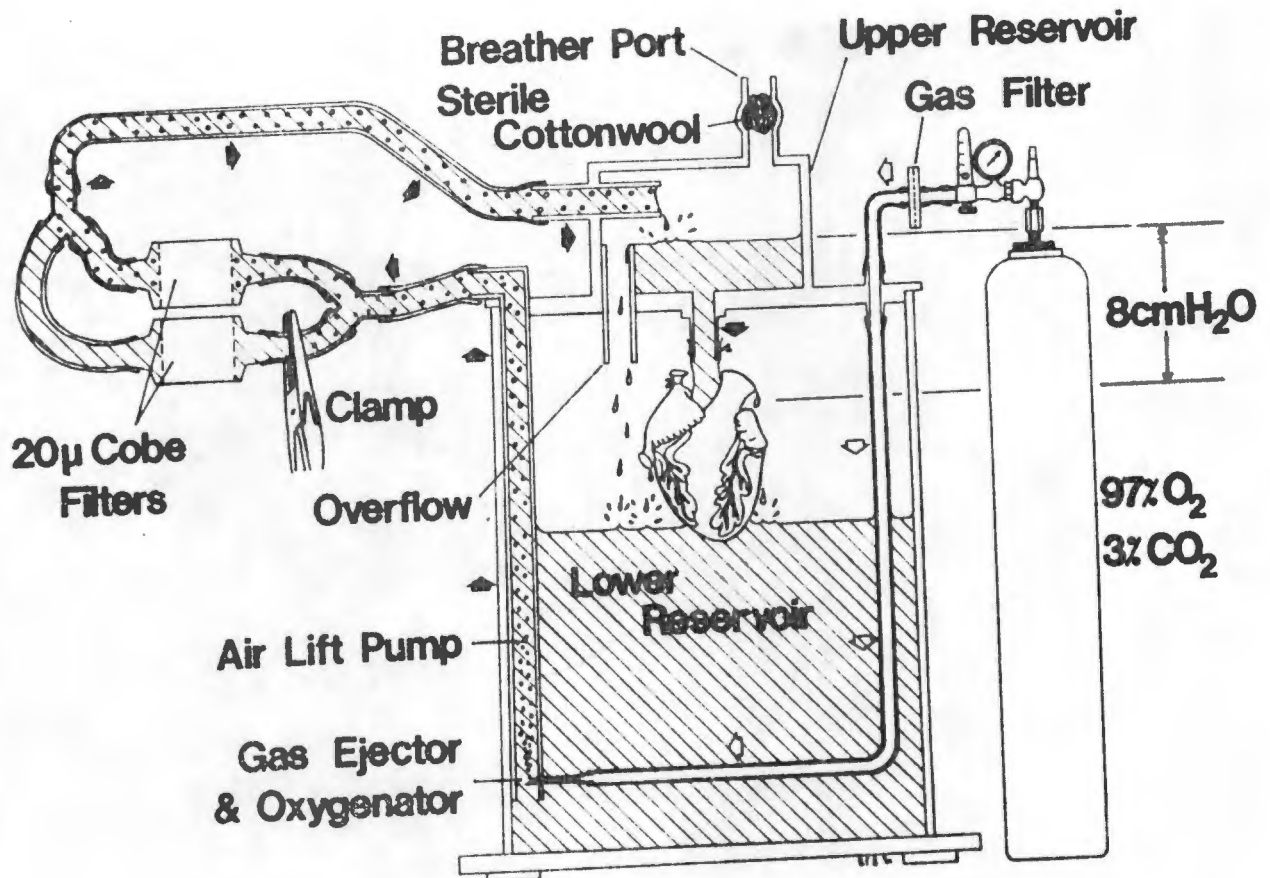


Fig. 11.1.

Diagram of the portable hypothermic perfusion unit. Perfusate is pumped from the lower reservoir (black arrows) through a 20 micron Cobe filter to the upper reservoir, from which the aorta is retrogradely perfused. Circulation of the perfusate is based on the airlift pump principle by which 97% oxygen and 3% carbon dioxide from a pressurised gas cylinder provides the driving force (white arrows).



## CHAPTER 12

### CLINICAL CARDIAC TRANSPLANTATION FOLLOWING STORAGE OF THE DONOR HEART BY A PORTABLE HYPOTHERMIC PERFUSION SYSTEM

#### 12. INTRODUCTION

On 2.12.67 the first human-to-human heart transplantation was performed at Groote Schuur Hospital, Cape Town. By 2.12.81, 57 heart transplants had been carried out at our institution.

In four cases the donor heart was excised and stored by continuous hypothermic perfusion before being transplanted, the periods of ischaemia ranging from 6 hrs 55 mins to 16 hrs 50 mins (table 12.1). This allowed time for tissue typing, lymphocytotoxic cross matching, transportation of the donor heart to the recipient hospital when necessary, and preparation of the recipient.

Neither preservation of the donor heart for periods in excess of approximately 24 hours nor the use of continuous hypothermic perfusion as a method of preservation appear to have been reported previously in the clinical situation.

##### 12.1.1. DONOR HEART EXCISION

All four hearts were removed from brain-dead, ventilated donors in whom the heart was still beating at the time of excision.

The donor chest was opened under sterile conditions by median sternotomy. The pericardium was opened longitudinally. The superior vena cava (SVC) azygos vein, inferior vena cava (IVC) and aortic arch were mobilised.

(Renal transplant surgeons then mobilised, but did not yet excise, both kidneys). Heparin was given (20,000 units i.v.). The azygos vein was doubly ligated and divided. The SVC was doubly ligated as far cephalad as possible and divided. A large needle, attached by sterile tubing to a litre bag containing cold (4°C) C<sub>2</sub> (table 2.1) cardioplegic agent was inserted into the ascending aorta. The IVC was clamped at the diaphragm and divided centrally. The arch of the aorta was clamped at the origin of the innominate artery.

One litre of cold cardioplegic agent was then infused rapidly into the root of the aorta, retrogradely perfusing the coronary arteries. This took approximately 47 - 75 seconds (mean 60 secs). At the same time cold (4°C) normal saline was poured over the heart to fill the pericardial cavity and cool the heart externally. (During this period of cooling excision of the kidneys was completed).

The heart was then excised, immersed in a bowl of C<sub>2</sub> cardioplegic agent (at 4°C) while two strong purse string sutures were placed around the distal ascending aorta and then transferred to the perfusion apparatus and suspended from it by means of the two sutures. The time interval between aortic cross clamping and the initiation of perfusion ranged from 23 to 30 minutes with a mean of 27 minutes.

#### 12.1.2. HETEROTOPIC TRANSPLANTATION

When the results of tissue typing and lymphocytotoxic cross matching were known, and when the heart had been transported to Groote Schuur Hospital (when necessary) and the potential

recipient prepared for operation, the recipient procedure was begun. Heterotopic transplantation was performed in all four cases. The operative technique has been described previously (Barnard and Losman 1975) and involved anastomoses between donor and recipient left atria, right atria and superior vena cavae, aorta and pulmonary arteries; this final anastomosis necessitating the insertion of a prosthetic graft.

Perfusion of the donor heart was maintained until the recipient was being supported on cardiopulmonary bypass and the right chest had been prepared to receive the transplant. After removal from the perfusion apparatus, the donor heart was immersed in cold (4°C) normal saline whilst the orifices of the IVC and both right pulmonary veins were closed by continuous suture. The heart was then placed in the right chest of the recipient and the anastomoses with the recipient heart carried out. Cold saline (4°C) was poured over the donor heart at 20 minute intervals throughout the operation until the restitution of myocardial perfusion with the recipient blood followed completion of the aortic anastomosis.

#### 12.1.3. OBSERVATIONS DURING HYPOTHERMIC PERFUSION

Initial coronary flow was variable (table 12.1). There was a slight reduction in coronary flow in all cases throughout the period of perfusion, with a mean percentage fall of 13.4%.

#### 12.1.4. CASE HISTORIES

Four recipients have received donor hearts stored by continuous hypothermic perfusions.

All four received immunosuppression consisting of azathioprine, methylprednisolone and rabbit antithymocyte globulin (Barnard et al 1981). Three patients received a low-dose steroid regime (32 mg/day), the fourth patient receiving an initial 64 mg/day which was reduced steadily to 30 mg/day by three months.

#### Case 1

The first patient received a donor heart which had been ischaemic for a total period of 12 hrs and 50 mins. It began beating spontaneously when reperfused with blood initially in 2:1 atrio ventricular block but, after a small bolus of i.v. adrenalin, reverted to sinus rhythm which was maintained. Its output was poor. However, the patient, after the discontinuation of cardiopulmonary bypass was being supported largely by his own heart, which required very considerable inotropic support consisting of isoprenaline and adrenaline. An external pulse trace of the femoral pulse carried out soon after the patient's return to the intensive care unit, showed much greater pulsation following ejection by the recipient heart than by the donor heart. There were no ECG signs of ischaemic damage. Though we were able to slowly reduce the inotropic support during the next few hours, donor heart function showed little improvement although sinus rhythm was maintained; there were no episodes of dysrhythmia. Nineteen hours after reperfusion of the donor heart had begun, there was a rapid improvement in donor heart function, for which no obvious reason was apparent.

Over the course of the next three hours, donor heart ejection increased dramatically until it was clearly the major support of the circulation; mean arterial pressure increased from 50 to 75 mm Hg. Twelve hours later the donor heart had taken over the circulation virtually entirely.

Though this patient has subsequently undergone three major rejection episodes in four months, the donor heart continues to function satisfactorily. Recent haemodynamic data obtained at cardiac catheterisation, and a recent endomyocardial biopsy, show no late signs of ischaemic damage.

#### Case 2.

Donor heart function deteriorated rapidly during the minutes before excision, the mean arterial pressure falling to 25 mm Hg despite fluid and inotropic support (dobutamine). The donor hearts total ischaemic period was 8 hours 7 minutes. On reperfusion spontaneous beats occurred, again initially in 2:1 block, and were reverted to sinus rhythm by the administration of adrenalin. Once again initial output was poor, the recipient heart providing the major support of the circulation, although the donor heart was ejecting moderately well. Substantial inotropic support was required. The two hearts shared support of the circulation for some 20 hours, by which time the pulse trace showed the donor heart to be dominant. Despite initial poor function, there were no ECG signs of ischaemic damage.

After four major rejection episodes requiring massive immunosuppressive therapy, donor heart function remains satisfactory, the patient showing no signs of low cardiac output. Histological examination of biopsies show evidence of continuing acute rejection but no significant late features of ischaemic damage.

(This patient has, however, developed toxoplasmosis, for which he is receiving specific therapy; we have serological evidence that confirms that this was transferred from the donor).

### Case 3.

This patient, an American with cardiomyopathy, had developed strong multi-specific antibodies against T lymphocytes after rejection at five weeks after a first heterotopic transplant in 1979. The donor heart was excised and the patient survived on his own heart though he maintained extremely limited exercise tolerance and required considerable antifailure therapy. These antibodies disappeared after 29 months. A test transfusion of one unit of whole blood was associated with the return of strong lymphocytotoxic antibodies to T cells (to 98% of a panel). Two months later, however, a donor became available in Port Elizabeth, some 750 kms from Cape Town. The heart whilst being perfused, was flown to Groote Schuur Hospital and stored whilst T cell lymphocytotoxic crossmatch was performed. This proved negative at all temperatures, though the B cell cross-match was strongly positive at all temperatures.

The operation proved difficult due to adhesions and a degree of fixity of the right lung and recipient heart, limiting exposure, and the ischaemic time of the donor heart during insertion (110 mins) was almost twice as long as usual. Total ischaemic time of the donor heart was 16 hours 50 mins. The donor heart started beating spontaneously and stabilised in sinus rhythm, though, as in the previous cases, output was poor, the patient requiring considerable inotropic support. There were no ECG signs of ischaemic damage to the donor heart. Some improvement occurred during the first 48 hours, though the donor heart at no time took over full responsibility for the circulation. Signs of acute rejection then became apparent (rising white blood count, rise in T cell rosettes, fall in QRS voltage on the ECG) and by the fifth day, despite increased therapy, donor heart function had ceased altogether. This was confirmed by cardiocatheterisation and angiography; a myocardial biopsy showed severe acute rejection. At this stage the recipients serum demonstrated a very strongly lymphocytotoxic antibody against donor T cells from stored donor spleen. Immunosuppression was discontinued, the heart was subsequently excised and the patient survives once again on his own heart with the help of an atrioventricular sequential pacemaker for sinus bradycardia.

#### Case 4

The patient, an Italian, received a donor heart which had been ischaemic for 6 hours 55 minutes. After electrical defibrillation, sinus rhythm ensued; immediate function was excellent, the donor heart taking over responsibility for the circulation without difficulty.

Minimal initial inotropic support was required. The patient suffered no major rejection episodes and donor heart function remains excellent after three months. An endomyocardial biopsy shows minimal features of acute rejection with no other abnormality.

#### 12.1.5. DISCUSSION AND CONCLUSION

The donor heart in Case 1 would clearly have been unable to support the circulation initially if orthotopic transplantation had been performed. The patient relied heavily on his own heart and very considerable inotropic support during the first 19 post-operative hours. During this period our only encouragement that this heart was not irreversibly damaged was the fact that it continued throughout in sinus rhythm; it seemed likely that severe, irreversible ischaemic damage would have been associated with rhythm or conduction disorders. The dramatic recovery of this heart over a period of three hours some 19 hours after coronary reperfusion was begun cannot be completely explained. The donor in this case, however, had been supported for several hours by a dopamine infusion, and there is evidence that high doses of dopamine lead to depletion of intramyocardial catecholamine reserves (Leier et al 1978). We have subsequently confirmed poor function after storage of pig hearts taken from animals pretreated for four hours with a dopamine infusion. It seems likely, therefore, that this heart may have required a period of recovery following transplantation, while its catecholamine reserves were replenished; the period of recovery may have been prolonged by the further use of dopamine in the immediate post-operative period.



We have also noted this need for a recovery period in hearts, stored simply for 2 - 3 hours in cold cardioplegic solution, which had been taken from donors who had received dopamine support. We have subsequently tried to avoid the use of dopamine in the potential donor.

The donor in Case 2 did not receive dopamine and the initial poor function was attributed to excessive constrictive support given at a time when it was not required. In our extensive animal studies, immediate function of baboon hearts after 20 - 24 hours hypothermic perfusion has been good, and orthotopically transplanted hearts have had no difficulty in supporting the circulation with only minimal inotropic support during the initial two hours (see section 10.4.3). There is a considerable difference in donor status between a healthy, freshly anaesthetised baboon and a clinically brain-dead donor with diabetes insipidus who is receiving various pharmacological agents such as inotropes and possibly vasopressin. The many factors which might influence subsequent donor heart function clearly require clarification.

We remain uncertain as to the extent of recovery which might have occurred in the donor heart in Case 3. This patient developed strong antibodies soon after transplantation and donor heart function was almost certainly being affected by severe, acute rejection at an early stage. Following our experience in Cases 1 and 2, we were encouraged to believe that full functional recovery of the donor heart was possible, for at no time was there any dysrhythmia or conduction abnormality.

Full recovery did not take place, however, and we remain in doubt as to the relative effects of storage, the long period required for insertion during which the heart was protected only by intermittent cold saline, and of early severe rejection.

In Case 4, storage was unquestionably successful, though the storage time was relatively short.

The effect of storage on the long-term function of these hearts remains uncertain, but we are encouraged by the continued good function after periods of 2 - 5 months of the hearts in Cases 1, 2 and 4 despite several severe rejection episodes in two patients.

The slight reduction in coronary flow observed during the period of hypothermic perfusion in all four cases are in agreement with our observation in experimental animals. A reduction in coronary flow of more than approximately 50% would be regarded as a rather poor prognostic sign, as in our experience this can, though not always, suggest inadequate preservation.

Further attention clearly needs to be paid to the effects of the several insults received by the hearts in brain-dead patients, particularly if these are to be followed by long periods of storage. We have, however, been sufficiently encouraged by this initial experience to continue our use of this storage system in the immediate future, particularly as it will enable us to obtain donor hearts throughout South Africa or even abroad, and thus helps solve the chronic problem of donor supply.

CORONARY FLOW AT THE BEGINNING AND END OF THE PERIOD OF  
PRESERVATION OF FOUR HUMAN HEARTS

| PATIENT | CORONARY FLOW (ml/min)       |                        | PRESERVATION PERIOD (H) | % REDUCTION IN CORONARY FLOW |
|---------|------------------------------|------------------------|-------------------------|------------------------------|
|         | At beginning of preservation | At end of preservation |                         |                              |
| 1       | 109                          | 100                    | 7                       | 8.26                         |
| 2       | 50                           | 42                     | 17                      | 16.00                        |
| 3       | 25                           | 21                     | 8                       | 16.00                        |
| 4       | 30                           | 26                     | 13                      | 13.33                        |

TABLE 12.1

## CHAPTER 13

### EVALUATION OF PROGRESS

13

#### SCIENTIFIC MESSAGE

An important feature of this work has been the achievement of successful 24 storage of the heart, an accomplishment which has not been achieved previously. The combined effect of cardioplegic arrest, hypothermia and the perfusate were responsible for adequate preservation over the 24 hour period. This thesis does not claim formulation of an ideal perfusate for 24 hours hypothermic perfusion of the heart because that would require numerous tests reaching far beyond the scope of this presentation. Various tests were carried out to evaluate both the extent of myocardial injury and the intracellular site of damage following perfusion. The former included physiological measurements, microscopy, functional and biochemical evaluation and orthotopic transplantation, whereas the latter included detailed biochemical analysis. By suitable deductions from the above determinations, modification to the perfusate was made, with improved function anticipated. By careful selection of chemicals and drugs a perfusate evolved which maintained myocardial viability for 24 hours under specific conditions of hypothermia and perfusion.

Oedema formation appeared throughout the experiments in chapters 1 to 8, and being unable to prevent it, a new approach was adopted; this approach was based on an hypothesis that progressive oedema formation would dilute intracellular constituents of the myocardium, protecting against increasing concentrations of calcium caused by either passive flux or calcium redistribution. The diluting effect would reduce the

concentration of toxic metabolic end products and also maintain a relatively constant acid-base balance. The net effect would be the prevention of enhanced resting myocardial tension and energy conservation for later use at the time of warm blood reperfusion. The hypothesis was indirectly tested by inducing myocardial fluid retention by the addition of small osmotically active molecules (chapters 9, 10 and 11) that could easily diffuse into myocardial tissues during preservation and similarly diffuse from the tissues at the time of warm blood reperfusion. An 80% gain in heart weight resulted, which was associated with excellent subsequent myocardial function, both on in vitro functional evaluation and following orthotopic transplantation.

The importance of the chemical constituents of the perfusate have been discussed in the preceding chapters, but some essential constituents require further emphasis.

1. Verapamil: a constituent of the cardioplegic solution (C2) that brought about the significant increase in coronary flow after 24 hours preservation. The development of enhanced resting myocardial tension was also absent following the use of this drug.
2. Taurine: an amino acid that dramatically lowered the release of LDH into the perfusate.
3. Chlorpromazine and Phenoxybenzamine: evaluation of these drugs in pilot studies showed that they were able to further improve myocardial protection. Chlorpromazine has vasodilator properties and numerous metabolic inhibitory effects (section 8.1.1) that conserve both structural integrity and myocardial energy reserves.

Phenoxybenzamine has similar stabilizing effects as well as vasodilator properties.

4. Osmotically active molecules: these include glucose, sucrose, glycerol, and phosphate and sulphate anions. The combined effect of these molecules in the perfusate resulted in the increased oedema formation. The hypothesis further required that these molecules be able to rapidly diffuse from the myocardial tissue at the onset of reperfusion, with subsequent reduction in the degree of oedema.

### 13.1

#### UNSOLVED PROBLEMS STEMMING FROM THIS THESIS

##### 1. Hypothermia

Most methods of preservation resort to the use of hypothermia to reduce or inhibit metabolic activities. Although effective in most cases, hypothermia changes the entire metabolic status of the tissues and these changes (physiological and biochemical) need further investigation.

##### 2. Perfusion

The superiority of hypothermic perfusion over simple ice storage has been obtained both from the literature and confirmed by experimentation in this dissertation. However, perfusion is a more complex method of preservation, and simple ice storage, if successful, would be easier, cheaper and less problematic. Further work on simple ice storage is indicated.

##### 3. pH

The ideal pH for hypothermic preservation has not been established. Some investigators believe in elevated pH levels to support further metabolic activity, whereas others reduce pH levels to inhibit metabolic activities. This problem requires clarification.

#### 4. Oxygenation

Oxygenation of perfusate is still controversial and more definitive experiments are required to finally answer this question. The knowledge that kidneys can be stored in ice for 48 hours without oxygenation or aeration suggests that oxygen may be unnecessary. Pilot studies in our laboratory showed no functional difference between aeration and oxygenation of the perfusate.

#### 5. Oedema Formation

Oedema formation has hitherto been considered damaging to tissue viability. In the final preservation solution presented in this dissertation, oedema formation was considerable, yet function excellent. It has not been conclusively proved that the presence of reversible oedema during the preservation period is, in fact, beneficial to preservation. The myocardium may function equally well with less or no oedema.

#### 6. Perfusion Solution

Optimizing of constituents is required by performing dose-response curves; further additions or eliminations may be necessary. For example, phosphate buffering may be superior to bicarbonate buffering.

#### 7. Brain-death

Hearts removed from healthy baboons under ideal conditions and preserved for 24 hours by hypothermic perfusion always functioned immediately. In four clinical cases of hearts removed from brain-death patients, immediate post transplantation function was, however, only observed in one case, with delayed function observed in the remaining three patients. It therefore seems likely that myocardial damage

was sustained before excision of the donor heart and that the organ required a period of recovery. This observation may demand further perfusate changes with the aim of preventing injury or assisting recovery. For example, the addition of myocardial substrates, both for energy production (glutamate) and DNA synthesis (ribose), may be of value.

#### 8. Air Transit of the Portable Preservation Unit

The pumping action of the portable unit was dependent upon the environmental pressure, thus changes in cabin pressure and altitude altered subsequent pump volume output. The apparatus therefore require further modification such that the pumping action is completely independent of pressure changes.

#### 13.1.2 SUMMARY OF HYPOTHESES TESTED

The table below ( table 13.1) outlines the hypotheses tested in this experimental work, mainly related to the perfusate, but also relating to:- (1) hypothermia, and (2) perfusion, as methods of preservation, (3) functional testing as a means of predicting subsequent cardiac performance following orthotopic transplantation, and (4) whether biochemical tests of myocardial viability correlated well with functional testing and orthotopic transplantation.

Although this thesis reports some progress in the field of organ preservation, it is clear that there are many problems which require further investigation.



TABLE 13.1

| CHAPTER    | HYPOTHESIS                                                                           | PERFUSATE MODIFICATION       | MAJOR OBSERVATIONS                                                                                                                                  | FUNCTIONAL TESTING (FT) AND/OR ORTHOTOPIC TRANSPLANTATION (OT) | CONCLUSION                                                                                                              |
|------------|--------------------------------------------------------------------------------------|------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|
| 3 (Soln 1) | Evaluation of hypothermic perfusion system using Krebs and Henseleit solution (K.H.) |                              | wt gain (Oedema) - 21%<br>CF - low<br>LDH & CK - high<br>ATP & CP - low                                                                             | FT : Negligible function                                       | Inadequate system and/or solution                                                                                       |
| 4 (Soln 2) | Taurine improves preservation                                                        | + taurine                    | wt gain - 23%<br>LDH - normal                                                                                                                       | FT : Improved but inadequate                                   | Taurine improved preservation. LDH release reduced                                                                      |
| 5 (Soln 3) | Effect of Hearse-based solution for 24 hours preservation                            | Multiple (Hearse et al 1976) | wt gain 33.8%<br>Op normal<br>EM-disrupted myofilaments                                                                                             | FT : Significant improvement                                   | Hearse-based solution superior to K.H. solution as perfusate                                                            |
| 6 (Soln 4) | Dextran reduces oedema formation                                                     | + dextran (4g/l)<br>-ATP     | wt gain 41.4%<br>HP - Haemorrhage and capillary damage<br>EM - Dilated T-tubules and disrupted myofilaments                                         | FT : Improvement but not significant                           | Dextran did not reduce oedema formation                                                                                 |
| 7 (Soln 5) | Dextran has no effect on oedema formation                                            | + dextran (2g/l)             | wt gain - 45.4%<br>glucose - low<br>lactate - normal<br>glycogen - low<br>ATP & CP - normal<br>lysosomes - high release<br>HP - Contraction banding | FT : No further improvement<br>OT : No survivors               | Dextran ineffective in preventing oedema formation over 24 hours preservation. Myocardium still inadequately preserved. |

| CHAPTER     | HYPOTHESIS                                                                       | PERFUSATE MODIFICATION                                                                                               | MAJOR OBSERVATIONS                                                                                                            | FUNCTIONAL TESTING (FT) AND/OR ORTHOTOPIC TRANSPLANTATION (OT)  | CONCLUSION                                                                                                                                                                                                                                                 |
|-------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 8 (Scin 6)  | Membrane stabilisers/vasodilator drugs improve preservation                      | - dextran<br>+chlorpromazine<br>+phenoxylbenzamine                                                                   | wt gain - 47.3%<br>CF - low<br>lactate - normal<br>glycogen - slightly increased<br>ATP & CP - normal                         | FT : Significant improvement<br>OT : No long term survivors     | Membrane stabilisers/vasodilators improve preservation                                                                                                                                                                                                     |
| 9 (Scin 7)  | Progressive oedema formation improves myocardial function following preservation | +Glycerol<br>Increased Na <sup>+</sup><br>Decreased K <sup>+</sup>                                                   | wt gain - 42%<br>CF - significant increase<br>LDH - high<br>ATP - normal<br>CP - low                                          | FT : Function good on modified apparatus<br>OT : 53.8% survival | Improved survival after OT. Oedema formation unchanged.                                                                                                                                                                                                    |
| 10 (Scin 8) | Reversible oedema formation improves myocardial function following preservation  | - insulin<br>- KCl<br>+ KH <sub>2</sub> PO <sub>4</sub><br>- Mg Cl <sub>2</sub><br>+ Mg SO <sub>4</sub><br>+ sucrose | wt gain - 81.3%<br>CF - normal<br>lactate - "<br>glycogen - "<br>LDH - "<br>ATP & CP - "<br>lysosomes - "<br>OP - "<br>SR - " | FT : Excellent<br>OT : Excellent (100% survival)                | Adequate preservation obtained.                                                                                                                                                                                                                            |
| 11 (Scin 8) | Evaluation of portable hypothermic perfusion system using soln 8                 | -                                                                                                                    | wt gain - 79.2%<br>CF - normal                                                                                                | OT : Excellent (100% survival)                                  | Adequate preservation obtained                                                                                                                                                                                                                             |
| 12 (Scin 8) | Clinical evaluation                                                              | -                                                                                                                    | CF - normal                                                                                                                   | Heterotopic transplantation                                     | System preserved 3 out of 4 hearts adequately, but delayed return of good function in 2 hearts; possibly related to haemodynamic instability before excision. The success of preservation in the 4th heart was difficult to assess due to early rejection. |

Abbreviations:-

OP = oxidative phosphorylation  
HP = histopathology  
EM = electron microscopy

## APPENDIX 1

### THE MITOCHONDRION

The mitochondrion is a membrane bound organelle that lies free within the cytoplasmic matrix. Its most important functions are providing the cell with widely used energy compounds, adenosine triphosphate (ATP) and controlling the level of calcium ions in the cytoplasmic matrix. In cultured cells observed with phase contrast optics the mitochondria are seen to be actively mobile and display a perpetual wringing and twisting motion. However, in cells with a high degree of internal organisation such as striated muscle and distal convoluted tubule of the nephron they tend to be restricted within cytoplasmic compartments immediately adjacent to the site of high energy demands.

Mitochondria vary in shape from spherical to thread-like forms. The internal organisation consists of two inner compartment separated by a convoluted inner membrane, called the cristae. The density of a cristae within a cell reflects the overall energy demand of the cell. For example, in cardiac muscle the ATP requirement for myofibrillar contraction is high, the cristae are large and closely packed. The outer mitochondrial membrane which forms the boundary to the intra mitochondrial matrix is particularly impermeable. The transport of ions across this inner barrier thus requires specific energy dependent mechanisms. The intra mitochondrial matrix, which lies within the inner mitochondrial membrane usually has fine fibrillar structure and also contains variable numbers of dense so-called intramitochondrial particules. The matrix also contains ribosomes and fine strands of DNA.

Muscle cells obtain the major component of their energy requirements via respiration, the transfer of electrons from organic fuel molecules to molecular oxygen. The rest of respiration in the cell is the mitochondrion which contains the enzymes and co-factors required for the tricarboxylic acid (TCA) cycle. Pairs of electrons derived from the intermediates of the TCA cycle flow down a complex chain of electron-carrier enzymes of success-fully lower energy level until they reduce molecular oxygen, the final acceptor. During this process the free energy of the electron is converted in the form of the phosphate-bond energy of ATP.

The exact mechanism of oxidative phosphorylation is a subject of much controversy (Boyer et al 1977). The most plausible explanation has been given by Mitchell (1979) in which he postulates that a chemi-osmotic proton gradient generated by the active transport of protons across the inner mitochondrial membrane by the  $F_1F_0$  ATP synthase with the concomitant synthesis of ATP. As these processes are vectorial the requirements for an intact membrane system is essential.

## APPENDIX 2

### DYNAMICS AND ADVANTAGES OF THE MODIFIED FUNCTIONAL TESTING APPARATUS

The functional testing apparatus was modified with the intention of subjecting the working heart to a more physiological state than hearts tested in an earlier series of this study. The plastic tubing of the apparatus was rigid and only the windkessel was compliant. There was negligible elasticity in the circuit as the compliance was extremely low due to the rigid polyvinyl chloride tubing (figure 2.5).

In a series of pilot experiments, use was made of a mechanical pump with an adjustable stroke volume to evaluate the elastic response of various balloons. The balloon was used to mimic the elasticity (Elastance) of an aorta. For control values a pig's ascending aorta (12 cms long) was also evaluated. The pump was set at a constant rate and only the balloon and aortic pressures were measured after 5ml increments in loading volume. The pressures and diameters of both the balloon and aorta were measured (figure 1). The aorta and the chosen balloon had a compliance curve that was similar.

The non linear peripheral resistance consisted of a similar type and length of balloon. The balloon was compressed by the use of a cuff from a mercury manometer, exerting a constant pressure of 73 mm Hg over the entire surface area of the balloon. With each heart beat a fixed volume of fluid would inflate the compressed balloon at one end extending this progressively to the other end after which time the balloon deflates again.

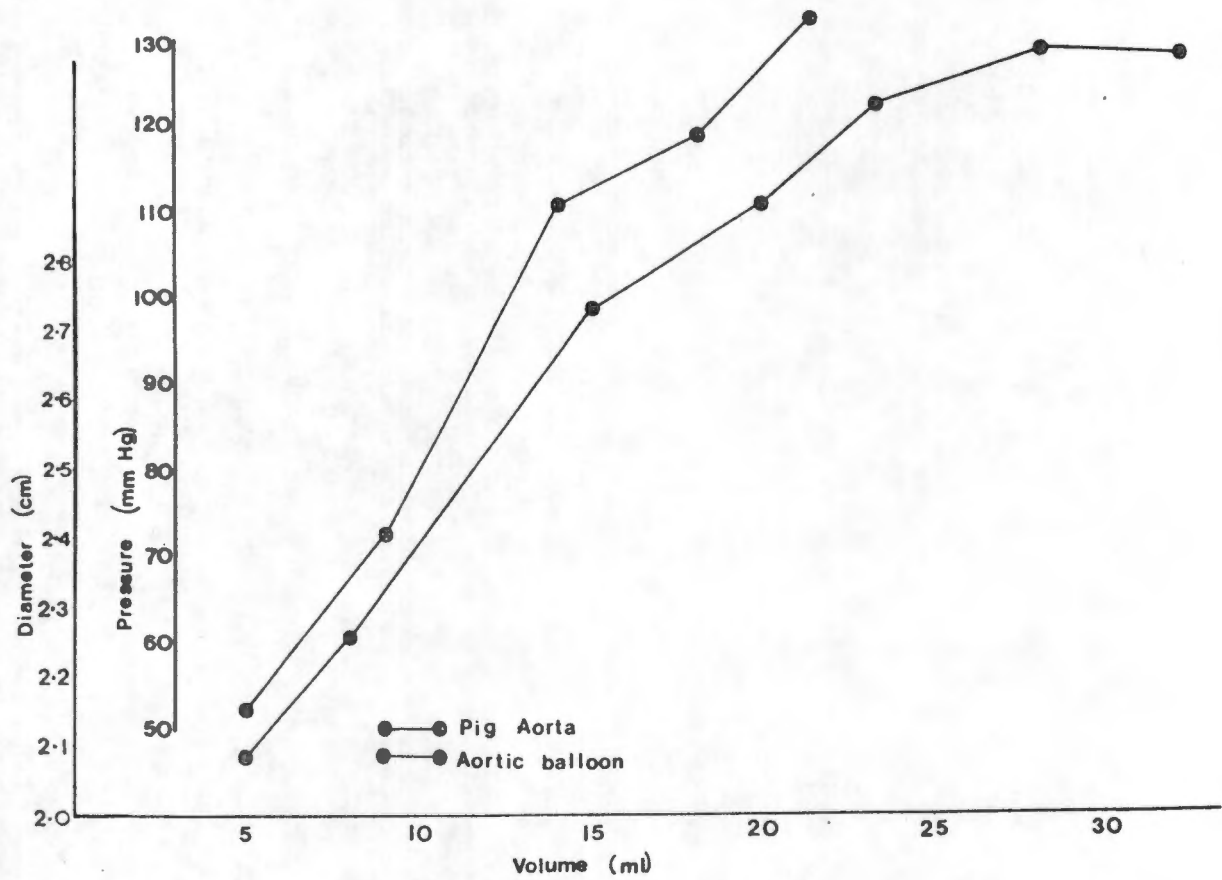
The advantages of the modified system were as follows:

The balloon had an acceptable compliance curve.

The elasticity of the apparatus provided a better myocardial diastolic perfusion due to local storage of energy in the elastic aortic balloon.

The aortic balloon and peripheral resistance together damped and prevented pressure overshoots resulting from the rigid *ex vivo* circuit.

Massage of the balloon provided sufficient pressure to the heart during Langendorff perfusion, if the perfusor pig was unable to maintain the pressure of the circuit.



Appendix 2

Fig.1.0 Compliance curve of an isolated pig's aorta and an elastic balloon. For details see text.

### APPENDIX 3

The lysosomal experimental procedures are discussed in detail in the text. These individual observations below (raw data) are given in order to clarify any conceptual complications arising from the text.

The total free acid phosphatase activity of each fraction was calculated by multiplying the measured optical density by the respective dilution factor.

The protein concentration of every sample in each fraction was then determined and the acid phosphatase activity expressed as activity/mg protein. (Tables 1, 2 and 3). From these activities the mean value was determined and subsequently used in the text. (Chapters 7 and 10).



THE TOTAL FREE ACID PHOSPHATASE ACTIVITIES AND PROTEIN  
CONCENTRATIONS IN FRESHLY ISOLATED CONTROL HEARTS.

GROUP A

| PARTICULATE                              |                  |                        |                                          | SUPERNATANT      |                        |                                          | NUCLEAR                                  |                  |                        |
|------------------------------------------|------------------|------------------------|------------------------------------------|------------------|------------------------|------------------------------------------|------------------------------------------|------------------|------------------------|
| Activity<br>units/L<br>( $\times 10^4$ ) | mg protein<br>ml | Activity<br>mg protein | Activity<br>units/L<br>( $\times 10^4$ ) | mg protein<br>ml | Activity<br>mg protein | Activity<br>units/L<br>( $\times 10^4$ ) | Activity<br>units/L<br>( $\times 10^4$ ) | mg protein<br>ml | Activity<br>mg protein |
| .1070                                    | 15.5             | 0.069                  | 14.63                                    | 6.5              | 22.4                   | 3.540                                    | 3.540                                    | 8.51             | 4.1                    |
| .1900                                    | 17.2             | 0.110                  | 15.00                                    | 4.1              | 36.6                   | 5.249                                    | 5.249                                    | 3.3              | 16.1                   |
| .0170                                    | 17.0             | 0.222                  | 27.57                                    | 2.5              | 110.3                  | 4.128                                    | 4.128                                    | 8.6              | 4.8                    |
| .3612                                    | 17.2             | 0.210                  | 24.00                                    | 5.0              | 48.0                   | 4.040                                    | 4.040                                    | 10.1             | 4.0                    |
| .0280                                    | 14.0             | 0.020                  | 23.70                                    | 5.0              | 47.4                   | 4.092                                    | 4.092                                    | 6.6              | 6.2                    |
| .1288                                    | 16.1             | 0.080                  | 25.37                                    | 6.1              | 41.6                   | 3.852                                    | 3.852                                    | 10.7             | 3.6                    |
| .4598                                    | 24.2             | 0.190                  | 39.15                                    | 4.9              | 79.9                   | 7.857                                    | 7.857                                    | 8.1              | 9.7                    |
| .1764                                    | 25.2             | 0.070                  | 39.31                                    | 5.6              | 70.2                   | 7.820                                    | 7.820                                    | 9.0              | 8.8                    |

Free lysosomal acid phosphatase activities of three differentially isolated fractions (particulate, supernatant and nuclear) expressed in the final form of activity/mg protein. These fractions were isolated from freshly arrested control hearts (Group A).

APPENDIX 3. Table 1.

THE TOTAL FREE ACID PHOSPHATASE ACTIVITIES AND PROTEIN CONCENTRATIONS  
IN HEARTS PRESERVED BY HYPOTHERMIC PERFUSION FOR 24 HOURS.

GROUP B

| PARTICULATE                                |                  |                        | SUPERNATANT                                |                  |                        | NUCLEAR                                    |                  |                        |
|--------------------------------------------|------------------|------------------------|--------------------------------------------|------------------|------------------------|--------------------------------------------|------------------|------------------------|
| Activity<br>units/L<br>(X10 <sup>4</sup> ) | mg protein<br>ml | Activity<br>mg protein | Activity<br>units/L<br>(X10 <sup>4</sup> ) | mg protein<br>ml | Activity<br>mg protein | Activity<br>units/L<br>(X10 <sup>4</sup> ) | mg protein<br>ml | Activity<br>mg protein |
| .0666                                      | 3.0              | 0.222                  | 19.30                                      | 0.5              | 386.1                  | 4.216                                      | 0.4              | 105.4                  |
| .2927                                      | 2.8              | 1.046                  | 17.99                                      | 0.5              | 359.8                  | 3.940                                      | 0.5              | 78.0                   |
| .1197                                      | 3.0              | 0.399                  | 18.21                                      | 0.6              | 303.6                  | 4.060                                      | 0.5              | 81.2                   |
| .0929                                      | 2.6              | 0.357                  | 18.52                                      | 0.4              | 463.0                  | 4.008                                      | 0.6              | 66.8                   |
| .0935                                      | 3.5              | 0.267                  | 24.90                                      | 1.9              | 131.1                  | 6.976                                      | 0.8              | 87.2                   |
| .2244                                      | 6.6              | 0.340                  | 29.40                                      | 2.0              | 147.0                  | 3.264                                      | 0.6              | 54.4                   |

Free lysosomal acid phosphatase activities of three differentially isolated fractions (particulate, supernatant and nuclear) expressed in the final form of activity/mg protein. These fractions were isolated from hearts preserved for 24 hours by hypothermic perfusion (Group B).

THE TOTAL FREE ACID PHOSPHATASE ACTIVITIES AND PROTEIN CONCENTRATIONS IN HEARTS PRESERVED BY  
HYPOTHERMIC PERFUSION AND SUBSEQUENTLY REPERFUSED FOR ONE HOUR AT NORMOTHERMIA.

GROUP C

| PARTICULATE                                |                  |                        |                                            | SUPERNATANT      |                        |                                            |                  | NUCLEAR                                    |                  |                        |
|--------------------------------------------|------------------|------------------------|--------------------------------------------|------------------|------------------------|--------------------------------------------|------------------|--------------------------------------------|------------------|------------------------|
| Activity<br>units/L<br>(X10 <sup>4</sup> ) | mg protein<br>ml | Activity<br>mg protein | Activity<br>units/L<br>(X10 <sup>4</sup> ) | mg protein<br>ml | Activity<br>mg protein | Activity<br>units/L<br>(X10 <sup>4</sup> ) | mg protein<br>ml | Activity<br>units/L<br>(X10 <sup>4</sup> ) | mg protein<br>ml | Activity<br>mg protein |
| .1859                                      | 11.0             | 0.169                  | 16.80                                      | 3.5              | 48.0                   | 3.227                                      | 3.5              | 9.2                                        |                  |                        |
| .2533                                      | 8.5              | 0.298                  | 19.75                                      | 2.5              | 79.0                   | 3.380                                      | 1.0              | 33.8                                       |                  |                        |
| .1197                                      | 7.0              | 0.171                  | 16.15                                      | 1.0              | 161.5                  | 2.655                                      | 1.5              | 17.7                                       |                  |                        |
| .1735                                      | 5.0              | 0.347                  | 15.60                                      | 3.0              | 52.0                   | 3.096                                      | 1.8              | 17.2                                       |                  |                        |
| .1065                                      | 5.0              | 0.213                  | 14.63                                      | 3.5              | 41.8                   | 3.864                                      | 4.0              | 9.6                                        |                  |                        |
| .1470                                      | 10.0             | 0.147                  | 14.25                                      | 5.0              | 28.5                   | 4.000                                      | 4.0              | 10.0                                       |                  |                        |
| .1200                                      | 15.0             | 0.080                  | 19.60                                      | 2.5              | 78.4                   | 3.314                                      | 3.5              | 9.4                                        |                  |                        |
| .0663                                      | 8.5              | 0.078                  | 18.90                                      | 3.5              | 54.0                   | 3.300                                      | 2.0              | 16.5                                       |                  |                        |
| .0927                                      | 9.0              | 0.103                  | 11.62                                      | 3.4              | 34.2                   | 4.816                                      | 4.3              | 11.2                                       |                  |                        |
| .1072                                      | 9.4              | 0.114                  | 2.550                                      | 0.6              | 42.5                   | 3.136                                      | 4.0              | 7.8                                        |                  |                        |
| .1467                                      | 9.0              | 0.163                  | 17.19                                      | 4.5              | 38.2                   | 3.680                                      | 2.0              | 18.4                                       |                  |                        |
| .1617                                      | 7.0              | 0.231                  | 17.40                                      | 1.5              | 116.0                  | 3.820                                      | 2.0              | 19.1                                       |                  |                        |

Free lysosomal acid phosphatase activities of three differentially isolated fractions (particulate, supernatant and nuclear) expressed in the final form of activity/mg protein. These fractions were isolated from hearts preserved for 24 hours followed by one hour warm blood reperfusion (Group C).

APPENDIX 3. Table 3.

APPENDIX 4

In order to briefly assess various perfusate constituents, screening tests were performed on preserved hearts using 2 hearts in each group.

The parameter measured was cardiac output, and the hearts preserved in the solution yielding the highest cardiac output were suggestive of which perfusate to choose for future preservation and possible further modification.

| SOLUTION | MODIFICATION (Addition or removal) | n | Cardiac Output |
|----------|------------------------------------|---|----------------|
| 2        | 2mM taurine                        | 2 | 539            |
|          | 4mM taurine                        | 2 | 588            |
| 6        | 0mM calcium                        | 2 | -              |
|          | 2.25mM calcium                     | 2 | 1655           |
|          | 1.10mM calcium                     | 2 | 1729           |
|          | 0g/L chlorpromazine                | 2 | 1995           |
|          | .005g/L chlorpromazine             | 2 | 2419           |
|          | .020g/L chlorpromazine             | 2 | 1965           |
|          | 0g/L Phenoxybenzamine              | 2 | 2396           |
|          | 0.01g/L Phenoxybenzamine           | 2 | 2653           |
|          | 0.02g/L Phenoxybenzamine           | 2 | 2447           |
| 7        | 136mM Glycerol                     | 2 | 2789           |
|          | 272mM Glycerol                     | 2 | 2625           |

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