# THE DEVELOPMENT AND STUDY OF AUTOLOGOUS LUNG OXYGENATION FOR CARDIAC SURGERY- THE DOUBLE RESERVOIR TECHNIQUE

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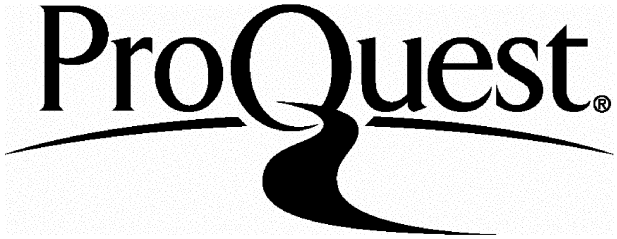


ABSTRACT

Cardiopulmonary bypass for closed heart operations has been steadily improving over the last forty years. It is still associated with a significant morbidity and is capable of further design improvement. Autologous lung oxygenation was a technique available in the early days of cardiac surgery but was soon eclipsed by the development of external oxygenators. There are however many inherent advantages associated with the use of the patients own lungs and this study has tried to reevaluate its role. The project started to look at existing levels of knowledge and then to use modern materials and methods to improve the old system. The study has operated at a variety of levels. Firstly, to show that the method could be used safely in a clinical setting; this involved the testing of a variety of cannulae, tubing materials, prime solutions and of perfusion techniques. Secondly, to show that efficient gas exchange was possible using the system. Finally, the study has looked at the effects of using autologous lung oxygenation on the "quality" of the bypass. Measurements of numbers of circulating

gaseous microemboli, the platelet function, the level of complement and other humoral cascades have all been looked at. The conclusions are that this technique produces significantly fewer microemboli than conventional bypass, that platelet handling is as good as membrane oxygenators but that complement activation is lower with autologous oxygenation. The study has not been directed to answering the question as to whether this is clinically significant and further, whether its clinical use should be encouraged. The study concludes with a look at the many questions that remain unanswered and attempts to indicate the direction of future investigations.

CONTENTS

DIRECTORY OF PLATES, FIGURES AND TABLES

PLATES



FIGURES

FIGURES CONTD.

TABLES

## AIM OF THE PROJECT

The aim of this project has been to explore and develop the double reservoir technique as an alternative method of cardiac bypass; to study and compare its effects with those of conventional bypass.

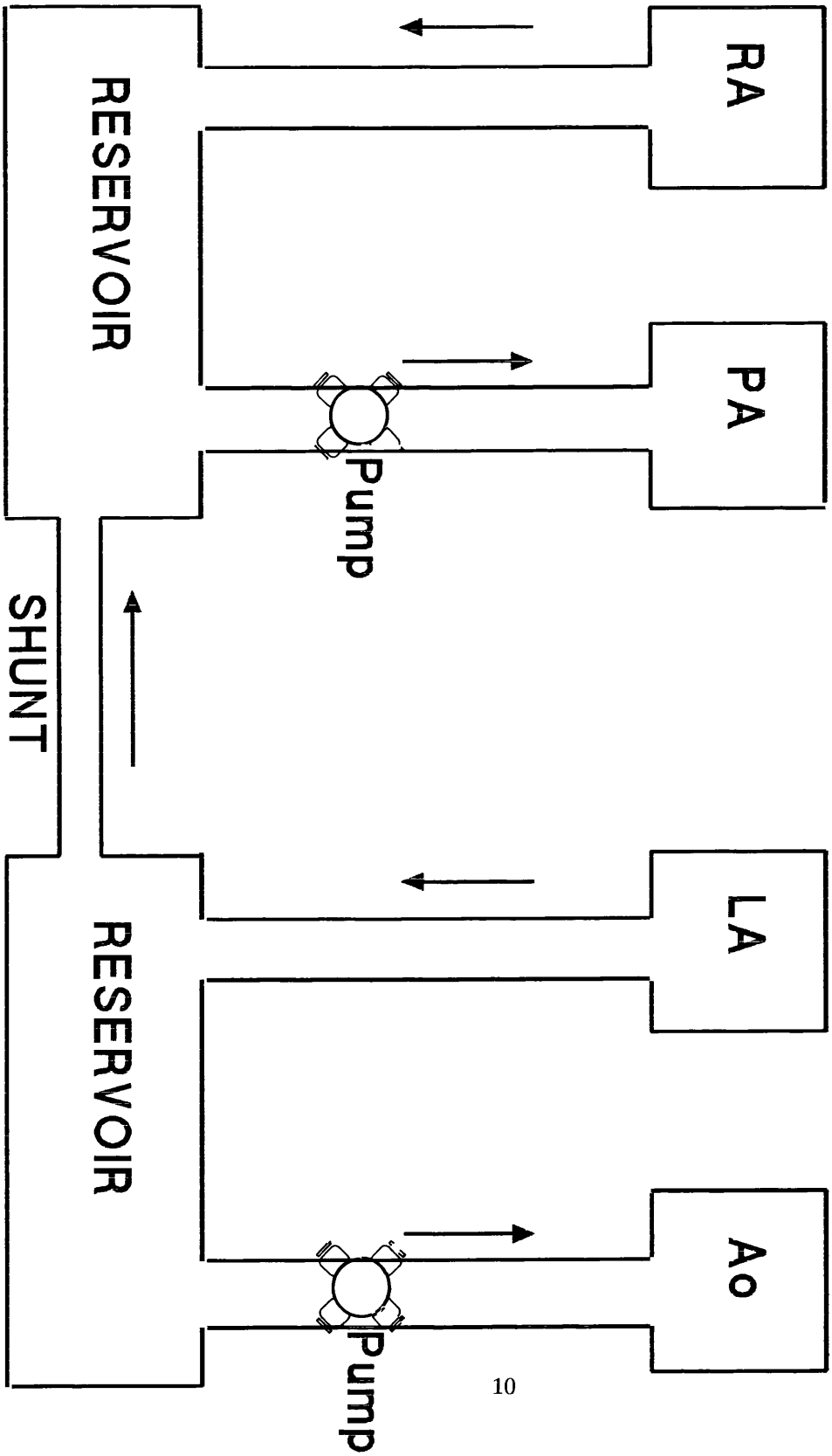
## DOUBLE RESERVOIR / AUTOLOGOUS LUNG OXYGENATION - DEFINITIONS

The double reservoir technique uses pumping equipment for the bypass of the cardiac chambers to allow surgery to be undertaken and uses the patient's own lungs to oxygenate the blood. The term "double reservoir" refers to the fact that there are two distinct circuits used; one for the right side of the heart and the other for the left (Fig. 1). Blood is taken from the right atrium and delivered to the lungs for oxygenation via the pulmonary artery. The oxygenated blood is then collected from the left atrium and pumped into the aorta. This obviously differs from conventional bypass which drains blood from the right atrium and pumps it via an artificial external oxygenator into the aorta.

The term " autologous lung oxygenation " indicates that the source for gas exchange is the patient's own lungs and that an external oxygenator is not required.

Figure 1. THE DOUBLE RESERVOIR CIRCUIT

RA = RIGHT ATRIUM PA = PULMONARY ARTERY LA = LEFT ATRIUM Ao = AORTA



# INTRODUCTION

One of the trends in perfusion for cardiac surgery has been to improve the current oxygenator technology to reduce many of its perceived problems. It's improvement in the ease of use both for the surgeon and the perfusionist has been dramatic, but of at least equal importance is the effect of the bypass on the patient.

The question must be asked why after around forty years with the external oxygenator, with its evolution through disc systems, bubble oxygenation, membranes interfaces, hollow fibres etc. should one want to look again at an old technique that with notable exceptions (Drew et al 1959), fell into disuse?

There are at least four different aspects to the answer (Table 1). Firstly, there has been a major change in the type of cardiac surgery undertaken particularly in the last twenty years; the number of closed heart operations, particularly coronary artery bypass grafts performed is around 75% of all major cardiac

procedures. The double reservoir, unless it is to be used only to provide profound hypothermia for circulatory arrest (Drew et al 1959), is restricted to closed cardiac procedures. In the early days of the development of the oxygenator, there were no coronary grafts and almost no closed procedures; therefore it is only recently that the technique could have widespread appeal.

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Table 1

Justification for renewing interest in double reservoir.

1 Change in surgery from predominantly open heart surgery for valvular and congenital conditions to majority of operations being closed procedures for ischaemic heart disease.

2 Critical evaluation of external oxygenators with increasing knowledge of pathophysiology and measurement of morbidity.

3 Using the knowledge from 2 to see potential advantages with the double reservoir system.

4 The double reservoir studies could improve our knowledge of conventional bypass effects and provide a unique experimental model.

The second and third reasons are linked in that there is currently critical evaluation with even the newer oxygenators and realisation that they do not universally provide a "safe bypass" and that the double reservoir may provide the opportunity to improve the quality of the bypass. Journals throughout the world are full of increasing evidence both experimentally and clinically of the pathophysiology of cardiopulmonary bypass and the morbidity that is associated with it. Finally, the techniques of the double reservoir allow its use as an experimental model to study metabolic functions of the lungs and to evaluate types of materials for bypass. That conventional oxygenators are a difficult medium to use for the testing of equipment has been previously demonstrated (Colson et al 1987).

Clearly, there is with all techniques a risk benefit equation that must be evaluated. The potential disadvantages seem to relate principally to the execution of the bypass by the perfusionist, by the anaesthetist and by the surgeon. The potential disadvantages may be seen in Table 2 but are discussed below and in more detail

in the clinical chapters. Having used the system in an animal model and in a clinical enviroment, most of the list of disadvantage can

Table 2

Potential Disadvantages of the Double Reservoir System

| |
|---|
| Anaesthetic Considerations |
| Perfusion skills |
| Potential to entrain air |
| Extra cannulation sites |
| Limited mobility of the heart |
| Interference from lung movements |
| Need to provide emergency bypass |
| Time considerations |

be countered. They are however always the first questions to be asked about the system and some of them are real disadvantages that must be overcome by training, care and practice.

Anaesthetic considerations include decisions of the choice of gases and the risks of gases coming out of solution; this can be

overcome by avoiding nitrous oxide in the peribypass period. Other factors include the need for good gas exchange with low tidal volumes; again in practice it is surprising how good levels of oxygenation and carbon dioxide removal can be achieved.

The perfusionist who is not familiar with the double reservoir technique may at first be daunted by it. The fact that there are two pumps looks difficult but the presence of a shunt between the two reservoirs makes balancing straightforward. The ability to pump air is obviously also present but can easily be overcome by level sensors or collapsing boots as well as by vigilance. A skilled perfusionist will soon realise the flexibility that the system affords especially weaning from bypass.

The potential to entrain air occurs not just at the perfusion level but more particularly with the surgeon. The most important site to consider is the left atrial cannulation site and again this can be overcome with existing techniques and cannulae with training; this is discussed in more detail in the clinical chapters.

That there are more cannulation sites is of itself a slight danger. In turn the right atrial cannulation is identical and can be single or double cannulation according to existing preference. The pulmonary artery cannulation is often new to surgeons but is identical to the aortic site (except at a lower pressure). The left atrial cannulation is skilled and is potentially the most dangerous part of the double reservoir technique and requires immense care. The aortic cannulation is identical to normal practice. Decannulation is also routine although there are additional sites to check prior to closure of the chest.

Concern has been expressed that with the multiple cannulae in place that lifting the heart for a circumflex graft would be much more difficult. This is not borne out in practice and mobility is identical to normal practice. The movement of the lungs are also not a problem in that the tidal volumes are so small that the concerns tend to be the surgeon checking that the patient is indeed being ventilated.

All of these potential problems are easily overcome when the patient is stable and there is no time pressure on the team. The procedure does need modification when bypass needs to be established in a hurry. There are two distinct approaches to this; one involves attaching a conventional oxygenator to the circuit (the design of the equipment allows for this to be done quickly). The other is to establish right atrial drainage and aortic return, establish partial bypass and allow some blood to pass through the lungs for oxygenation. The technique which will be used is surgical preference, but the strategy needs to be discussed in advance.

Finally, it might sound from the formidable list of potential problems, that the technique would take an inordinate amount of time. The best answer to that is in the times actually taken in the clinical study which includes our learning curve; the times for the procedure were identical to conventional bypass following adherence to the protocol outlined in the clinical chapters.
The advantages can be thought of in two ways; to see what

advantages are unique to the system and also to compare it with the best conventional equipment available. This study has not delineated every aspect of the technique and cannot answer all of the questions; it does however postulate what advantages may expect to accrue from its use. These are detailed in Table 3.

Table 3

Potential advantages of the double reservoir technique

| |
|---|
| Low rates of haemolysis |
| Low rates of gaseous microemboli |
| Low rates of complement activation with reduced pulmonary dysfunction |
| Platelet function preservation and normal coagulation |
| Preservation of metabolic functions of the lung during bypass |
| Elegant weaning for poor ventricular function |
| Built-in ability for long term assist |
| Easy system for heparin coating |
| Cost saving over membrane oxygenation |

Low rates of haemolysis have been demonstrated with the double reservoir technique and even extend over a 24 hour period as shown in the animal studies chapter. These rates indicate that good occlusion settings on conventional roller pumps are not traumatic to blood. The use of centrifugal pumps may improve

things still further, although this has not been demonstrated in this study. Low rates of gaseous microemboli have again been shown compared with any external oxygenator. The reasons for this will be discussed later, but may include not only less generation of the bubbles but also their preferential flow into the pulmonary rather than systemic circulations. The complement cascades have again been shown to have a lower circulating level than with membrane oxygenation. This may be because actual generation is less or that because the lungs are in circuit, they can "neutralise" their effects. Further studies need to be performed to elucidate this, but elastase levels are dramatically lower for the double reservoir system. This should be translated into fewer pulmonary complications but no studies have been undertaken to prove this.

Platelet counts and function are dramatically better with membrane oxygenator rather than their bubble predecessors. Studies with the double reservoir could not differentiate between it and membrane oxygenation. It is increasingly realised that the

lung has important metabolic functions as well as its gas exchange function. The double reservoir method does not render the lungs ischaemic during the bypass and this may for instance allow the homeostatic mechanisms for prostaglandin/ thromboxane levels to remain intact (Faynmonville et al 1986).

Perfusion aspects have shown great flexibility for the double reservoir technique. Weaning of patients with poor ventricular function can be achieved in an elegant manner by differentially weaning right and left sided bypass. This weaning can also be extended if necessary over many hours without major haematological consequences, in contradistinction to many external oxygenators. In particular, the simple surface without any foreign meshes also would allow heparin coating which may afford further advantages. Cost is not the most important decider for the choice of an oxygenator although it is obviously important; the double reservoir system is by its nature cheaper than sophisticated oxygenators. To show that it provides a high quality bypass would be welcome in tandem with cost reduction.

This can be compared with the state of knowledge of conventional cardiopulmonary bypass; naturally some of this morbidity will be shared with the double reservoir technique. The information available about cardiopulmonary bypass is however crucial to the understanding and comparison of alternative systems.

## CONVENTIONAL CARDIOPULMONARY BYPASS - MORBIDITY

The morbidity of cardiopulmonary bypass takes many forms and research has been directed at the haemolysis of blood, damage to other elements such as platelet counts and function, to the effects of circulating gaseous microemboli, complement activation, prostacyclin imbalances, pulmonary and neurological dysfunction as well as many other aspects. It is important to focus on some of the known effects at this stage, to later put in perspective the results achievable with the double reservoir technique.

The majority of operations performed using conventional cardiopulmonary bypass proceed uneventfully and grossly at least, the patients appear not to have any deleterious effects. However, this is not true in all patients and there are an increasing number of studies being produced to show that, for instance, the psychoneurological impairment is more severe and affects more people than was at first thought (Bojar et al 1983, Aberg et al

1983, Sloggoff et al 1982, Shaw et al 1984). There are obviously some "risk" factors that increase the likelihood of problems. The duration of bypass is certainly important and the probability of structural or functional damage seems to increase as the perfusion period extends beyond three hours (Kirklin et al 1983). The age of the patient is also important and studies from the same unit in Birmingham, Alabama (Kirklin et al 1981) indicate that the damage increases in patients under six months of age and even more so under three months. The other factors include the type of oxygenator and type of pump used, the perfusion flow rates achieved, temperature considerations and the amount of cardiotomy suction employed. The flow rates in the microcirculation are of equal importance (Kirklin et al 1987) and poor microperfusion can prevent neutralisation of the deleterious effects of the inflammatory mediators. These problems may manifest themselves in a variety of ways which range from the subtle to the gross; there may an increased tendency to bleed postoperatively, the patient may retain a high systemic vascular resistance and thus be poorly perfused or there may be pulmonary,

neurological or psychological impairment which may be permanent or temporary.

The exposure of the blood to an unphysiological surface can affect any of its constituents; viz. the red blood cells, the platelets, the white cells and other unformed elements including the plasma proteins. These in turn, when part of the various amplification systems i.e. complement, fibrinolytic, coagulation, kallikrein and bradykinins can affect body organs at the cellular level. It is thought that the damage produced is greater the larger the surface of blood / foreign material interface and therefore one would expect the lack of an oxygenator to have major benefits since the surface area is reduced at least five-fold. Heparin coated equipment certainly reduces complement activation (Nilsson et al 1990a) although this may be due to a reduction in the amount of heparin administered. The more complex the membrane structure, the more difficult it is to coat with heparin; this is unlike the simple structure of the double reservoir design.

The damage to the erythrocytes is largely caused as a result of the shear stresses which is unlikely to be much altered using the double reservoir system. There is some evidence (Solen et al 1978) that some damage however is caused by exposure to a foreign surface. The damage can to some extent be measured by an estimation of the plasma free haemoglobin levels, which result from the haemoglobin released by damaged red cells. Haemoglobin thus released, is initially carried in a protein-bound form and then as free unbound haemoglobin as plasma binding capacity is exceeded (Lathem and Worley 1959). Damage can occur as a result of the gas/blood interface and in bubble oxygenators, large gas flows through small orifices may cause shear stresses in bubble formation resulting in haemolysis (Ferbers et al 1958). Even in membrane oxygenators, it has been shown (Ward et al 1974) that microbubbles of air have a strong tendency to cling to the membrane surface so that the surface is more complicated than expected. The damage that occurs not as a result of the oxygenator but with the cardiotomy suction system should not be forgotten (de Jong et al 1980). One more source of

haemolysis, is from the oxygen radicals released as a result of neutrophil activation (Vercellotti et al 1985), further demonstrating the interaction between many of the different blood elements.

The platelet involvement is more complex to explain and to measure the effects. It is seen that platelet numbers decline during cardiopulmonary bypass (Kalter et al 1979) and also that their function is impaired (Born 1984). Part of the decrease in numbers and function is due to the fact that as a result of foreign material exposure, the platelets form clumps which adhere to the materials in the oxygenator and also in the lungs. However studies have shown (Hope et al 1981) that with high flows, the numbers of adherent platelets to the biomaterials are not high. One way to measure the ability of platelets to function is to measure their ability to clump with a given stimulus. This has been shown (Born 1984) to occur using ADP to cause the stimulus for platelets to aggregate, and he demonstrated a significant deterioration during cardiopulmonary bypass. Numerous studies

have demonstrated the drop in platelet numbers (Kalter et al 1979, de Leval et al 1972) and others (Addonizio et al 1978, Longmore et al 1979, Radegran and Papaconstantinou 1980) have tried to reduce the damage by infusions of prostacyclins to reduce their ability to clump. The study by Longmore showed that not only was a heparin prostacyclin combination better for platelet counts and function than heparin alone, but also that there was much less fibrinogen consumption and little in the way of arterial filter deposition. Addonizio showed that the reduction in the numbers of platelets circulating that have good function may well be the most important factor in postoperative bleeding and that preservation with PGE1 would actually shorten the postoperative bleeding times. That the platelets are also involved in the humoral amplification and their degradation involve tissue damage has since been demonstrated in many studies (Niewiarowski et al 1981, Martin et al 1978, Polley and Nachman 1979,1983 and Chesney et al 1972).

The damage that occurs as part of the humoral amplification is

cannot be simply explained, not only because each system is still poorly understood but also because the various parts interact with each other. The initiating event appears to be the meeting of blood with a foreign albeit so-called biocompatible material. Much of the early work in this field, was undertaken in haemodialysis patients; it was noted (Kaplow and Goffinet 1968) that the white cell counts were very low soon after starting haemodialysis. It was thought this was probably due to sequestration onto the cellophane membrane but later work, showed a more important site of sequestration to be the lung. Craddock (Craddock et al 1977) observed the high incidence of pulmonary dysfunction in patients undergoing haemodialysis and queried the possibility of the plugging of pulmonary vessels by white cells, linking this white cell sequestration with complement activation. This proved to be the start of a fundamental understanding for the complement role in cardiopulmonary bypass and has subsequently been repeated and amplified by other papers (Howard et al 1988). In this latter study, lung biopsy specimens showed trapping of polymorphonuclear leucocytes and helped

explain the higher white cell counts recorded from the right atrium when compared with the left. Although subsequently seen to be less important, it was shown (Fehr and Jacob 1977) that the white cells would non-specifically adhere to plastic as well as the pulmonary bed. This has been put beautifully into perspective with later observations on the mechanisms involved in a review by one of the early workers in the field (Jacob 1983).

Complement is activated by a wide variety of stimuli; in response to antigen challenge, injury or foreign-body exposure. There are two pathways for complement activation, the classical pathway and the alternate pathway (Fig. 2). The classical pathway can be activated by a variety of activators including bacterial and viral surfaces (Loos et al 1978 and Cooper 1985) or antigen-antibody interaction with C1. The alternate pathway is activated by foreign materials when C3b is bound to an activating surface (Pangburn and Muller 1980). Many of the manoeuvres we perform not associated with the bypass, will activate complement, including the induction of anaesthesia (Kutsal et al 1989). It is important not

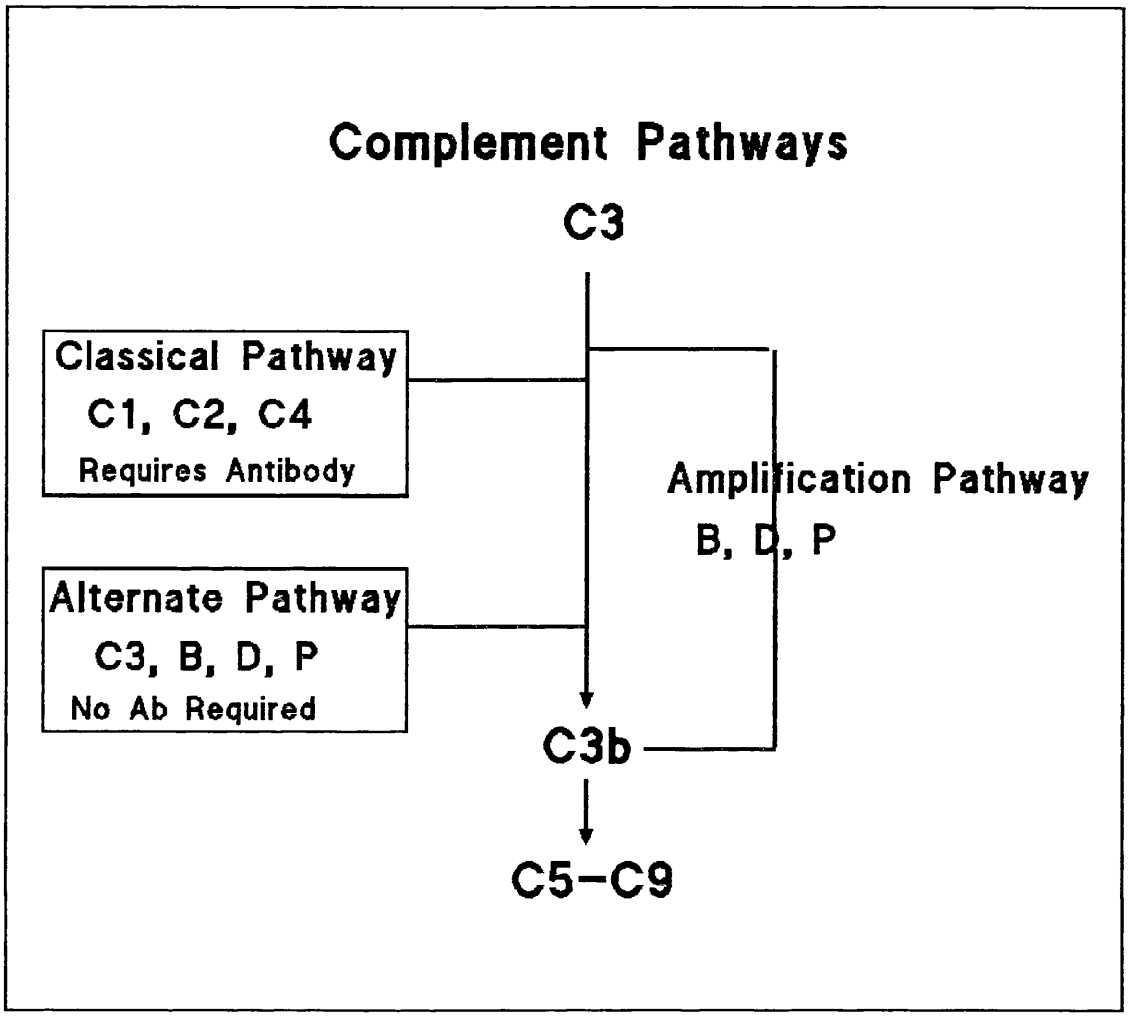



Figure 2

to study just one element of the complement pathway because this can easily lead to false conclusions. This was shown in a comparison of bypass and non bypass operations (Fosse et al 1987a). They showed that nonbypass operations e.g. abdominal aneurysmectomy could stimulate C3dg production although there were no granulocyte changes; they concluded that the bypass

activates the terminal pathways of complement and assays detecting initial and terminal parts should be included.

The administration of heparin itself may activate both arms of the complement cascade (Bonser et al 1990a) as can protamine at the end of bypass (Weiler et al 1990). The final product of complement activation, following its now well documented cascade is a complex of glycoproteins C5 to C9; these can bind to cell membranes causing lysis and the production of powerful anaphylatoxins called C3a and C5a (Fig. 3). The term anaphylatoxins was first used as long ago as 1910 by Friedberger and later studies showed that these can cause further enzymatic release, more white cell aggregation, platelet effects and an increase in vascular permeability (Grant et al 1975, Goldstein et al 1973 and Virella et al 1983). These two studies showed, that this could be mediated by triggering selective lysosomal enzyme release, particularly from basophils in the case of histamine release. It was also demonstrated that the effects resulted mainly from the activation of the alternate complement pathway.

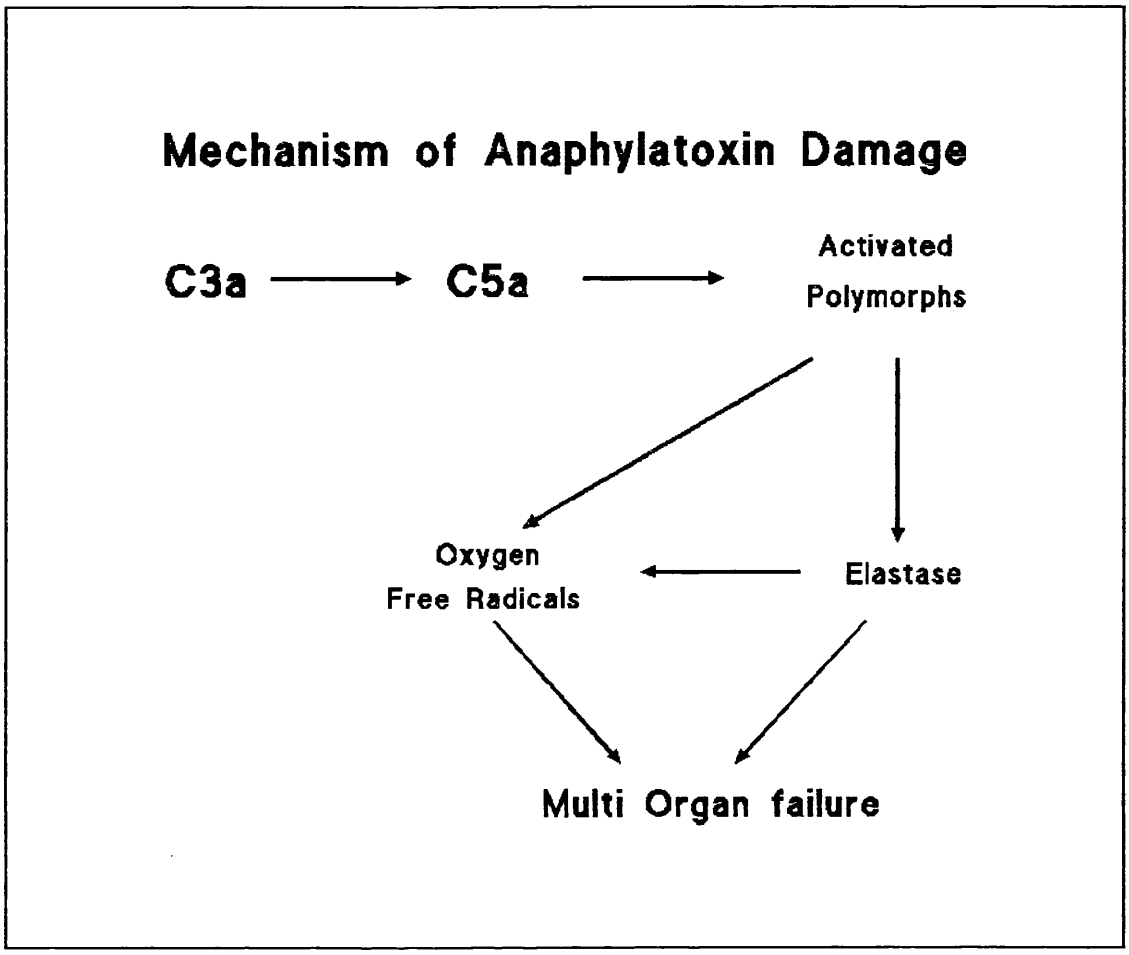



Figure 3

In 1981, two studies were published looking at complement activation in patients during cardiopulmonary bypass rather than haemodialysis (Chenoweth et al 1981 and Hammerschmidt et al 1981). Chenoweth measured complement activation in 15 patients and showed that C3a increased at the beginning of cardiopulmonary bypass and continued to increase. Significant

transpulmonary neutropaenia was seen. They used radioimmunoassays to follow events but failed to detect significant C5a levels. It was subsequently determined that the binding of C5a to white cell membranes was so high that there was little circulating that could be measured. Hammerschmidt and workers postulated in their own study, that the neutropaenia resulted from complement activation and that this may be the cause of postperfusion lung. Animal studies (Bando et al 1990) have shown that lung injury can be actually be reduced by depleting circulating white cells using a leucocyte filter in the bypass circuit. Leucocyte counts were depleted by 97% as compared to 60% of the controls. Free radical generation was significantly reduced in the depleted group, lending more support to the theory of lung damage.

The next step in the link between complement activation and pulmonary damage, was to prove that there is lysosomal enzyme release during cardiopulmonary bypass and its origin. Two successive studies (Gnanadurai et al 1977 and Gnanadurai et al

1978) showed that there was lysosomal enzyme release but the initial report could not identify its source with any certainty. The 1978 report however, by using the technique of selective superior vena cava and comparative arterial samples of N-acetyl B-glucosamide showed the lung to be the probable source. High levels of neutrophil elastase and evidence of oxidative activity has been demonstrated in patients with respiratory distress syndrome (Merritt et al 1983 and Cochrane et al 1983), a condition which appears very similar to the post perfusion lung. Other studies (Havel et al 1984) have demonstrated a peak in elastase after the release of the aortic cross-clamp. The lysosomal release is accompanied by generation of toxic free radical species : superoxide, hydroxyl radical and hydrogen peroxide. One paper (Royston et al 1986)in a careful study linked the sequestration of white cells to the production of free radicals. They also discussed the role of release of the cross clamp and the relative ischaemia of the lung (which does not occur with the double reservoir technique. There are antidotes such as superoxide dismutase and glutathione, but they tend to be specific for a type of free- radical

and therefore may have limited therapeutic value (Westaby 1987). The theory therefore starting with foreign material activating complement, which then sequesters cells that release enzymes that in turn damage the lung, although not proven is beginning to gain credence (Westaby 1986). Conflicting evidence (Handin et al 1977 and Kornecki et al 1986) is presented with a view that the free radical production may actually enhance platelet aggregation and help to modulate haemostatic reactions in areas of inflammation or vessel injury. Further evidence (Clancy et al 1983 and Goldman et al 1985) indicates that the role of the enzyme release in mediating the inflammatory response is at the least multifactorial.

These observations have in turn generated further studies to see if the type of oxygenator used was relevant and if so, whether the responses be modified pharmacologically. It has been assumed that bubble oxygenation would activate more complement than membrane oxygenation although one study (Videm et al 1990) in fact has shown the reverse. It postulates that the reason for this is the greater surface area in contact with blood with a membrane

as compared with a bubble. This would support reasoning for the finding of low complement cascades with the double reservoir. A Japanese study (Yamasaki M 1989) has postulated that bubble oxygenators activate the classical pathway for complement whilst the membrane oxygenators activate the alternate pathway. That this is too facile an approach is seen by a study (Videm et al 1989) looking at a variety of different membrane and bubble oxygenators. Their conclusions were that complement activation was low with a hollow fibre membrane and a soft shell bubble membrane. A capillary membrane, a non porous membrane, a sheet membrane and a hard shell oxygenator all induced similar high amounts of complement activation. It was interesting to note that the tubing set per se induced only minor activation.

Trying to look to the clinical implications of this activation, a Scandinavian paper, (Nilsson et al 1990b) demonstrated improved cardiac and respiratory function with membrane oxygenation but could not relate this to inflammatory activity during the bypass. Another study (van Oeveren et al 1985), which prospectively

compared many aspects of platelet function and complement activation in two groups of patients, one who had a bubble oxygenator for their bypass and the other group, a membrane. The results showed that whilst the membrane oxygenator reduced the haemolysis and the platelet count and the function were well preserved, the white cell trapping across the lungs and the complement activation was similar in both groups. A similar study (Cavarocchi et al 1986) added a third group of patients who were given steroids during bubble oxygenation. They showed that steroids could mitigate the C3a activation and white cell lung sequestration when using a bubble oxygenator, producing about the same results as in the membrane group. Other papers, (Bolanowski et al 1977) compared the ability of PGE1, dipyridamole and steroids to reduce pulmonary leucocytic aggregation during cardiopulmonary bypass; they showed that PGE1 was the most effective of the three. Contradicting these findings Fosse (Fosse et al 1987b) was unable to reduce complement activation by administration of steroids, although it did increase the granulocytes in peripheral blood postoperatively.

Trying to look at the possible clinical significance of many of these differences, a canine study performed (van Oeveren et al 1987) showed decreased host defence mechanisms against infections using bubble as compared to membrane oxygenation. That the bactericidal activity of polymorphonuclear cells may be related to their aerobic/anaerobic status and thus the role of free radical production complicates the issue (Mandell 1974).

In the neonatal situation (Merritt et al 1983) showed that elastase levels in the newborn could be reduced by the addition of pulmonary surfactant. Elastase levels were also reported reduced by the addition of continuous infusion nifedipine (Riegel et al 1988). Two studies showed reduced complement activation by manipulation of the priming fluid one by adding polygeline (Bonser et al 1990b) the other substituting dextran for plasma prime (Mellbye et al 1988). These are representative of many studies on white cell trapping and complement activation and show one line of thought to reduce the damaging effects by improving either the oxygenator, or the drugs used with it; in this

thesis, the approach has been to do away with the oxygenator entirely and see what beneficial effects if any will accrue.

Other problems to be considered are the methods used to determine complement fragments. Reports (Lamche et al 1988, Klos et al 1988 and Hack et al 1988), describe a simple radioimmunoassay based on charcoal separation, by enzyme-linked assays and by I125-C3 detection, rather than the commercial radio-isotope methods currently available; the levels between these three systems and the commercial kits are said to be similar but remain to be confirmed. The site of sampling for complement levels has also been investigated (Langlois and Gawry 1987), and the conclusion are that blood taken simultaneously from central venous catheters, arterial lines and the antecubital fossa all had identical complement levels.

Apart from complement activation, there is also involvement of the three other humoral amplification systems. Hageman factor (factor XII) which was first isolated in 1958 (Ratnoff and

Rosenblum 1958) is an important triggering factor for all of the systems (Ruddy et al 1972); it can generate bradykinins and initiate clotting and via cofactors, fibrinolysis by conversion of plasminogen to plasmin. The triggering factor for Hageman factor is contact with a foreign surface and has been detailed by Ratnoff (Ratnoff 1969) and others.

The activation of Hageman Factor, as can be seen (Fig. 4), leads to the activation of the coagulation cascade by converting thromboplastin to its active form and despite apparently adequate doses of heparin, a certain amount of microcoagulation continues (Verska 1977). Studies have shown the consumption of clotting factors (Kalter et al 1979) and elevated plasma Fibrinopeptide A (FpA) and Thromboxane A2 levels during the course of cardiopulmonary bypass (Davies et al 1980). FpA is a 16 amino acid peptide cleaved from Aa chains of fibrinogen by thrombin. It has been shown that radioimmunoassay of FpA is a sensitive index of in vivo thrombin activity. Thromboxane A2, a product of arachidonic

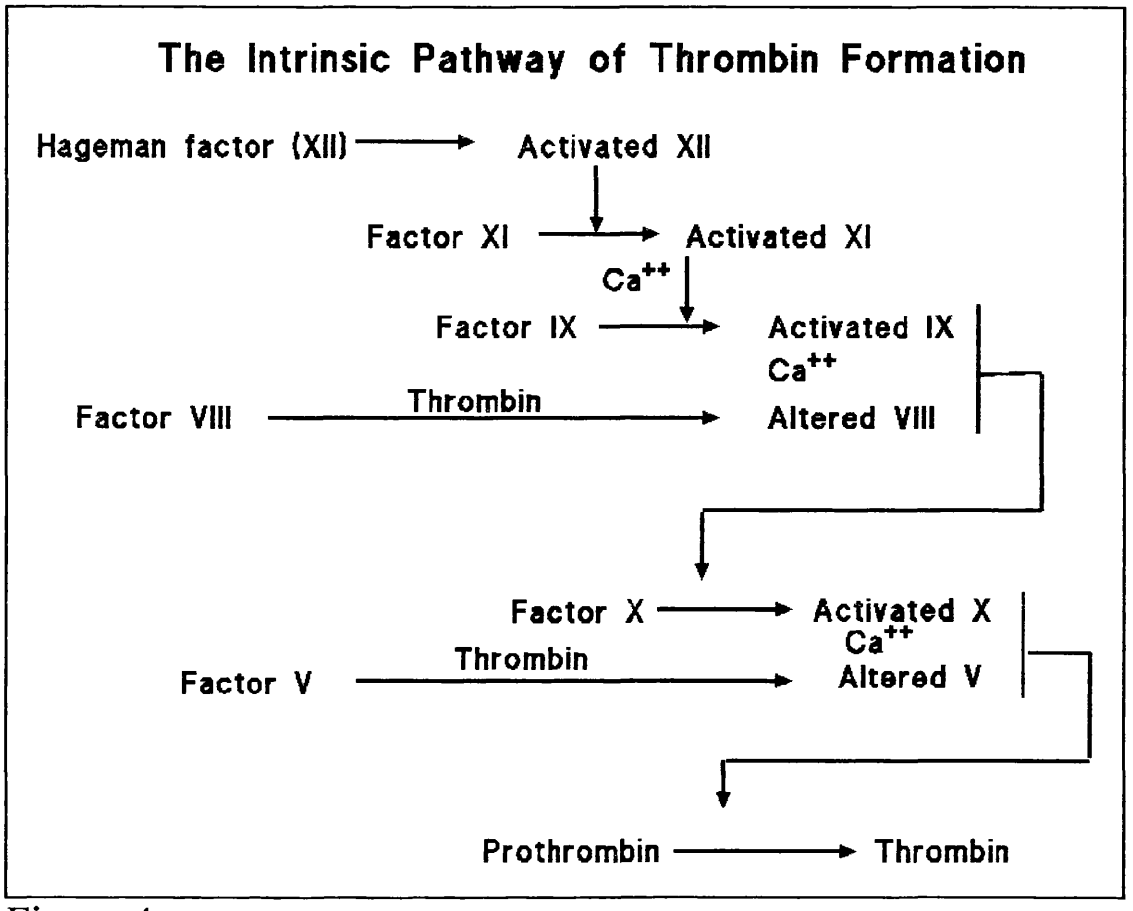



Figure 4

acid metabolism in platelets, is a potent inducer of platelet aggregation and of vascular and respiratory smooth muscle (Moncada and Vane 1981). Together with prostacyclin, it may play a role in the maintenance of vascular homeostatic mechanisms.

Prostacyclin and thromboxane thus have contrasting effects on vascular tone and platelet function but the variety and difficulty in measuring circulating levels renders them liable to artifact

(Fitzgerald et al 1983). Work on thromboxane receptor-blocking drugs e.g. AH23848 may help elucidate its role (Brittain et al 1985).

That this has clinical relevance has been clearly shown in many papers. One such (Bachmann et al 1975) in a review of 512 patients reported 5.6% who had severe coagulation problems and when the fibrinolytic abnormalities are included, the percentage is still higher. It has been shown (Lambert et al 1979) that some 20% of patients had hyperfibrinolytic bleeding and that most of these were successfully treated with E-aminocaproic acid. Jorgensen (Jorgensen et al 1970) showed in an animal study, that vascular damage can be produced by an infusion of ADP into the arterial system with an immediate extravasation of blood cells through endothelial gaps. They postulated that transient platelet aggregation in the microcirculation may be an important factor in causing vascular lesions. Paediatric studies (Greeley et al 1988) have demonstrated cardiopulmonary bypass induces high thromboxane values but they were unable to link this to pulmonary artery pressure changes. However in a series of case

reports (Morel et al 1987), there was data to support that high plasma levels of C5a anaphylatoxins and thromboxane is associated with pulmonary vaso and brocho-constriction induced by protamine reversal of heparin.

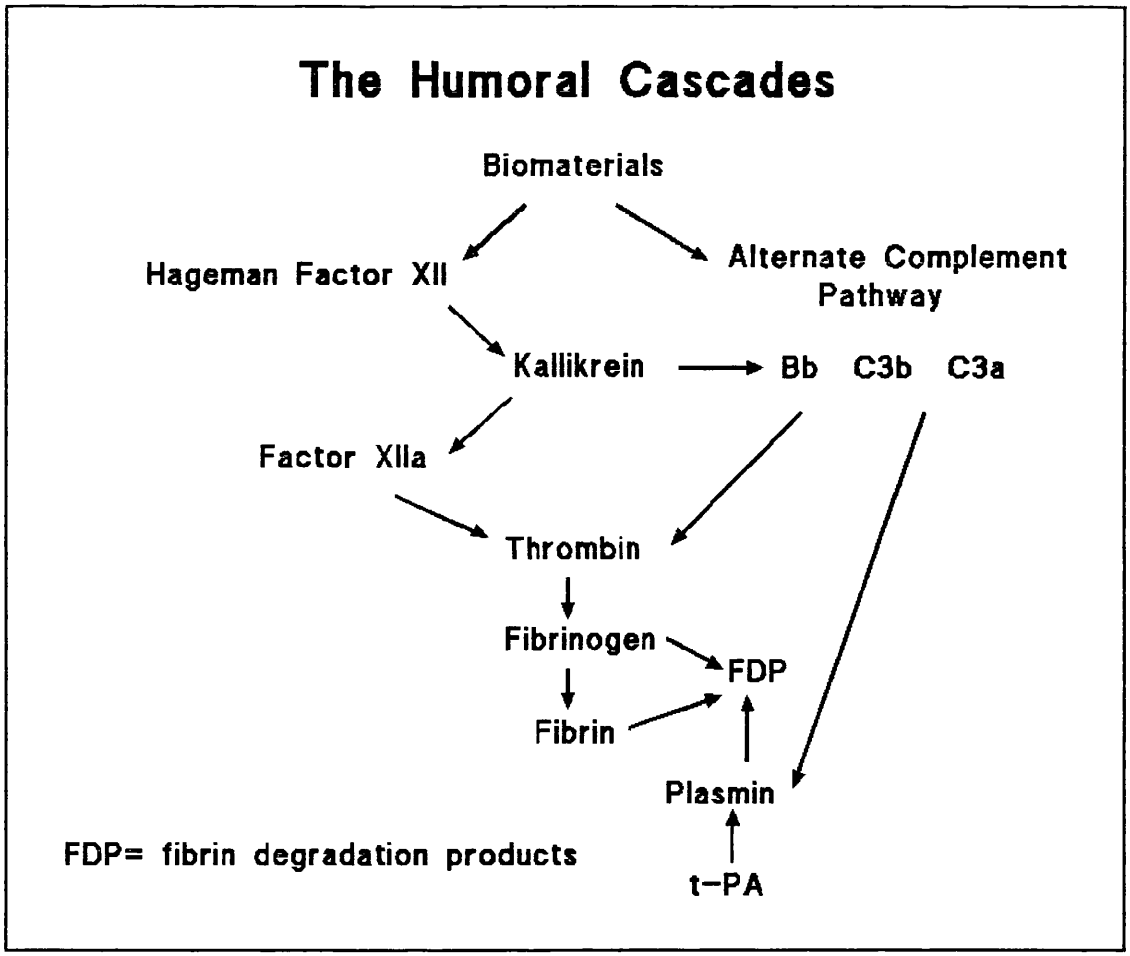



Figure 5

The last of the humoral amplification systems viz the kallikrein and bradykinins (Fig. 5) are also triggered by activated Hageman

factor (Heimark et al 1980). Bradykinins increase vascular permeability to proteins and water which produces vasodilatation. Kallikrein activates Hageman factor and activates plasminogen to form plasmin (Kaplan et al 1972). It is clear (Kongsgaard et al 1989), that the activation of the kallikrein-kinin system starts prebypass with the administration of heparin and may contribute to the previously mentioned heparin effects on complement. That bradykinin is produced in significant quantities during bypass, has been well demonstrated (Ellison et al 1980) and even hypothermia has been shown to cause increased levels (Pang et al 1979). The latter study demonstrated that whilst bradykinin levels increased with cooling, the levels dramatically increased after the onset of cardiopulmonary bypass. They hypothesized that the removal of the lungs from the circuit was probably the explanation for this. The important function of the lung in the metabolism of many of these factors has been demonstrated with regards the prostaglandin / thromboxane balance (Faymonville et al 1986). Their study concluded that the metabolic role of the lung in prostaglandin balance was important and when the lungs were

excluded as in cardiopulmonary bypass, there was a profound prostaglandin imbalance. That prostaglandin E2 is released in response to the heart-lung machine and cleared by the lung is suggested by a major increase in its concentration during pulmonary bypass and its decrease when the lungs are subsequently reperfused. They also showed that thromboxane is released from the pulmonary circulation from sequestered pulmonary platelets and microemboli. Thromboxane may then induce irreversible aggregation of platelets and further release of serotonin and ADP (Svensson et al 1976). The levels of thromboxane B2, which is a potent coronary vasoconstrictor in the coronary circulation, may play a major role in surgical ischaemic damage (Kobinia et al 1986). This has obvious implications for the double reservoir technique, because the lungs are never out of the circuit and thus able (temperature permitting) to function as a metabolic regulator; further, and indeed as mentioned as the fourth reason for the justification of the technique, it can provide a unique model for understanding conventional cardiopulmonary bypass. The fact that the lungs are continually being perfused may

help understand the role of the lung with regards not only metabolic functions but its ability to act as a homeostatic mechanism within the humoral cascades.

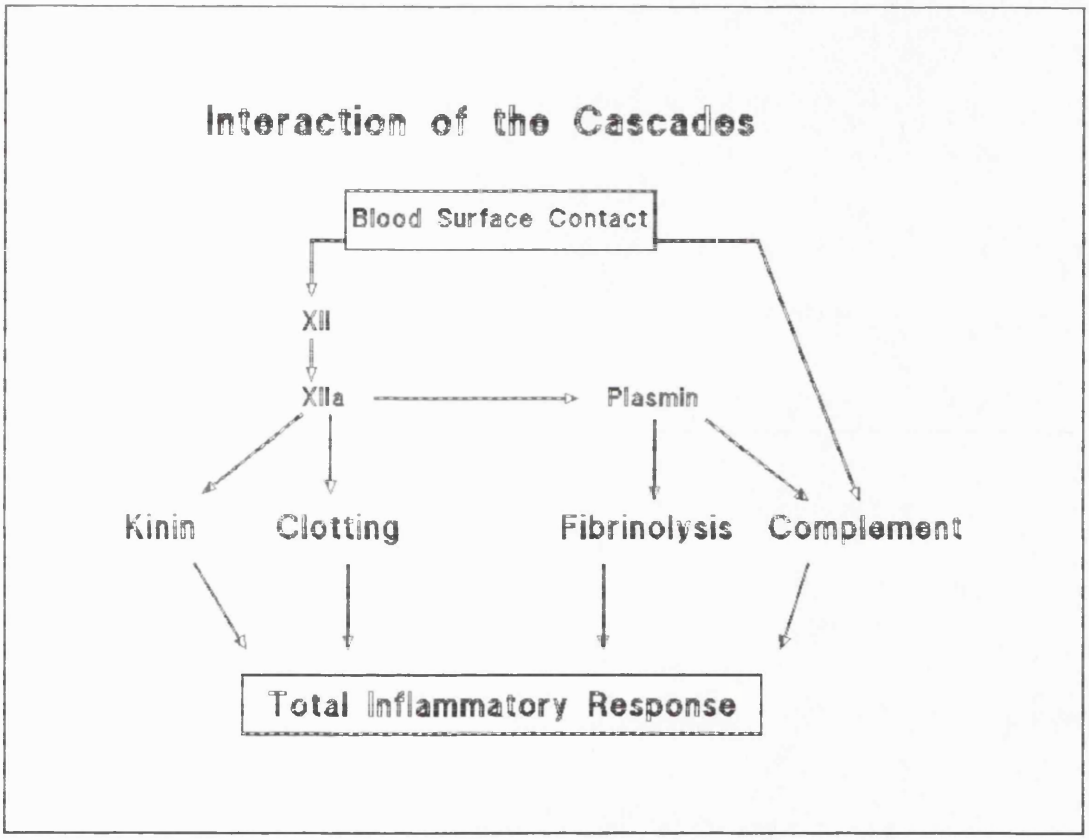



Figure 6

That all of the cascades interact (Fig. 6), make their study more demanding and the detection of which is the relevant factor all the more complex. Other mediators are obviously also involved because there are systemic reactions resembling inflammation

(Haeffner-Cavaillon et al 1989). Interleukin-1 (IL-1) is an endogenous pyrogen and a key mediator in inflammation and is generated by circulating monocytes within hours of cardiopulmonary bypass. This may explain the neutrophilia often seen after cardiopulmonary bypass (Dale et al 1975).

I have discussed and reviewed much of the current literature concerning the blood/foreign material interface in order to contrast this with the double reservoir system. Finally, I would like to discuss as part of its justification, the role of gaseous microemboli, previously alluded to, in producing neurological morbidity since microbubbles are a problem with almost all external oxygenators to a greater or lesser degree, and not with the double reservoir.

An early reference to this (Allardyce et al 1966), suggested that particulate microemboli from the disc oxygenator could damage the blood and went on to say that a filter should be developed to prevent this. A canine study (Brennan et al 1971), showed that

cardiopulmonary bypass caused a significant decrease in brain blood flow and metabolism and correlated it with titres of microparticles generated and demonstrated that it could be reduced by filtering the blood. In 1980 Taylor (Taylor et al 1980) reported evidence that in-line arterial filters reduced particle counts and in turn reduced postoperative cerebral dysfunction. The paper described a new experimental model of measuring CPK-B in cerebrospinal fluid, a marker (it is thought) of brain damage. It reported that it could prevent the rise of CPK-B during bypass with an in-line 40 micrometer screen filter. Two reviews warn of the dangers of embolisation and neurological deficit (Anon 1982, Taylor 1982), the former stating that heparin by itself stimulating platelet aggregation in certain situations, can cause problems.

Several papers attempt to quantify the incidence of neurological damage following bypass, although not all of it can necessarily be attributed to microemboli(Sorensen et al 1987). One study (Bojar et al 1983) reported a 2.8% mortality with a 1% incidence of



major neurological deficit and stated that they felt air emboli was an important factor. Another (Aberg et al 1983) found about a 7% incidence of low late intellectual function but a lower incidence in those patients undergoing valve replacement which they state justifies their theory of silent embolisation. In a prospective study of 204 patients (Slogoff et al 1982), their overall incidence of cerebral dysfunction was 16.2% for transient problems and 6.4% after the 10th day and again suggested air or particulate emboli as their major aetiological factor. Clearly the harder one looks, the more one finds, for in another prospective study (Shaw et al 1984), 78 of 100 patients undergoing coronary artery surgery showed some degree of early cerebral dysfunction. This must be put into perspective though and work has shown (Smith et al 1986) that whilst early morbidity for both neurological and psychoneurological deficit is indeed high, at two months the neurological deficit is relatively small. Moderate or severe neuropsychological problems were still present in 25 out of 64 patients at eight weeks postoperatively and this was associated with a long bypass time. It is also important to state, that the

source of microemboli both gaseous and particulate is not only the oxygenator and one study (Gallagher and Pearson 1973) looked at bubbles over the arterial line and the carotid artery using an ultrasound transducer and showed that operative manoeuvres such as arterial and atrial cannulation and decannulation produced significant gaseous microemboli (GME). That said, the numbers of GMEs produced, vary enormously from type to type (Pearson et al 1986) and low gas-blood flow ratios were not always associated with low GME levels. It was shown that the number of GME was lower with membrane oxygenation but their use does not guarantee that the arterial blood will be free of GME, with sources such as venous cannulation sites, cardiotomy suction being important. This has been reproduced elsewhere (Padayachee et al 1987); they reported low levels of GMEs using membrane oxygenation although again aortic cannulation produced readings over the middle cerebral artery in most patients.

Using the literature to compare a membrane oxygenator with the

double reservoir system, one might predict that both should be generate low GME levels and both have good platelet handling but the complement activation and thus possibly the pulmonary complications of cardiopulmonary bypass may be quite different.

# THE HISTORY OF THE DOUBLE RESERVOIR

The history of the double reservoir technique is very much entwined with the early history of conventional bypass. As well as working on various methods of achieving an artificial oxygenator, there were considerable efforts made to evaluate the use of cross circulations, heterologous isolated lungs, isolated homologous lungs and then various degrees of autologous oxygenation. These differing techniques were being developed and evaluated in parallel, often in the same units with experience from one going towards other projects. That the double reservoir technique largely failed to be continued in the sixties, resulted from its limitations for open heart surgery and the fact that oxygenators became simpler to use and were apparently safer.

When or to whom the honour of conceiving or producing the concept of bypassing the heart, is almost impossible to say but the French physiologist Nysten in the early 1800s was amongst the first to attempt to oxygenate animals deprived of their respiratory

function (Nysten 1811). He did this by injecting oxygen intravenously into animals breathing pure nitrogen and showed he could prolong their survival from four to twelve minutes (Galletti and Brecher 1962). This procedure was known as "endogenous oxygenation", because the oxygenation whilst not exactly physiological was nevertheless contained within the body. This was further refined as it became clear that smaller bubbles were more desirable, however, the greatest problem was how to cope with the resultant foam produced. The work never progressed sufficiently to become a clinical reality.

Another technique which was used clinically and up until fairly recently at that, was that of donor circulation. Fredericq, an early worker in the field used paired dogs or rabbits and made glass connections between the proximal carotid artery of each to the distal carotid of the other. Hedon used a metal cannula whose inner surface had a segment of fresh jugular vein stretched over it. This neatly avoided many of the problems of clotting around the cannulae. By 1920 Bazett and Quinby had reported a method

for the diversion of the entire cardiac output and made the fundamental observation that "the animal in better condition bleeds into the other". Later workers discovered the necessity of both controlling and balancing the flows to ensure that one side did not become hypovolaemic whilst the other became overloaded. To transfer this principle to man, a simple extracorporeal circuit was required where blood from the arterial system of the donor is pumped into the arterial system of the recipient. Meanwhile, the blood accumulating in the venous system of the recipient is diverted to the donor before reaching the right heart and thus cardiopulmonary bypass is achieved. This entails the donor coping with two circulations simultaneously and therefore the donor must be considerably larger than the recipient. This has been used with excellent results by Lillehei (Lillehei et al 1959), but the main limitations are the time factor with somewhat inadequate perfusion producing increasing levels of acidosis and the size difference necessary. The technique has no indications today.

In 1928 (Bayliss et al 1928) described a method of oxygenating

blood using inverted aluminium cones and there were other versions described (von Euler and Heymans 1932). Later, to attempt to reduce the froth that many methods produced, Cruickshank (Cruickshank 1934) used a magnetic oxygenator requiring ball bearings and an armature which was quite successful. The first system approaching clinical reality, was provided by Gibbon (Gibbon 1939) who detailed a method that not only stopped foaming but could cope with flow rates up to 600 mls per minute. From the Netherlands (Jongbloed 1949), came a spiral oxygenator and a full discussion of its clinical implications.

The same year, Gibbon reported (Stokes and Gibbon 1949) his early results on a canine model that also were good enough to look forward to clinical application. A short time later (Bailey et al 1951), discussions of mitral repair using a double pump system were published. By 1953 it was stated, with some justification, that "the stage is almost set for clinical use" (Melrose 1953). Indeed, the oxygenator constructed by Melrose had been extensively tested, the physiology monitored (Melrose et al 1953)

and prototypes for clinical use started.

158 patients in at least one centre had undergone cardiopulmonary bypass from March 1954 to 1956 for a wide range of conditions (DeWall et al 1956, Kirklin et al 1955, Lillehei et al 1955). There followed many more reports and reviews in the coming years (Gibbon 1954, Dennis 1956, Bahnson 1958, Gibbon 1959) and it became clear that the external oxygenator not only worked but was capable of rapid improvement even to the extent of using a disposable oxygenator (Lillehei et al 1956).

Other techniques were being developed at the same time and also had early beginnings from the late 19th. Century; one such was that of isolated lung oxygenation. This was called homologous oxygenation when animals of the same species provided the isolated lungs and heterologous if not. An elegant device for the oxygenation of blood from isolated animal lungs was provided by Jacobj working in Strasbourg. His original detailed description and diagrams (Jacobj 1895) showed circuits for venous and arterial

blood conducted through brass flasks, conduits and stopcocks to provide oxygenated blood from an isolated lung. By 1951, many refinements had been added and started to achieve periods of clinical significance, if not suitable reliability. The concept was somewhat amusingly presented as "Why not attach a lung to a dog's leg? Well why not?" (Potts et al 1951). They went on to describe a technique whereby anaesthetized dogs could be kept alive two to three hours entirely on the isolated lungs of another dog that had been freshly and carefully prepared. They stated, that they made this report to "record the fact that homologous lungs are effective respiratory organs". They went on however to suggest "dimly", that it could be a practical solution.

In 1952, another study (Weslowski et al 1952) experimental experience was presented with homologous oxygenation using dogs and also their pilot studies on heterologous lungs. They showed that for the good oxygenation and removal of carbon dioxide, meticulous attention to details of the technique, including complete exsanguination, heparinisation and many other things

were necessary, but then, the lungs functioned well for periods in excess of three hours. They felt that whilst the technique showed little promise from the point of view of transferring it to clinical practise, it was worth pursuing for the information it revealed about pulmonary physiology and cardiac bypass. Indeed their documentation of haematocrits, plasma haemoglobins, blood biochemistry during their studies is most impressive and ordered.

That homologous lungs provided a good match was obvious, it remained however an impractical solution for clinical work. For this reason after early experimental work using homologous lungs, Mustard (Mustard 1955) turned to the possibility of heterologous oxygenation. Knowing that rhesus monkey lungs were group O and Rhesus positive, Mustard used their lungs in thirteen clinical cases of transposition of the great vessels. All cases died, but this was felt to be due to unsuccessful correction; the oxygenation and filtration of the blood was apparently excellent. This prompted him to use the technique for a less serious condition and he operated on an eight month child with tetralogy of Fallot with

complete success. His conclusions were that the homologous lung provided a filter that is incomparable with any artificial filter requiring an equal amount of heparin, that the haemolysis was also negligible and that oxygenation was complete. Other workers in the field (Waldhausen et al 1957), also used heterologous oxygenation using dog lungs but failed to achieve success.

Despite sporadic success, the fear of oedema in the isolated lung and the potential for cross infection, this technique also failed to become widely used.

The next phase in development, were the techniques with isolated right and left heart bypass. The number of conditions where right heart bypass alone was indicated are limited, but Dodrill (Dodrill et al 1953) reported the first clinical case in a patient with congenital pulmonary stenosis. They used, as they had done in their canine models, right atrial cannulation and then pumped the blood to the right lower lobe pulmonary artery with the cannula pointing centrally. This proved to be successful.

Left heart bypass was attempted in a similar fashion, but the principle problem was that of the collection of oxygenated blood from the pulmonary veins or left atrium. One such system (Kantrowitz et al 1951), described the bypassing of the left atrium and ventricle to expose the mitral area. It differed from other systems in that it allowed for either a transatrial or a transventricular surgical approach of the mitral valve. The system consisted of "delivering normally oxygenated blood from the left lung, via cannulae in the pulmonary veins, into a glass chamber and thence pumping it into the aorta via the femoral arteries, while blood from the right lung is temporarily prevented from entering the left heart". They reported that the blood pressure could be adequately maintained and that haemolysis was insignificant. Other workers (Pierce and Southworth 1953) using somewhat similar methods found that none of the left heart pumps were quite as effective in maintaining the circulation as in their cross-circulation studies although the circuit was simpler to set up. In contrast to this, in 1955 there were reports (Jamison et al 1955) that left heart bypass from left atrium via the left

superior pulmonary vein to left subclavian artery could be successfully performed using the fairly efficient Gemeinhardt pump. Again however, the few indications and the ever present danger of air embolus ensured that it remained an experimental exercise.

All of these studies led naturally onto the autogenous lung oxygenation work and perhaps even though this work may have little relevance to today's practice, it could be argued that some of their observations that whenever they used biological rather than artificial filters or oxygenators, the blood trauma was always consistently lower with consequent benefit to the patient is still a general principle that continues to be true.

The studies on autologous oxygenation commenced around 1950; a paper in 1952 (Wesolowski and Welch 1952) explains the technique very well. They used right atrial and left pulmonary artery cannulae for the right sided bypass and the left atrial appendage to the left brachial artery for the left sided circuit.

They used various cannulae types including glass and early forms of polyethylene and whilst they clearly found the technique challenging, they certainly made it work, supporting their animals for over two hours.

In 1954, attention turned to what became known as the "azygous flow principle" in which the animal had the inferior vena cava occluded and the superior cava occluded above the level of the azygous vein. It was shown (Cohen and Lillehei 1954), that this flow was sufficient to support life in excess of five minutes although the timing was critical if brain damage was to be avoided. Then using this as the basis of their technique, they (Cohen et al 1954) combined this with a double pump system and since the forward flow was very small they utilised a single lobe of a lung. Their results showed that they could achieve 100% oxygen saturation of the blood, that haemolysis was low and their infective and thrombotic problems were also reduced. Encouraged by this, they performed the direct closure of a ventricular septal defect using this technique without problems.

The main limiting factor was time, in that the low flow was only tolerated for about 30 minutes.

A lot of attention was focused on the problems of adequate pulmonary venous drainage versus the risk of air embolus; this was addressed in an elegant study (Read et al 1956) comparing open drainage with a closed system using Penrose drains which were collapsible and provided good drainage with little chance of air entrainment. The practice however was complicated and again failed to reach popularity.

The principle of autologous oxygenation was by now accepted, and more units began to evaluate it for themselves. One such, (Blanco et al 1958) became happy with the technique in animals and satisfied with their results, undertook five aortic valve operations. They were cautious in their approach and connected an oxygenator in their circuit to be used if there were problems. Apart from one case, they used right atrial cannulation and a cannula through the right ventricle and past the pulmonary valve

for the right sided circuit and left atrial gravity drainage into a reservoir and femoral return for the left. Apart from learning how to use it, they had no significant problems and their patients all did well. The longest had a bypass time of nearly two hours with little blood damage which could not have been achieved in 1958 with an external oxygenator. Their results were thus excellent and encouraged still more surgeons to look at the technique.

Using open gravity drainage for their system, Cass and Ross (Cass and Ross 1959), developed a means of more easily balancing the levels of the two reservoirs. Since they used a open system, if the level of one reservoir dropped, air could easily be introduced. To avoid this, they used a small shunt between the two sides and set the right sided pump slightly faster than the left. This ensured a small left to right shunt, which made balancing the levels much easier and decreased the risk of introducing air. They used this technique experimentally on repairing atrial septal defects, but reported that air would enter the left system from the atriotomy.

This showed the greatest limitation of the technique, clearly making it unsuitable for the correction of intracardiac defects, which were the predominant type of problem amenable to surgical correction at the time.

Another solution to the problem of how to perform open heart surgery, was to use the technique to produce profound hypothermia and then circulatory arrest. Studies on a canine model were undertaken (Drew et al 1959) with cooling down to 10°C. They initially tried this with just left sided support but soon found that at that temperature bilateral support was needed. Their circuit was essentially the same as previously described. An important observation, and perhaps the one that prompted that team to continue for many years with the technique, was the absence of even minor pulmonary complications. They stated that this was in contrast to their experience using cardiopulmonary bypass, where they frequently observed bloodstained bronchial secretions. Further, they found no difficulty in restoring normal heart rhythm and no plasma electrolyte imbalance. The same

year the unit reported (Drew and Anderson 1959) their first three clinical cases. They used cooling down to about 15oC with circulatory arrest times of 45 minutes. They had good results but noted the fair amount of time that the rewarming period took.

Bjork (Bjork 1960) compared formally the system of profound hypothermia with and without an oxygenator. He found that without an oxygenator, there was less blood trauma or haemolysis. The oxygenation was better than in any oxygenator then available and there was no postoperative metabolic acidosis. On the other side, the disadvantages were found to be the time limit of 45 minutes circulatory arrest in adults, the handling of the bypass was more complicated and the method was more time consuming. His conclusions were therefore, that for his routine work, he preferred an oxygenator at normothermia especially in children, in whom he thought were at particular risk of brain damage with that technique.

Another comment was made in 1960 (Shabetai 1960) who reviewed the state of the art up until that time. He described many of the varying techniques for overcoming the various problems but refused to predict if the technique would rival the progress that was being rapidly made with the external oxygenator.

History, in the short term at least, showed that autologous oxygenation ceased to be used except by Drew. Other surgeons clearly felt that whilst the technique had some major benefits, for the type of surgery that most of them were practising, the problems outweighed the benefits.

The next twenty years showed no interest in even studying the technique until Ross at the National Heart Hospital, London thought it timely to re-evaluate the technique. There were many reasons for this; as previously stated, most surgery performed was no longer open and thus the double reservoir technique could be used without major danger of air embolisation. The cannulae

design in both shape and materials available had vastly improved, making the practice of cannulation less hazardous. Finally, the costs of the oxygenator with a large patient workload was ever increasing and perhaps this could be reduced by using the double reservoir technique. In addition to these reasons, it became clear to me that there were major advantages for the patients, in having the lungs perfused during the operation and by removing the oxygenator might remove an important stimulus for complement activation. It also became clear that it provided another important model for understanding conventional cardiopulmonary bypass. This can be directed to the bypass itself or to other variables such as temperature effects or materials testing. The simple surface also lends itself to heparin coating although that may negate any cost benefit.

A preliminary study (Bodnar and Ross 1983) refreshed the known effects of the technique and provided the impetus to conduct a detailed programme of animal investigations as a prelude to perhaps a clinical study. A unit in Sweden has also recently been

investigating the possibility of using autologous oxygenation for coronary artery surgery (G. William-Olsen, personal communication and Berglin et al 1986). They studied ten patients undergoing coronary artery surgery and used cold crystalline cardioplegia for myocardial preservation. Their study encountered no technical difficulties and in all patients, the postoperative course was smooth. They reported a reduced level of haemolysis compared with their own (unpublished) controls.

The following chapters detail the plan of research and the transition from the laboratory to the operating theatre. Knowledge of the history has proved to be not only interesting but also valuable, as many of the lessons, apparently new, are in fact old discoveries lost in the literature.

# ANIMAL STUDIES

Introduction

Choice of animal model

Selection

Anaesthesia

Preoperative preparation

Premedication

Induction of anaesthesia

Intubation

Ventilation

Monitoring

Intravenous fluids

Anticoagulation

Cardiovascular drugs

Muscle relaxants

Postoperative care

Investigations

Surgery

Bypass

Plan of Animal Studies

## Introduction

The previous chapters have outlined the background to the double reservoir technique and some of the justification to studying it further. It was decided to undertake this in a staged manner. The first phase would be a general investigation of the technique and becoming familiar with an animal model, next the animal model would be refined to study techniques of both cross clamp fibrillation and of cold crystalline cardioplegia and finally to assess its suitability for long term assist. After these had been successful, it was determined to proceed again to the clinical environment. The animal studies were approved under Home Office license under my name and were undertaken at the Cardiothoracic Institute, Brompton Hospital, London. Methods common to all of the animal procedures are detailed below.

The Animal Model - The choice of animal

A wide variety of animals have been chosen to use as the experimental work for cardiac procedures. Dogs were not chosen because they are extremely expensive to purchase and keep and it is difficult to obtain a Home Office permission for their use. Whilst pigs have been used with great success in many units, it was felt that the size range was not ideal. Calves have again been used with success and some of the problems associated with their use i.e. their rapid growth and perhaps a slightly unusual calcium metabolism when young, would not have been relevant to these experiments. They are however again, quite expensive to both purchase and then keep. Sheep were chosen for our projects despite the poor survival rates achieved by some workers (Dries et al 1983). Previous local experience suggested that handling, husbandry (Worden and Lane-Petter 1976) and supply were easy and that the proposed surgical procedures were feasible. Further, they were selected as the model of choice, because the expense was not high, they were freely available in a wide range of sizes

and the laboratory where the work was to be performed had gained a lot of experience in their care. This latter point is of considerable importance, since animals that will not feed well, tend not to do well either. Their physiology is quite well documented and many of the drug doses are known.

Selection

It rapidly became clear that some sheep used had had pneumonias whilst young, and although the disease was no longer active, their ability to oxygenate was impaired. These pneumonias were most likely pasteurella in origin and certainly fitted that description (Gilmour and Gilmour 1985). They had dark, usually black blotches on one or more surfaces of their lungs and the $pO_2$ obtainable was always relatively low. As a result, it became the practice to select every animal personally. Only male animals were used; this was on the advice that female animals were more difficult to operate on, since at certain times of their oestrous cycle, their tissues were more fragile. I had no untoward experiences to support that theory on the occasions females were used.

Anaesthesia

Full anaesthetic details have been reported (Macrae and Glenville 1988) , where a technique of anaesthesia and surgery used in sheep during insertion of ventricular assist devices is described. Extracts relevant to this study, in particular drug doses used, are detailed below.

Preoperative preparation

We obtained our animals a few days prior to their use, although this increased husbandry costs, it allowed the animals to become accustomed to the change in diet and environment and observed for coincidental disease. In addition, relevant laboratory investigations were then performed preoperatively and shearing, disinfection and premedication arranged.

There is no agreement in previous reports on the question of withholding food and water form sheep preoperatively. Some workers withhold food for up to 48 hours preoperatively, (Worden and Lane-Petter 1976 and Hecker 1984) whereas Green suggests feeding animals up to the time of anaesthesia (Green 1979). Our practice was to fast animals for 16 hours whilst allowing them free access to water up to 2 hours preoperatively. We followed Green's advice on the passage of a stomach tube to prevent ruminal tympany. In addition, the use of oral tetracyclines the

night before operation, appeared to reduce the amount of ruminal gas production.

Premedication

When accustomed to their new surroundings, sheep are usually quiet and cooperative and they will tolerate the swift induction of anaesthesia. More boisterous animals are easier to handle following premedication with diazepam 1-2 mg/kg intramuscularly on hour preoperatively (Green 1979).

We did not use atropine premedication routinely. Hall states that atropine is not effective in reducing salivation in sheep except in very large doses, which produce undesirable tachycardia (Hall and Clark 1983). However, Green discusses the problem and suggests that a dose of 0.5mg/kg IV both protects the heart from vagal responses and reduces salivation.

Induction of anaesthesia

The use of thiopentone in ovine anaesthesia is well established (Green 1979 and Sharma et al 1970). We used an injection of 2.5% thiopentone at a rate of 2mls/sec into an ear vein, until the animal became drowsy and sat in its pen. When a further 4mls (100mg) was given, the animal could be lifted onto the operating table and intubated immediately.

Alternative sites for induction are the internal jugular veins and superficial veins in the forelimbs. If venous access cannot be achieved, the use of ketamine 10-20mg/kg intramuscularly for induction has been described (Britton et al 1974).

Intubation

For intubation, sheep are best lain in the lateral position. We used a 25cms straight bladed animal laryngoscope made by Penlon (Oxford, UK). The techniques of laryngoscopy employed are similar to those used in man, the main anatomical differences from man being the large leafy epiglottis of the sheep and the greater distance from teeth to larynx (about 20cms).

Intubation was usually possible in the apnoeic period immediately following an induction dose of thiopentone. The alternative was to deepen anaesthesia by the administration of nitrous oxide and halothane in oxygen via a Magill attachment (Mapleson A) and a Hall pattern conical face mask, with the animal breathing spontaneously.

Ventilation

Anaesthesia was continued in our animals with a conventional nitrous oxide - oxygen - halothane technique until establishment of bypass. Equipment consisted of a Boyle pattern anaesthetic machine delivering gases to a Blease Brompton Manley ventilator with positive end expiratory pressure (PEEP) valve.

Following intubation, the animals were easy to ventilate without additional muscle relaxation. An appropriate tidal volume was 10 ml/Kg with a minute volume of 100-150 ml/Kg adjusted to maintain normocarbia by arterial blood gas analysis.

Once the chest was open, gross lung collapse with consequent hypoxia readily occurred. This was largely prevented by the use of the ventilator in the time-cycling mode, with an appropriate end inspiratory pause and the application of 10 cms of water PEEP. By employing these measures we have rarely required an $FIO_2$ of greater than 0.5.

Monitoring

ECG : Intradermal silver needles were used in all four limbs to provide a standard 6 lead ECG configuration.

Venous access and measurement of central venous pressure were obtained by percutaneous cannulation of the animal's internal jugular vein.

Arterial cannulation was performed in a number of ways, depending on our precise requirements. Firstly, for experiments which required post operative arterial blood sampling the carotid artery was approached surgically, lying as it does in the sheep, deep to the internal vein. A catheter placed in this position surgically and then tunnelled subcutaneously is secure in the post operative period, unlike devices placed in limb vessels which are easily dislodged by the animal. An alternative to a surgical cut down, which was performed successfully on a number of occasions, is a Seldinger approach to the carotid artery from a lateral approach, aiming caudally and deep to the internal jugular vein in

a medial direction.

Pulmonary artery catheters of standard human pattern are readily introduced in sheep via the internal jugular vein, through a valved introducer. The flotation technique is identical to that used in man. Readings of pulmonary artery pressures and cardiac output by the thermodilution method were performed in all cases.

Temperature was measured in our animals by a thermistor probe placed in the nasopharynx. Our animals were kept warm during surgical procedures by a thermostatically controlled electric heating blanket.

A TM8 bubble activity monitor (Technique Laboratories Ltd., Hampshire) was attached to either arterial line (usually the aortic) to measure the number and size of circulating gaseous microemboli. The device allows counting in the bubble size range of 10-1000 microns. The detecting head a continuous narrow beam of ultrasound is propagated into the tubing to be monitored.

As bubbles pass the beam, a receiving transducer picks up subtle modifications to the signal.

The pressures required to ventilate the sheep were displayed on the ventilator and recorded.

Intravenous fluids

Sheep produce copious amounts (500 mls/hr.) of saliva and gastric secretions. In these they suffer a net loss of bicarbonate and potassium which our studies have shown result in a metabolic acidosis and hypokalaemia if not replaced. Replacement of K+ at a rate of 10-20 mmols/hr and 100 mls of bicarbonate over the first two hours maintained balance. Failure to correct led to haemodynamic instability and arrhythmias and also failure to establish an adequate cardiac output after cardiopulmonary bypass.

Volume replacement for blood loss may initially be carried out with a plasma expander such as Haemaccel. However the nature of experimental cardiac surgery is such that massive blood loss is not unknown and means of coping was available to the anaesthetist. Donor blood was either given by the same animal over a two week preoperative period or from a laboratory sheep donor. In the double reservoir experiments, the use of donor

blood was almost never used. There are said to be two blood groups in sheep, the RO groups and that they are not compatible (Melby and Altman 1976); others (Schalm 1965) say that cross-match is unnecessary. Our own experience suggested that there are two significant groups but mismatch only occurs on very fast transfusion e.g. in the prime of the extracorporeal circulation.

Anticoagulation

In view of the relative resistance to heparin of ovine blood (Hecker 1979 and Shen et al 1978), we chose to use a dose of heparin of 4mg/kg for bypass procedures. This was satisfactory as has reversal of the heparin effect at the end of bypass with 8-10 mg/kg of protamine. Monitoring of coagulation was carried out using a Haemochron ACT method.

Cardiovascular drugs

Various parts of the operative procedures on sheep were best performed with moderate hypotension e.g. aortic cannulation. Small doses of nitroglycerin (0.1-0.3 mg) which in man usually have visible effects cause no change to systemic arterial pressure in sheep. Larger doses e.g. 1-1.5mg. as a bolus provided an appropriate dose. Phentolamine 1-2 mg. had similar effects within the human dose range.

Muscle relaxants

Sheep are easily intubated and ventilated without muscle relaxants, however during thoracotomy with cutting diathermy appropriate increments of relaxants worked well to prevent kicking; and provided an appropriate relaxant and dose was chosen (Borrie and Mitchell 1960 and Cass et al 1976), reversal was successful. Pancuronium 1-1.5 mg/kg or atracurium 0.5-0.75mg/kg were loading doses found to be satisfactory. Reversal of relaxants with atropine 1.2 mg and neostigmine 2.5-3.75 mg. were effective. Other units state that on no account should relaxants be used since the animals rarely recover; this has not been our experience.

Postoperative care

Following intercostal nerve blocks, skin closure and reversal of neuromuscular blockade, spontaneous ventilation was established. The tracheal tube was removed once the animal started swallowing. The sheep was standing and often eating and drinking within half an hour of finishing the operation.

Pain relief was sometimes necessary for the following 24 hours, usually a small dose of intramuscular opiate. It was found that the quickest way to provide a good recovery was to return the postoperative animal to the pen with the other sheep early in the recovery period.

Investigations

All animals had regular blood gas analysis performed in the laboratory. The haematology performed was somewhat limited. The measurement of haematological data can present problems, in that the indices for sheep red cells and platelets are sufficiently different from those of man to deceive Coulter counter analysers (Mitruka and Rawnsley 1977). Manual counts and not resetting the Coulter counter indices (Dries et al 1983) were our solution. The haematology measurements therefore consisted of the haemoglobin, the white cell and platelet count and the haematocrit.

The platelet function was performed by myself and technicians experienced with ADP function tests. The protocol used was as follows: 9 mls of blood were added to 1ml of 3.8% sodium citrate. The sample is then centrifuged at 1200 rpm for 10 minutes to obtain platelet rich plasma. This platelet rich plasma (prp) is used for the aggregation studies. The remaining portion of blood

from a few tubes is spun at 2800 rpm for ten minutes to get platelet poor plasma (ppp). This ppp is used as the blank for calibrating the Payton dual chamber aggregometer. ADP 0.05 mls of $10^4$ Molar solution stored at -20°C is used. This is brought to room temperature before use. The plasma is left for 15 minutes prior to reading. The samples were all performed within three hours of sampling but preferably earlier.

For control of heparinisation, we successfully employed the Haemochron Activated Clotting Time method and haematocrits were measured with a Hawksley capillary centrifuge.

Biochemistry was more straightforward and a large range of factors were looked at. These included urea and electrolytes, liver function tests, cardiac enzymes, calcium and phosphate estimations. The plasma free haemoglobin was estimated using the benzidine technique modified by orthotolodine. This gives results that are intermediate between the high values obtained from the standard benzidine technique and the lower values from

scanning spectrophotometric methods (Wright 1896). The radioimmunoassays for the complement pathway (C3a etc.) have not been raised for ovine work. There has been a lot of work in which both complement levels and white cell sequestration across the lungs have been measured (Cavarocchi et al 1986) and it seems likely that high white cell trapping is associated with high complement activation. On bypass with the controls animals, this was not always reliable, but just postbypass, good samples were obtained.

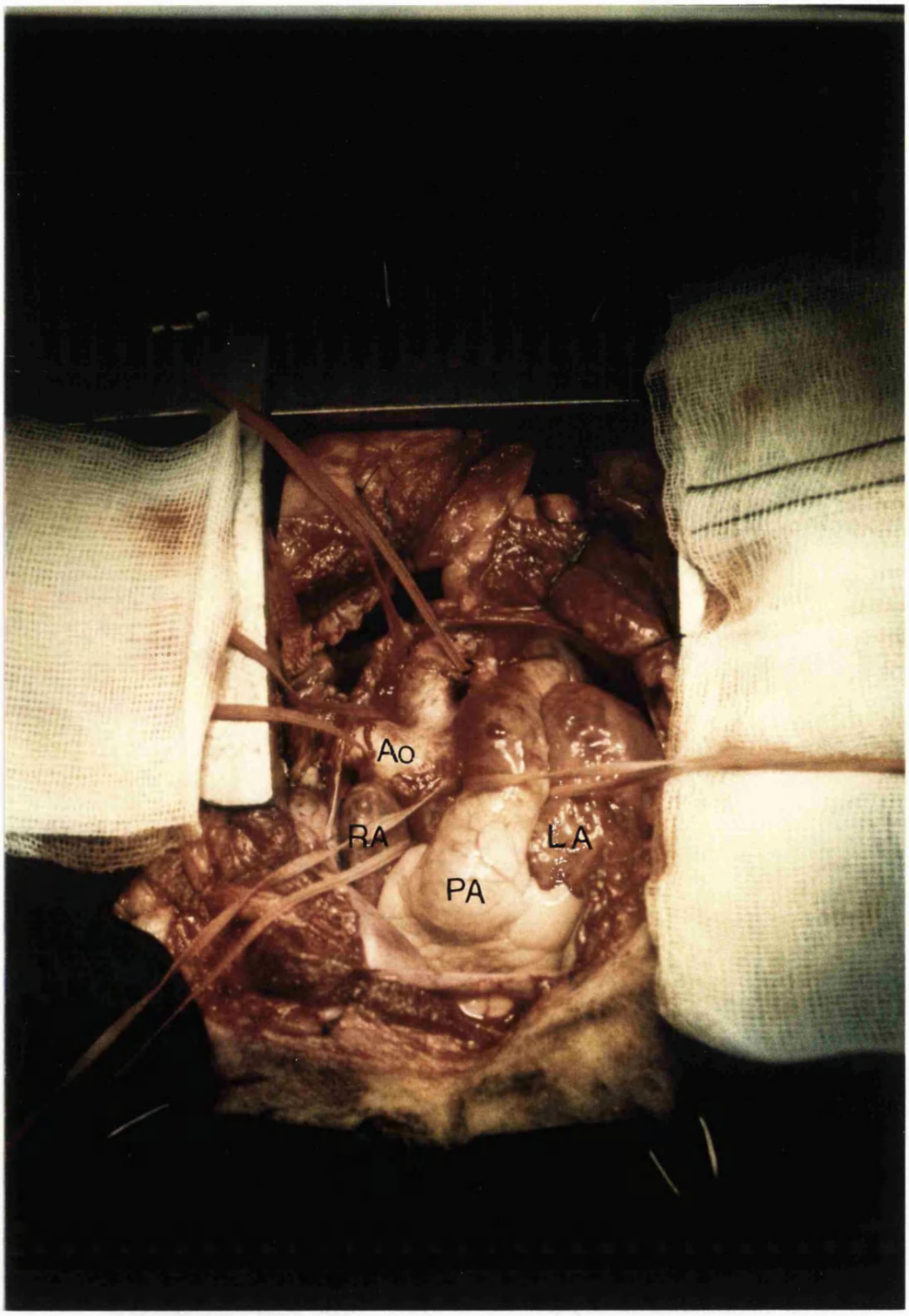
Surgery

Basic anatomy of the sheep is described (Sisson and Grossman 1953), but cardiac anatomy is not really dealt with. However, access to the chest was provided by a left posterolateral thoracotomy through the 5th. intercostal space. In this position, only the left atrium and pulmonary artery are clearly visible. Since access to all four chambers is required, alternative approaches were tried. The median sternotomy is not ideal; the shape of the chest meant that the heart is very deep in the chest and access is very difficult. It is also said that for survival studies, this approach is unstable and could retard the animal's progress.

It was thus necessary to provide a dissection under the pulmonary artery to reveal a length of ascending aorta and arch (see Plate 1). The anatomy of the sheep is such that only one major vessel leaves the arch which progressively divides to supply the head and forelimbs. The aorta is extremely thin and delicate; the standard

PLATE 1. DISSECTION OF THE ANIMAL MODEL

RA = RIGHT ATRIUM PA = PULMONARY ARTERY LA = LEFT ATRIUM Ao = AORTA



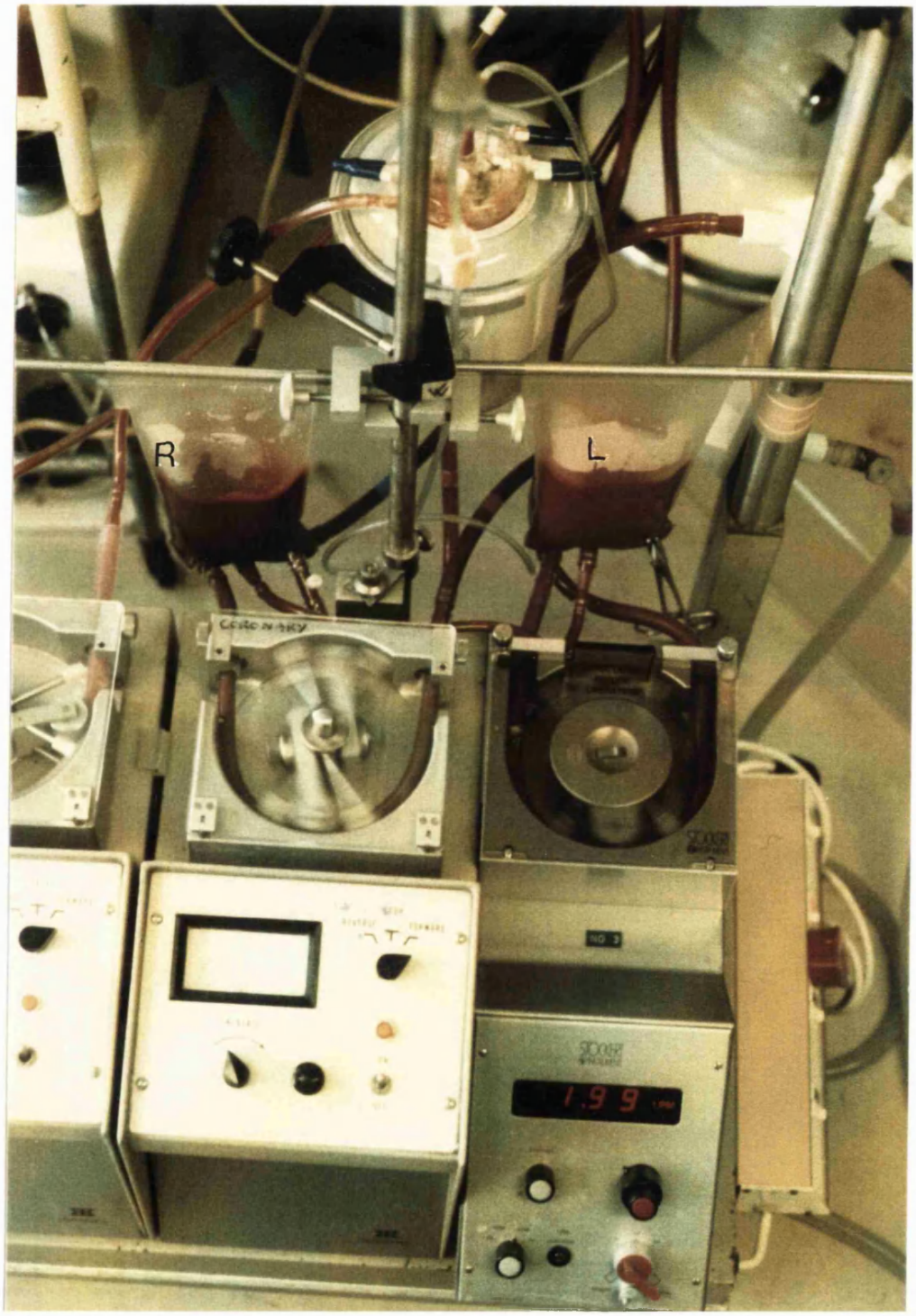
technique of prolene purse-strings is unreliable and will occasionally tear the aorta. Instead, a double purse-string with two small teflon pledgets was used and the pressure reduced with nitroglycerin for cannulation. The pulmonary artery site was both superficial and robust. The left atrium was also superficial but care was needed not to tear this very thin structure, but with care and fine sutures, it proved suitable both for the left atrium and via the mitral valve for the left ventricle. The right atrium could be delivered into the wound with some cautious traction and proved to be reliable and easy to handle. Decannulation had no special techniques although again hypotension was used for the aorta. The chest closure was identical to clinical practice and usually only one intercostal drain was used which was removed within 24 hours.

Bypass

The equipment used for providing the bypass were Travenol Roller pumps, double tubing sets 3/8th. inch throughout of PVC provided by Wessex Medical. The reservoirs were Polystan 1500mls reservoirs with a 3/8th.inch tubular shunt (Plate 2). These reservoirs were standard equipment and not modified; this meant that we had the ability with the built-in heat exchangers to connect either or both sides to a heat exchanger. We chose to connect the left sided circuit because of the flexibility it gave to running the bypass, but we were aware that if profound cooling was used, care would be needed to avoid large gradients that might cause bubbles to come out of solution. Pre-warmed Sodium Compound Lactate was used to prime; the volume decreased with experience to under two litres. The order of events would be after heparin administration, to cannulate the aorta and left atrium and initiate left sided bypass with the shunt closed. Whilst cooling or full bypass was being achieved, the right

PLATE 2. THE ANIMAL DOUBLE RESERVOIR CIRCUIT

R = RIGHT CIRCUIT L = LEFT CIRCUIT



heart cannulae could be placed and right heart bypass could be started. Full flow was considered in the range of 2.4L/min/$m^2$ although the surface area was perforce an estimate.

The first few cases experienced an immediate and dramatic rise in the pulmonary artery pressure, usually accompanied by rises in the airway pressures necessary to ventilate the sheep. This had therefore all the features of some broncho/vasospastic attack and attempts were made to understand the aetiology. A study of this was made during the early evolution of the technique (Aletras et al 1960), but this international group's conclusion was that in contrast to other workers, that the problem just did not exist.

We initially thought that the problem might have been that the priming solution that hit the lungs was too cold and we repeated this using prime at about 20°C. This did not provoke the raised pressures. We did find that the rate of onset of bypass was important however, in that with a rapid rate of onset with effectively only prime reaching the lungs, the pressures would

almost invariably rise. By going on more slowly this could be avoided entirely and was presumably due to good blood / prime mixing. Simple measurements of haematocrit taken every five seconds after going on showed that the rate should initially be very slow but could then quickly be increased. We used this technique on all of the animals after that and all perfusionists using the technique clinically were warned to ensure thorough mixing, particularly with the right sided bypass.

Once full bypass was achieved on both sides, the shunt between the two reservoirs was opened and the pump speeds could be adjusted to provide a small left to right shunt. This did not measurably reduce the arterial saturation, but it made balancing the levels easy. When either side of bypass was stopped or reduced in weaning, then the shunt was clamped.

The venous drainage with properly placed cannulae was excellent and inverting the heart to mimic circumflex artery surgery showed that the drainage remained excellent. Prior to inverting of the

heart, the azygous vein which enters via the pericardium directly into the coronary sinus, was tied off and divided.

The procedure for weaning from bypass was the reverse of going on; as soon as the heart was beating, the right heart bypass was stopped after clamping of the shunt. Rewarming was continued and left heart bypass stopped when required. To see how this system functioned over a long period, three operations were performed each involving 24 hours of bilateral support.

Plan of the animal studies

The figure below (Fig 7) details the plan undertaken for this project. After the initial few animals that were used to pass the learning curve both for surgery, anaesthesia and perfusion, a detailed plan was followed.

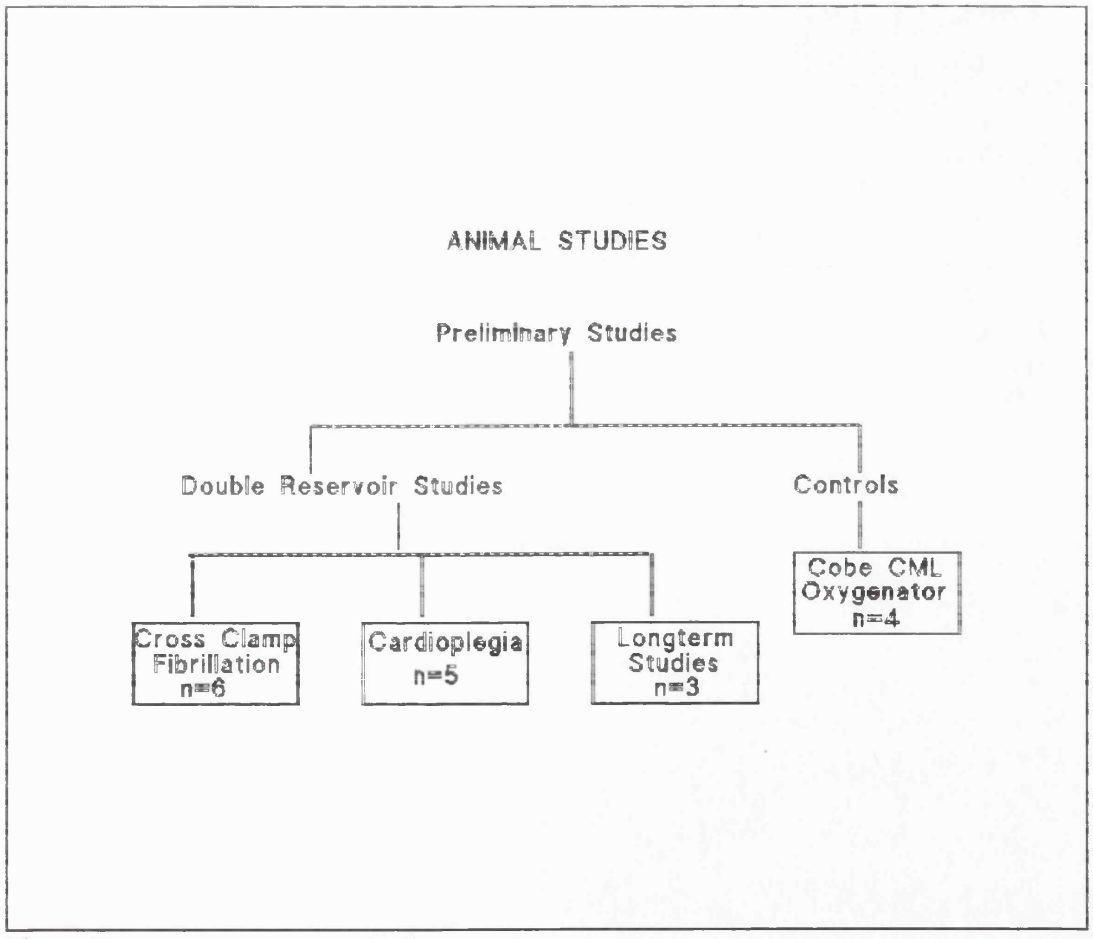



Figure 7

It was decided at an early stage that the aim of this phase of the

work was to show that the techniques were safe. Many of the complement studies were unavailable for sheep and we did not want to use large numbers of animals unnecessarily. The data although compared between groups, is not put up for detailed statistical analysis. That said, a certain number of control animals were thought to be essential to provide a baseline for many of the investigations performed.

## CROSS CLAMP FIBRILLATION STUDIES

Following on from many of the lessons learnt in the preliminary study, a series of six animals underwent an investigation using cross-clamp fibrillation to enable coronary artery surgery to be performed.

The reason the experiments started with cross-clamp fibrillation was threefold; firstly, it was likely to have fewer complications from electrolyte imbalance than with cardioplegia and secondly the cooling would only be to 32.5°C. Both of these factors, it was hoped, would lead to a better chance of obtaining survivors. The last reason was that some surgeons who had previously used only cardioplegia, were starting to reevaluate the use of cross-clamp fibrillation. This study took no views as to which method was superior, rather that the double reservoir technique could be used with either or both.

## METHODS

Six sheep were used, five male and one female; the weights ranged from 27kg to 36kg, mean 30.2kg. The standard procedure, previously described, was used i.e. establish left heart bypass, cool to 32.5°C and whilst cooling, right heart bypass was established. The procedure was well tolerated and the sheep were able to be weaned easily from bypass in all cases. In addition to mimic problems and facilitate rewarming, the sheep were left on bypass for at least another 60 minutes. The pericardium was hitched with stay sutures to the wound edges; under these circumstances, the lung movements were controlled and did not interfere in any way with the surgery field. The heart was also tipped to reveal the circumflex territory and despite that, the venous drainage was maintained.

At this time, in all but one case, the left atrial cannula was advanced into the left ventricle to obviate the need to vent. There then followed three periods of aortic cross clamping, each

lasting twelve minutes with five minute intervals where the heart was DC converted to sinus rhythm. No blood was used during the entire procedure on any animal so that all the platelet function and other tests would relate to the animal itself. Cardiotomy suction was also kept to a minimum to avoid extraneous damage (deJong et al 1980). In previously reported studies (Bodnar and Ross 1983) , the experiments were stopped as soon as the animal had been off bypass. In this study, after bypass, the protamine was given and the aortic cannula removed. The chest was closed after intercostal bupivocaine block and drainage. In some animals, venous access was maintained and fluids or diuretics given according to their fluid status and haematocrit, but this was stopped after six hours. The intercostal chest drain was removed the following day and the animals returned to their enclosure. The animals were terminally anaesthetized at one week.

Four further sheep had a standard bypass using a Cobe CML membrane oxygenator. So that these animals could serve as a control for this and later studies, no cross clamping or any other

procedure was performed. These animals were not recovered.

The bubble counts were measured by a TM8 bubble activity monitor with the setting at ten microns, to enable all bubbles that the monitor was capable of detecting to be counted.

The platelet counts are corrected according to the formula $PC_c = PC_m(PCV_1)/PCV_2$ where $PC_c$ is the corrected count, $PC_m$ is the measured count, $PCV_1$ is the initial PCV and $PCV_2$ is the PCV at the time of the $PC_m$. The error bars on all the figures show Standard Error of the mean.

RESULTS

All animals survived the procedure except one who died from exsanguination from an accident during decannulation of the aorta.

It was evident that clot easily formed and there were no bleeding problems. The postoperative course was uneventful. They were extubated within 30 minutes of chest closure and standing, eating and drinking within another 60 minutes.

Fig. 8 shows the ability of the system to oxygenate well and to remove carbon dioxide. The maximum concentration of inspired $O_2$ ($FiO_2$) was 0.5. Range 0.33 - 0.5 (all but four of the readings were taken with the FiO2 below 0.4). In the early phase of bypass, it was necessary to manipulate the tidal and minute volumes but this was usually by way of reduction to prevent very high oxygen levels. At the end of bypass, there was no deterioration in the ability to oxygenate and this continued in the postoperative period. The airway pressures were unchanged in all cases throughout the anaesthetic. Other parameters indicating

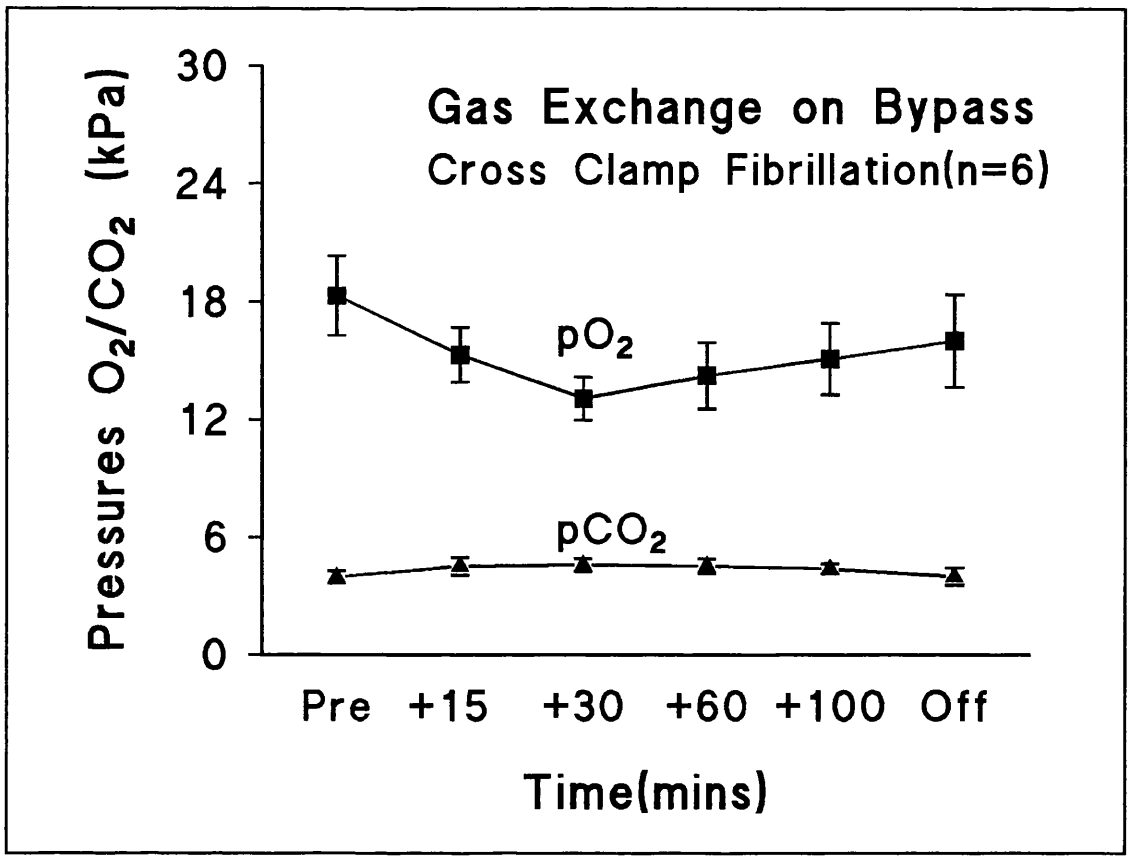


Figure 8

adequacy of bypass included the lack of acidosis on bypass.

The pressures over the course of bypass can be seen in Fig. 9. The systemic mean pressures show a typical pattern of a gradual fall during the bypass followed by a steady rise to prebypass levels at the end of rewarming. The pulmonary pressures showed no change throughout the cases; this was achieved by ensuring that the right sided bypass had a well mixed prime. In the early cases,

it was found that clear prime sent to the lungs provoked a severe broncho and vasospastic reaction.

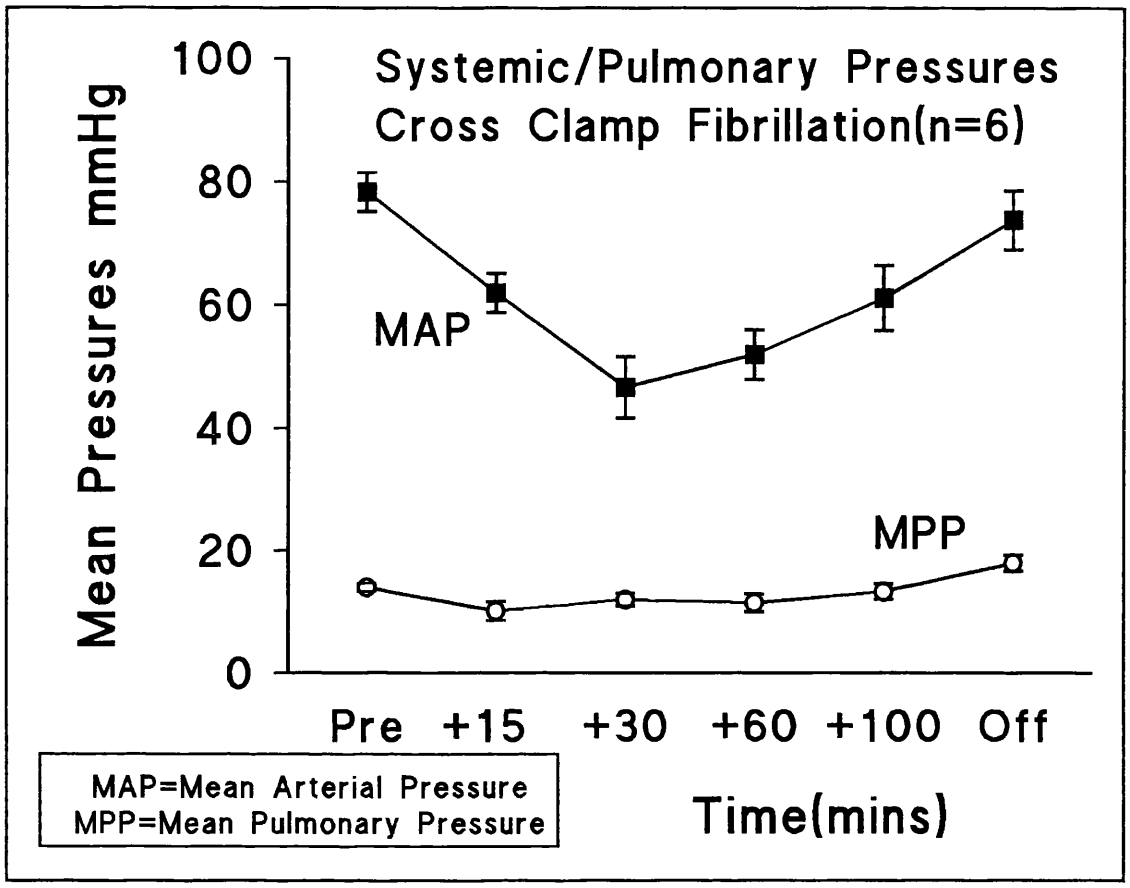



Figure 9

The fact that the double reservoir technique uses two roller pumps raised the possibility that the red cell haemolysis would be at unacceptable levels. Typical values found on bypass (Wright 1986) are in the range of 30 to 40 mg/dl at the end of bypass. Other researchers have found that the addition of a further roller pump adds 5-8 mg/dl to the single pump values (KM Taylor 1991,

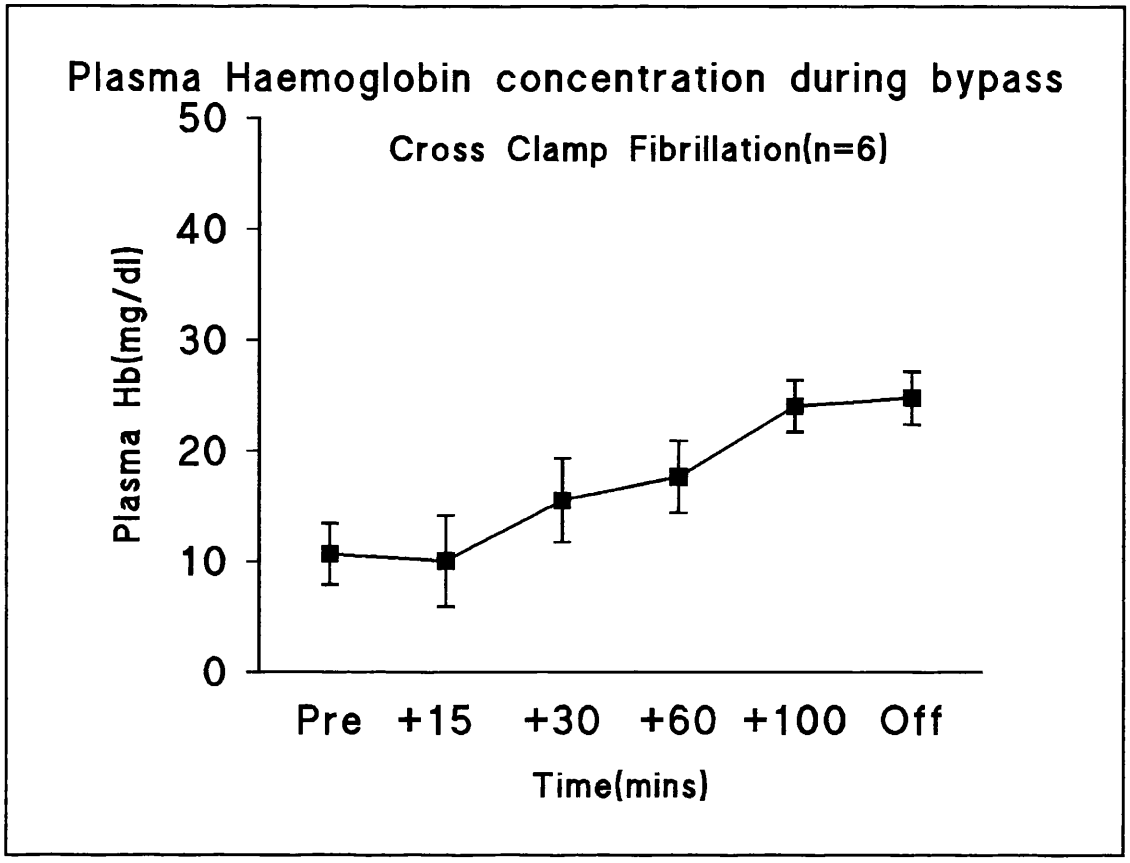



Figure 10

personal communication). The values seen in Fig.10 show a small and steady rise over the bypass that failed to reach 30 mg/dl at the end. This was an important finding and one that was repeated in subsequent studies. The numbers are small in the groups but comparing with bypass results obtained in the

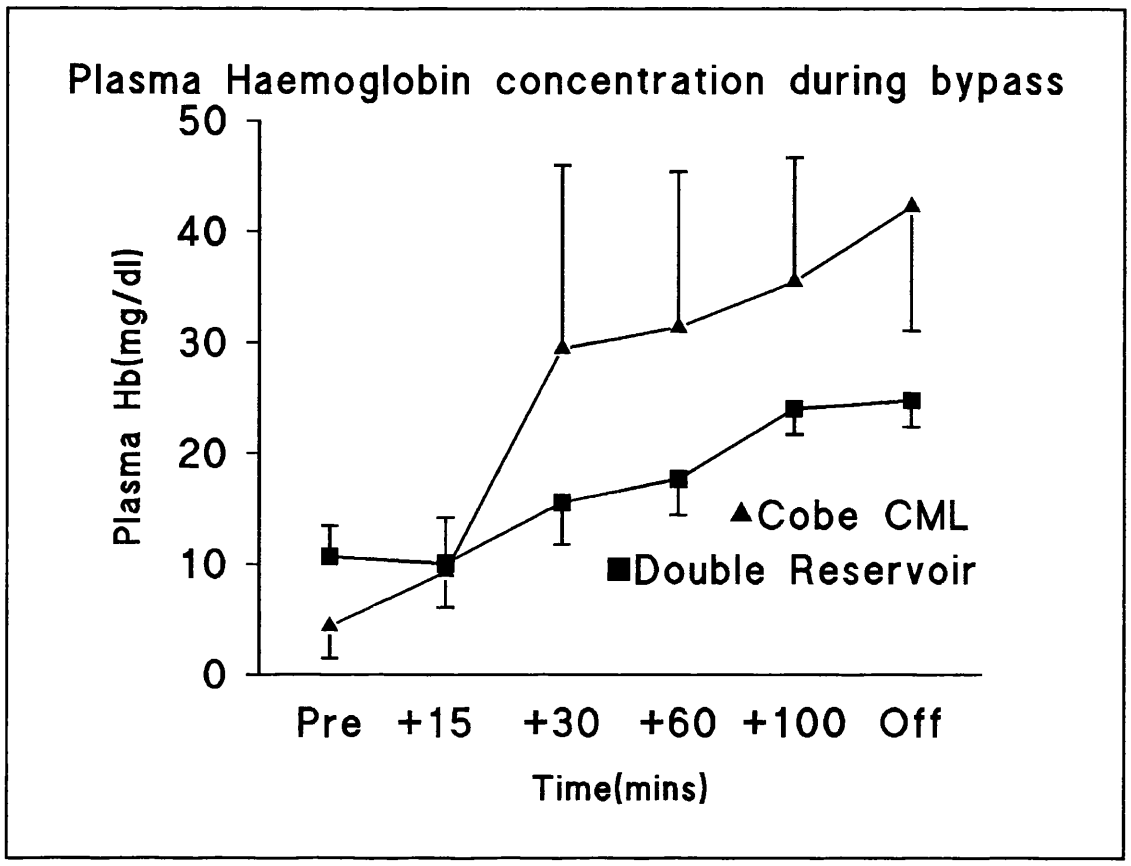


Figure 11

sheep with a Cobe CML membrane Fig. 11 the values are at the least no worse.

Platelet results are also presented in Fig. 12 and show that numbers of platelets are preserved throughout the bypass. As with clinical cases, the platelet numbers on subsequent postoperative days, either returned to preoperative levels or

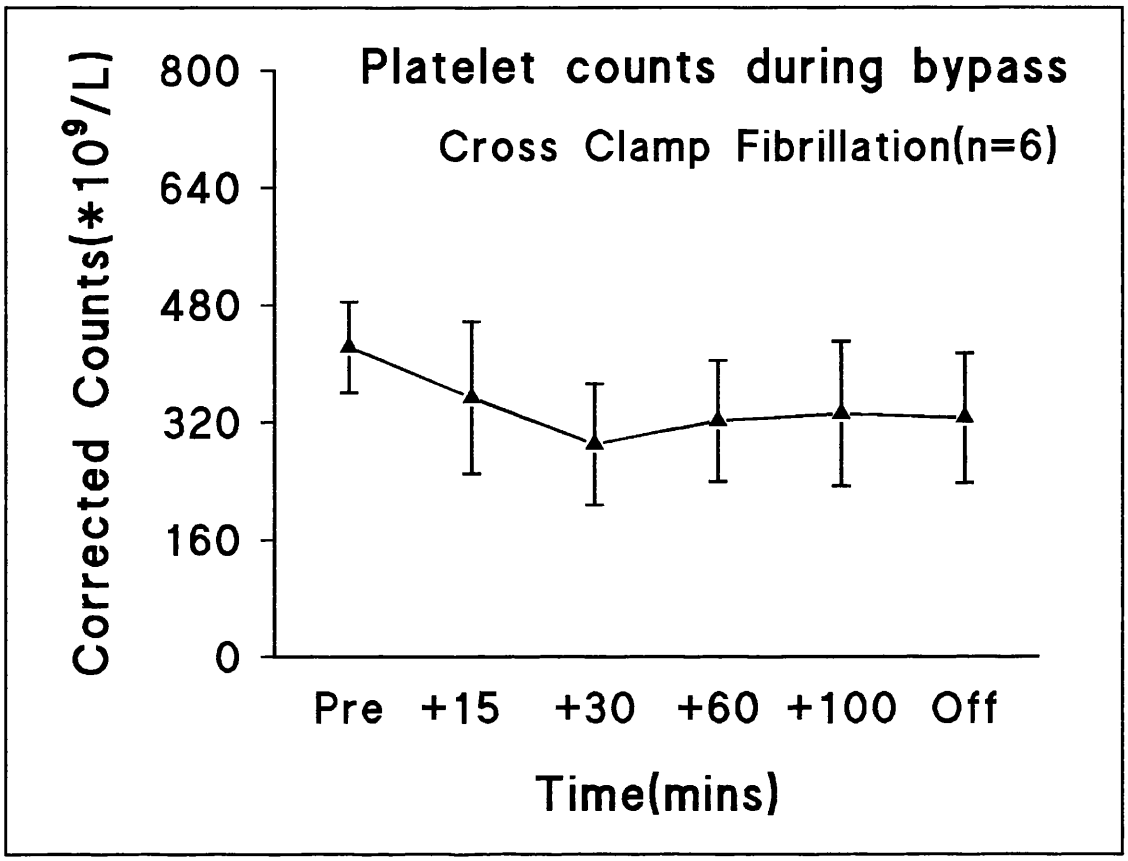


Figure 12

slightly higher. The corrected platelet counts seen in Fig. 13 again show perhaps slightly lower levels as the bypass proceeds.

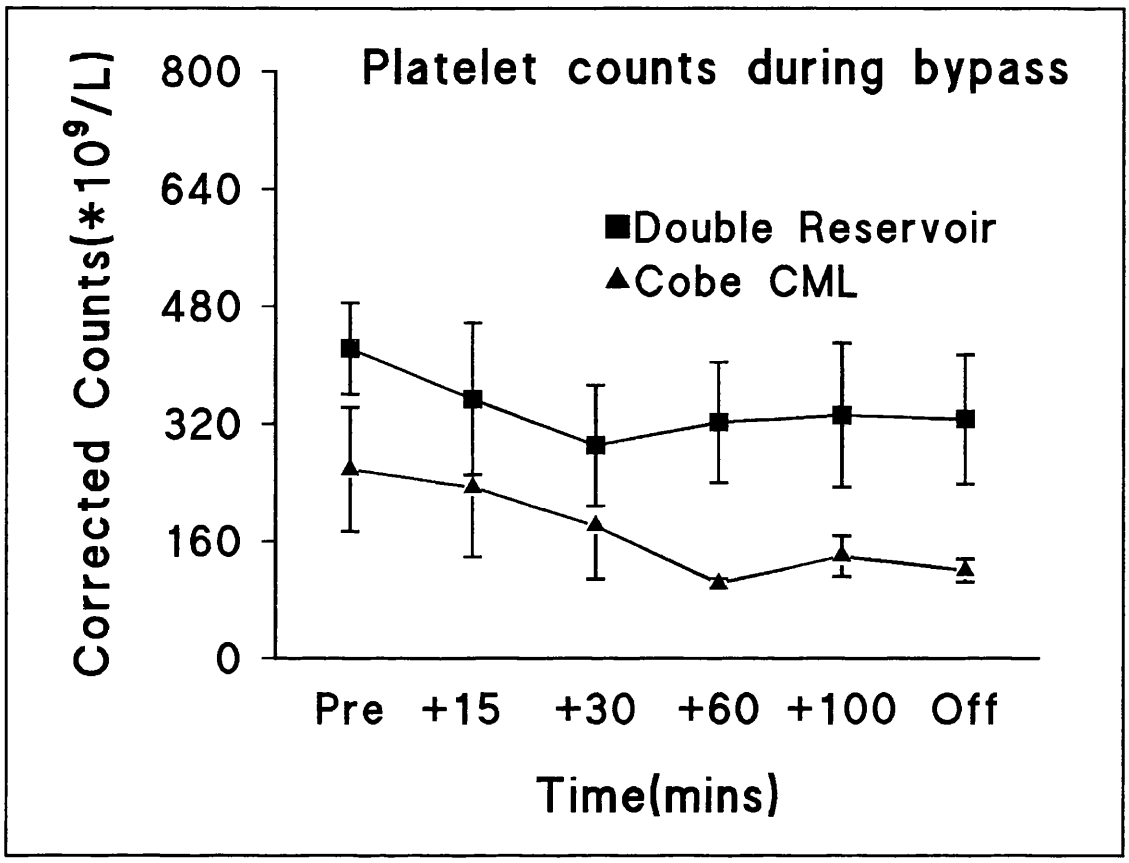


Figure 13

Platelet function as assessed by ADP aggregation is displayed in Fig.14. It shows that platelet function which dips during the cooling period to about 55% is restored after warming to 87.3%(SEM 4.97). The results were consistent over the cases, which was encouraging considering the problems associated with

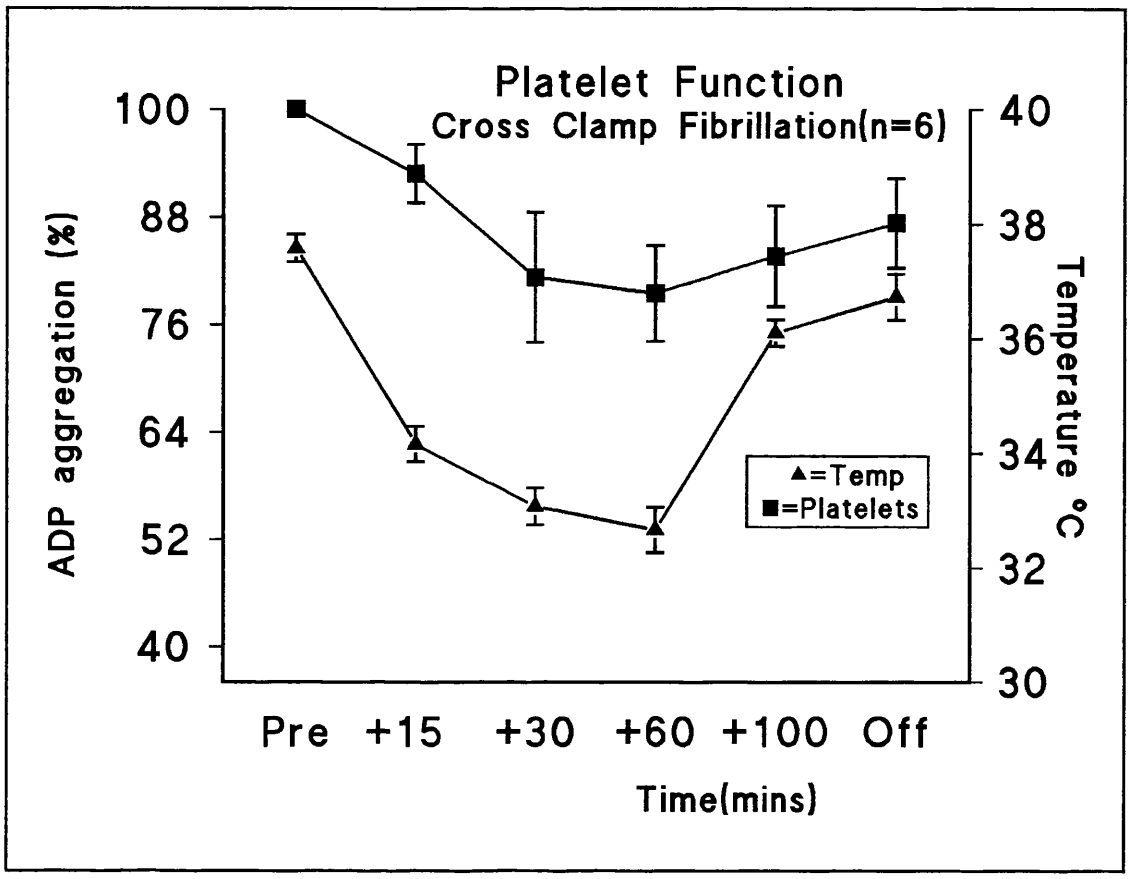


Figure 14

reproducibility of ADP aggregation. The platelet function in the membrane sheep was about the same with return to about 78% of the prebypass function.

Bubble counts results, using the TM8 bubble activity monitor were consistently zero for all of the cases. The exceptions to this were the few moments after going on left heart bypass and at times when the levels in the reservoirs were allowed to drop to very low

levels. At these times, there was obvious turbulent flow and small numbers of 10 micron size bubbles were generated. This illustrates the problems of cannulation (Pearson et al 1986) and of keeping adequate volumes in the reservoirs. That said, the levels were so low i.e. zero for the rest of the bypass in all cases that checks were continually made to ensure the TM8 was actually working. The TM8 probe was principally placed on the systemic arterial line since the systemic circulation appears the most important from a morbidity point of view. When it was placed on the pulmonary arterial line, low levels of counts in the range of 5- 10 counts/second were occasionally encountered. It was postulated from this that any of the bubbles entrained from the bypass equipment or other sources were being drawn across the shunt to the right side. Whether this is correct or not, it remains that almost no circulating gaseous microemboli get to the systemic circulation using the double reservoir technique.

## CARDIOPLEGIA STUDIES

These studies were undertaken to see if the double reservoir technique could work with cardioplegia. The potential problems were purely in the animal context as to whether one could keep the haematocrit high enough during the bypass without giving any donor blood. If the haematocrits were to go very low, then the results may not have been meaningful. Secondly, there was some doubt as to whether the cooling would make recovery experiments impossible. Finally and perhaps crucially from a clinical point of view, it was important to see whether atrial warming from the cannulae, particularly on the left side, would render the cardioplegia ineffective.

## METHODS

Five male sheep were used with weights 33 to 45 kg.(mean 37.6). The bypass techniques were identical to that previously described. In addition, the sheep were cooled to nasopharyngeal temperatures of 25°C. At this time, after cross clamping the aorta, a first dose of St. Thomas' cardioplegia was given in the dosage of about 15mls/kg. Myocardial temperature probes were placed around the left atrium and one deep in the intraventricular septum. A repeat dose of about 7-10 mls/kg cardioplegia was given as the septal temperature rose to over 20°C. The cross clamp was removed after at least 40 minutes and rewarming was commenced. At full rewarming, the sheep was taken off bypass and then recovered in the manner previously described. The same measurements were taken as before and in addition, the temperature readings of the various probes were recorded.

## RESULTS

All animals came off bypass without difficulty and recovered from the procedure and were extubated. They were generally much more sleepy than their cross clamp fibrillation counterparts and did not stand for about two hours. One animal cooled down over the course of the next 12 hours and was not a longterm survivor.

The ability of the double reservoir technique to provide gas exchange is fundamental. Fig. 15 demonstrates that good oxygenation was obtained throughout the bypass as was carbon dioxide removal. Attention was given to the haematocrit during the bypass and was rarely allowed to drop below 20. There is a trend to lower oxygenation over the course of the bypass which raised the possibility that longterm bypass may not be suitable. This was shown not to be so. Further the levels were still very acceptable on low fractions of inspired oxygen and the animals were extubated and breathing spontaneously on room air at the end of the operation.

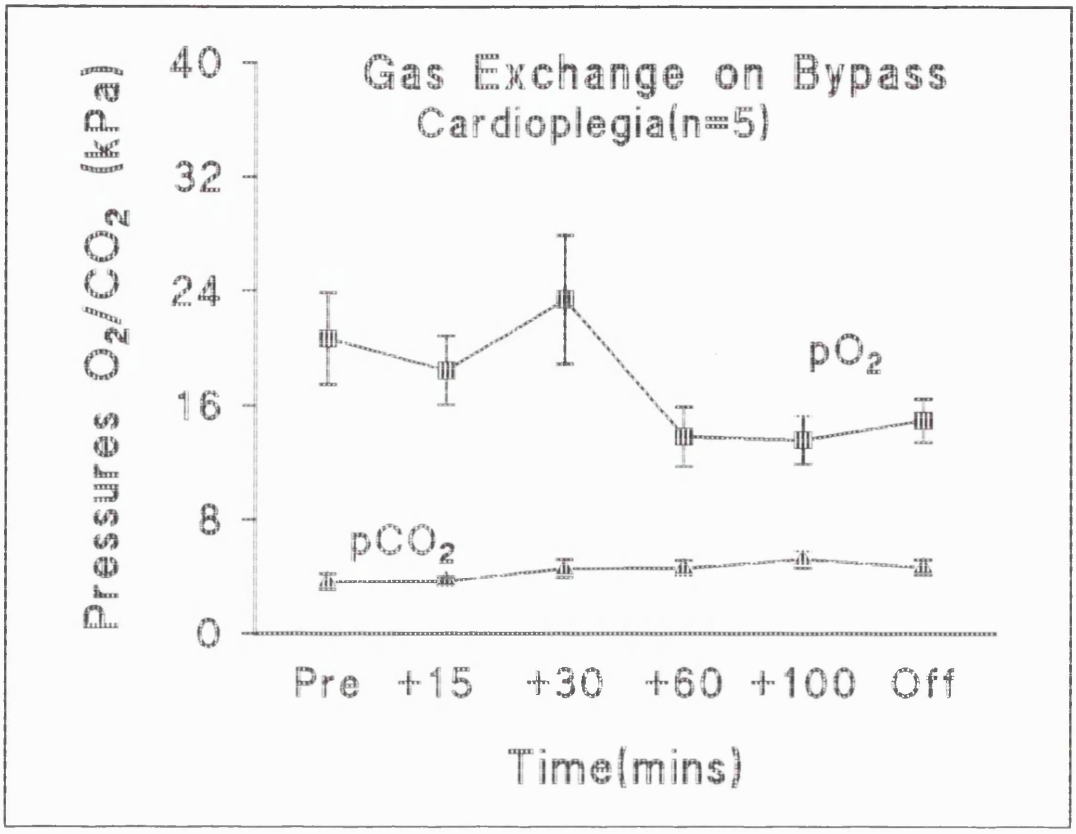



Figure 15

These animals were also monitored with arterial lines and Swan Ganz catheters and the results can be seen over the page in Fig.16. The systemic pressures show the typical pattern and again, the pulmonary pressures were relatively constant throughout the bypass. This was also reflected in the airway pressures recorded from the ventilator which were consistently low.

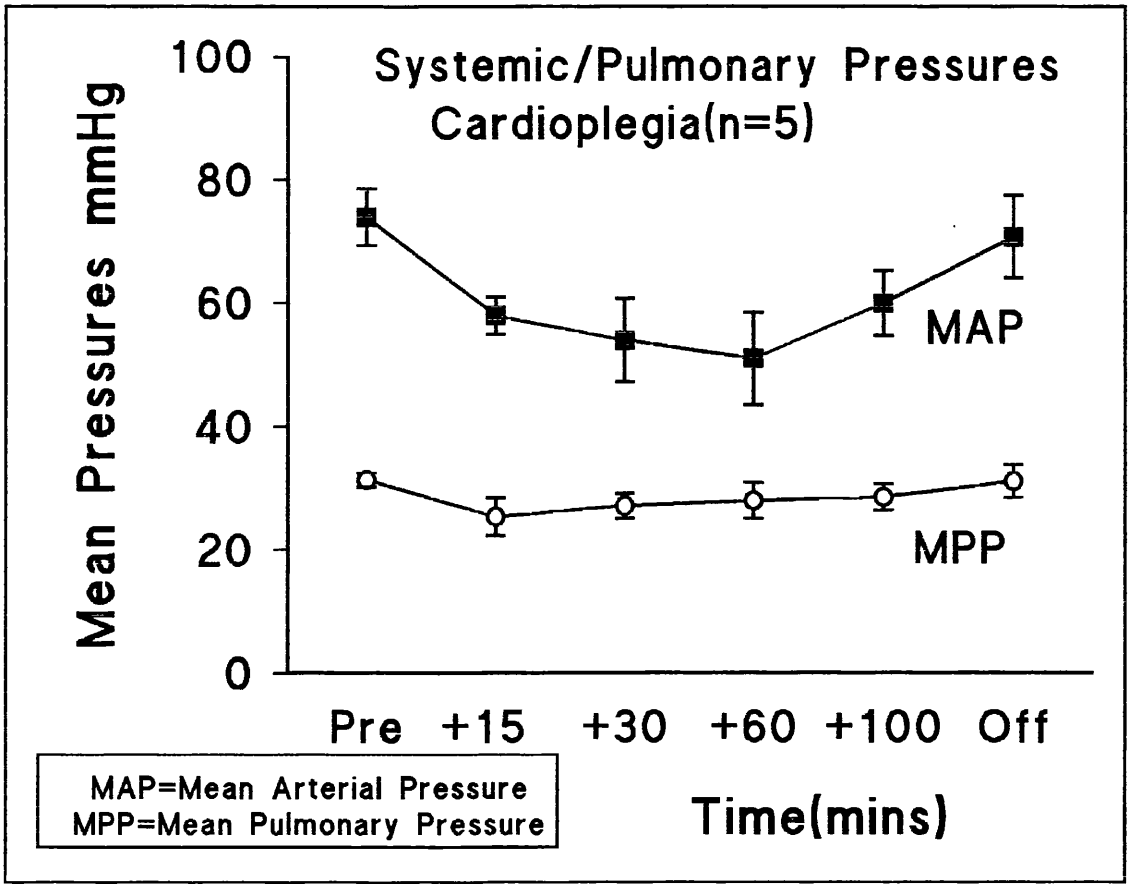

Systemic/Pulmonary Pressures
Cardioplegia(n=5)
Mean Pressures mmHg
100
80
60
40
20
0
MAP
MPP
Pre
+15
+30
+60
+100
Off
Time(mins)
MAP=Mean Arterial Pressure
MPP=Mean Pulmonary Pressure

Figure 16

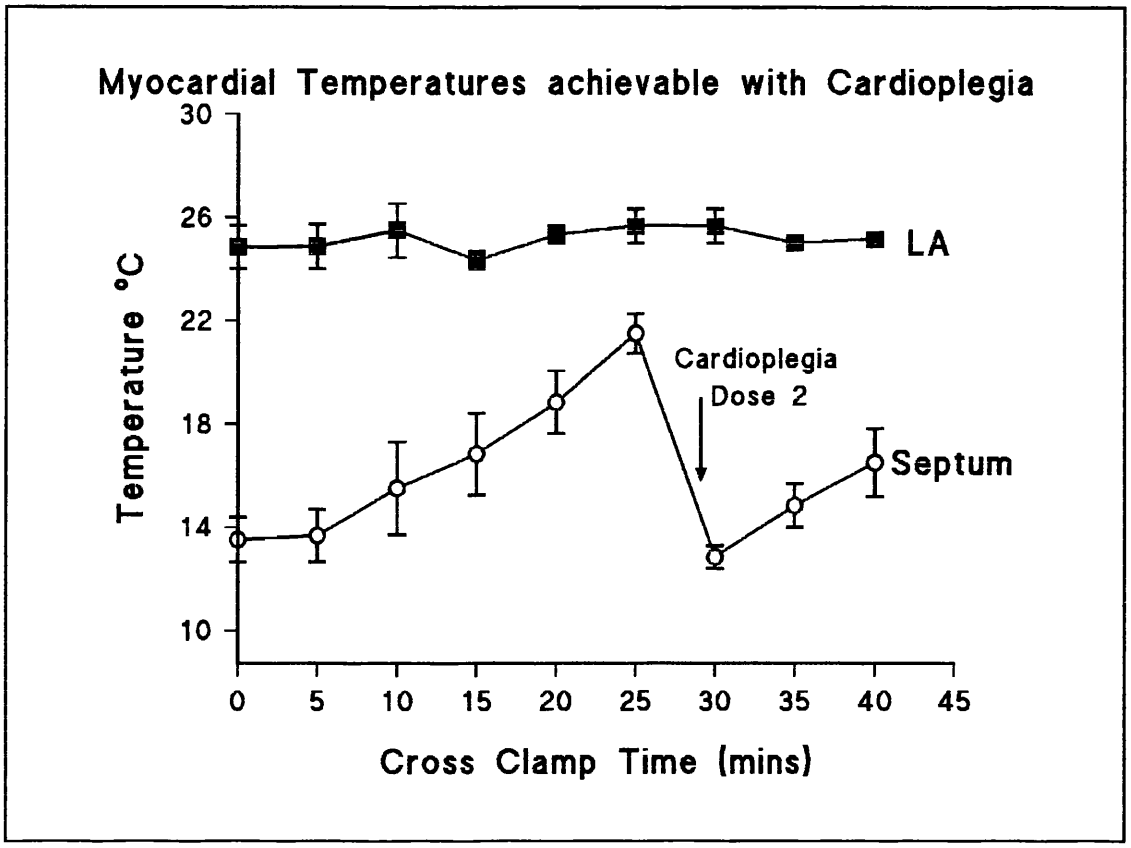


Figure 17

The importance of the timing of giving further doses of cardioplegia is of obvious importance for clinical cases. It was therefore carefully investigated in these cases using myocardial temperatures as a marker. The results of this can be seen in Fig. 17. The core cooling temperature of 25°C was maintained throughout the cross clamp time. The first dose of cardioplegia allows the septal temperature to cool to 13°C. No topical cooling was used during the cross clamp period. The temperature can be

seen to rise with time and by 25 minutes has exceeded 20°C. At this point a further, but smaller, dose of cardioplegia was given. The temperatures again fell to 12°C and rose in a similar manner.

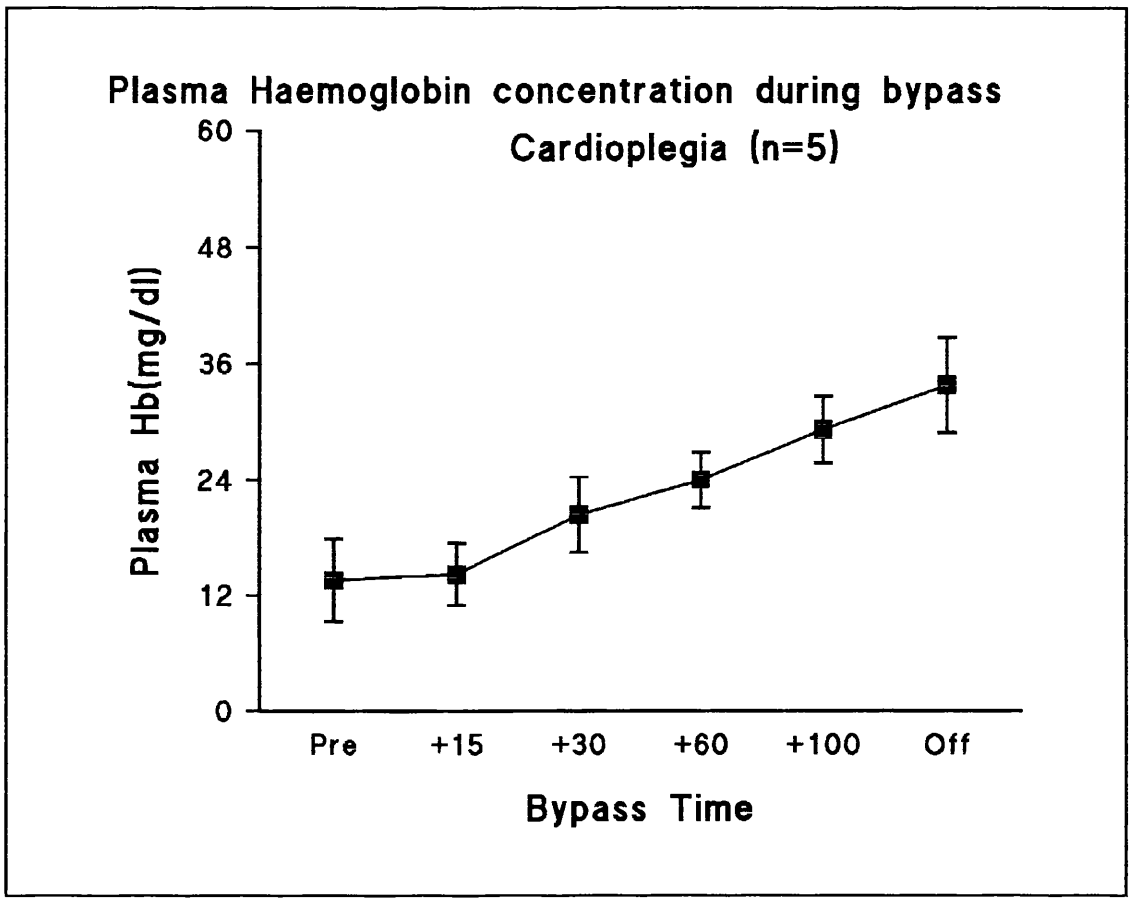



Figure 18

The plasma haemoglobin levels were recorded during the course of the bypass in Fig. 18. The levels rise throughout the bypass and do not appear to have reached a peak. The highest levels reached are still not excessive but again, the need to perform long term

studies is seen. It should be noted that the peak levels are below 36mg/dl.

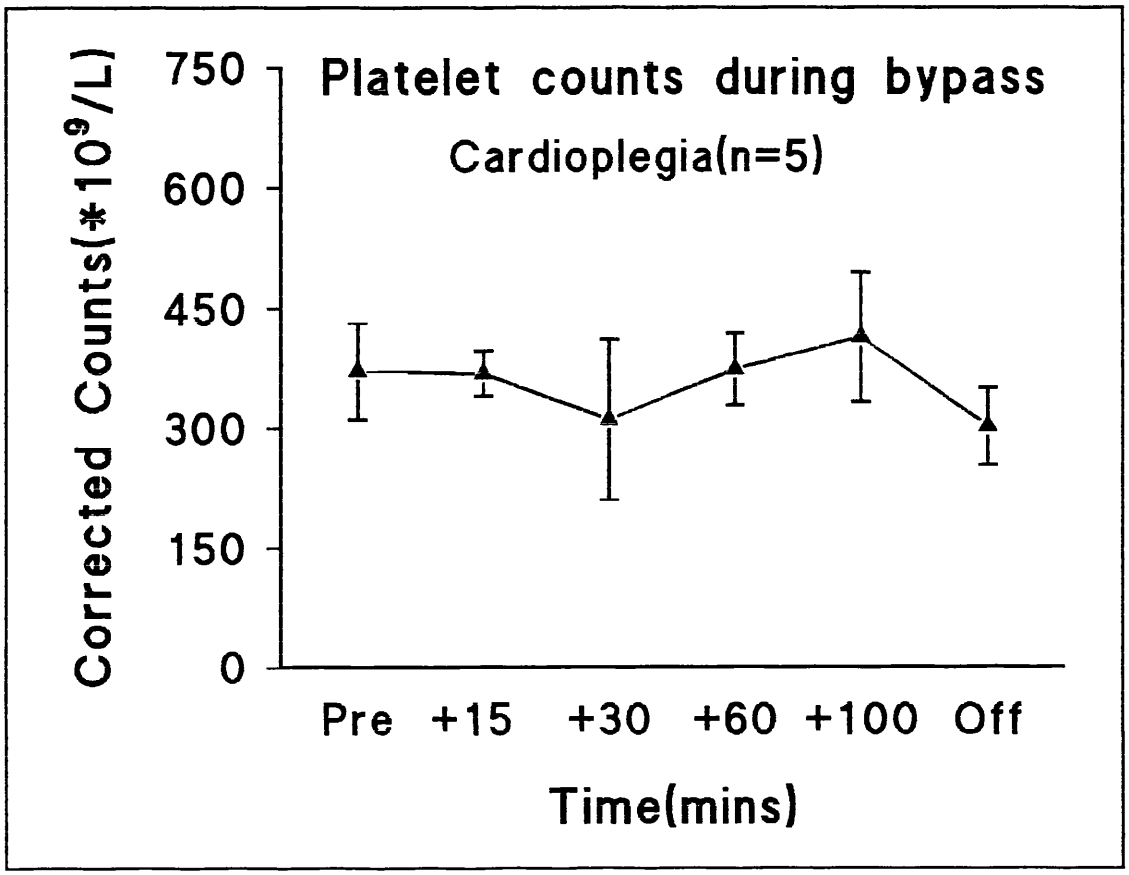



Figure 19

The ability to form clot at the end of bypass was impressive in all cases. As soon as protamine was given, the surgical field appeared extremely dry. It was easy to observe that the raw serous surfaces normally seen on cardiopulmonary bypass were absent during the bypass and postoperative bleeding was minimal. This is reflected in the platelet counts and platelet ADP

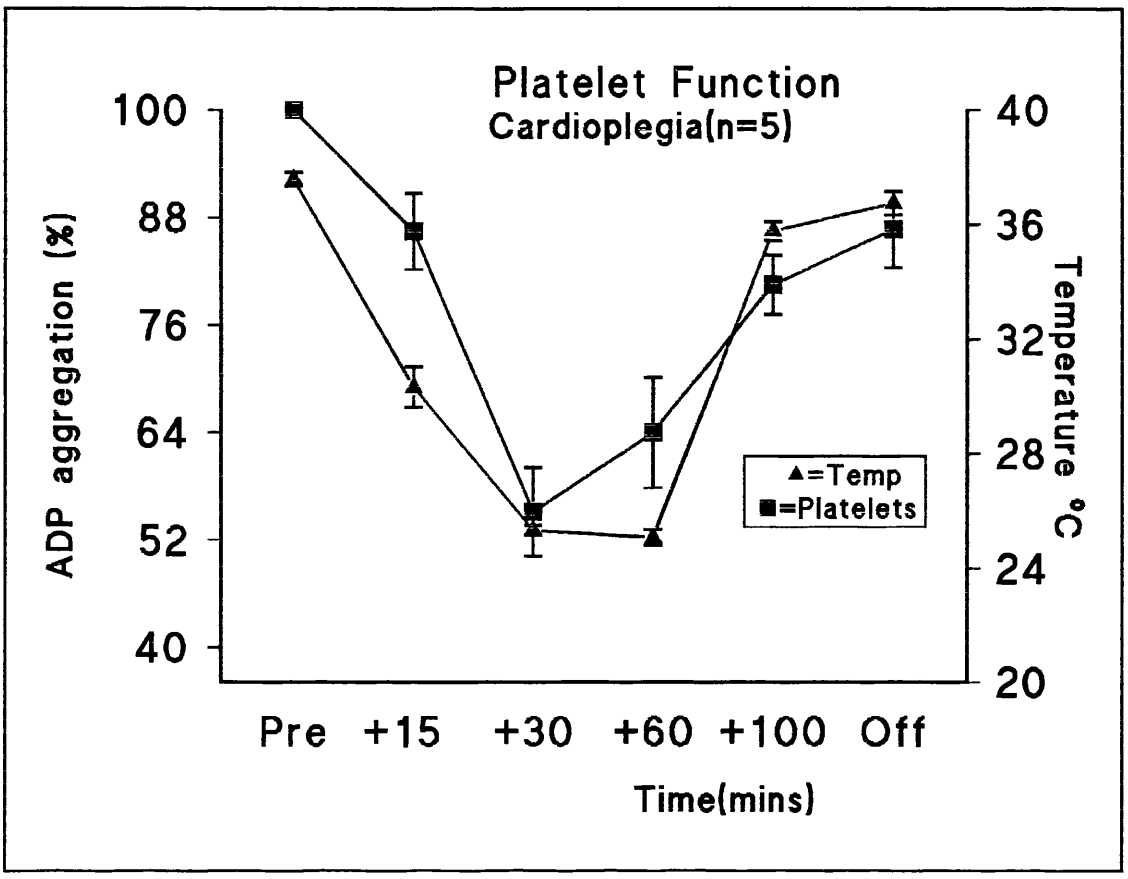


Figure 20

aggregation studies that are detailed in Figs. 19 and 20 respectively. The corrected platelet counts according to the above formula showed very little fluctuation over the bypass. At the end of bypass, the level is the same as prebypass. Finally, despite the obvious and severe effects of cooling, platelet function is restored to 90% of prebypass levels after rewarming and after protamine administration. The Error bars show Standard Error of the Means and show very consistent results over all the animals.

## LONGTERM STUDIES

The previous studies have demonstrated the ability for the double reservoir technique to provide good gas exchange, but raised the question as to whether the good results can be sustained over a two hour bypass. They have shown that systemic pressures follow a predictable course and are always within an acceptable clinical range without major pharmacological manipulation. The pulmonary pressures have shown excellent consistency over both sets of cases, again stressing the good mixing of the right sided bypass and the slow rate of going on bypass on that side. The plasma haemoglobin levels have also been well within the physiologically acceptable range but have not peaked during the period of the bypass. Finally, the clinical ability to form clot together with the platelet counts and platelet function tests, have demonstrated good haematological performance in the short term.

Encouraging though all these results are, they left sufficient

numbers of questions unanswered to permit proceeding to the next phase of the project i.e. clinical studies. The requirement was to find out if these many parameters could be sustained over a longer period. If a clinical case met problems coming off bypass, it was important to predict what the likely effects of the bypass were going to be. Therefore it was decided to perform long term bypass experiments where animals would be kept on bypass for 24 hours and the same parameters measured.

METHODS

Three male sheep 45 to 63 kg. were selected. They were placed on bypass in the standard manner, allowed to drift down to about $33^{0}C$ and kept on bypass for 24 hours. At the end of this time, they were fully rewarmed and taken off bypass.

To obviate the need to watch the bypass levels over a 24 hour period, the use of centrifugal pumps was considered. Roller pumps and shifts of perfusionists were employed however to allow comparison with the previous studies.

The fluid requirement over a 24 hour period was important. None of the animals in the previous studies received blood either peri or postoperatively; this allowed clear interpretation of the haematological data. It was not possible for this long period of time, so the sheep each had two units of blood taken off in the weeks prior to their operations. This was transfused when their haematocrits dropped below 20. Although preserved blood, it was at least their own and therefore there were no grouping problems.

RESULTS

The three sheep were each on bypass for 24 hours. At the end of that period, all of the sheep were weaned from bypass without any difficulty. One animal required a small dose of adrenaline as a single bolus, but was not repeated. All animals were decannulated and had protamine administered after which time, they maintained excellent pressures and arterial gases. As an indication of the adequacy of the bypass, one animal had no infusions of bicarbonate on bypass, one required 100 mls and the third only 150 mls 8.4% sodium bicarbonate infusion.

Gas exchange was good over the course of the bypass period as seen in Fig. 21. After about ten hours of bypass the $FiO_2$ was increased to0.9 or 1.0 but by the end of the bypass period and after an aggressive diuresis, this was reduced to between .6 and .75. The removal of carbon dioxide was not impaired throughout the period. Postoperatively, there was no problem with extubation and good gases were obtained initially. These deteriorated as the

animals continued to be sleepy and the study was then stopped.

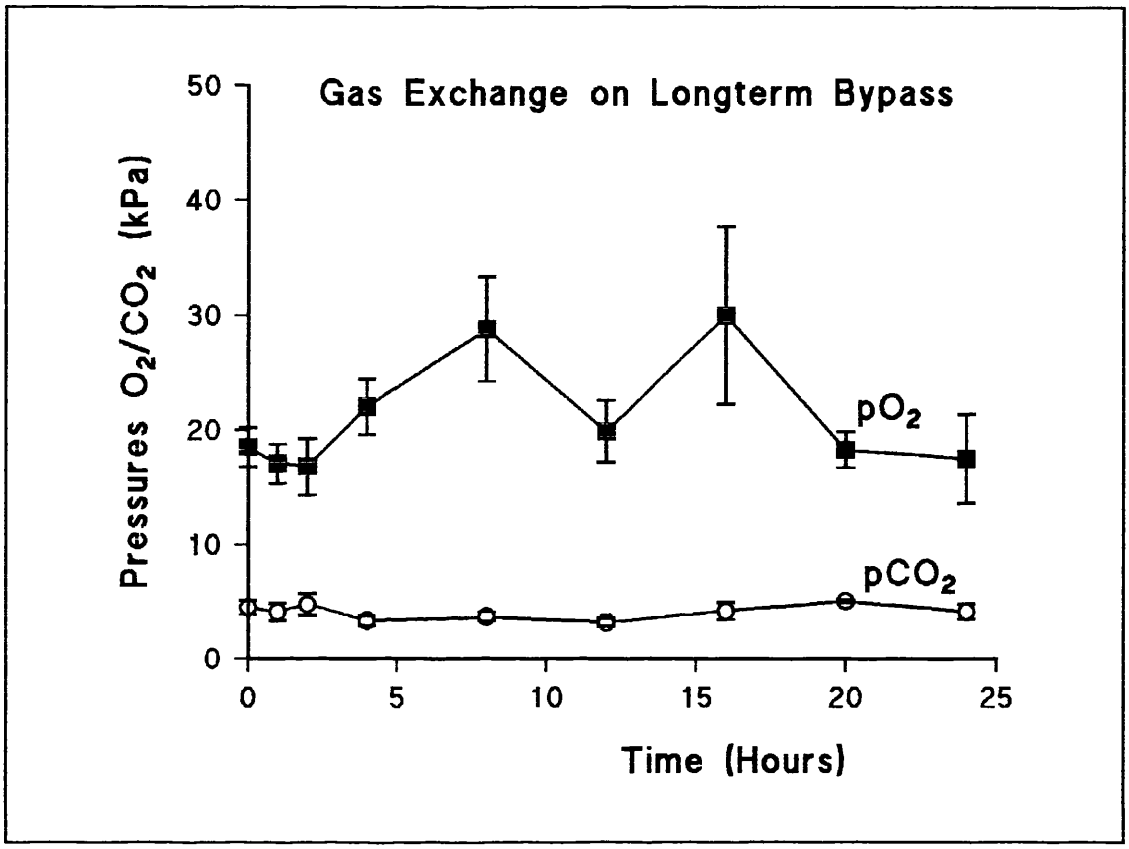



Figure 21

Two animals were recovered for a short time, but although standing and occasionally drinking, were too drowsy to maintain their airways and the experiments were therefore stopped.

The systemic pressures are seen together with the mean pulmonary levels on Fig.22. They are identical with the two previous studies. The figure shows the standard vasodilatation

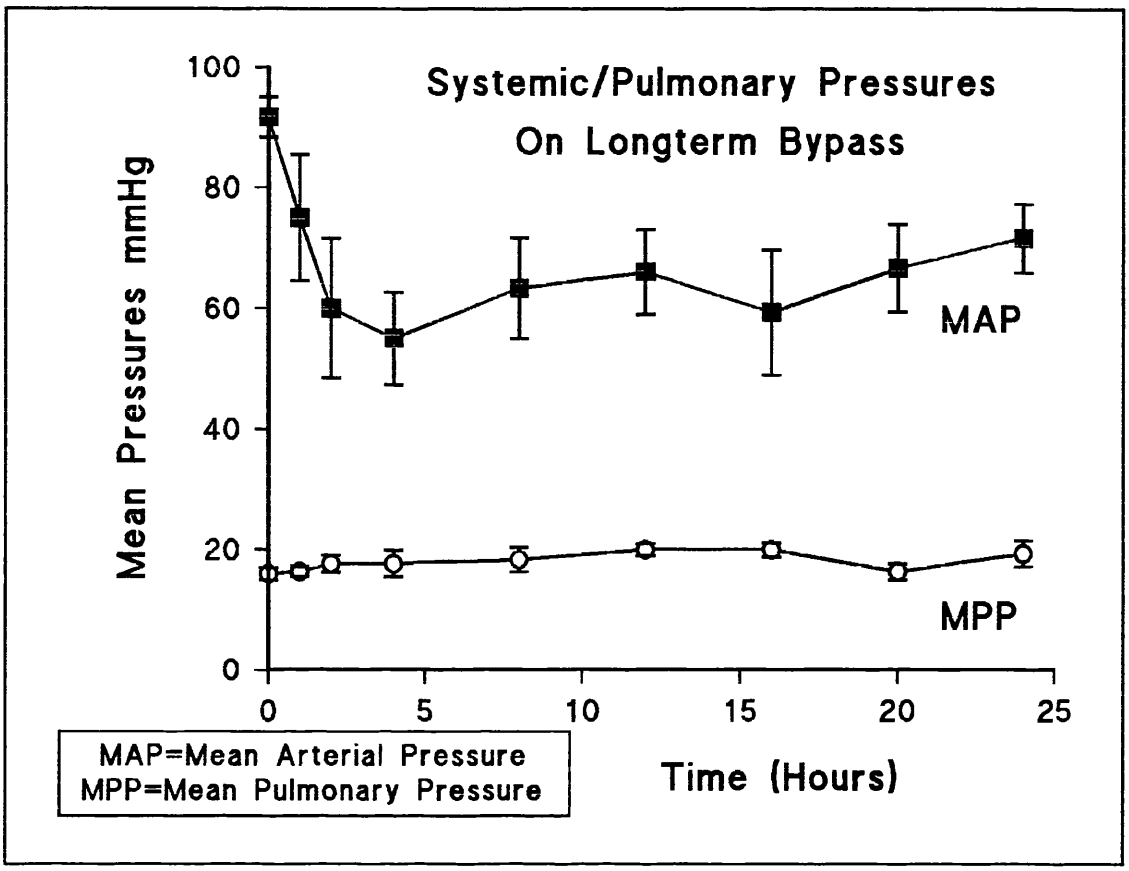


Figure 22

over a three to four period followed by a gradual rise to prebypass levels. There were no rises in the pulmonary vascular circulation and again this was mirrored in the ventilatory airway pressures.

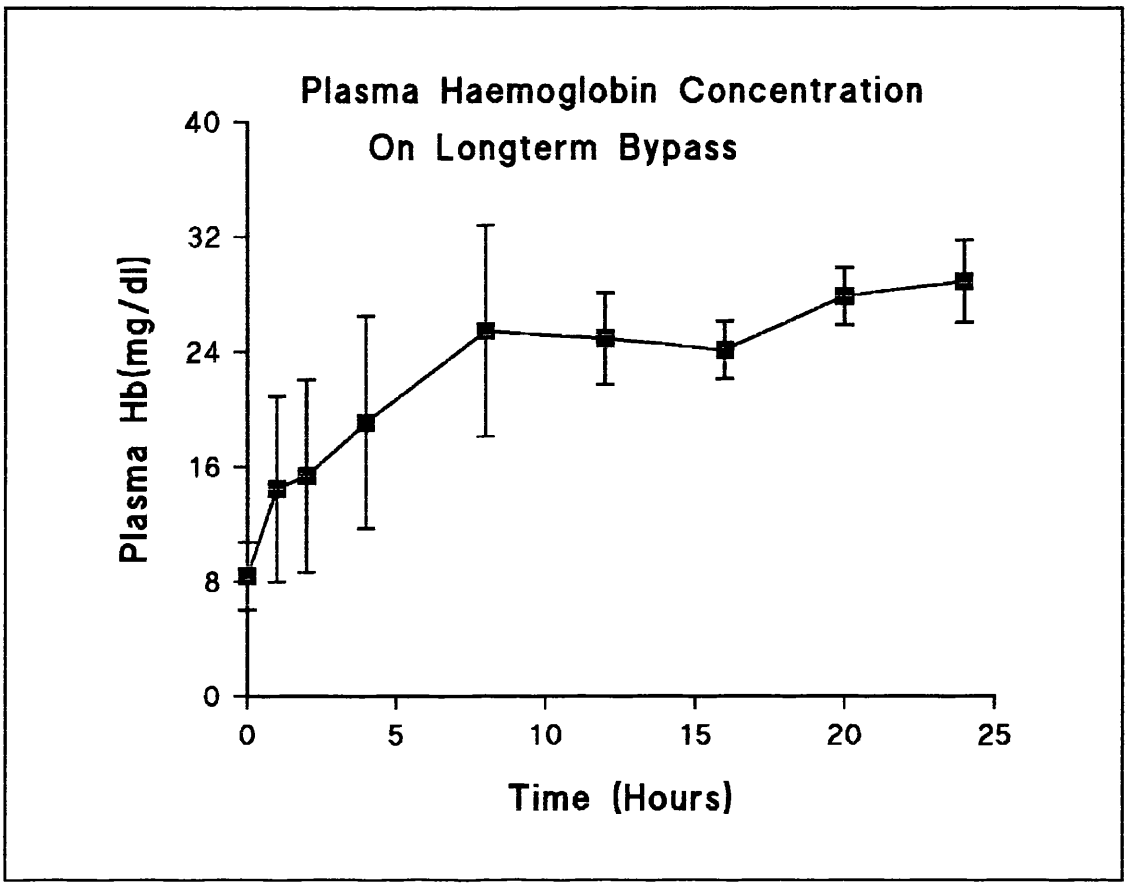


Figure 23

The plasma haemoglobin levels were quite surprising. They can be seen in Fig.23; the initial studies had led one to predict that the levels would continue to climb over a long period but this was not confirmed. The levels even at 24 hours are fractionally above 30 mg/dl and show no sign of climbing higher. The initial fairly steep climb stopped at 10 hours and after that there is a gentle rise in levels.

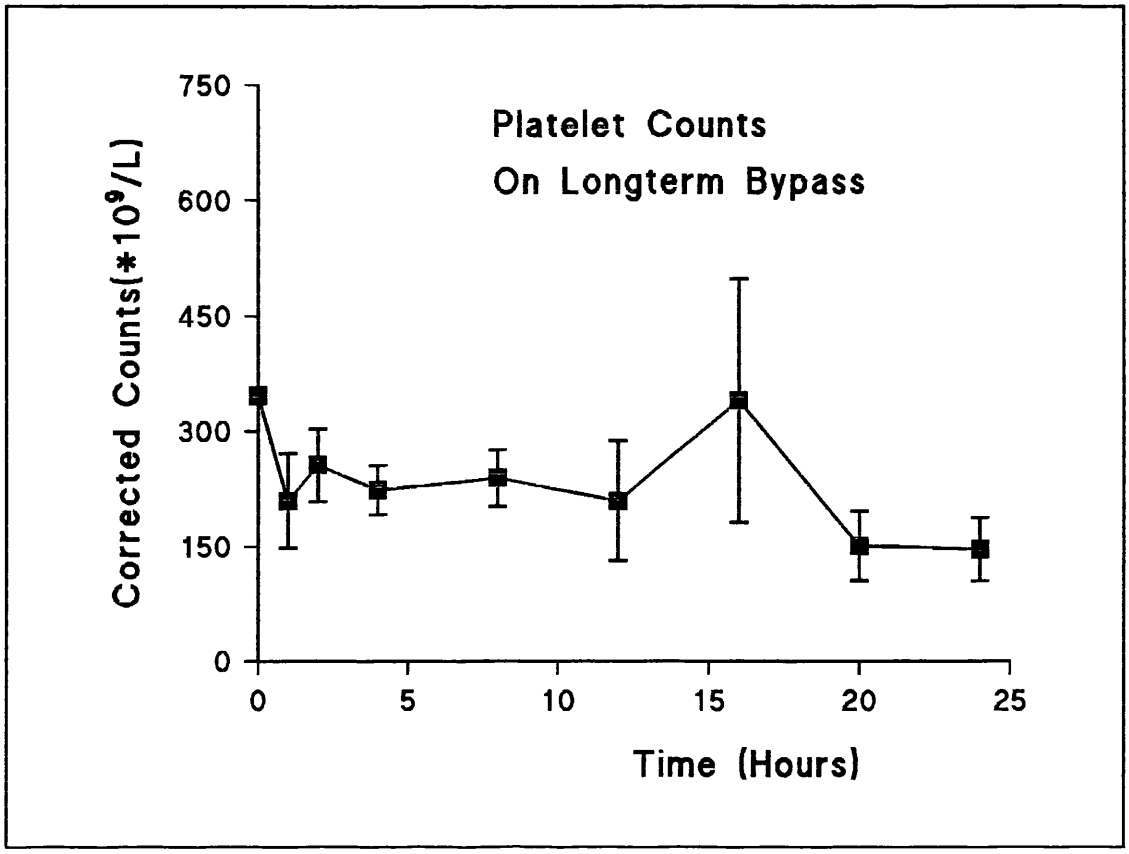


Figure 24

The haematological data was also very encouraging. The sheep formed excellent clot after protamine administration. The platelet counts on Fig. 24 show a gentle decline over the 24 hour period but were still greater than 40% of prebypass levels. Their function was only assessed by ADP aggregation in one case, it dipped to 50% at 12 hours but was back over 80% post protamine administration.

## DISCUSSION

The fundamental question that was asked of these three projects, was whether a clinical study was indicated. Previous clinical work (Drew 1959) had not used the double reservoir technique for coronary surgery and the intention was not to produce profound hypothermic arrest. Ross (Ross 1986, personal communication) had used the technique on a few patients in 1984 with mixed results. Although the perfusion seemed acceptable for those cases, there was felt to be insufficient data to proceed further.

We chose to look at the double reservoir technique on several levels; the ease of use for the surgeon, anaesthetist and perfusionist, the ability to oxygenate and remove carbon dioxide and perfuse the patient, the basic haemodynamic effects, the haematological and biochemical values as well as gaseous microemboli and other factors that might provide a beneficial bypass for the patient.

The surgeon obviously has two extra cannulations to perform. The

pulmonary artery cannulation, although perhaps unfamiliar, is easy to perform with a cannula identical to the aortic cannula normally used; the only caveat is to ensure that the collar is fairly short so that only the main pulmonary artery is cannulated and blood flows through both lungs. The left atrial cannulation needs more care because of the potential to introduce air. We found that the simple manoeuvre of hand ventilation ensures a temporary high left atrial pressure at the time of cannulation.

The actual time involved was not greater than with conventional bypass since the cannulae are placed sequentially and the right sided cannula are placed when already on bypass and cooling is taking place.

The perfusionist's role is perhaps greater than with conventional bypass. The setting up is also more complicated, but that is only because at that time, there were no custom made sets. Confidence in running the system led to smaller and smaller prime volumes. The running of the bypass does require a clear understanding of

the technique and a certain amount of practice. The fact that the perfusionists did not control the degree of oxygenation took a little adjustment, but they were the first ones to spot a falling $pO_2$; deoxygenated blood in the soft shell reservoirs was easy to spot. The use of the shunt is also a concept that has to be learnt to exploit it to the full.

The positive side was to see the perfusionist who understood what extra flexibility and control they have gained, run a case. The weaning is particularly important for the fragile ventricle with a low ejection fraction and the double reservoir can give the perfusionist amazingly fine control.

The individual blood results are presented in their appropriate chapters, but certain generalisations can be made. The haematocrit was the same as with conventional bypass. The need for heparinisation, whilst not formally compared with matched controls, appeared to be also identical, as was the reversing dose of protamine. The white cell counts do not drop as much as in

conventional bypass, corrected for haemodilution. As previously stated, this is due to the fact that the leucocytes do not get sequestered in the pulmonary circulation. The lack in drop in the platelet count is perhaps even more pronounced and even after protamine administration approached corrected values of 90% of the prebypass value.

The plasma haemoglobin was measured in the animal studies; in the more carefully controlled conditions of the laboratory, the plasma free haemoglobin levels were impressively and consistently low. This was true in the long term studies, as well, where the bypass times were around 24 hours. This answers many questions as to what contribution to blood trauma two pump heads make rather than one. It seems likely, that well adjusted roller pump heads are relatively non-traumatic to erythrocytes, whereas an external oxygenator be it bubble or membrane, may be after many hours.

The biochemical results presented failed to show any differences

between the two methods of non-pulsatile perfusion. ADP aggregation studies were performed in many of the animal studies. Whilst a clear trend to platelet preservation is demonstrated with the double reservoir technique, there are clearly many deficiencies in using this technique as an index of platelet function. This has been a recurring theme in many studies where documentation of platelet function has been attempted. Initially, people were happy to quote changes in platelet number and then realised that this was too crude an index. The ability of the platelets to aggregate to a variety of stimuli has been investigated and now certain factors of platelet metabolism e.g.beta 3 thromboglobulin are being evaluated. Certainly, the method of using ADP is important and there should be a minimal delay in taking the samples and then analysing them. The ADP should be stored at the appropriate temperature and if possible a technician with a lot of experience in using a dual chamber aggregometer should perform the analysis.

It is obviously desirable that the level of gaseous microemboli

(GME) is zero apart from the inevitable flurry after going on bypass but this can now almost be matched by many membrane oxygenators (Pearson et al 1986). As discussed in the introduction, whilst it is clearly desirable to have low levels of GMEs, it is equally important to prevent other particulate embolisation as well. It remains to be seen whether the reduction of particulate matter is equally dramatic and could thus reduce the need for arterial filters, again reducing the foreign material challenge.

The results of these three studies were quite clear. All markers of an adequate perfusion system were in place as well as certain unforseen advantages. Clinical cannulae and bypass equipment had to be thought about and the technique refined, however the basic system was sound.

The ability of the system to provide good gas exchange was demonstrated in all three studies. The ability to keep an animal on bypass for 24 hours and still extubate it was ample evidence of

its efficacy. The problem if any for the routine cases was not to have too high a $pO_2$ during the case and this required frequent monitoring to reduce the ventilatory settings. This was done so effectively that despite good $CO_2$ washout, the lungs could barely be seen to move and could not have interfered with the surgical field. The pressures on the systemic side were identical to those expected for standard cardiopulmonary bypass and may say something for the fact that the flow was non-pulsatile. It has been said that the problem with the technique was the pulmonary vasoactive phenomenon associated in the early bypass period. The early experimental studies addressed this problem and found that the problem was not temperature but that sending clear prime to the lungs with full flow, often provoked such a reaction. The above studies were after that finding. All cases had the prime mixed with blood from the left side and the rate of going on on the right side was initially cautious. This resulted in the uniform finding of low mean pulmonary pressures on bypass and low airway pressures extending even to the 24 hour studies. There was little acidosis as seen by the negligible requirement for sodium

bicarbonate infusions except when the animals had high losses of bicarbonate rich fluid from their stomach tubes.

The other indices measuring adequacy of the bypass and potential damage were the haematological data. The plasma haemoglobin levels for all three studies were at or below that expected for standard bypass (Wright 1986). The few oxygenator studies that were undertaken showed the double reservoir favourably; perhaps of most importance were the results obtained on the 24 hour study. Their low readings over a long bypass time indicate that the system is not severely damaging and moreover that the system may actually be ideal for long term support. That two roller pumps would not be haemolytic was an unexpected finding but a valuable one for the clinical cases; should it be difficult to come off bypass, the system would be adequate for support over an extended period. The bypass also might not have to be bilateral in that by weaning down and monitoring pressures, it would be possible to see which side of the heart needed most support and adjust accordingly. The problems of assessing the degree and site

of failure have been well documented (Pennington et al 1985) and the outcome clearly depends on accurate diagnosis. Here is a system that allows accurate diagnosis as part of its intrinsic make up.

The other indices including platelet numbers and function as assessed by their ability to aggregate to an ADP stimulus further support the relative biocompatibility of the system. It was realised at the outset that no system would be perfect, but it was important to be able to demonstrate that the system was no worse than the best available oxygenators. That excellent clot could be formed after 24 hours support after a standard dose of protamine was an achievement that fulfils those requirements.

The final conclusions relate to the flexibility of the system to be used for either cold crystalline cardioplegia or for cross clamp fibrillation. As previously stated, this study took no views as to which might be superior, rather that it could be used for either of them. The findings were again quite clear. All of the animals

came off bypass well and although these were healthy hearts with no known disease, they were cross clamped for adequate periods of time and performed well. The cross clamp fibrillation studies were unequivocal in their findings that the double reservoir provided a good environment for coronary bypass grafting. The cardioplegia studies were also encouraging although the fundamental finding was that repeat doses of cardioplegia were necessary after about 25 minutes. For an average cross clamp time, this would mean one repeat dose of cardioplegia as was mimicked in the studies. The question that remained unanswered from these studies was the effect that atrial warming might have clinically. There is much evidence (Braimbridge and Darracott 1980) that there is physiological deterioration of the right ventricle without changes on the left during conventional cardiopulmonary bypass and that this may lead to supraventricular tachycardias (Smith et al 1983). This would potentially be compounded by having both atria warmed but could be partially offset by the application of topical cooling. This however is a major study in its own right and has not been addressed here;

however, the response of the animals off bypass and their subsequent postoperative days, did not indicate any serious problems.

The result of all of this information was sufficiently encouraging to start planning a clinical set of studies and the following chapters detail their results.

# CLINICAL STUDIES

## INTRODUCTION

The next phase of the double reservoir project was to extend this into the clinical field. Two studies were undertaken , the first at the National Heart Hospital in London with Mr. Donald Ross used cross clamp fibrillation as the technique for coronary artery bypass grafting. The second study with Mr. Rex Stanbridge at St. Mary's Hospital in London, used cold crystalline cardioplegia. There were no contraindications to the randomisation of the patients with the exception of restriction to closed procedures. Patients with ventricular aneurysms or undergoing concomitant valve operations were therefore excluded. No patient was excluded on the basis of ventricular function or respiratory status. All patients signed informed consent forms after receiving verbal information.

The exact technique used varied from the initial animal studies to

the clinical cases and is still being refined, but the original concept is largely intact.

## BYPASS DESIGN TECHNIQUES

In both animals and patients the right atrium is cannulated using a metal caged basket or a plastic two stage cannula, which provide excellent gravity drainage. The drainage basket is connected via tubing to a reservoir and a pump then propels the blood forward to a cannula in the main pulmonary artery(PA). Studies in the past (see history of double reservoir), have used differing techniques for achieving pulmonary artery cannulation. These have included access via a right ventriculotomy and also direct cannulation of either right or left pulmonary artery divisions. On consideration of these approaches, the option of opening the right ventricle just below the pulmonary valve seemed both unnecessarily mutilating and also perhaps more complicated and was not used in either study. Much early work used just one lung for oxygenation, in practice both in the animals and clinically, it was easier to insert a cannula into the main pulmonary artery. The blood is then pumped into the main pulmonary artery using a roller pump. Here again there are other alternatives including

the use of vortex or centrifugal pumps but the plan was to use as little special equipment as possible and therefore traditional roller pumps were used. The blood was then collected from the left atrium; in animals the cannula was designed to pass through the mitral valve into the left ventricle; this was considered less desirable clinically since there was potential for valve damage. Early clinical work employed the atrial appendage as the source of blood; since this led to ventricular arrhythmias in a few cases and involved manipulation of the heart upwards, this route was not favoured. Instead, the route chosen was the right superior pulmonary vein - left atrial junction. The roof of the left atrium below the aorta has also been considered but was not tried. Prior to cannulating the left atrium, the simple manoeuvre of hand ventilation, ensures that a positive pressure can be guaranteed when the atrium is open. This is potentially the most dangerous cannulation and it is essential that no air is entrained. The oxygenated blood is then drained into another reservoir and then pumped out into the ascending aorta. As can be seen from the diagram, there is a shunt connecting the two reservoirs; the

concept behind this, (Cass and Ross 1959) was to allow easier balancing of the two reservoirs. By maintaining the right heart pump about 10% faster than the left, the blood shunts minimally from left to right ensuring no desaturated blood enters the systemic circulation. The design of the shape, size and entry ports of the reservoirs proved very important not only to the ease of priming and controlling the bypass but also assisted in the reduction of circulating gaseous microemboli to zero.

Naturally an important feature of the technique is that the lungs continue to be ventilated. Practice has again varied between anaesthetists in clinical and animal settings. Most have used 100% oxygen and adjusted the tidal and minute volumes according to the arterial blood gas analysis, but others have used a small percentage of carbon dioxide, particularly at the lower temperatures.

An oxygen / nitrous oxide mixture has also been employed, but some clinical anaesthetists have not used this for fear of the

theoretical complication of bubbles coming out of solution. That said, gases were checked to prevent very high pO2 values with its potential problems. The postoperative ventilatory management has been identical to normal practice.

The sequence of events (Table 4) both clinically and experimentally has been to prepare the patient, give heparin and then cannulate the aorta and left atrium. Left heart bypass is then started and the appropriate cooling can commence. Whilst this is proceeding the right heart bypass can be undertaken; this routine therefore takes little or any additional time over conventional bypass. Towards the end of the procedure, as soon as the heart is beating, the shunt can be clamped and right heart bypass stopped. If there is good right heart action the left atrial return will be maintained. When the patient is fully rewarmed and ready to come off bypass, left heart bypass can be stopped and protamine administered and decannulation performed in the usual way. Apart from the saving in time, this proved to be an excellent method for weaning from bypass. It can identify at an

early stage if there is a problem with the right side of the heart. When left heart bypass is stopped, because the right heart is already ejecting, the changes are not so abrupt and weaning can be very gentle for the cases with poor ventricular function. Finally should there be a problem with either side of the heart, the means for unilateral or bilateral support to be continued are there.

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Table 4

Bypass procedure

Give heparin

Cannulate ascending aorta

Cannulate left atrium

Establish left-heartbypass and cool (shunt closed)

Cannulate pulmonary artery

Cannulate right atrium

Establish right-heartbypass (shunt now open)

Perform distal grafts

Clamp shunt and stop right-heartbypass

Continue warming and stop left-heartbypass when ready to wean.

(Proximal anastomoses can be performed with or without right-heartbypass)

In the animal studies, pressure measurements of all chambers of the heart were continually monitored to see if any unexpected changes may have occurred. In particular, it was thought important to watch pulmonary artery and left atrial pressures as indicators of problems. It was found that with certain precautions, there were no major changes and the clinical cases were performed with no extra monitoring. These precautions included ensuring a thorough blood / prime mix and the rate of establishing bypass was not too fast. Features such as a manometer line built into the tip of the left atrial cannula were however designed and occasionally used.

In studying what is effectively a completely different form of bypass, the ideal would have been to study every aspect of the current state of knowledge of the pathophysiological effects of bypass. In a study performed by a very small team, this was obviously not possible. It was therefore necessary to identify the most important factors. This has meant that certain aspects have been largely ignored although they would provide valuable

information. Studies have focused on the gaseous exchange, the formation of gaseous microemboli, haematological data including red and white cells, platelets and coagulation and complement pathways. Detailed studies on water shifts in the lungs and the psychological and neurological morbidity of the double reservoir method are some areas that are important to conduct, but have not been conducted in this study.

The conclusions that can therefore be drawn on all of these studies are limited, although if the fundamental data is encouraging, one would feel justified in extending the field of study. Potential advantages can be identified and in selecting certain aspects for evaluation, they can be specifically studied and compared with cardiopulmonary bypass using the "best" oxygenators currently available. Table 5 identifies some of the potential or looked for benefits with the double reservoir technique; the later text will discuss which of these are proven and which remain to be studied.

Table 5

Potential advantages of the double reservoir technique

Low rates of haemolysis

Low rates of gaseous microemboli

Low rates of complement activation with reduced pulmonary dysfunction

Platelet function preservation and normal coagulation

Preservation of metabolic functions of the lung during bypass

Elegant weaning for poor ventricular function

Built-in ability for long term assist

Easy system for heparin coating

Cost saving over membrane oxygenation

Model for studying cardiopulmonary bypass methods and materials

## METHODS

Those methods that are common to both studies are detailed below. Individual variations to take account of the different coronary techniques are described individually. The bypass equipment was different for the two studies and is individually described.

The anaesthetic techniques were similar for the two techniques and differed little from standard cardiopulmonary bypass except for ventilating during bypass with and oxygen and occasional carbon dioxide mix. Induction agents were identical in each centre for the double reservoir and control groups.

Sampling of blood was taken at the pre-heparin time, 5 minutes after the onset of bypass, 20 minutes after the onset of bypass and then at 20 minute intervals until bypass was stopped. A final sample was taken ten minutes after protamine administration. All samples were taken from a wide bore venous line either directly in the right atrium or superior vena cava except for left atrial samples. The latter were taken either from a sampling port from

the perfusion line coming out of the left atrium or direct left atrial or peripheral arterial for the conventional cases depending whether on bypass or not. White blood cell drops across the lungs i.e. sequestration were calculated by subtraction of simultaneous sampling form right and left atrial samples.

Haematological examination was performed using a Coulter counter and in addition there was quality control of manual white cell and platelet counts. All platelet counts have been corrected for haemodilution according to the formula on Page 108. Plasma haemoglobins and ADP aggregation were performed in an identical manner to the description on Page 92.

Elastase release was quantitated in complex with alpha 1-proteinase inhibitor by enzyme linked immunoabsorbent assay (Merck, Darmstadt, Germany).

2.5 mls of blood was collected into beta thromboglobulin (BTG) collecting tubes to determine BTG concentrations. The BTG assay

was performed with a radioimmunoassay (Amersham Int., UK) according to the manufacturer's instructions.

3 mls of blood, anticoagulated with EDTA (0.01M) was centrifuged and stored at -70°C for c3a assays. C3a des arg concentrations were determined by radioimmunoassay (UpjohnCo, Kalamazoo, Michigan).

Extrinsic (Tissue-type) Plasminogen activator was assessed by an indirect assay (Verheijen et al ) based on the parabolic assay of Drapier. The system contains activator, plasminogen, the synthetic plasmin substrate H-D-Val-Leu-Lys-pNA (S-2251, Kabi) and a mixture of soluble fibrinogen fragments prepared by treatment of fibrinogen with cyanogen bromide. The addition of these fibrinogen fragments considerably enhances the sensitivity and specificity of the method owing to specific stimulation of the plasminogen activator.

The number of microbubbles entering the systemic circulation was estimated by the use of the TM8 (Technique Labs, Hampshire)

bubble activity monitor. The TM8 probe was placed around the aortic line.

## STATISTICAL METHODS

Mean differences between double reservoir technique patients and the controls were analysed using the two-sample t-test. The humoral cascade data was analysed in two ways; omitting the post protamine level (which occurred at different time intervals with each patient), the mean of individual regression lines for each patient was compared. The first and last readings of the cascades was analysed by the student t-test. Readings comparing the white cell counts or their differences as an index of sequestration, were analysed as paired or non paired t-tests where appropriate. Significance has been expressed either by quoting the p value concerned or by being within the 95% confidence interval limits. The graphical displays have been prepared on Fig P software package (Fig P Software, Durham, USA). The graphs show a descriptive rather than numerical X axis because the pre heparin time and the post protamine time were not constant times relative to the bypass intervals and therefore were out of context independent variables. The error bars in all graphs show the

standard error of the mean.

The individual statistical tests employed for any one set of data, were used on the advice of the Division of Medical Statistics at the University of Newcastle upon Tyne. The basis of the advice came from studies performed at the University and their evaluation of the dangers and problems of the analysis of serial measurements in medical research (Matthews et al 1990).

## CROSS CLAMP FIBRILLATION STUDIES

This was a controlled trial of ten patients using a double reservoir method and ten patients with a Cobe CML membrane oxygenator (Cobe Laboratories Inc, Colorado). Gas exchange, gaseous microemboli production, plasma haemoglobin, white cell counts, platelet counts and function and C3 levels were all measured.

METHODS

The bypass circuit for the double reservoir system was a Polystan 1500 ml reservoir (82910 Polystan, Denmark) for each side of the heart joined by a shunt. The heat exchanger was connected to the left-sided circuit. The circuit, 3/8th inch on all routes, was primed with 2 litres of compound sodium lactate. Cannulae used were Wessex aortic cannulae (Wessex, Sussex) for aortic and pulmonary cannulation, a Ross basket single atrial cannula for the right atrium and an angled wire wound cannula (Polystan, Denmark) for the left atrium.

The revascularisation proceeded with the intermittent cross clamp fibrillation technique, whereby a distal anastomosis is completed with the heart fibrillating and the aorta cross clamped. The proximal anastomosis is then performed with the aorta unclamped and the heart beating at $32^{0}$C.

Tidal volumes for the DR group were adjusted for the size of the patient and the blood gases obtained, but were typically around 550 mls; the minute volume was usually 5.5 Litres.

RESULTS

The ten double reservoir (DR) patients had a mean age of 55.4 years (range 44 to 79 SD 10.7) and the Cobe CML (Cobe) patients had a mean age of 57.5 years (range 46 to 71 SD 7.81). The bypass times were also statistically similar mean 83.3 minutes for the double reservoir (range 60 to 115 minutes SD 15.5 ) and 85.1 minutes for the oxygenator group (range 57 to 102 minutes SD 14.3). The number of grafts was 3.9 (SD .568) for the DR and 3.6 (SD .516) for the Cobe patients.

The gas exchange data (Fig. 25) was quite different from the pattern seen in the animal studies. The levels of the $pO_2$ seen on bypass were higher in the double reservoir patients. This is because the anaesthetists were understandably reluctant to reduce tidal and minute volumes lower than they were comfortable with. That is however despite the clear data showing that $CO_2$ washout was good and that levels could have been safely lowered. Ways of overcoming this could include $0_2$/air mixes.

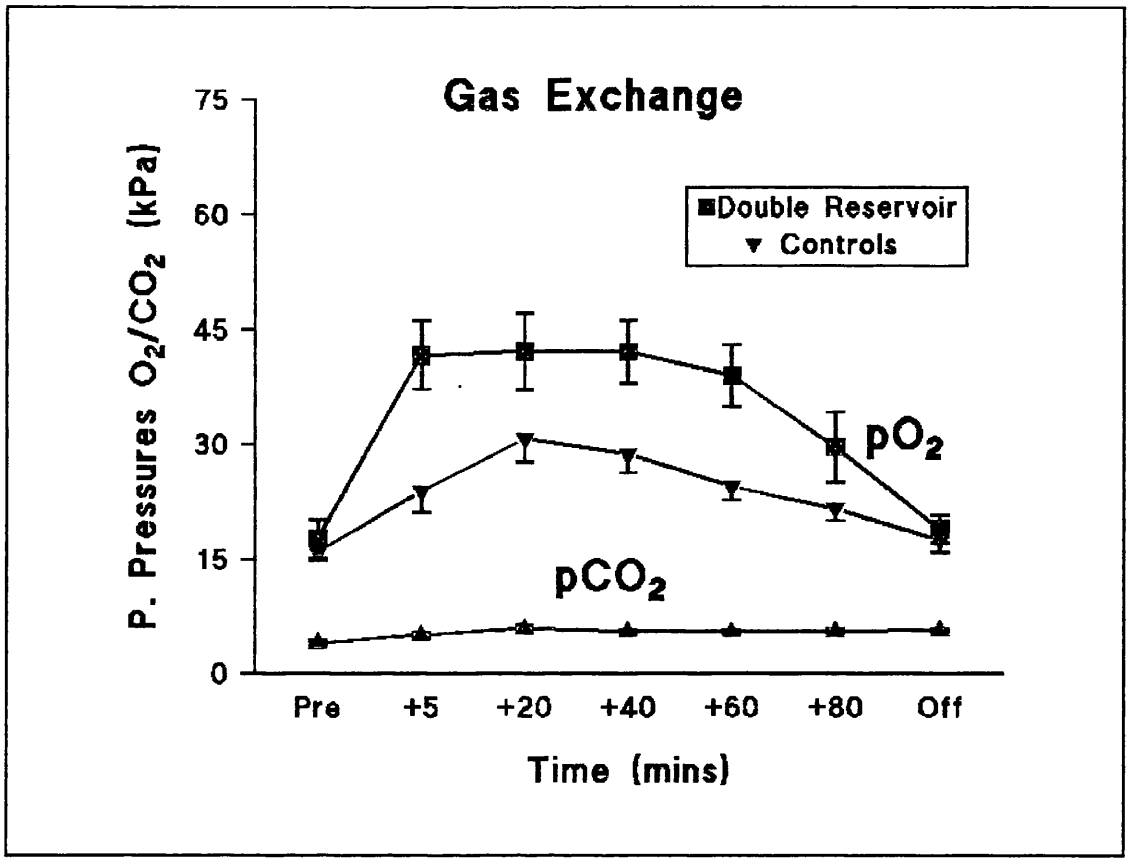
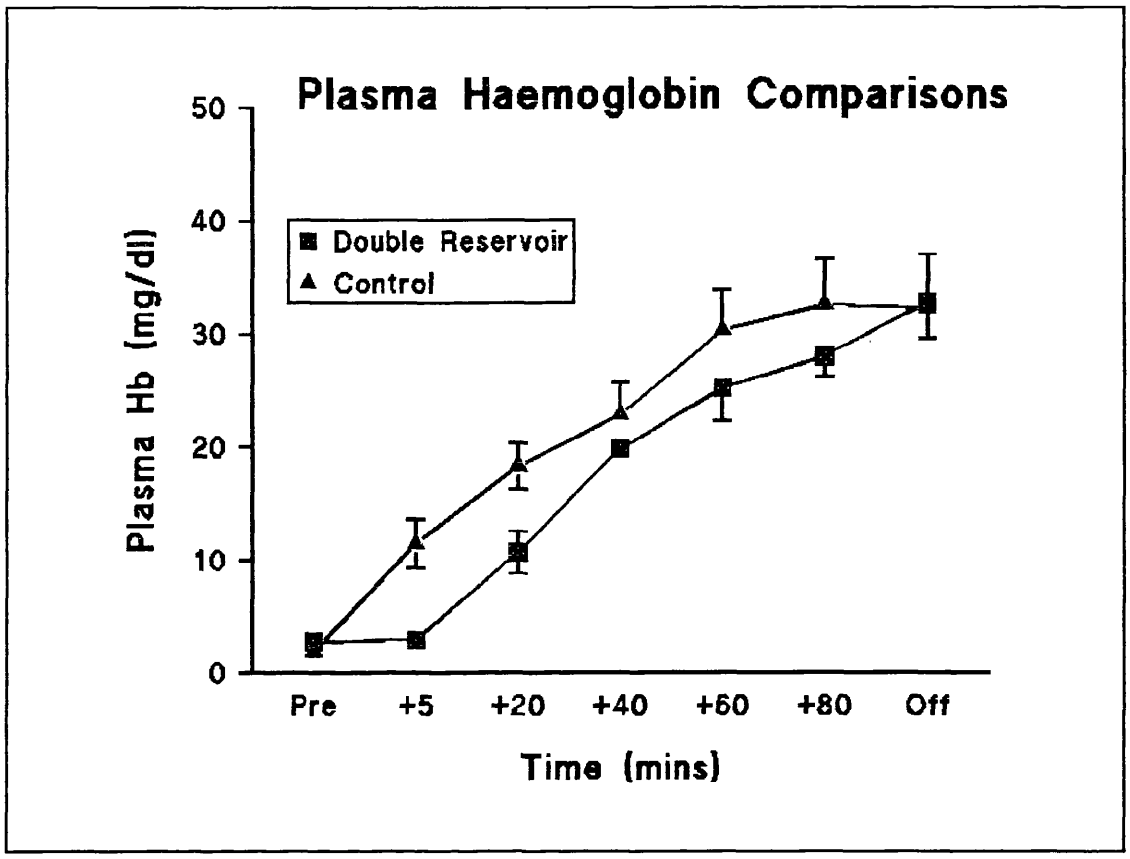


Figure 25

Parameters of the quality of the bypass included assessment of the plasma haemoglobin levels (Fig. 26). In common with the animal studies, the plasma haemoglobin levels, were low and did not rise to pathological levels over the bypass. Analysis both by end point t-tests and whole curve regression analysis confirms the visual appearance that there is no difference between the DR group and Cobe group.


Plasma Haemoglobin Comparisons
Plasma Hb (mg/dl)
50
40
30
20
10
0
Double Reservoir
Control
Pre
+5
+20
+40
+60
+80
Off
Time (mins)
Figure 26

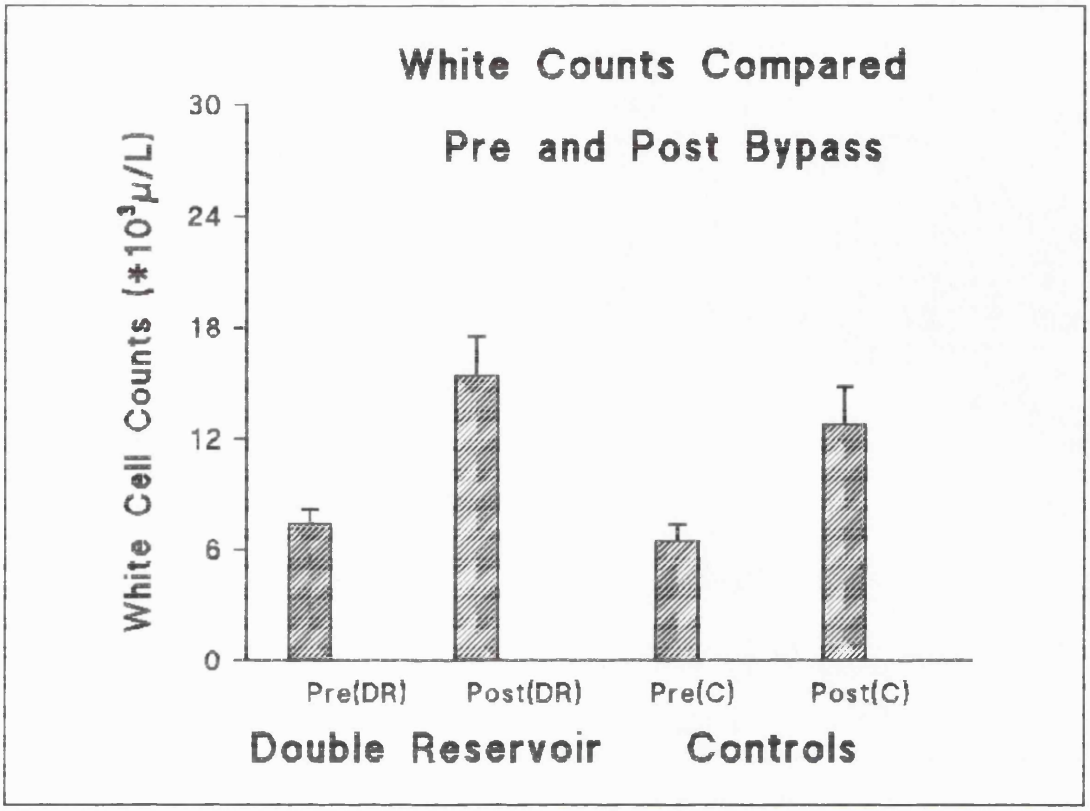



Figure 27

The data above (Fig. 27) shows that in both groups there was a significant leucocytosis when pre and post bypass levels are analysed. This leucocytosis is seen in many studies (Cavarocchi et al 1986). There was no significant difference however between the two groups either pre or post bypass. In this study, although

drops across the lungs were measured for the DR group and found to be low ($3.67*10^9$/L SEM 0.155 p=0.045), they were not recorded in this first study.

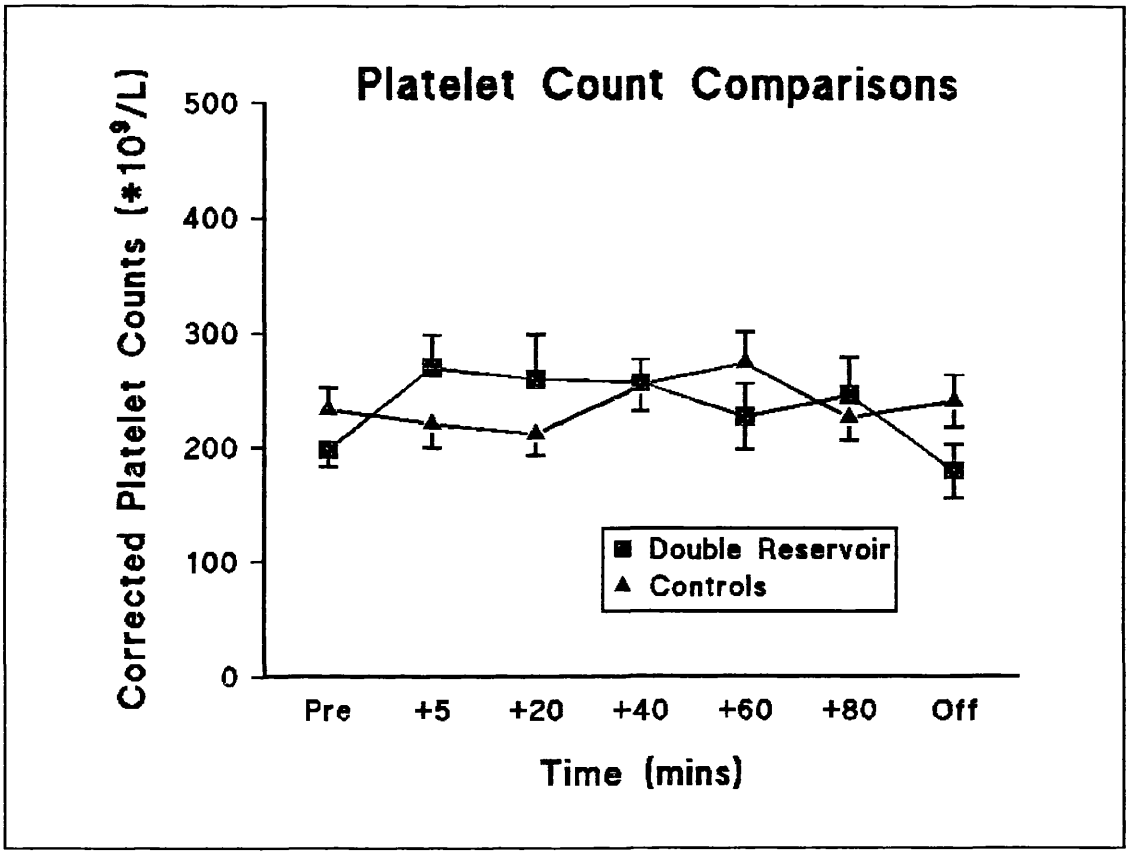



Figure 28

The platelet counts were compared over the bypass between the two groups (Fig. 28). Pre heparin counts were $198*10^9$/L SEM 14.6 for the DR group and 233 SEM 19.2 for the Cobe group. Post protamine levels were 180 SEM 23.4 and 241 SEM 22.7 respectively. None of these figures are statistically different,

confirming that the corrected counts for both groups are very little changed from their prebypass levels.

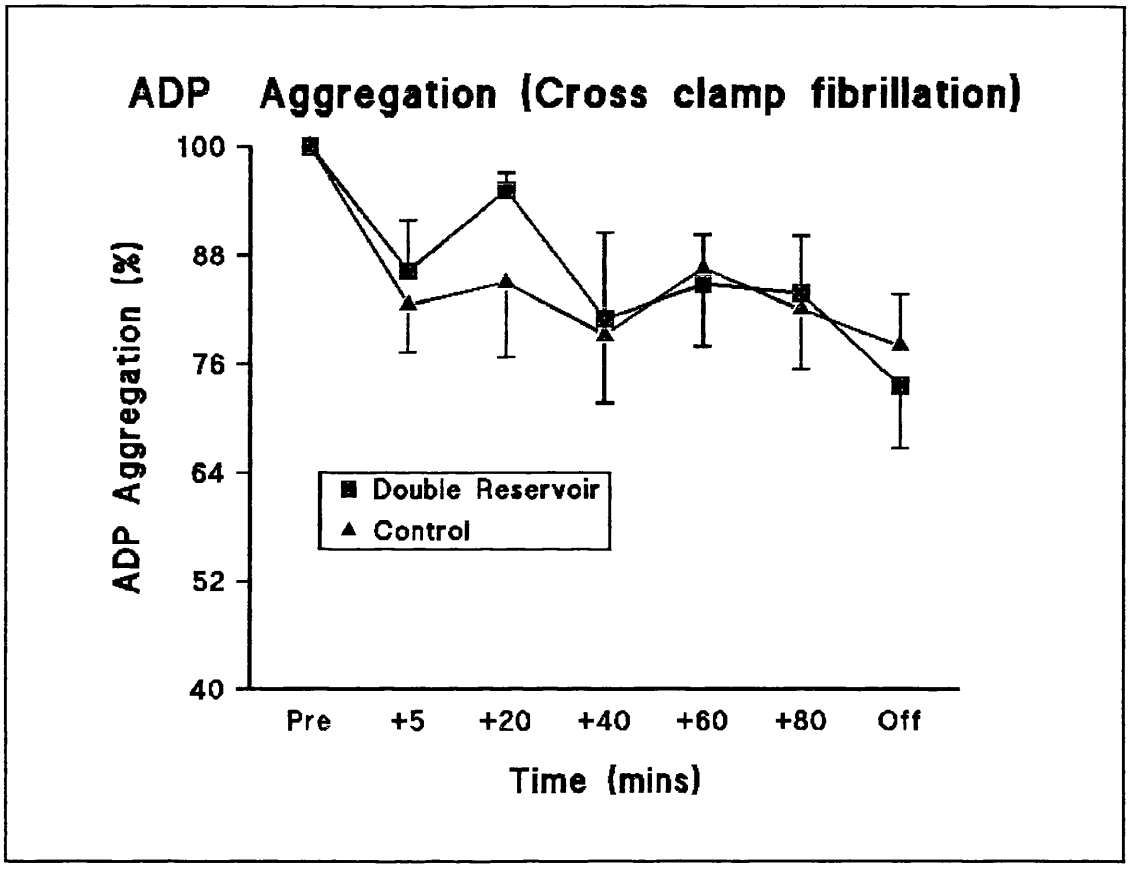



Figure 29

Platelet counts are important, but it is their function which is more important. The above graph (Fig. 29) shows the ability of the platelets to aggregate when given a stimulus of ADP. Post protamine levels show that the DR group showed 73.6% activity SEM 6.87 and the Cobe group showed 78% SEM 5.68. This end point and indeed other points along the time scale are not

statistically different. This confirms that numbers and performance of platelets are similar between a membrane and the DR group.

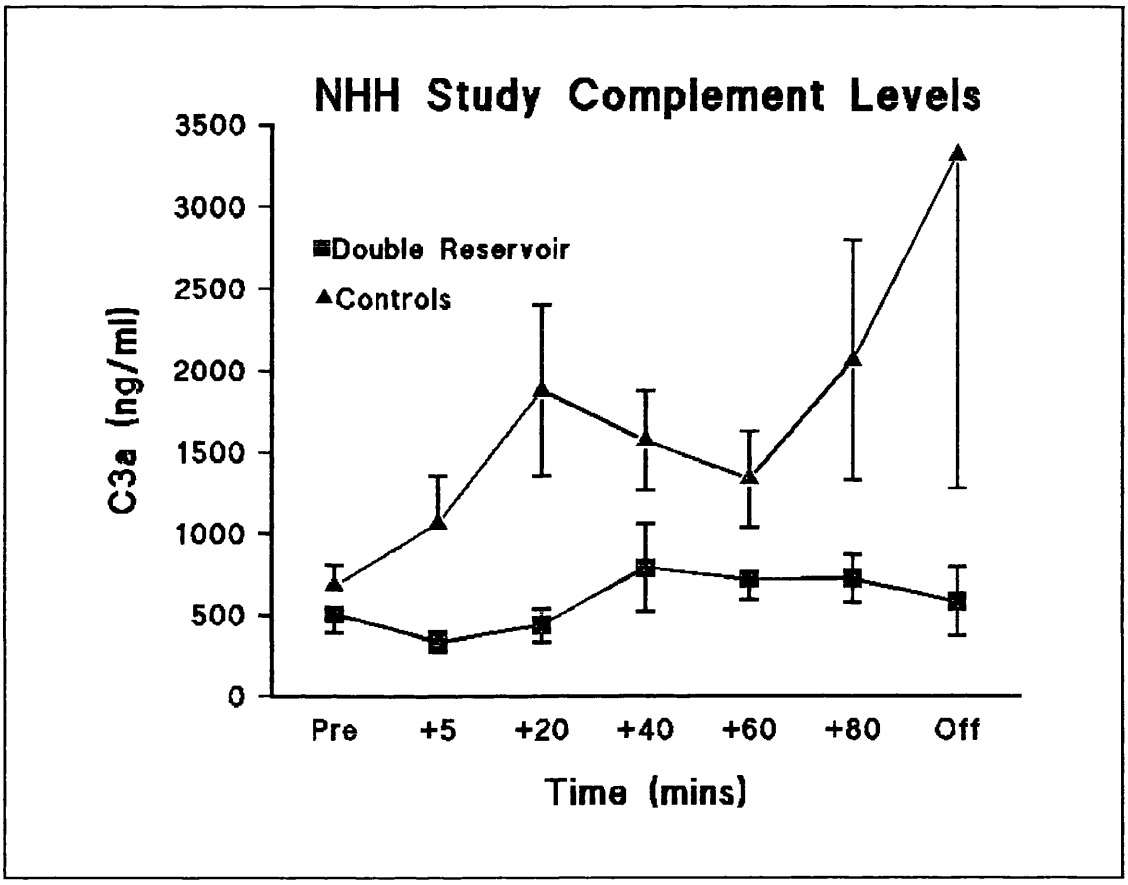



Figure 30

The final graph in this study (Fig. 30) shows the comparative C3a levels measured between the two groups. The data despite wide variations in the standard errors and deviations show two distinct trends. The regression characteristics have been compared over comparable time intervals (i.e. omitting post protamine data).

The findings are that both groups show an increase in C3a levels over the bypass period. The DR group significantly increases over time, the average slope was 4.64 (SD 4.13) $p=0.04$. The Cobe group increases over time with an average slope of 16.06(SD=9.03) $p=0.016$. The rates of increase are statistically very different with the Cobe group C3a levels increasing at a greater rate $p=0.02$.

Other parameters looked at between the two groups did not lend themselves to either statistical analysis or graphical display. The most important of these was the number of circulating gaseous microemboli (GME). In the DR group, as in the animal studies, the GME counts were consistently zero apart from the immediate post bypass period. In the Cobe group, despite published data (Pearson et al 1986) which also recorded very low levels, there were continual GME counts in the range of 10-20 counts per second with the TM8 probe set on reading the smallest bubble size i.e. 10 microns.

The amount of sodium bicarbonate needed to be given over the course of the DR bypasses was exceedingly low as a reflection of the absence of acidosis. The requirement for the Cobe group was more consistent although there was no formal comparison between the groups.

In the group that constituted this study, there were no major complications. One minor complication was a reopening for bleeding from an inadequately controlled pulmonary artery cannulation site. There were no deaths and all patients were weaned from the ventilator within 24 hours according to normal practice.

## CARDIOPLEGIA STUDIES

This study was conducted in a similar manner to the cross clamp fibrillation patients except that myocardial protection was with cardioplegia. The animal studies allowed an estimate of a safe interval for repeating the doses of cardioplegia in a clinical setting. The membrane oxygenator used for the comparison in this study was a Shiley M2000 (Shiley Corp., Berks).

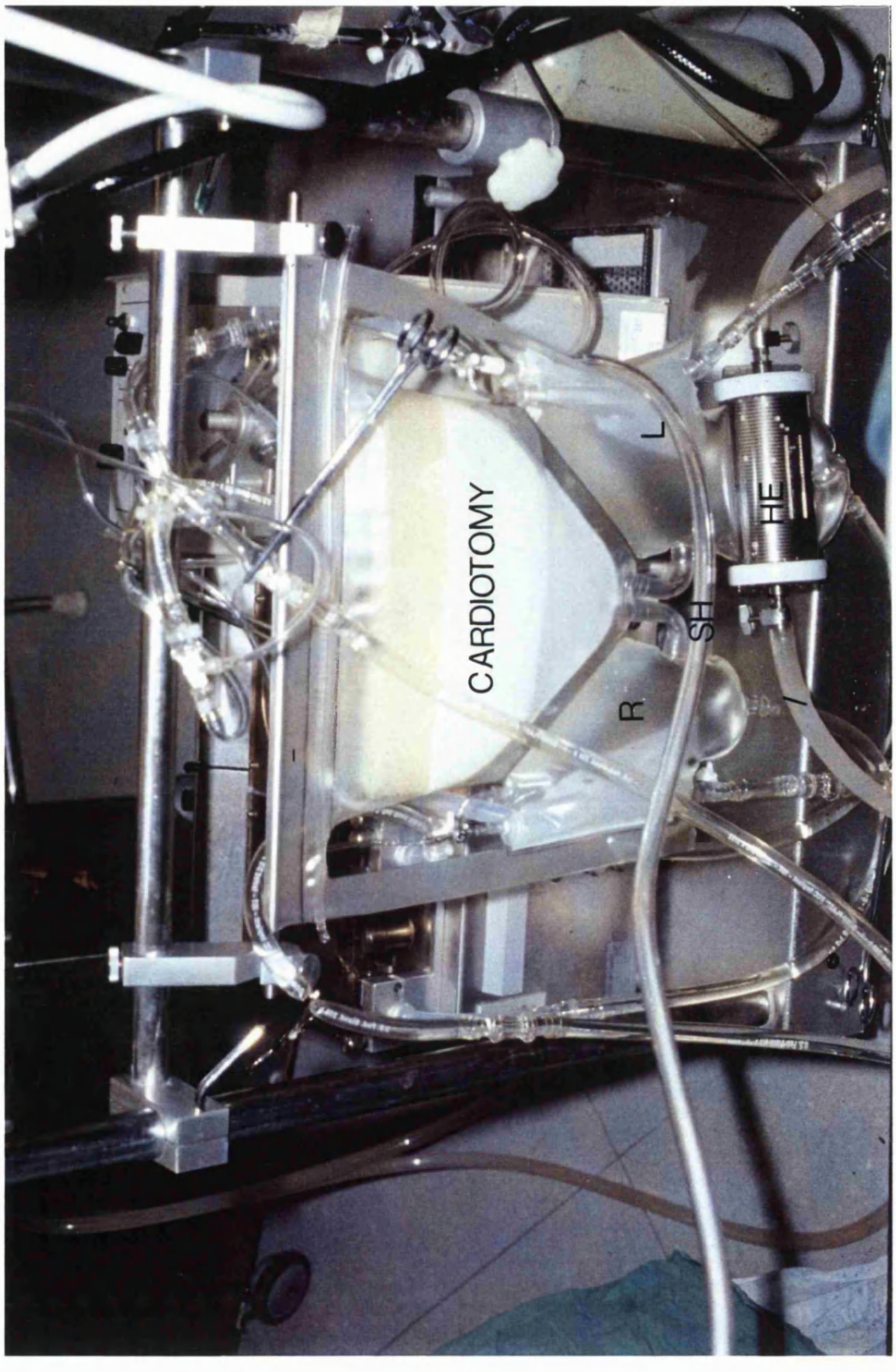
## METHODS

Eleven patients used the double reservoir perfusion system and eleven used a Shiley M2000 membrane oxygenator. The double reservoir system for this study was purpose built (Auto-ox, Polystan, Denmark). As can be seen from Plate 3, it was a soft shell system that incorporated two 850 mls reservoirs, a cardiotomy reservoir with a 40 micron filter size and a heat exchanger incorporated within the left sided reservoir; as with previous designs, a shunt was made between the two sides that could be clamped at will. The design allowed cardiotomy return to be directed to either reservoir. Ports were incorporated to allow sampling, to allow temporary access to the atmosphere and also to allow a conventional oxygenator to be connected in case of catastrophe.

An identical protocol was followed except that cooling was to 28$^{0}$C and after cross clamping one litre of crystalline cardioplegia (St. Thomas solution) was instilled into the aortic root. Further doses

PLATE 3. THE CLINICAL DOUBLE RESERVOIR CIRCUIT

R = RIGHT CIRCUIT L = LEFT CIRCUIT HE = HEAT EXCHANGER SH = SHUNT (obscured)



of cardioplegia were given every 30 minutes; in addition topical cooling was used. Cannulae used, were a two stage cannula for the right atrium, Wessex (Wessex, Sussex) cannulae for aorta and pulmonary artery and an angled spiral tipped catheter (DLP) for the left atrium(Plate 4). The prime used was Hartmanns solution.

Sampling intervals and methods were as previously described. In addition to the parameters studied in the National Heart study, mean white cell drops across the lungs , complement and elastase estimations were made. The other humoral cascades were investigated by estimation of tissue plasminogen activator (t-PA) and beta thromboglobulin (BTG) levels. Postoperative blood loss was also recorded.

PLATE 4. CLINICAL CANNULAE FOR THE DOUBLE RESERVOIR

RA = RIGHT ATRIUM PA = PULMONARY ARTERY LA = LEFT ATRIUM Ao = AORTA

## RESULTS

The two groups double reservoir (DR) and Shiley M2000 (Control) were comparable groups in a number of ways. The mean age of the DR group was 57.4 years SD 6.7 against 62.6 years SD 9.1 for the Controls were not significantly different at the 95% confidence interval limits. The bypass times were also statistically similar, 102.4 minutes SD 22.6 for the DR group and 96.6 minutes SD 23.7 for the controls. all other parameters were also statistically similar including body surface area (DR 1.91 SD 0.08, Control 1.96 SD 0.15),number of coronary grafts performed (DR 3.9 SD 0.8, Control 3.7 SD 0.8). The cross clamp time was also not statistically different (DR 45.3 minutes SD 13.5, Controls 44.6 minutes SD 11.5). The postoperative blood loss measured from the drainage bottles in the intensive care unit looked different (DR 447 mls SD 122.2, Controls 962.6 mls SD 749.9)but probably because of the large standard deviation in the Control group, it failed to be significantly different with a p value of 0.06 and not reaching 95% confidence intervals.

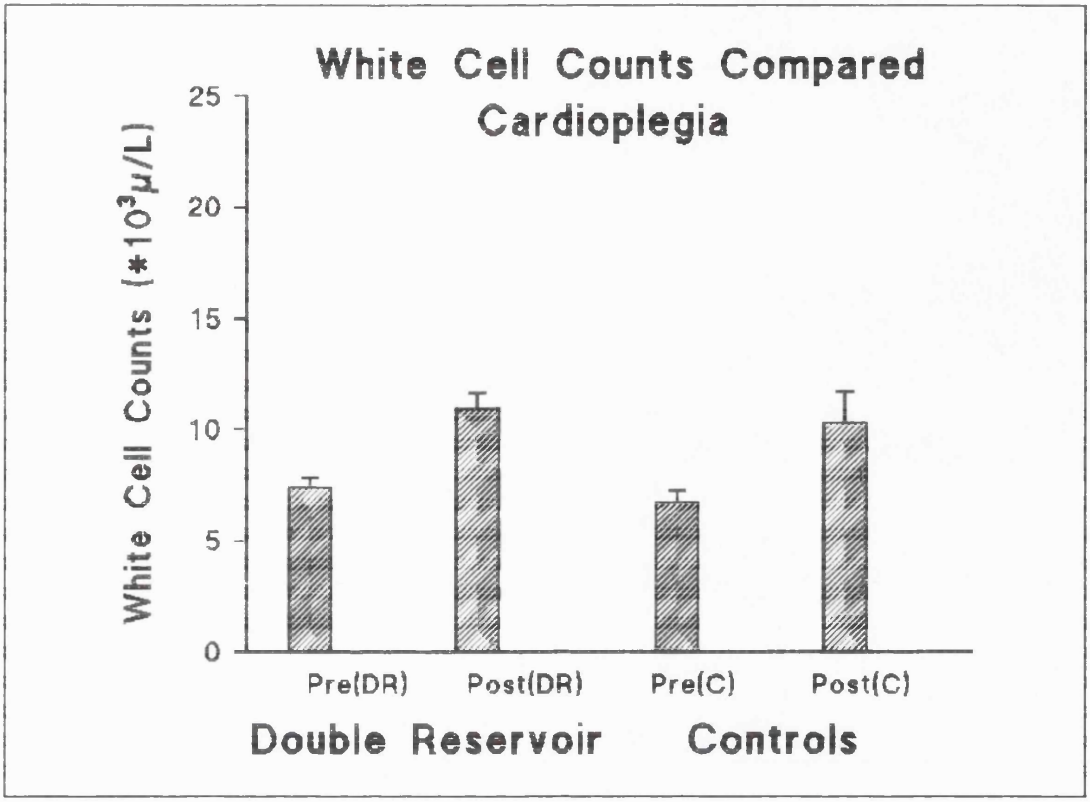



Figure 31

The white cell counts (Fig. 31) showed a similar pattern to the cross clamp fibrillation study. In both groups there was a significant leucocytosis over the course of the bypass. The DR group showed a mean increase from $7.3*10^3$/L SD 1.4 to 10.9 SD 2 and this increase was significant with a p value of 0.0004. The Control group showed an increase from 6.7 SD 1.5 to 10.2 SD 4.2

which was also significantly larger with a p value of 0.02. There was no significant difference between the two groups.

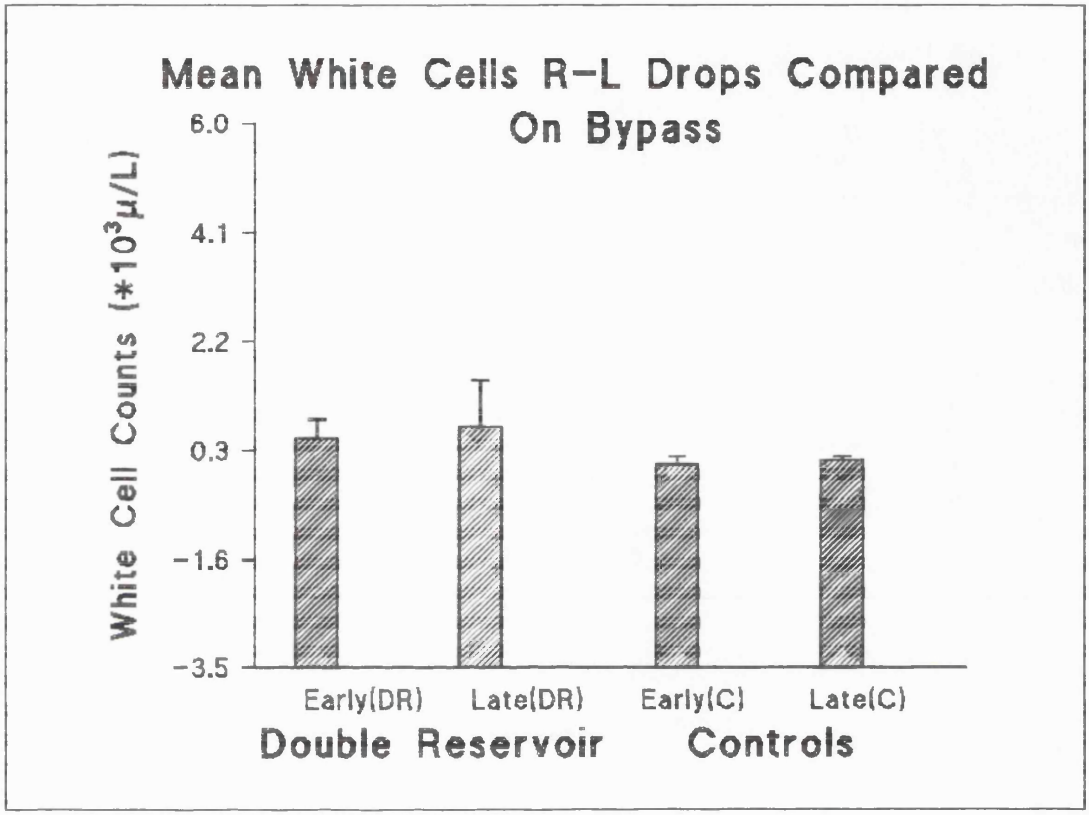



Figure 32

Mean cell differences were recorded between the two groups (Fig. 32). The sequestration levels of both groups were very low. The early levels was the first recordings made between the right and left atria on bypass and the late differences were taken as the last sample taken whilst still on bypass. No differences could be seen either between the two groups or at the different bypass times.

The values were corrected for haemodilution and also just the neutrophil proportion were looked at; these different aspects also failed to show any differences.

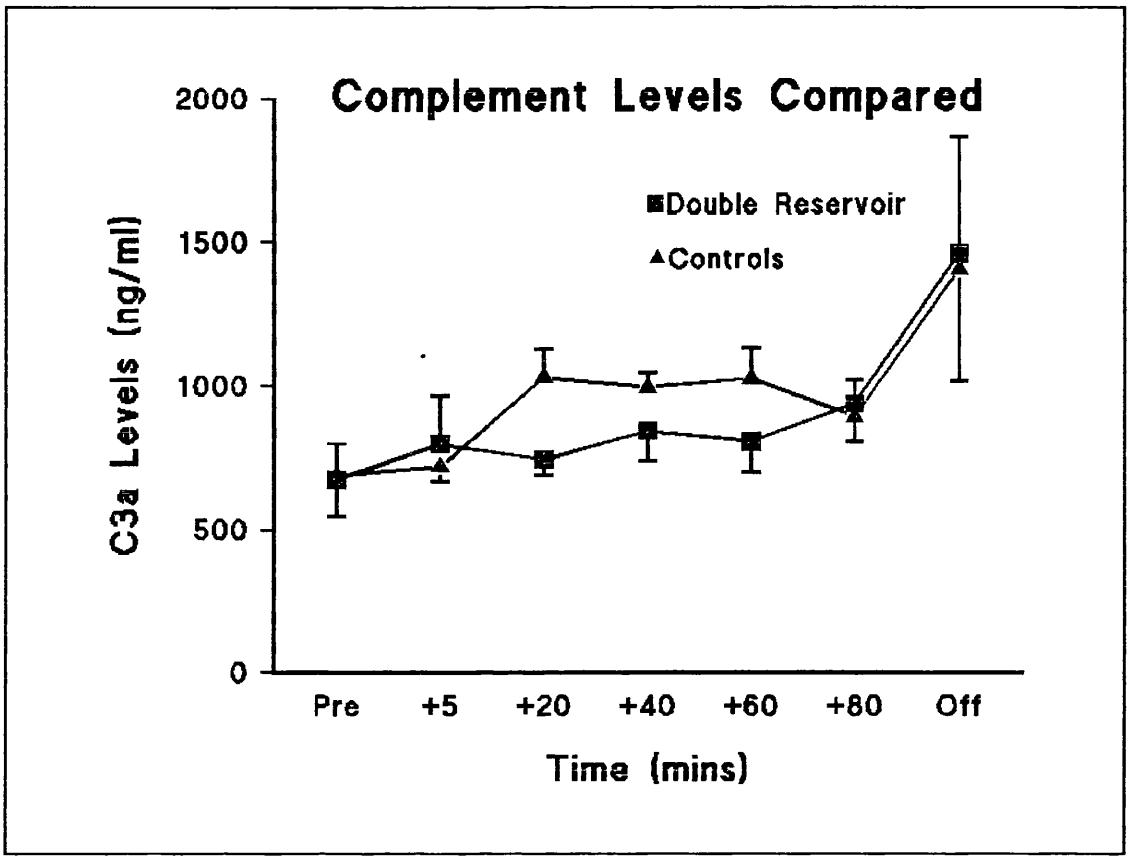


Figure 33

In the previous study, a clear difference could be seen in the estimates of the C3a levels. In this study (Fig. 33), this was not demonstrated. The increase over the bypass was not statistically different (DR 242 SD 505 and Controls 157 SD 179). The analysis of the regression lines, which demonstrated a clear difference in the rise and rate of rise of C3a levels in the previous study, was unable to demonstrate statistical differences.

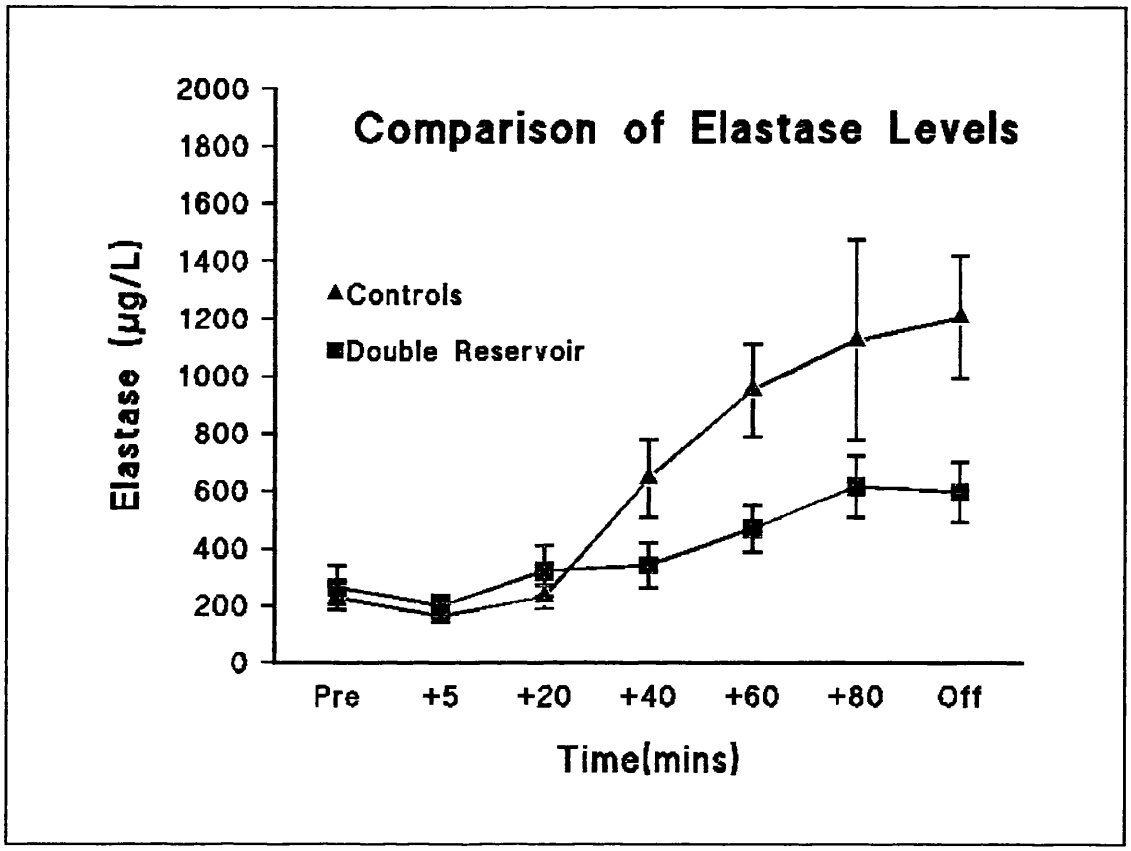



Figure 34

Although the complement levels were not statistically different in this study, the enzyme released as a consequence of complement activation, elastase (Fig. 34), showed very clear results. The increase in elastase levels in the DR group was 267.4 microgram/L with a SD 324.2 but in the Control group the increase was 914 with a SD 720.1. This was significant with a p value of 0.036 and also using 95% confidence intervals. The mean values for each group of regression values also showed that the rate of increase

was greater in the Control group.

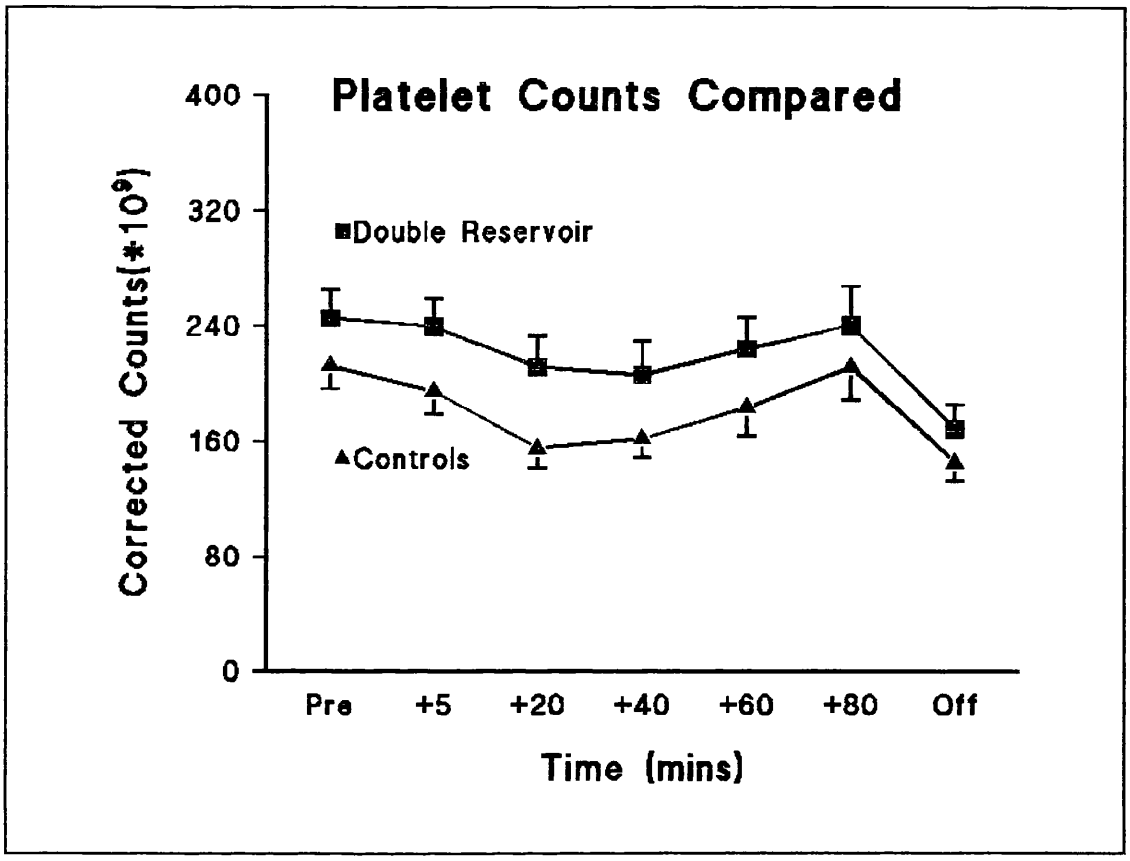



Figure 35

The platelet counts corrected for haemodilution were compared between the two groups (Fig. 35). As the graph shows, they are not statistically any different at any point in the bypass. Comparing pre and postoperative counts, the numbers for both groups declined by about 10%. There is however some evidence for platelet sequestration in the Control group, looking at mean

platelet count differences between right and left atrial samples. As the bypass proceed, the sequestration increases and by 20 minutes, the controls have shown drops across the lungs to around 18 $*10^9$/L (p value 0.013) and comparison of the two groups is also significantly different when tested against each other (p 0.03). There is no evidence for sequestration of platelets at any stage with the DR group.

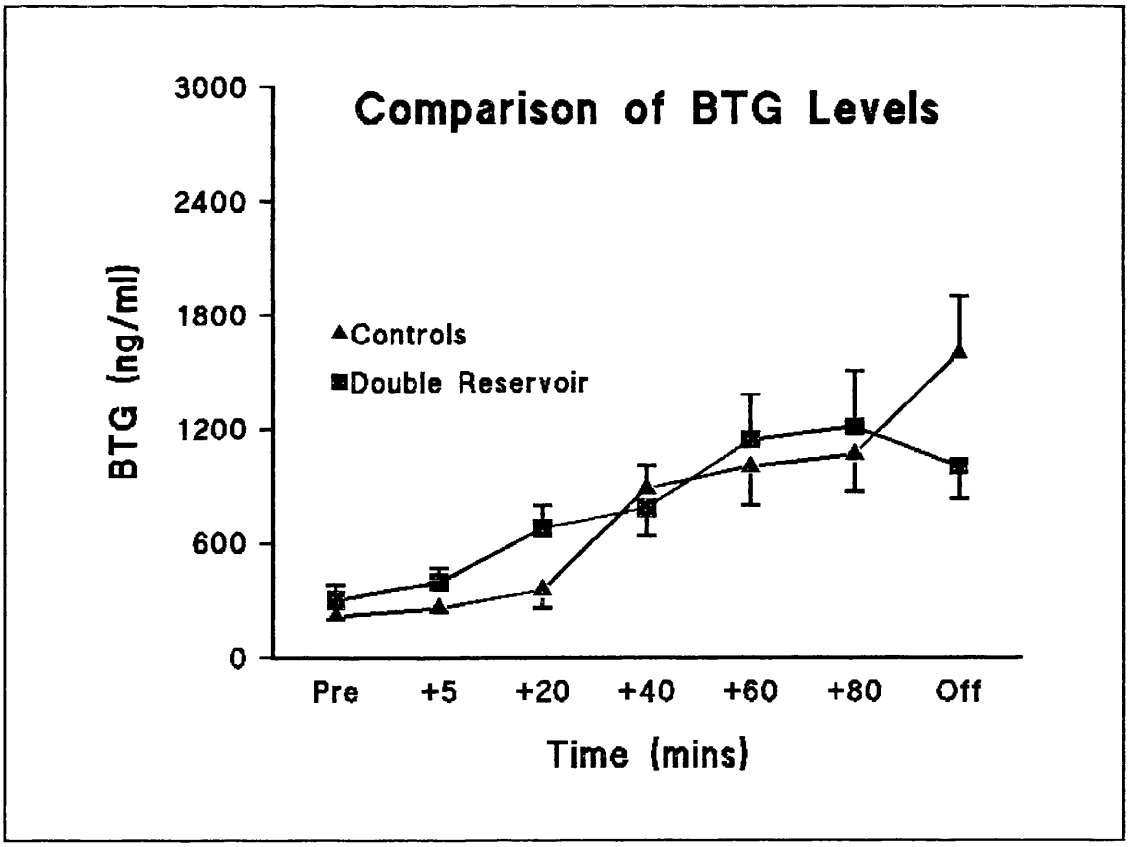



Figure 36

Fig. 36 shows Beta Thromboglobulin levels throughout bypass and

shows a steady rise in both groups. although the control level appears higher at the end of bypass, mean DR 1002 SD 170 and Controls 1593 SD 304 ,the difference fails to reach significance at the 95% confidence interval.

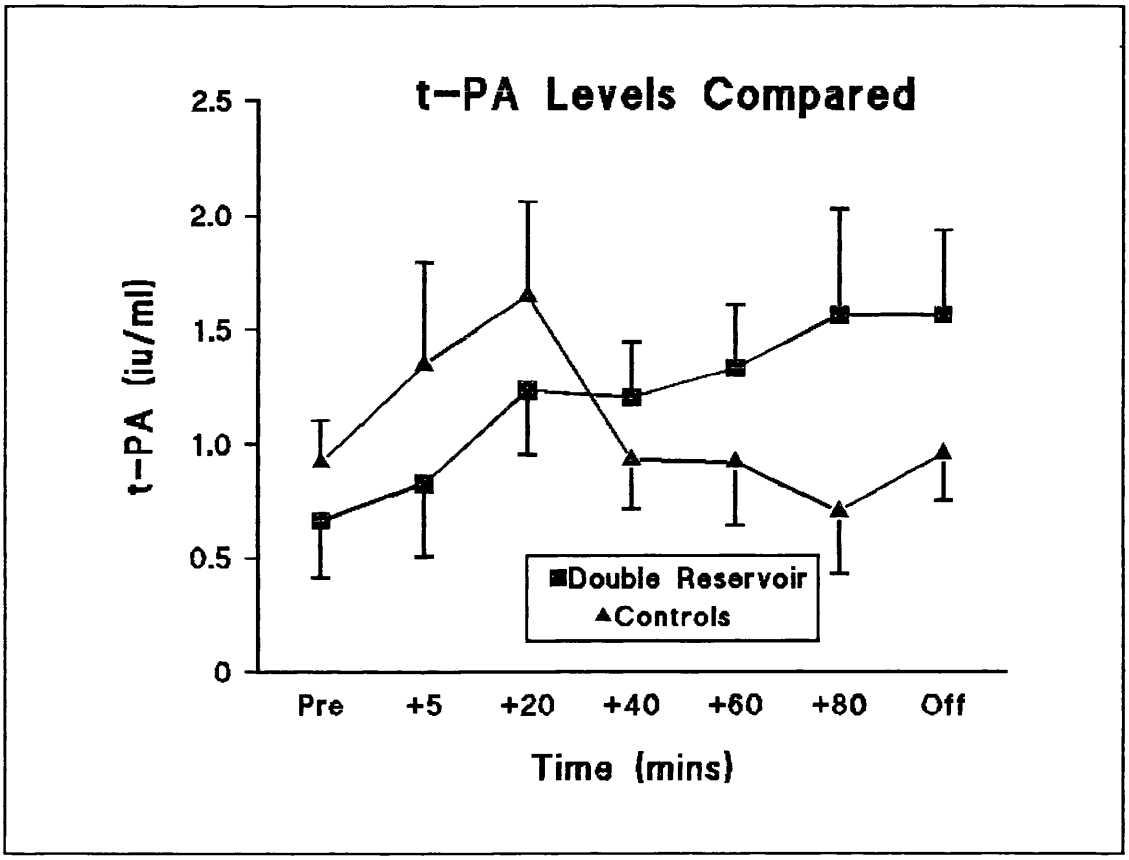



Figure 37

Fig. 37 shows the tissue plasminogen activator (t-PA) activity. There was no significant difference between the two groups over the bypass although, converting the data to logarithmic values,

does show a significantly higher value for the DR group at the end of bypass.

All patients in the study had immediate uncomplicated recoveries; this included one patient in which air had been entrained from the shunt of the reservoir. Another patient, suffered a cerebrovascular accident on the ward three days postoperatively, having had a standard recovery; he subsequently died. The bleeding in the post operative period was low but not statistically different from the Control group. There were no ventilatory problems and extubation was timed on normal criteria.

## DISCUSSION

The double reservoir project has been a long and cumulative study. It started as the possible revival of a technique that fell into disuse because it was complex to use and not suitable for the then common open heart operations. The decision to look anew was taken because of the improvement in bypass equipment available, because most cases are now closed cardiac operations and because there is still significant morbidity associated with cardiopulmonary bypass.

In the animal laboratory, techniques and expertise was built up to allow a study to be undertaken. It started looking at cannulae, at bypass procedures and at a basic physiological level. When the model had become reasonably successful, it progressed to a formal study assessing its role for cross clamp fibrillation coronary artery bypass grafting. This study demonstrated some of the basic requirements for any perfusion system; it provided good oxygen uptake and carbon dioxide removal. The pulmonary and vascular resistances over the bypass were such that little pharmacological

manipulation was necessary. Airway ventilatory pressures were low and no acidosis was encountered. Assessment of haemolysis was also performed and showed the range was certainly acceptable over a two hour bypass. Platelet counts and function were well preserved and levels of circulating gaseous microemboli were exceedingly low.

This study was then repeated for the technique of cold crystalline cardioplegia where these findings were repeated. In addition, timing of further doses of cardioplegia was investigated and estimated for the forthcoming clinical work. The final phase of the animal study, had three animals on the double reservoir circuit for 24 hours each. This valuable study confirmed that many of the features including gas exchange, lack of acidosis, platelet function, lack of severe haemolysis and the ability to form good clot after an extended period, still pertained after 24 hours. This therefore also added the possibility of using the system for a short to medium term assist for the failing heart. All of these conclusions, led one to feel that a new clinical evaluation should be performed.

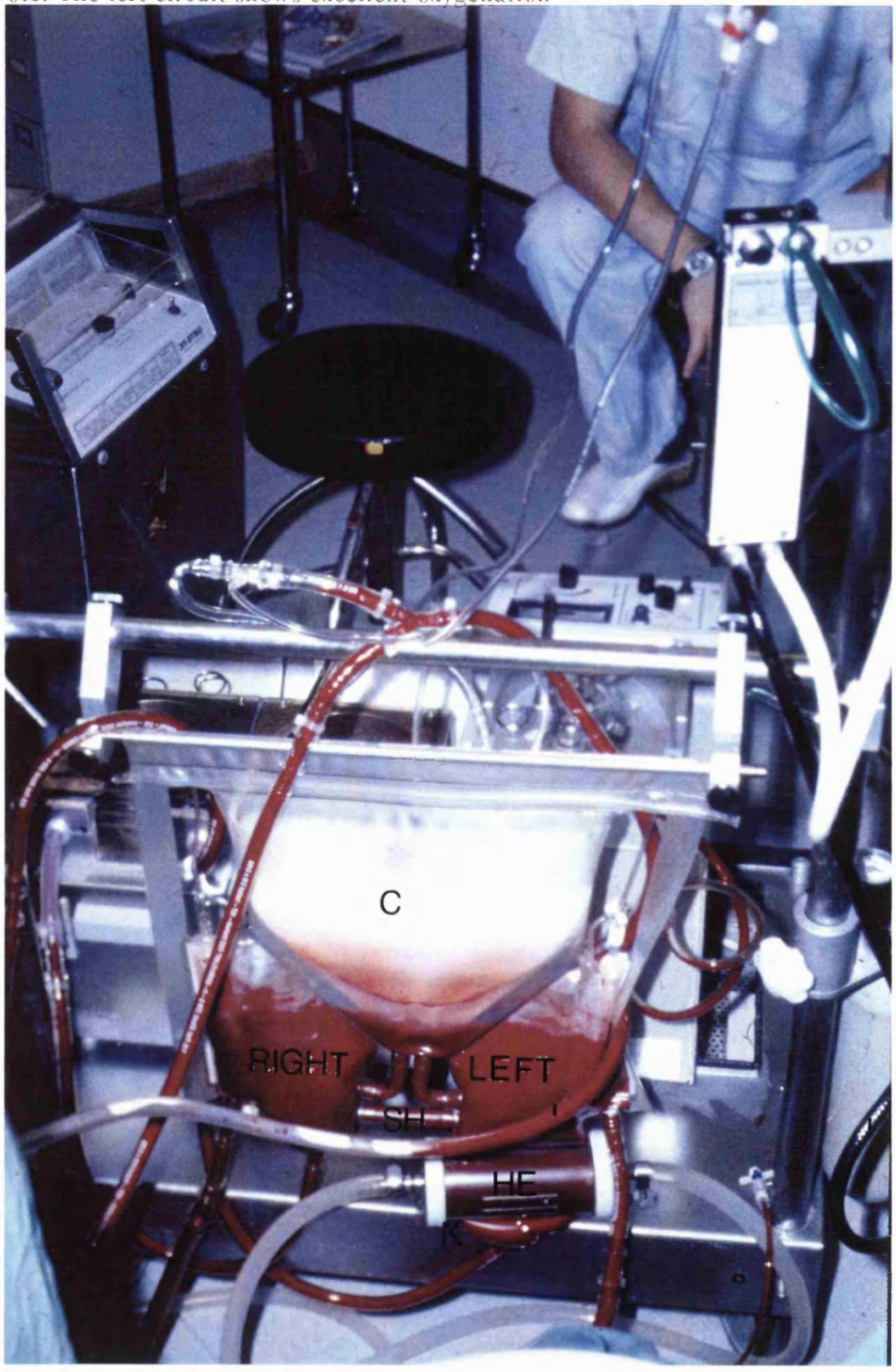
The next phase was to undertake a study for each of the coronary artery grafting techniques. The important lessons on the conduct of the bypass had been learnt in the laboratory and therefore it was not surprising to see in these studies that bypass times for the double reservoir technique were no longer than their controls. The number of grafts was comparable and the use of the Internal Mammary artery the same in both groups. The findings of the animal work were largely confirmed although there were differences. Gas exchange was again excellent, although there was the potential danger of allowing the $pO_2$ levels to become too high. To avoid this, lower ventilatory settings could be achieved, but this required anaesthetic confidence. However, should the $pO_2$ drop, the perfusionist looking at the soft shell reservoirs (Plate 5), will be able to detect it as fast as an in-line oxygen monitor. When the settings were reduced, it is important to note, they were still adequate for carbon dioxide washout.

The measurements of gaseous microemboli were also reconfirmed in the clinical setting, leading to the hope of a low incidence of

PLATE 5. THE DOUBLE RESERVOIR CIRCUIT ON BYPASS

RIGHT=RIGHT CIRCUIT LEFT=LEFT CIRCUIT C=CARDIOTOMY RESERVOIR SH=SHUNT HE=HEAT EXCHANGER

Note: The left circuit shows excellent oxygenation



neurological morbidity post bypass. This was particularly encouraging in the setting of high oxygen partial pressures on bypass as there is a clear association between the two (Pearson et al 1986). That the use of ultrasonic detection of GME in extracorporeal circuits has many limitations (Moulinier and Mausurel 1978) and relates not just to the type of oxygenator used but the gas/blood flow rate, reservoir levels and so on,is well known, but it remains one important indicator. Others have argued (Padayachee et al 1987) that measurements directly over the cerebral circulation is also important.

Measurement of plasma haemoglobin levels over the bypass were also in the "acceptable" range. The values obtained by whatever assay method are only an indication of the situation; other methods of expressing the results include a haemolysis index which is related to flow. However, one view is that in vitro testing remains the only satisfactory way of comparing one system with another (Wright 1986).

The leucocytosis seen over the bypass in both studies was similar to that published elsewhere (Cavarocchi et al 1986). This also includes the data on white cell sequestration in the lungs, which was comparable with membrane oxygenation or bubble oxygenators combined with steroid. This level was quite small and differences at that level may be difficult to demonstrate. If this was well correlated with high complement levels, activation of polymorphonuclear cells, release of elastase and subsequent lung damage (van Oeveren and Wildevuur 1987b), this was not shown in the double reservoir studies. In the first study, a clear difference in C3a levels was demonstrated with the DR group having barely changing levels over the bypass.

Studies have shown a variety of findings at the complement fragment level although there is more unanimity with elastase levels. One study (Antonsen et al 1987) showed that while C3d rise through bypass was linear, the elastase showed an exponential increase. The role of cross clamping may also be crucial to white cell sequestration as well as complement split products. The cross

clamp fibrillation techniques although cumulative cross clamp times are in the 40 minute range, they are achieved with periods of perfusion after each period of clamping. The cardioplegic techniques involve one continual period of cross clamping. This may affect the ability of the lungs to "mop up" complement fragments, although the results support the theory that they still do not have the damage effects as seen by low elastase levels in the DR group. An alternative explanation is the different design of the two systems used for the double reservoir groups; in the first study, the cardiotomy reservoir was separate although manufactured by Polystan and in the second study the cardiotomy was integral and perhaps of differing blood contacting characteristics.

In the second study there was no clear difference in the C3a levels, but the elastase profile which may go on to cause the lung damage (Cochrane et al 1983) was much lower than the control group. The role of protamine for reversal of heparin is also seen to be a potent producers of both C5a and of thromboxane (Morel

et al 1987). In the cases they reported, this was associated with both broncho and vaso pulmonary constriction. However, although a steep rise was seen in both control groups after protamine administration, no clinical changes were noted.

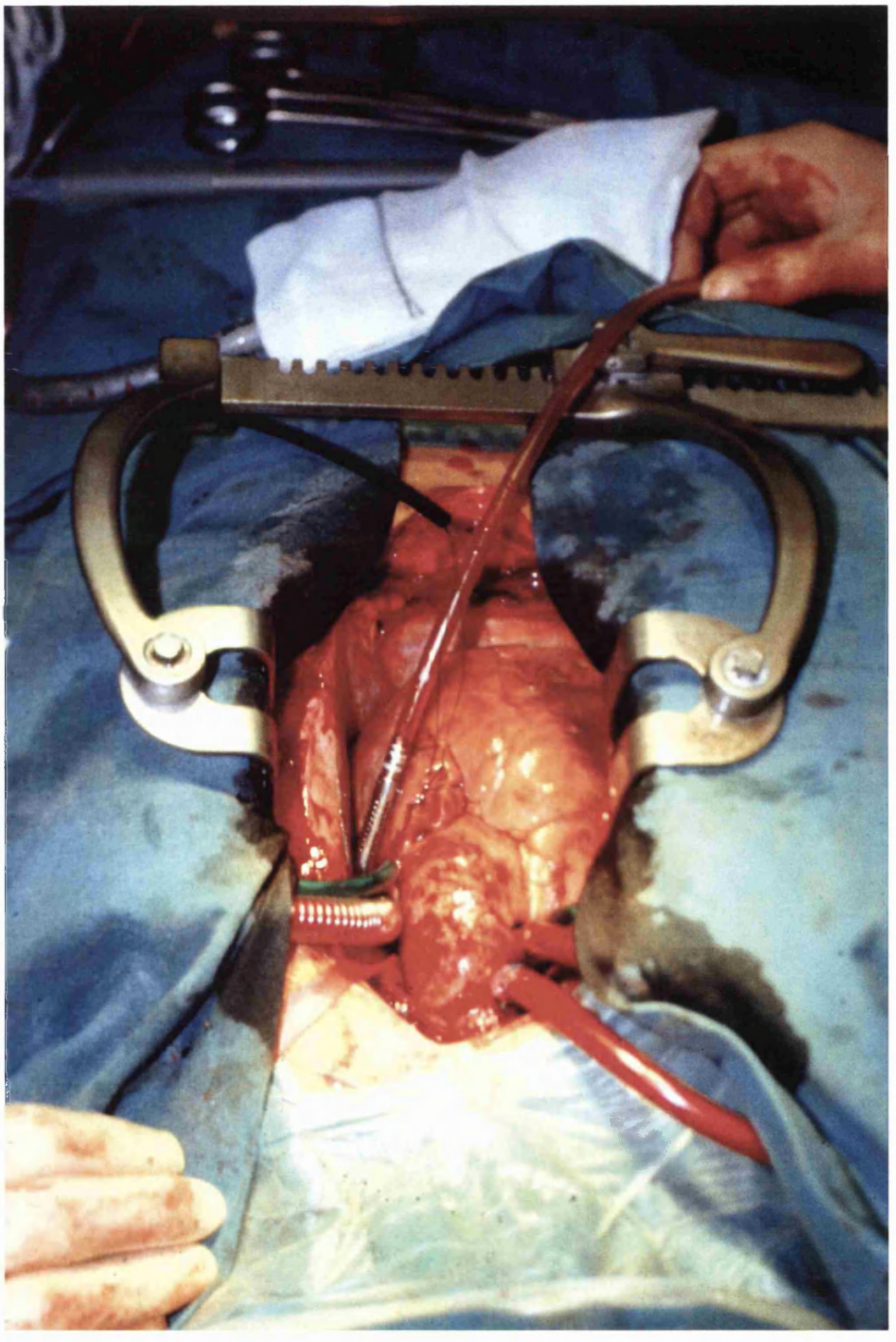
Platelet counts and function were compared between the DR group and membrane oxygenation. None of the data has shown any differences between the groups except that early in the bypass there may be some platelet sequestration in the lungs in the membrane group. Specific platelet release markers including beta thromboglobulin (BTG), triggered by cardiotomy suction and other factors, was also identical in the DR and control groups.
In the animal studies, it was possible to exclude cardiotomy suction when looking at the damage caused by cardiopulmonary bypass. This is difficult to achieve in clinical practice and therefore has additional affects on the results (deJong 1980). Cardiotomy suction, is also likely to explain the levels of tissue plasminogen activator (t-PA). The t-PA level, along with fibrinopeptide A and fibrin degradation products, cause further

amplification and damage to the coagulation and fibrinolytic systems (Davies et al 1980). The levels were high in all groups studied and this further reinforces the importance of cardiotomy suction and design of the equipment. Clot formation was seen to be excellent and therefore may have had limited clinical importance.

The above discussion has related principally to the patient; whilst this is obviously the most important part of any system, it has to be used by surgeons and perfusionists in a manner that is both safe and not cumbersome. The surgical aspects have been fully discussed, but the conclusions are perhaps that three of the cannulations are routine. The left atrial cannulation is however, the most difficult and potentially dangerous; it remains the weak point of the double reservoir technique. Mobility for surgery is however normal (Plate 6) and circumflex grafting is no different to conventional bypass. Knowledge of the bypass principle and practise is perhaps more important than with conventional bypass. If there are problems, one must be able to issue clear instructions

PLATE 6. THE OPERATIVE VIEW WITH THE CANNULAE IN POSITION

Note: Full mobility of the heart is retained



to the perfusionist.

The perfusionist must also be comfortable with the bypass and for this design at least, soft shell reservoirs. If the shunt is not closed off appropriately, whilst weaning right heart bypass for instance, there is a real possibility of entraining air fast. Mechanisms like double booted tubing or level alarms can of course overcome this. The ability in the design to connect an oxygenator in case of catastrophe is considered important. When the system is understood, the perfusionist gains control of the patient's circulation in a way that is never achievable with cardiopulmonary bypass.

The double reservoir technique has many theoretical advantages to the patient over conventional bypass; this study has shown somw of them and how the technique can be carried out safely. There are many directions as to demonstrating these in a clinical and experimental setting which are discussed in the following section.

# FUTURE DIRECTION

The limitations of the project are all too clear, however there are several future projects that could take the state of knowledge further, both for conventional cardiopulmonary bypass as well as autologous oxygenation.

The role of cross clamping in allowing high levels of activated complement fragments to accumulate is unclear. Does the act of cross clamping render homeostatic mechanisms ineffective, even if there is a full five litre right sided flow through the lungs? The answers could be provided from an animal model.

The complement and elastase levels were encouraging in the double reservoir technique, but are they going to be reflected in a reduced incidence of adult respiratory distress syndrome (pump lung etc.)? Clinical studies correlating lung water, radiological appearances, gas exchange with the complement pathway may help answer this. This would also link in with careful studying of the

kinetics and aetiology of platelet and white cell sequestration. Similarly, does the fact that the double reservoir technique either generate or at least divert GMEs from the systemic circulation mean that post operative neurological morbidity will be reduced? Studies in centres that have a special interest in that field could cooperate to compare conventional and autologous oxygenation.

There is also room for improvement in the design of the DR equipment including safety features to lessen the chances of air entrainment. These would also be directed to the flow characteristics to ensure that GMEs stay low and perhaps to heparin bonding to still further improve its biocompatibility.

Finally, there is a unique model to study many aspects of bypass; effects of temperature of materials and of drugs. This can be carried out without the major influence of the oxygenator itself.

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