An Investigation Into New Materials for Extracorporeal Life Support. Including: Mechanical Properties, Blood Surface Interactions and the Inflammatory Response to Bypass

Thesis submitted for the degree of

Doctor of Medicine

at the University of Leicester

by

Giles John Peek

MBBS (London)

FRCS (England)

March 1998

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would like to thank the following for their invaluable help: Firstly my supervisor Richard Firmin. I would also like to thank Hilliary Moore (ECMO), Richard Scott (Perfusion), Martin Jarvis (Cardiothoracic Surgery), Mike Jackson (Department of Surgery), Derek Forbes (Biomedical Services), Nick Taub (Epidemiology and Public Health) and Colin Morrison and Kim Wong (Department of Engineering) for their expert and patient assistance and support.

I am grateful to Professors Lunec (Biochemistry), Bell (Surgery) and Whalley (Immunology) for allowing me the run of their laboratories, and their respective staff for putting up with me.

The staff of the ECMO programme especially Linda Evans and Guy Cadwallader, and Biomedical Services deserve special thanks. I would also like to thank Richard Reeves and Andy Thompson of Glenfield Hospital Department of Perfusion, Hussein Mulla and Roman Mychajluk of Glenfield Hospital Pharmacy and all the staff at the Leicester Royal Infirmary Departments of Haematology, Biochemistry and Histopathology. Kate Radford of the Department of Medical Illustration at the Leicester Royal Infirmary expertly handled the artwork and graphics, and I am most indebted to her.

For provision of funding and equipment I would like to thank the British Heart Foundation, Glenfield Hospital Research and Development Fund, The Sir Samuel Scott of Yews Trust, Pall Biomedical, Sorin Biomedical, Norton Performance Plastics, Rehau GmBh, Eschmann, Derby Royal Infirmary (Dept of Cardiothoracic Medicine) and Sally Garner (Glenfield Hospital, ITUs).

Last, but not least, I would like to thank my wife Sally for her support during this project.

ABSTRACT

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF MEDICINE AT THE UNIVERSITY OF LEICESTER

by

Giles J Peek MBBS FRCS

An Investigation Into New Materials for Extracorporeal Life Support. Including: Mechanical Properties, Blood Surface Interactions and the Inflammatory Response to Bypass

INTRODUCTION: Extracorporeal Membrane Oxygenation (ECMO) causes coagulation and inflammation. Also pump tubing can rupture. Therefore new tubing is needed.

AIMS: To compare mechanical properties and biocompatability of two potential ECMO tubings (LVA and SRT) with Tygon (current tubing). To develop a novel porcine veno-venous ECMO model. To review ECMO results at Glenfield Hospital.

METHODS

I) Mechanical: Durability; roller pump and test rig. Wear; electron microscopy. Spallation; laser diode particle counter.

II) In-Vivo Biocompatability: 5 pigs for each material, 48 hours veno-venous perfusion, samples: Blood count, blood gases, Prothrombin, Thrombin and Activated Partial Thromboplastin times, Lactoferrin, C3adesarg and Thromboxane B2. Lung neutrophil immunohistochemistry, histology, and lung water.

III) In-Vitro Biocompatability: a) 5 circuits of each material recirculated for 6 hours, human blood. Samples as above plus fibrinogen, C5b-9 instead of C3adesarg.

b) I^{125}Fibrinogen uptake with and without albumin washing.

IV) Clinical Review: Retrospective.

RESULTS

I) Mechanical: Tygon was unpredictable, but better than LVA and SRT.

II) In-Vivo Biocompatability: The porcine model was successfully established. The only significant difference between groups was higher haemolysis with Tygon compared to SRT. Animals developed "ARDS" and thrombocytopenia.

III) In-Vitro Biocompatability: a) SRT and LVA, increased coagulation. LVA increased haemolysis. b) Untreated Tygon, lower fibrinogen uptake, no differences after albumin.

IV) Clinical Review: (n) and % survival: Respiratory; Adult (99) 63%, Paediatric (81) 77%, Neonatal (134) 75%. Cardiac; Adult (8) 38%, and Paediatric (28) 61%. Results for the first 50 adult respiratory patients: mean PaO_2/FIO_2, 65 $\pm$ 36.9 mmHg, mean Murray score 3.4 $\pm$ 0.5 and 66% survival. Compared to historical controls (55.6% & 42% survival) p=0.036 & p=0.0006.

CONCLUSIONS: Neither SRT nor LVA are mechanically adequate for ECMO. SRT and LVA are less biocompatable than Tygon, causing more coagulation and haemolysis. The porcine model was technically successful but needs larger numbers to discriminate between materials.

PREFACE

The subject of this thesis is Extracorporeal Membrane Oxygenation or ECMO. ECMO is a relatively new treatment for fulminant respiratory and cardiac insufficiency and uses modified cardio-pulmonary bypass technology to provide cardio-respiratory support for prolonged periods in the intensive care unit. ECMO can be used for patients of any age from 34 weeks gestation up to 60 years old, suffering from any form of potentially reversible respiratory or cardiac failure.

ECMO is usually considered when conventional treatment has failed, and patients are at very high risk of dying (80% to 100% estimated mortality). Survivals with ECMO in these moribund patients range from in excess of 95% for meconium aspiration, to around 30% for adult cardiac support. The apparent survival advantage of ECMO over conventional treatment for severe neonatal respiratory failure has been proven by the UK Collaborative ECMO Study, which was fully randomised. However adequate controlled trials have not yet been performed for paediatric and adult respiratory ECMO or for cardiac support, which remain controversial areas.

Possible reasons for scepticism regarding the efficacy of ECMO are, firstly its technical complexity, and secondly the potential for bleeding. Taken together these factors can produce horrendous complications if accepted management protocols are not followed, such complications are often fatal. Notwithstanding the potential for error, the successful use of ECMO in hundreds of hospitals around the world in over 14,000 patients, indicates that properly trained teams can perform ECMO safely and with a minimum of complications.

Unfortunately there are two factors which are inherent to the way ECMO is currently practised, which could impair patient survival. Both factors are related to the material from which the extracorporeal circuit tubing is manufactured. Firstly the mechanical properties of a tubing material must allow prolonged compression by a roller pump without either rupturing, or producing excessive spallation. Such "raceway" rupture is very rare, but carries a 50% mortality, and is one of the most feared mechanical complications of ECMO. Secondly the material must be inert or biocompatible, and produce a minimum of activation of the inflammatory and coagulative pathways in the blood, such a material would not cause the pulmonary consolidation seen with current circuits. Also the reduced activation of platelets and the coagulation system would mean a reduced transfusion requirement for blood products, and perhaps the possibility of heparin free ECMO.

The currently used raceway tubing is a modified PVC, Tygon S-65-HL. It is far more durable that any other commercially available material, and has acceptable bio-compatibility.

Nevertheless, improvements in durability and biocompatability could theoretically result in improved survival, especially in patients at risk of bleeding, or who require surgery whilst on ECMO. In addition such improvements could allow ECMO use to be extended to patients who have previously been contra-indicated because of the risk of haemorrhage, especially premature neonates, and patients with recent trauma.

The aim of this thesis is therefore to examine the mechanical properties and biocompatability of two new potential ECMO tubing materials, LVA (a silicone rubber) and SRT (a poly-olefin) and compare these to Tygon, as a control. A combination of in vitro and in vivo experiments were conducted. A novel porcine model of prolonged extracorporeal circulation was developed to compare the materials under clinical conditions.

In addition to this, the clinical practice and results of ECMO at Glenfield Hospital, Leicester is also described, and compared to published series of patients receiving conventional intensive care, as well as to patients reported in the international Extracorporeal Life Support Organisation (ELSO) registry.

CHAPTER 1

EXTRACORPOREAL MEMBRANE OXYGENATION (ECMO), AN OVERVIEW OF CLINICAL PRACTICE

INTRODUCTION

Extracorporeal membrane oxygenation or ECMO uses a modified cardiopulmonary bypass circuit to provide prolonged cardiorespiratory support in the intensive care unit, usually by means of extra-thoracic cannulation. Patients suffering from a wide range of potentially reversible conditions causing respiratory, cardiac or combined cardiorespiratory failure may benefit from ECMO support. Examples would be severe meconium aspiration in the new-born infant, meningococcal septicaemia in the child, and ARDS in the adult. ECMO is usually considered when a patient is deteriorating despite maximal conventional therapy, and their estimated mortality exceeds 80%, ECMO can convert this to a survival of approximately 50 to 95% depending on the diagnosis and age group. In this chapter we will discuss the history, indications and principles of extracorporeal life support, based on our own experience, the Extracorporeal Life Support Organisation (ELSO) registry, and the published literature.

HISTORY

The concept of using a pump to support the function of the heart and lungs is not new. Benjamin Ward Richardson first experimented with such a system in 1865 (Richardson, 1865), but his efforts were hampered by the immediate coagulation of the blood upon contact with his experimental apparatus. The discovery of heparin in 1916 by the medical student Jay McLean at Johns Hopkins (McLean, 1959) was a necessary pre-condition to the successful development of extracorporeal circulation. However it was the

death of a young woman from pulmonary embolism witnessed as an Intern by Gibbon that inspired him to develop a viable cardio-pulmonary bypass machine (Gibbon, 1939). Although the early film oxygenators were not suitable for prolonged perfusion as severe haemolysis occurred after a few hours (Gibbon, 1937), they did allow the circulation to be supported for the short periods necessary to carry out intra-cardiac surgery, demonstrated by the first successful clinical use of the pump oxygenator by Gibbon to close an atrial septal defect in 1953 (Gibbon, 1954). The whole new speciality of open cardiac surgery blossomed following this notable achievement, but it was not until the silicone rubber membrane oxygenators were developed by Kolff (Kolff and Effler, 1956), Kolobow (Kolobow and Bowman, 1963) and Bramson (Bramson et al. 1965) in the late 1950's and early 1960's that prolonged circulatory support could be contemplated. The separation of the blood and gas phases in the membrane oxygenator greatly reduced the blood damage, particularly haemolysis and complement activation produced when there was direct contact between gas and blood, and allowed perfusion to continue for days and weeks rather than mere hours without denaturing the blood, save for a predictable mild thrombocytopenia.

The laboratory and early clinical development of ECMO resulted in the first clinical success in 1972 (Hill et al. 1972) when Hill et al used femoro-femoral veno-arterial ECMO to support a 24 year old man who sustained an aortic transection and multiple fractures during a road accident, he developed ARDS on the fourth post operative day and was supported on ECMO for 75 hours: he survived.

Hills' success generated a wide interest in the use of ECMO in adults which lead to the American National Institute of Health (NIH) sponsored ECMO study in the mid 1970's (Zapol et al. 1979; Hill et al. 1978). This was a national multi-centre trial in which all patients with respiratory failure, as defined as positive pressure ventilation with an inspired oxygen concentration of greater than 50%, were entered into a data base. Patients with

severe respiratory failure who fulfilled certain "ECMO Criteria" (Fast entry: PaO2<50mmHg with FIO2 1 & PEEP>5cmH2O. Slow entry: PaO2<50mmHg for >12 hours with FIO2>0.6, PEEP>5 cmH2O and shunt fraction > 30%) were randomised to either receive ECMO or continue on conventional treatment. The trial was curtailed prematurely because of high mortality in both treatment groups (91.7% conventional and 86.4% ECMO).

Although interest in ECMO waned considerably after the publication of the NIH study, two groups of researchers, Gattinoni et al in Italy, and Bartlett et al in the USA believed that the fundamental principle of ECMO was correct and that greater understanding of its application and more appropriate case selection could result in better outcomes. Gattinoni, in collaboration with Kolobow, developed a low flow veno-venous system to provide extracorporeal CO2 removal to adult patients and recorded survivals of 48.8% (Gattinoni et al. 1986), which he attributed to his use of the low pressure, low frequency ventilation which minimised barotrauma. He developed a concept of ARDS, based on CT scans of the lung, that conventional ventilation merely over ventilates the small area of residual normal lung or "baby lung", rather than recruiting the diseased lung (Gattinoni et al. 1987). Bartlett held similar beliefs about the importance of lung rest, but also believed that the neonatal lung had greater recuperative power than the adult. He reported the first series of successful neonatal ECMO patients in 1982 (Bartlett et al. 1982). Following this lead other groups began to experiment with neonatal respiratory ECMO. By the mid 1980s it had become accepted practice for severe neonatal respiratory failure in the USA. Following 2 modified design trials in the USA (Bartlett et al. 1985; O'Rourke et al. 1989) and a fully randomised UK trial (Anonymous. 1996) it is now accepted therapy in this age group in the UK. Bartlett and other workers also began to extend their practice to include paediatric patients and, now again tentatively, adults using the lessons learnt from

the neonatal age group; namely low level heparinisation, avoidance of haemorrhage, lung rest and selecting patients with potentially reversible pathology. The use of veno-venous perfusion became accepted as the mode of choice for isolated respiratory failure (Klein et al. 1985; Bartlett, 1995). Concurrently active ECMO programmes were experimenting with ECMO as a cardiac assist both as an elective and emergent procedure. Although the high level of technical difficulty, haemorrhage and poor results (Moreno-Cabral et al. 1994) & Vide Infra in adult patients with predominantly left ventricular problems caused disenchantment with the technique. The efficacy of ECMO in the paediatric age group with its predominance of pulmonary hypertension and right heart failure has become gradually more apparent as experience has grown (Fausles, 1995) & Vide Infra. The limitations of ECMO in terms of raised left ventricular afterload in the adult have also been realised and now teams can compensate for this by using a left sided vent or balloon counterpulsation to off load the left ventricle, or choose a ventricular assist device (VAD).

The development of expertise in the safe prolonged use of ECMO for respiratory support is usually a pre-requisite for effective cardiac ECMO, as teams who do it infrequently generally do it badly. It has been shown from analysis of the ELSO registry that 10-20 cases per year is a minimum requirement to maintain consistent performance. Cardiac support is also the most challenging type of ECMO as patients often develop a coagulopathy following cardio-pulmonary bypass, have active or potential bleeding sites, and frequently have anatomical considerations which make cannulation more difficult (Firmin et al. 1995). As cardiac ECMO is indicated in less than 3% of cardiac surgical cases, few surgical centres could support an ECMO programme based on their cardiac cases alone. Units that have experimented with cardiac ECMO in the absence of an ongoing respiratory ECMO programme have usually been disappointed with their results.

INDICATIONS & CONTRA-INDICATIONS .

The indications for ECMO support are in principle the same irrespective of the disease being treated, the age group of the patient, and even which organ systems require support. Patients may benefit from ECMO if they have developed severe respiratory or circulatory failure (or both) that is refractory to maximal conventional treatment, providing that the underlying disease process is potentially reversible, and that they do not have an absolute contraindication. The question of reversibility or otherwise of a disease may be further influenced by the availability of donor organs when ECMO is used as a bridge to transplant. An additional caveat for the post cardiac surgical patient is that the cardiac repair must be technically satisfactory for ECMO to have any chance of being effective. ECMO should be used to save life rather than prolong death. Several indices can be used to define when a patients condition has reached sufficient severity for ECMO to be considered, and these will be discussed below, but it is important to note that individual assessment of each patients' condition, in particular the rate of deterioration, is often more useful than arbitrary guidelines when it comes to decision making. Contra-indications fall into three main groups, firstly concerning the reversibility of the disease, secondly the projected functional status and quality of life for the patient should they survive, and thirdly contra-indications to prolonged heparinisation.

INDICATIONS FOR NEONATAL RESPIRATORY ECMO: The oxygenation index (OI) is the most commonly used measure of the severity of neonatal respiratory failure, it is a particularly useful parameter as it contains an expression of airway pressure. The OI may be calculated as follows:

$$OI = \frac{\text{Mean Airway Pressure x } FIO_2\ (\%)}{\text{Post- Ductal PaO2 (mmHg)}}$$

Once the OI exceeds 40 then ECMO is indicated (Anonymous. 1996), there has been little work to assess the impact of novel "conventional" therapies such as oscillation

or inhaled nitric oxide (iNO) on the OI, but it would be sensible to assume that a given OI during oscillation indicates worse lung disease than the same OI in a baby on intermittent positive pressure ventilation (IPPV). When considering using iNO or oscillation on a baby with respiratory failure one must first consider what facilities exist to support the patient if they fail since it may not be possible to convert the child back to IPPV for transfer to an ECMO centre, and it is currently not practible to transfer patients on oscillatory ventilation. Transport of patients on iNO could be potentially dangerous to all members of the transport team in the event of cylinder disruption, and probably should not be considered.

The common thread that unites neonatal respiratory failure is the development of hypoxic pulmonary vasoconstriction resulting in the re-opening of right to left shunts via the foramen ovale and ductus arteriosus (persistent fetal circulation or PFC) leading to further hypoxia and increasing pulmonary hypertension, this vicious circle can often only be broken with ECMO. The most frequent indications for neonatal respiratory ECMO are meconium aspiration syndrome, infant respiratory distress syndrome, congenital diaphragmatic hernia and idiopathic persistent pulmonary hypertension of the new-born, relative numbers of these diagnoses and outcomes are given in the discussion of ELSO results below.

CONTRA-INDICATIONS TO NEONATAL RESPIRATORY ECMO: Gestational age less than 34 weeks and birth weight less than 2 kg are both important contra-indications to neonatal ECMO as the risk of intra-cranial haemorrhage in this group of patients is unacceptably high (Revenis et al. 1992). A pre-existing intra-cranial haemorrhage greater than Grade I is also a contra-indication (Taylor et al. 1989). Ventilation with high pressure and high oxygen concentration for longer that ten days results in irreversible ventilator lung injury and is an absolute contra-indication (Short, 1993b). The coexistence of congenital abnormalities is a relative contra-indication , and depends on the nature and severity of the abnormality, however, often the exact extent of such an abnormality cannot be discerned whilst the baby is critically ill and hypoxic, and in this situation it is usual to initiate ECMO treatment to stabilise the patient prior to more a detailed assessment. Coagulopathy and an extra-cranial focus of haemorrhage (i.e. a chest drain in the lung) are also only relative contra-indications as these problems can usually be controlled by transfusion of blood products and surgery.

INDICATIONS FOR PAEDIATRIC RESPIRATORY ECMO: The development of severe, potentially reversible, acute respiratory failure refractory to conventional treatment is the indication for ECMO support in children, just as it is in the neonate and adult. Conventional treatment can be considered to have failed when a-A Oxygen gradient exceeds 450mmhg for over 24 hours (Tamburro et al. 1991), especially if high peak inflation pressures (i.e. > 30 cmH_2O) or high PEEP (> $5cmH_2O$) is required, the presence of hypercapnia (excluding permissive hypercapnia) and the nature and speed of onset of the condition must also be taken into account as must the co-existance of failure of other organ systems. Individual patient assessment is paramount in deciding if a child will benefit from ECMO support, and there are no hard and fast criteria in contrast to the neonatal group. Common diagnoses include pneumonia which may be bacterial, atypical or viral (especially Respiratory Syncitial Virus, RSV) and the Acute Respiratory Distress Syndrome (ARDS) which may follow sepsis, massive transfusion , smoke inhalation or trauma. Often patients with sepsis present in multi-organ failure with impaired tissue oxygen delivery despite inotropic support, this group of patients may benefit from veno-arterial (VA) ECMO, rather than veno-venous (VV) which is used more often for respiratory failure. VA ECMO can provide circulatory support to reverse septic shock, improve perfusion, and allow inotropes and pressors to be weaned off, the improved renal perfusion will often reverse oliguria, but haemofiltration is frequently required in this group.

CONTRA-INDICATIONS TO PAEDIATRIC RESPIRATORY ECMO: Irreversible ventilator lung injury occurs in children after approximately 7 to 9 days depending on the age of the child, with younger children being more resilient. The presence of intra-cranial bleeding is more difficult to diagnose than in the neonate as ultra-sound scanning is no longer possible after the fontanelles have closed, however if a high index of suspicion is present, for example, in the child with a head injury, intra-cranial bleeding should be excluded with CT or MRI scanning prior to the initiation of ECMO. Relative contra-indications include the presence of a disease incompatible with a reasonable quality of life such as severe broncho-pulmonary dysplasia or cystic fibrosis, and irreversible diseases, such as disseminated tumours. Again extra-cranial bleeding sources are usually amenable to surgical treatment.

INDICATIONS FOR ADULT RESPIRATORY ECMO: These are identical to the indications for paediatric respiratory support, severe but potentially reversible respiratory failure refractory to optimal conventional management. Patients usually present with either pneumonia or ARDS, obviously the causes are different in this age group, as can be seen in the results section below. In the adult population conventional treatment can be considered to be failing when an FIO_2 of > 80% is required, the peak airway pressure exceeds 35 cmH_2O, or if severe lung injury (Murray score >2.5) (Murray et al. 1988) is present (Vasilyev et al. 1995). Just as in the paediatric population the importance of individual patient assessment cannot be over emphasised. Also it is essential to interpret the ventilatory parameters in light of the diagnosis and speed of onset of the patients condition. For example, varicella pneumonitis can render a previously ambulatory patient almost unventilatable in only a few hours, whilst ARDS following trauma usually causes deterioration over several days.

CONTRA-INDICATIONS TO ADULT RESPIRATORY ECMO: High pressure ventilation for longer than seven days is the most important contra-indication, closely followed by intra-cranial bleeding. Relative contra-indications are similar to the paediatric group, with the addition of age greater than 60 years. Good outcome can be achieved with ECMO in the over 60's provided that they are previously fit and present with single system failure.

INDICATIONS FOR CARDIAC ECMO: These are similar for all age groups. The University of Michigan Criteria, can be used to select patients who may benefit from Cardiac ECMO (below). The survival figures for adult patients receiving cardiac support (29-33%, (Joyce et al. 1990)), contrast sharply with the often excellent outcome for paediatric cardiac ECMO (61%). However, it is still worth considering cardiac ECMO or VAD use in adult patients provided the cardiac lesion has been corrected, some groups obtaining 50% survival in these patients (Pennington et al. 1989; Pennington and Swartz, 1995). The differences in outcome between the adults and children may be related to the prevalence of coronary and peripheral atherosclerosis in the adult patients and also the

pattern of ventricular failure seen in the two groups. Children who require post operative cardiac support often have predominately right ventricular failure, usually with super-added pulmonary hypertension. VA ECMO reduces right ventricular preload and afterload, and left ventricular preload very effectively, but leads to high left ventricular afterload. Adults tend to develop left ventricular failure more commonly and the left ventricle is less likely to recover if it is not off-loaded by either venting or intra-aortic counterpulsation. This hypothesis is supported by the efficacy with which ECMO can be used to support adult patients with normal left ventricles and isolated right ventricular failure, for example those with massive pulmonary embolism (Davies et al. 1995).

When considering using ECMO for cardiac support in a patient who will not wean from bypass it is essential to ensure that the cardiac repair is technically adequate, otherwise ECMO support is unlikely to be effective.

THE UNIVERSITY OF MICHIGAN CRITERIA FOR CARDIAC ECMO

CARDIAC INDEX	< 2 L/ SqM/min for 3 hours.
METABOLIC ACIDOSIS	Base Deficit > -5 for 3 hours.
BLOOD PRESSURE & OLIGURIA (for 3 hours)	Neonate MAP < 40mmHg Infant MAP < 50 mmHg Child MAP < 60 mmHg Urine < 0.5 ml / Kg / hr
POST-OPERATIVE	Failure to wean from bypass.

CONTRA-INDICATIONS TO CARDIAC ECMO

Again the University of Michigan Criteria serve as a useful guide:

In cardiac arrest (relative contra-indication depending on cause and duration)

Prior cardiac arrest with unknown neurological status

Actual or possible major brain injury

Conditions incompatible with normal health

Prolonged period of shock: Metabolic acidosis > -5 for 12 hours
Oliguria < 0.5 ml/kg/hr for 12 hours
Prolonged hypotension

ECMO is contra-indicated if the long term outlook or quality of life will be poor, if there is a contra-indication to heparinisation, or if the patient has been ventilated too long and too hard (ten days for neonates and seven days for adults).

RESULTS OF PATIENTS TREATED WITH ECMO.

Most active ECMO centres report their cases to the Extracorporeal Life Support Organisation, ELSO, in Ann Arbor, Michigan, USA. These data are entered into a data base which contained 13,974 cases at the end of 1995. The results of ECMO at Glenfield hospital up until the end of 1995 will be given below together with the results from the ELSO registry for the same period, for comparison. The survival of patients in the different age groups will be taken solely from the ELSO registry, our own data will be presented in chapter 3.

OVERALL RESULTS.

	ELSO		GLENFIELD	
GROUP	N	% Survival	N	% Survival.
Neonatal Respiratory	11011	80%	97	72%
Paediatric Respiratory	1067	53%	59	71%
Cardiac Support (Paed).	1650	43%	28	61%
Adult	246	46%	65	63%

NEONATAL RESPIRATORY RESULTS.

Diagnosis	N	% Survival
CDH	2226	58%
MAS	3978	94%
PPHN	1485	82%
IRDS	1135	84%
Pneumonia/ Sepsis	1712	77%
Other	475	75%

OI-Oxygenation Index, CDH-Congenital Diaphragmatic Hernia, MAS-Meconium Aspiration Syndrome, PPHN-Persistent Pulmonary Hypertension of the New-born, IRDS-Infant Respiratory Distress Syndrome.

PAEDIATRIC RESPIRATORY RESULTS.

Diagnosis	N	% Survival
ARDS	87	53%
Bacterial Pneumonia	93	44%
Viral Pneumonia	336	57%
Other Pneumonia	105	61%
Other	446	50%

PAEDIATRIC CARDIAC RESULTS

Diagnosis	N	% Survival
Transplant	15	53%
Left to Right Shunt (ASD/VSD/PDA/AV Canal)	227	42%
Left Sided Obstructive Lesions (Aortic Stenosis/Mitral Stenosis/Coarctation)	84	39%
Hypoplastic Left Heart	44	25%
Right Sided Obstructive Lesions (Pulmonary Stenosis or Atresia/Tricuspid Atresia)	77	48%
Cyanotic Increased Pulmonary Flow (Truncus Arteriosus/TGA/TGV)	273	36%
Cyanotic Increased Pulmonary Congestion (TAPVR/PAPVR)	148	49%
Cyanotic Decreased Pulmonary Flow (TOF/DORV/Ebstein)	250	43%
Anomalous Left Coronary Artery	29	62%
Post Operative (Fontan)	48	35%
Other	80	36%
TOTAL	1275	41.3%

ADULT RESULTS

Diagnosis	N	% Survival
Bacterial Pneumonia	28	39%
Viral Pneumonia	27	67%
Aspiration Pneumonia	8	38%
ARDS	59	51%
Other Respiratory	43	49%
Peri-Transplant	18	33%
Cardiac	63	38%

ECMO PATIENT MANAGEMENT

PERSONNEL

Although it may seem strange to start a discussion of patient management by considering personnel it is actually the most crucial factor in achieving a satisfactory patient outcome. Successful ECMO is a "Team Game", and all team members must have complementary skills and be prepared to work together. The most important member of staff in this respect is the person at the bedside. It is essential that there is a member of staff at the patients bedside twenty four hours per day who is not only competent in managing the ECMO circuit, but can also perform emergency repairs if (when) it malfunctions. This person must also be conversant with the different conditions being treated and work closely with the patients' nurse to manage the patient holistically. Operating room perfusionists are experts at managing short term bypass, but, unless they have also studied prolonged extracorporeal support in some detail, are not necessarily the ideal people to perform this function. However, involvement of perfusionists is, in our view, needed for the safe running of an ECMO programme as they have superior skills for rapid circuit priming, establishing the patient on ECMO, and performing circuit repairs or modifications.

Perfusion support is also helpful for haemofiltration, which is also often required. Using perfusionists to look after the circuit is sub-optimal utilisation of a scarce resource, and will interfere with their primary commitment in the operating theatre. All ELSO registered ECMO centres use "ECMO Specialists" to perform this function. The specialist is usually an experienced intensive care nurse, although they may equally be a perfusionist, physiotherapist or doctor who has received additional training in ECMO circuit and patient management. They are usually loaned from their base speciality to the ECMO programme on a part time basis depending on the number of patients on ECMO at any given time. Specialists must practice emergency circuit repairs on saline filled ECMO circuits (water labs) to maintain their level of skill.

The majority of ECMO centres also have a Co-ordinator. Whilst the specialist is the most crucial team member on a minute to minute basis, it is the co-ordinator who oversees the ECMO programme as a whole, including staffing, safety, training, equipment, finance, administration and is important to continuity of care.

The ECMO Director must be a doctor, although the individual speciality of that doctor is probably not important, as effective directors include neonatologists, paediatric general surgeons, cardiac surgeons and intensivists. However, regardless of base speciality, they must retain clinical control and responsibility for patients receiving ECMO. Whichever speciality the director comes from he must be supported by other specialities. If the director is not a surgeon, he must enlist the help of his surgical colleagues, preferably having a named surgeon as an integral part of the team. This is essential to ensure safe, slick, cannulation and decannulation, and also to manage complications that may arise in these critically ill patients. Having the same surgeon or small team of surgeons involved allows them to build up experience and expertise with ECMO and will result in better patient outcomes. If the programme is busy the director is often supported by a deputy and

a clinical fellow (or several). Although not essential, it is probably sensible to have several specialities represented within the ECMO medical team itself.

CANNULATION

Whilst there are differences in cannulation technique dependent on the mode of ECMO to be employed and the age of the patient, there are a number of principles common to all ECMO cannulations. Firstly it is important to maintain the patients current level of treatment until they are established on ECMO as they are often so unstable that even a few seconds without their inotropes or without heavy ventilation can result in destabilisation or even cardiac arrest. Secondly patients must be anaesthetised and paralysed to reduce the risk of air embolism, except in exceptional circumstances when they are cannulated awake and can perform a Valsalva manoeuvre. If the patient is not already anaesthetised we have found a combination of Ketamine 1-4 mg/Kg IV and Atracurium 0.5-1 mg/Kg IV to provide adequate anaesthesia with excellent haemodynamic stability. Once the target vessels have been either exposed or initial vascular access obtained (if percutaneous cannulation is being used) a loading dose of heparin must be given and allowed to circulate for 2 minutes before the cannulae are placed, 50 u/Kg is usually sufficient to give an Activated Clotting Time (ACT) of 200-250 seconds which is adequate for cannulation. If there is delay in establishing extra-corporeal flow ACTs should be checked and further heparin given if indicated.

VENO-VENOUS CANNULATION: Veno-venous or VV ECMO is the mode of choice for respiratory failure. The relative benefits and disadvantages of VV versus VA perfusion will be discussed separately below. Let us first consider VV cannulation in the neonate. The circulation is accessed via the right internal jugular vein, it is not practicable to use the femoral vessels for access in the neonate as they remain very small until the child starts to walk. There are several methods of cannulation described (Moulton et al. 1993; Firmin et al. 1995), which all have advantages and disadvantages. We will describe our method of

cannulation using the semi-Seldinger technique (Peek et al. 1996a) which does not require ligation of the internal jugular vein, and obviates the need for re-exploration of the neck for de-cannulation.

Double lumen cannulae are suitable for patients between 3 and 6 Kg in body weight. Cannulae are available from Jostra (Hirrlingen, Germany) sizes 12 and 15 FG and Kendal (Mansfield, MA 02048, USA) in size 14 FG. The Jostra cannula comes with a guidewire and dilators for percutaneous insertion, but in our experience is actually almost impossible to place percutaneously, especially quickly, this is due a number of factors including the large size of the initial needle (18 SWG) and the geometry and inflexibility of the dilator. Despite this the Michigan group has begun to place them percutaneously; avulsion of the jugular vein has occurred in one patient, and the procedure remains experimental (personal communication R Bartlett). The Kendal cannula does not have any dilators, and these must therefore be taken from another Seldinger dilator set.

The patient is positioned supine with the head turned to the left, and a roll under the shoulders. The jugular vein is exposed via a transverse incision over the lower 1/3 rd of sternocleidomastoid. It is usually necessary to divide the belly of omohyoid. All dissection is carried out using electro-cautery, and haemostasis must be painstaking. Once the vein has been exposed it should be assessed as to the size of cannula it will accept, and a sling is passed around the vein proximally, this is clipped but not tied. A small stab incision is now made cephalad to the main incision, this should be sized so that the incision is tight around the cannula. Heparin (50 iu/Kg) is then given. The introducer needle is passed through this incision and into the vein, entering the vein as cephalad as possible. The guidewire is inserted through the needle, which is then removed. The dilator and cannula are then introduced over the guidewire into the vein. The proximal ligature may be used to provide counter traction at this time. The cannula is advanced into the right atrium (usually 6-8 cm), with the perfusion (arterial) lumen aimed at the tricuspid valve i.e. antero-medially. The guidewire and dilator are removed and the lumens connected to the circuit. Extracorporeal flow is slowly established. The ligatures are removed, the wound is packed with a small amount of Surgicel (Johnson & Johnson, Ascot, UK) and Tisseel glue (Immuno AG, Vienna, Austria) is instilled into the wound. The wound is then closed in layers. The cannula is secured with a drain stitch (2-0 Mersilene), and a chest X-ray is taken to confirm correct cannula placement, in the mid right atrium. If the cannula position is not satisfactory it may be adjusted by simply advancing or withdrawing the cannula without re-opening the incision. However care must be exercised not to withdraw the

cannula so far that the drainage side holes are outside the vein. Minor oozing from the wound can usually be controlled by pressure for ten minutes, and bleeding around the cannula as a result of an inadvertently large stab incision can be staunched with a simple purse-string suture. When the patient is ready to be de-cannulated they are sedated and paralysed, a mattress suture is placed around the stab incision, the lumens are clamped, and the cannula is quickly withdrawn by an assistant whilst the suture is tied. It is not necessary to press on the neck to achieve haemostasis.

Of 87 neonates treated with ECMO at our hospital since 1989 we have used a double lumen cannula in 43. The technique described above being used for the last 12 patients. We have had no catheter related complications. Double lumen cannulae have also been used in 14 infants under 6 Kg in weight. We have also used this technique to recannulate two infants with recurrent RSV infection, who required a second ECMO run several months later. In both cases the jugular vein was seen to be intact and unobstructed. Doppler ultrasonography has confirmed jugular vein patency post decannulation in a number of patients.

The facility to adjust the cannula position, and the ease with which decannulation is achieved makes this technique very convenient, much more so than the previous technique where the cannula and vein are both secured with a ligature. Another advantage of non-ligation is the absence of a constriction of the cannula from the ligature, and the reduced incidence of kinking of the cannula when it can move freely for short distances within the vein. Other short term advantages of non-ligation of the jugular vein include the drainage of de-oxygenated blood down the ipsilateral jugular vein and directly into the cannula, thereby reducing recirculation, and possibly a reduction in intracranial venous pressure which may have important advantages in terms of further reducing the incidence of intra-cerebral haemorrhage (Walker et al. 1996). Long term advantages remain to be seen, as do the long term patency rates.

Whilst it may be possible to design an efficient percutaneous double lumen cannula for neonatal ECLS it is notable that the 12 FG cannula may often only just be accommodated in a 3 Kg baby, and the importance of being able to watch the vein directly whilst it is being dilated, in order to prevent its tearing or avulsion must not be underestimated. The semi-Seldinger approach is also quicker than percutaneous access, unless a line is already present in the right jugular vein. Severe bleeding from the cannulation site has not occurred thus far with this technique, but any severe haemorrhage should prompt exploration as, in our experience with other cannulation techniques, such

haemorrhage usually indicates a mechanical problem, such as a side hole outside the vessel.

Patients smaller than 3 kg in whom the 12 FG cannula cannot be accommodated will usually require VA cannulation (see below), as will patients larger than 6 kg who have not started walking yet, as their femoral vessels will be too small to allow VV ECMO via two cannulae. An alternative option for these patients, until a wider range of double lumen cannulae are available, is the French AREC (Assistance Respiratoire Extra-Corporeal) system which provides VV ECMO via tidal flow through an 8 to 10F single cannula using a non-occlusive roller pump and gating system (Chevalier et al. 1993; Durandy et al. 1990).

Patients who have begun to walk are usually suitable for two cannula veno-venous access, which can usually be established percutaneously. Although there are theoretical advantages in terms of lower re-circulation with femoral drainage and atrial return the total blood flow obtainable is lower with this configuration, and therefore there is reduced capacity for extracorporeal gas exchange. It is thus prefferable to drain blood from the atrium. The total flow rate in the circuit is determined by the resistance in the venous cannula and the height of the venous syphon (how high is the bed), Pousseilles law (below) shows us that optimal drainage is achieved using a short, fat cannula:

$$\text{FLOW} \propto \frac{\text{Radius}^4 \times \text{Pressure}}{\text{Length}}$$

Thus the best venous drainage can be obtained by the most direct route, i.e. the right internal jugular vein, which will allow the insertion of the largest and shortest drainage cannula possible. The atrium can also be accessed using a long cannula from the femoral vein, or in rare circumstances via the left jugular vein. Care must be taken if using the left jugular not to insert such a large cannula that the innominate vein is obstructed as cerebral venous congestion can result. These other routes should only be used when the right jugular approach is not possible. The return cannula can be inserted in either femoral vein. Jugulo-femoral cannulation will usually result in approximately 20% recirculation, i.e. 20% of the blood in the venous drainage line will be oxygenated blood from the return cannula. Femoro-femoral cannulation results in higher re-circulation than this (up to 60% as measured by oximetry and concurrent Doppler cardiac output monitoring in some of our

patients) but satisfactory extracorporeal gas exchange can still be maintained. In some patients the optimal matching between blood flow and oxygen delivery is obtained by reversing the direction of flow, i.e. draining from the femoral vein and re-infusing into the jugular vein, this does not seem to be predictable (PB Rich, Personal communication).

Most surgeons and physicians are conversant with the Seldinger technique (Seldinger, 1953), but there are some important details which become crucial if very large cannulae are to be placed safely. The target vessel must first be entered with a small cannula up which the guidewire can be passed. We find the 3 ¼ in 16 SWG Angiocath (Becton-Dickinson) ideal for this purpose as it is easy to insert and will accept the 0.038" guidewire from the introducer set. It is usually best to insert the Angiocath into the target vessel as a separate procedure before prepping and draping the patient, in this way sterility does not have to be compromised if the target vessel cannot be entered with the Angiocath, as an alternative site can be used without having to remove all the drapes and start again, this saves vital minutes in these very hypoxic and unstable patients. Having inserted the Angiocath its position should be confirmed using a pressure transducer, the aspiration of dark blood at low pressure is not sufficient evidence of venous cannulation, as these patients are, by definition, hypoxic and may often be hypotensive as well. Inadvertent arterial cannulation has occurred once in our hands prior to adoption of this simple checking procedure. For paediatric cannulation the target vessel can first be entered with a small cannulae i.e. 22 or 20 SWG Jelco (Ethicon), a 0.021" guidewire is passed, and then a 6 FG sheath introducer (Cordis) is inserted into the vessel over the wire. The 0.038" guidewire from the cannula set will fit up the 6 FG sheath. Again, cannula placement should be verified by pressure transduction before the large ECMO cannula is inserted. Percutaneous ECMO cannulae are available from a number of manufacturers (Research Medical, Biomedicus), we use the DLP cannulae as we believe the insertion system to be the safest. Cannulae may be sized using the following table as a guide, or by using the M-number (Sinard et al. 1991; Montoya et al. 1991) which is an index of potential cannula flow.

Approx. Age	Approx. Weight	Drainage Cannula	Return Cannula
18 months	15kg	17FG	14FG
5 years	25kg	21FG	17FG
>16 years	60kg	21FG	21FG
>16 years	70kg	28 FG	21FG
>16 years	>100kg	2 x 28 FG	28 FG

Intermediate sizes of patients can be accommodated by using two smaller drainage cannulae. Cannulae should be able to drain up to 120ml/kg/min to provide full gas exchange on VV ECMO, although in practice 60-80 ml/kg/min is often sufficient.

As stated earlier, having inserted the small cannulae into the target vessel the patient should be anaesthetised, paralysed, prepped and draped, heparin (50u/Kg) is then given. Guidewires are inserted, the presence of ectopic beats during guidewire insertion, confirms venous placement and also indicates that the guidewire should be withdrawn slightly. The track must now be dilated up to accept the cannula, first a small stab incision is made at the entry point of the wire through the skin, this should be slightly smaller than the cannula itself to ensure haemostasis. Secondly, a pair of artery forceps are inserted into the wound and spread to dilate the subcutaneous tissues. Then the dilators are passed, we use both DLP and Research Medical dilator kits, the Research Medical kit has the advantage of larger dilators (up to 16 FG), it is essential that the guidewire does not become kinked at this point, otherwise the dilator may be pushed outside the vessel, this can be prevented by always ensuring that the guidewire and dilator move independently of one another. The ECMO cannulae may now be inserted, the DLP cannulae come with soft, flexible introducers which are threaded over the guidewire, and then the cannula is threaded over the introducer, this makes guidewire kinking and vessel disruption less likely. The principal of the guidewire moving independently during dilatation, is especially applicable during cannula insertion, the assistant should slowly withdraw the guidewire and introducer whilst the surgeon advances the cannula forward, in this way the introducer and guidewire remain still in relation to the patient whilst the cannula advances into the vessel. It goes without saying that someone should have hold of the guidewire and introducer at all times. Firm resistance is felt during cannula insertion, but as long as progress is smooth and the introducer and guidewire still move independently then it is usually safe to proceed.

Having successfully placed the cannula the introducer and guidewire are removed and the cannula is connected to the circuit and secured to the skin with sutures. It may be

realised at this point that the skin incision is too big, this can result in bleeding around the cannula, which can be prevented with a purse-string suture placed around the cannula.

When initiating VV ECMO low flow should be used until mixing of the patients blood and prime has occurred, otherwise infusion of large amounts of hyperkalaemic, citrated blood prime will result in hypotension or hypocalcaemic, hyperkalaemic cardiac arrest, this should be treated with calcium in addition to the usual dose of adrenaline and cardiac massage, often the first sign of impending arrest is the presence of bright red oxygenated blood in the venous return line. This is a result of increased recirculation as the native cardiac output falls, a sharp reduction in extracorporeal flow coupled with a dose of calcium can usually prevent any further problems at this point.

VENO-ARTERIAL CANNULATION: Veno-arterial or VA support is indicated in the presence of haemodynamic instability, when VV cannulation is not technically possible, and for Cardiac support. Extra-thoracic cannulation is preferable as the incidence of bleeding is lower. However trans-thoracic cannulation may be necessary in patients who fail to wean from cardio-pulmonary bypass and need to be transferred from the operating theatre on ECMO. In this circumstance it is usual to connect the ECMO circuit to the bypass cannulae. If left ventricular function is impaired it may be necessary to vent the left side of the heart via the left atrium, left ventricle or pulmonary artery, but this is not usually required for isolated right ventricular failure.

Extra-thoracic VA access can be provided via the right carotid artery and jugular vein or the femoral vessels, or a combination of both. Other routes such as the brachial, subclavian and illiac arteries have been described, but are more difficult to use (Moulton et al. 1993). The carotid approach is obligatory in the neonate as the femoral vessels are too small. The right common carotid artery and internal jugular vein are exposed via a transverse incision over the lower third of the neck. All dissection is carried out either bluntly or with electro-cautery to reduce the risk of haemorrhage. The platysma is incised to expose the sternomastoid which is retracted laterally. The omohyoid is divided and the carotid sheath is opened. The jugular vein and carotid artery are encircled with no.1 non

absorbable ligatures proximally and distally, and heparin is given (if the patient is not already anti-coagulated). The cephalad ligature on the artery is now tied and the cannula is inserted via an arrowhead arteriotomy. The caudal ligature is now tied around the vessel and cannula to achieve haemostasis. The cannula is further secured with the cephalad ligature. The tip of the arterial cannula should lie at the orifice of the innominate artery, and not in the arch or ascending aorta, this usually equates with a distance of 2-3 cm from the arteriotomy in the neonate. The venous cannula is inserted in exactly the same manner, up to a distance of 6-8 cm in the neonate, so that the cannula tip and side holes lie in the right atrium. Wire-wound neonatal cannulae are available from Medtronic (Grand Rapids, Michigan, USA) and Research Medical (Midvale, Utah, USA), 8-12FG arterial and 10-14FG venous cannulae, cannulae are sized against the vessel. If a cannula larger than 14 FG is needed, the DLP (Medtronic) range (17-28FG) can be used.

Because the carotid is not an end artery it is possible to tie it off without any short term sequelae in the majority of cases (Streletz et al. 1992), the collateral circulation from the left carotid and vertebral arteries being usually sufficient. Data from the UK Collaborative ECMO Trial, in which VA cannulation with carotid ligation was used in 56% of cases (Anonymous. 1996) shows that the incidence of neurological impairment is similar in ECMO survivors (26%) and survivors of conventional treatment (29%) with 2% (1 patient) of cases in each group suffering severe impairment (Griffiths DQ<50). Given that the overall survival in the ECMO group was much higher than in the conventionally treated patients , 68% vs. 41% survival ($p=0.0005$) this supports the hypothesis that carotid ligation is consistent with an acceptable morbidity. When lesions do occur following carotid ligation they are more likely to be right sided (Schumacher et al. 1988). Risk factors for such a lesion include cardiac arrest, severe hypoxia and hypotension at the time of cannulation (Schumacher et al. 1988) which results in a loss of cerebral autoregulation

(Short et al. 1994b) and watershed infarct during the 3-5 minutes that it takes for cerebral perfusion to be re-established from the left side (Klein and Whittlesey, 1994), other studies with fewer patients cannulated whilst in extremis show that the majority of right sided lesions could be avoided (Lazar et al. 1994), except for approximately 10% of patients who do not have a complete circle of Willis (Anonymous1989a).

Opinion regarding carotid and jugular reconstruction is divided (Levy et al. 1995) (Lohrer et al. 1992) with good arguments on both sides. It is our practice to primarily re-construct only those vessels which look healthy and non-friable at the time of de-cannulation, as most vessels are very fragile the majority are ligated. There is some evidence to suggest that we should be more concerned about jugular ligation than carotid ligation (Walker et al. 1996) as high venous pressures are thought to contribute to cerebral ischaemia by decreasing cerebral perfusion pressure. The use of veno-venous ECMO using the double lumen cannula with non-ligation whenever possible obviates this problem. An alternative method is to use distal perfusion cannulae to establish antero-grade flow and venous drainage (Weber and Kountzman, 1996), but these are technically difficult to insert and manage, and offer little advantage for the vast majority of patients.

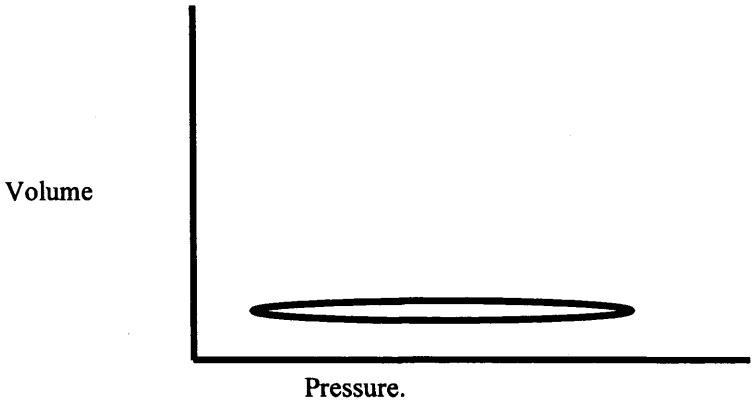
The femoral arterial access approach is familiar to most cardio-thoracic and vascular surgeons but is anatomically less suitable than the carotid as the femoral is an end artery, if it is ligated a distal perfusion cannula is mandatory, the same is true of the femoral vein. This problem can be circumvented by cannulating the artery via a purse string and the vein either via the saphenous bulb, or using a semi-Seldinger technique (Peek et al. 1996a; Peek et al. 1996b). Another useful approach is to insert the venous drainage cannula using the standard percutaneous technique at a different site from the arterial cannula, i.e. venous in the right jugular or right femoral vein and arterial in the left carotid or left femoral artery. Entirely percutaneous access can be used on the femoral

artery, but if done emergently can have an unacceptable level of complications, with 42% of 389 patients sustaining complications in one series (Moreno-Cabral et al. 1994), and in any event the artery must be exposed at decannulation in order to repair it. Percutaneous access to the carotid should not be attempted because of the risk of embolism.

When initiating ECMO flow with VA cannulation full flow (100 ml/kg/min) can be established much more rapidly than with VV ECMO because VA perfusion provides circulatory support. However, with both VV and VA ECMO care should be taken not to immediately correct hypercarbia as sever cerebral vaso-constriction will result (Walker et al. 1994), it is preferable to correct the $PaCO_2$ over several hours.

ADVANTAGES AND DISADVANTAGES OF VV & VA ECMO

Advantages of VA ECMO include "instant" haemodynamic stability with the ability to provide circulatory as well as respiratory support, the absence of recirculation is another advantage. Disadvantages of VA ECMO may be considered as those related to the cannulation which have been already discussed above, and the physiology. When the majority of the venous return is drained from the patient into the pump the right atrial pressure is reduced, and therefore also the right ventricular preload , pulmonary blood flow and right ventricular afterload. Because the pulmonary blood flow is reduced so also is the return to the left atrium, and therefore the left ventricular preload is low. However the arterial cannula is pumping blood back into the aortic arch at systemic arterial pressure, and therefore the left ventricular afterload is high. If almost all of the patients venous return is being drained then they are effectively on full bypass and the left ventricle will not eject and the arterial pressure waveform will be non-pulsatile. However, if the left ventricle is healthy it will continue to contract with iso-volumetric pressure loops being generated:



Iso-Volumetric Left Ventricular Contractions

Gradually the left ventricle will fill from the small amount of pulmonary venous return, and veni-cordi minimi, until it has sufficient end diastolic volume to eject against the raised after-load imposed by the pump. Problems may arise, however, if left ventricular contraction is impaired i.e. by myocarditis or coronary artery disease as the heart will not be able to eject against the raised left ventricular afterload, and then the left ventricle will become distended with blood causing further damage. This can be prevented either by venting, or by reducing LV afterload by balloon counterpulsation.

Much has been written about "myocardial stun" on VA ECMO (Holley et al. 1994; Martin et al. 1991b; Martin et al. 1991a; Martin and Short, 1988), and it remains a controversial area. It can be defined as a reversible impairment of left ventricular function related to VA ECMO. There is no doubt that stun occasionally occurs and a combination of high afterload, distension and myocardial hypoxia is probably to blame. To make a diagnosis of myocardial stun one must demonstrate impaired myocardial function, and it is not possible to do this when the patient is on full bypass as echocardiographic criteria for reduced circumferential shortening cannot be applied to a ventricle which is contracting iso-volumetrically . We have performed serial echocardiograms on infants during weaning from VA ECMO and demonstrated what appears to be a poorly contracting ventricle whilst

on full flow, but 5 minutes later on minimal or no flow, contracts normally. Thus care must be taken to interpret echocardiograms in light of the pump flow when on VA ECMO, as the heart may just be responding to abnormal loading conditions. This is equally true of shunting which may dramatically reverse when pump flow is reduced. Therefore echocardiograms to assess LV function and presence and direction of shunting should be performed with the VA ECMO flow as low as possible, or with the patient clamped off if they have recovered sufficiently.

Myocardial hypoxia was mentioned as a potential cause of stun, this may seem paradoxical as oxygenated blood is being pumped into the aorta, but experiments with micro-spheres (Kinsella et al. 1992) show that blood entering the coronary ostia whilst on VA ECMO comes largely from the left ventricle. If there is little underlying lung function the left ventricular blood will be poorly oxygenated, and the ventricle becomes hypoxic (Murata et al. 1996), increasing pump blood flow until all the venous return is captured and the left ventricle is no longer ejecting will result in pump blood entering the coronaries, but this may have adverse effects in terms of reduced pulmonary blood flow, or if there is a large ductus, increased left to right shunting and pulmonary oedema. Low pulmonary blood flow is also thought to be responsible for the pulmonary infarcts and fibrosis seen in the post mortem material in the NIH ECMO study (Zapol et al. 1979).

The presence of a large ductus arteriosus may cause problems on VA ECMO once the patient starts to recover. As the pulmonary vascular resistance falls shunting across the ductus will become left to right and if it is large can result in very high pulmonary blood flow and pulmonary oedema. This is an indication for ligation of the ductus on ECMO.

In spite of all these problems VA ECMO remains the method of choice for inexperienced users and unstable patients particularly if circulatory support is required, and is especially good for right ventricular impairment.

Veno-venous ECMO is the mode of choice for respiratory failure. It provides no circulatory support but can nevertheless still be used when there is moderate inotrope requirement (up to approximately 10mcg/kg/min of dopamine or dobutamine). The reason for this is three-fold. Firstly institution of extra-corporeal gas exchange allows the airway pressure to be greatly reduced, thereby removing the tamponade like effect of positive pressure ventilation from the heart and improving its function. Secondly, further improvements in myocardial function occur as the pulmonary vascular resistance (PVR) falls, thereby reducing right ventricular afterload. The reduction in PVR is secondary to the infusion of oxygenated blood into the venous system, oxygen being the physiological pulmonary vasodilator. Thirdly, the left ventricular blood on VV ECMO has a much higher oxygen content than seen on VA ECMO. As the coronaries are supplied largely from left ventricular blood (Smith et al. 1989) myocardial oxygenation is better on VV ECMO than on VA.

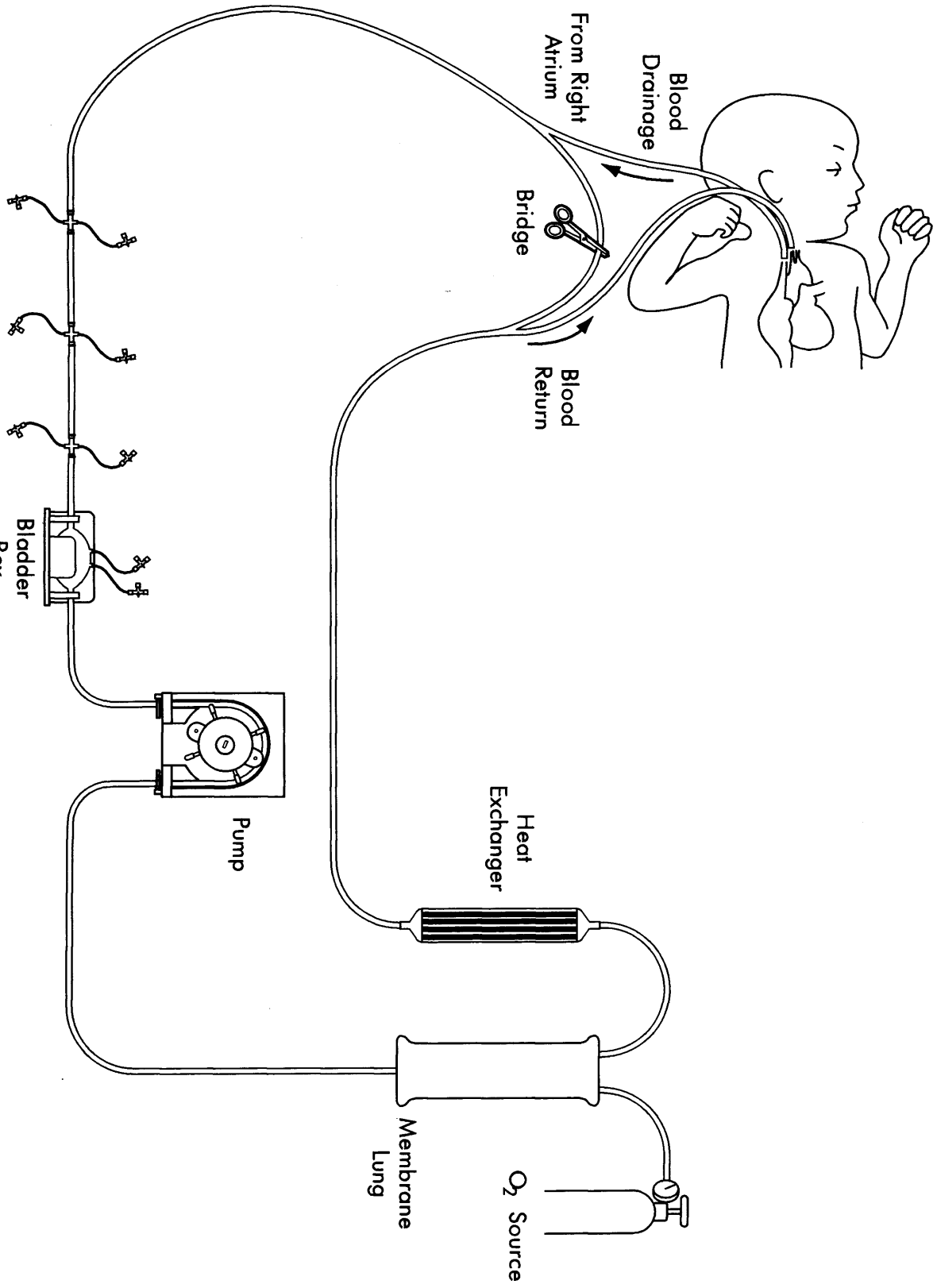
Another important fact about VV ECMO is that pulmonary blood flow is maintained, which is thought to prevent pulmonary fibrosis and infarction (Zapol et al. 1979), also the loading conditions of the heart are un-altered. Other advantages of VV ECMO include percutaneous cannulation in older children and adults, and the preservation of both the Carotid artery and Jugular vein in neonates. Trialing off ECMO is also simpler on VV ECMO (see below). Disadvantages include the lack of cardiac support, the occurrence of re-circulation and positional cannula flow when using the double lumen cannula. These are the main reasons that in neonatal ECMO it is recommended that VA ECMO is used initially until teams are experienced, In paediatric and adult ECMO it would be safe to use VV cannulation from the outset.

ECMO CIRCUIT DESIGN, EQUIPMENT & PRIMING

ECMO circuits vary between institutions but the majority of centres use occlusive roller pumps, usually Stockert, servoregulated with a Seabrook bladder box, Avecor silicone membrane oxygenators and Tygon S-65-HL tubing. Pressure measurement pre and post oxygenator is also essential, but other sensors such as flowmeters, perfusate temperature, haematocrit and oxygen saturation probes are optional. Below are diagrams of our neonatal (Fig. 1.1), and adult (Fig.1.2) ECMO circuits.

The choice of individual circuit components will be discussed individually. Firstly the choice of pump. Roller pumps were the first pumps commercially available for cardio-pulmonary bypass, and therefore they are familiar to most Perfusionists and Cardiothoracic surgeons. They are reliable, and can be run for 500-1000 hours between services. Roller pumps, if properly calibrated and handled, cause an acceptably low level of haemolysis (Moon et al. 1996; Cappelletti et al. 1961). There are two main disadvantages of occlusive roller pumps for ECMO use; tubing wear and their ability to create large negative or positive pressures if improperly used. Tubing wear can result in raceway rupture. There are 152 Raceway ruptures reported in the ELSO registry, with a mortality of 53% . In order to decrease the risk of raceway rupture 44% of centres "walk" their raceways, that is periodically move an unworn portion of tubing into the pump boot. This procedure is not without risk as the pump must be turned off to walk the raceway, and the patient may become hypoxic and hypotensive during this period. It is possible to walk the raceway with the pump still running, albeit at reduced speed, tubing being slowly inched forward, or withdrawn against the direction of rotation. The former method is difficult to perform safely, and may result in raceway rupture during water lab practice. Duplex circuits with two pumps are used in some centres (Borg et al. 1996) in this way flow can be maintained via one pump whilst the raceway is walked on the other, this is ideal in terms of raceway

Fig. 1.1 Neonatal ECMO Circuit



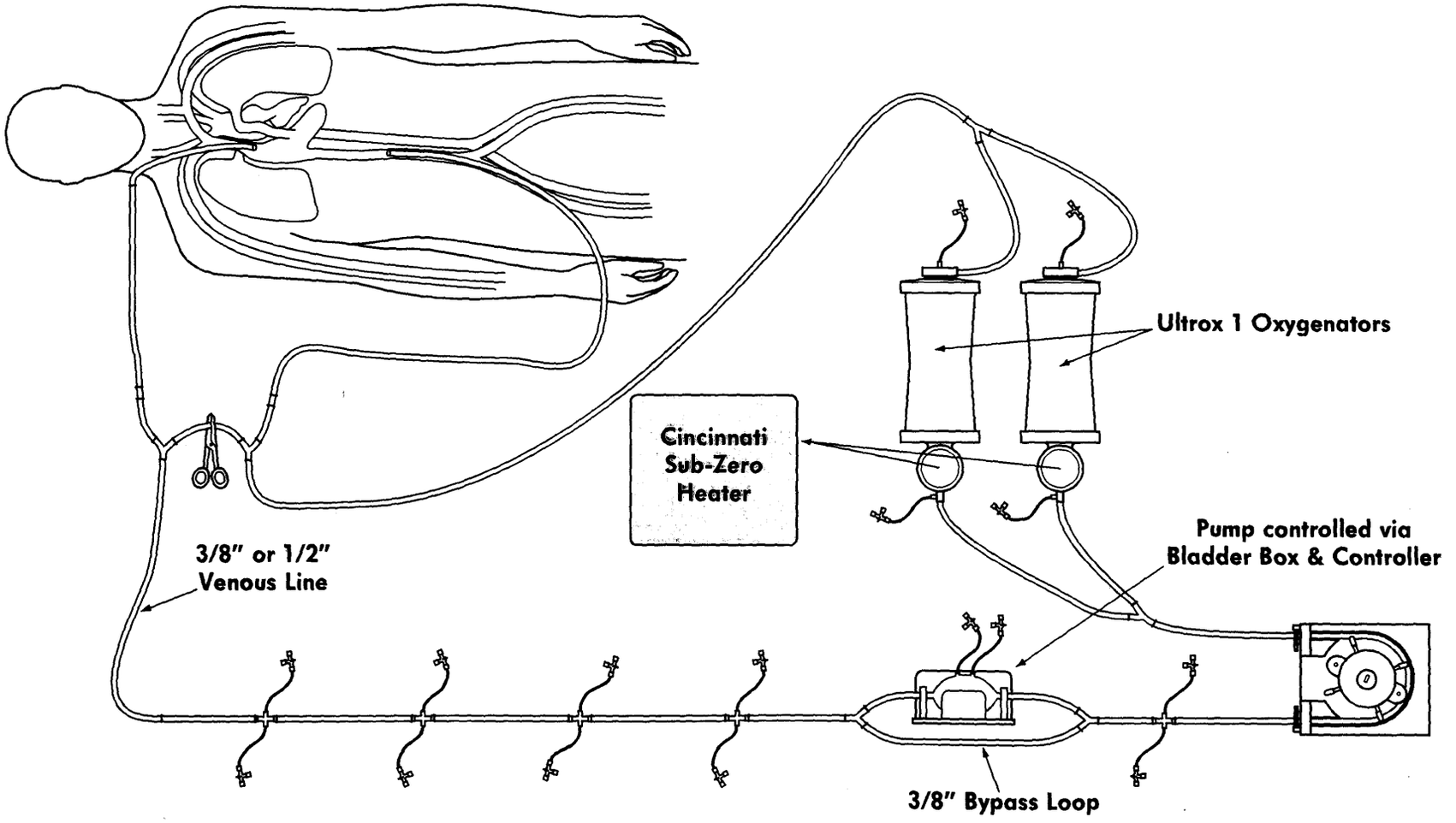


Fig. 1.2 Adult ECMO Circuit

maintenance, but increases the complexity and cost of the circuit.

The only suitable raceway tubing for use in an occlusive roller pump is Tygon S-65-HL (Norton Performance Plastics, Akron, Ohio, USA), although the precise durability of this tubing during an ECMO run is uncertain. Because of recent litigation in the USA there is little enthusiasm for the development of safer materials because of the perceived legal implications. In reality raceway rupture is a rare although dramatic and potentially lethal complication.

Because a roller pump can generate large negative or positive pressures certain safeguards are required during prolonged use. Negative pressure results in cavitation, with gas coming out of solution and forming bubbles, which can result in airlocks, or embolise into the patient. This can be prevented by assuring adequate fluid supply (venous drainage) to the pump at all times. During cardiopulmonary bypass this is achieved with a venous reservoir, the Perfusionist watching the level at all times. A large reservoir is not practicable during ECMO because the level of heparinisation is insufficient to prevent clotting in a large volume of stationary blood. Instead a small (60ml) haemodynamically profiled collapsible silicon bladder is used. The bladder is held against a micro-switch, and thus acts as a sensor of venous drainage. When the venous return is inadequate the bladder empties, trips the micro-switch, triggers a relay in the bladder box and sounds an alarm. The pump turns off, allowing the bladder to fill and preventing the generation of negative pressure and cavitation. The function of the bladder box can be replaced by electronic servoregulation from a pressure sensor in the venous return line, as is available on the Stockert CAPS pump. This is said to be reliable by centres using it. The capacity of the roller pump to produce high positive pressure can literally blow the circuit apart if obstruction occurs. This risk can be reduced by the measurement of pre and post

oxygenator pressures with an audible alarm system, and also by ensuring that all connectors and other circuit components are secured with double cable-ties.

Other pumps that can be used for ECMO include the French non-occlusive tidal flow Rhone-Poulenc / AREC system which is only suitable for neonates and is discussed in detail elsewhere (Chevalier et al. 1993; Durandy et al. 1990)), and the Biomedicus centrifugal pump. Advantages of centrifugal pumps include the fact that they cannot pump large volumes of air, although gas bubbles are still a risk, or generate large positive pressures, and therefore do not require servoregulation and such close supervision as a roller pump. Disadvantages include the need to change the rotor periodically, as they are only licensed by the FDA for 24 hours. Despite this many centres routinely use them for up to 4 days (Medtronic, Technical Support). Another disadvantage is higher haemolysis than a roller pump during ECMO use. The haemolysis is a result of the capacity of centrifugal pumps to generate high negative pressures and shear stress rates if the venous drainage is impaired, therefore the inlet pressures should be monitored (Pedersen et al. 1997). Centrifugal pumps are suitable for use during ECMO transport because of their compactness and simplicity during short term use. Non-occlusive roller pumps will soon be commercially available for ECMO and bypass use. These have specially contoured raceways and rollers which minimise shear stress, resulting in a low index of haemolysis. They are also inherently safe, being unable to pump air, or generate large positive or negative pressures.

The majority of centres use solid, spiral wound silicone membrane lungs (oxygenators) (Avecor). These are available in a range of sizes from 0.8 SqM for neonates up to 3.5 SqM for adults, the larger oxygenators have integral heat exchangers, but the smaller devices need a separate heat exchanger to maintain normothermia. Heat exchangers should be stainless steel rather than aluminium, as aluminium corrodes when in prolonged

contact with oxygenated blood. Maintenance of normothermia is essential if good results are to be achieved. To determine the size of oxygenator for a given patient the rated flow is used. This is the volume of blood at venous saturation (65%) that can be raised to 100% saturation by the oxygenator per minute. The rated flow of an oxygenator should be higher than the maximum anticipated flow for a given patient (~120 ml/kg/min). We use two oxygenators in parallel for our adult patients to provide adequate capacity for oxygenation. Oxygenators are arranged in parallel rather than in series to maximise oxygenation, as this provides the maximum gradient of oxygen tensions in the blood in a counter-current system.

Because transfer of carbon-dioxide across the oxygenator is diffusion limited rather than flow limited like oxygen (Bartlett and Cilley, 1993) maximum carbon-dioxide transfer can be achieved by arranging two oxygenators in series, for this reason low blood flow $ECCO_2R$ circuits have the oxygenators in series (Gattinoni et al. 1986), however this configuration has limited oxygen transfer capability and is not the best design for patients with little or no lung function, or for patients requiring high extracorporeal blood flow.

Micro-porous oxygenators are in wide use for cardio-pulmonary bypass, they are made from polypropylene, and are more efficient than solid silicone oxygenators, having a higher rated flow per unit of surface area, thus priming volumes and resistance to blood flow are lower. The difference in material allows heparin bonding, as silicone is a difficult material to bond. Unfortunately the only available heparin bonded microporous oxygenator for ECMO use (Maxima, Medtronic) develops a severe plasma leakage limiting its operating life to between one and five days (Rossaint et al. 1992) . This is expensive in time and oxygenators and also may be stressful for the patient depending on the circuit design. A silicone oxygenator will usually function for several weeks without a problem, and so microporous technology needs to improve to this level before it becomes

more widely adopted. Oxygenator function is checked twice daily by means of a blood gas sample taken from the post oxygenator tubing, acceptable function is indicated by a pO_2 > 50 KPa. The development of a steadily increasing trans-oxygenator pressure gradient, haemolysis or thrombocytopenia may also indicate a failing oxygenator.

Circuits are first flushed with Carbon Dioxide to displace air, this makes the fluid priming of the circuit easier as CO_2 bubbles will dissolve better than the nitrogen in air. Circuits are then washed with 20% Human Albumin Solution to coat the tubing to achieve some degree of passivation (Tsai et al. 1988). Following this a balanced electrolyte solution such as Plasmalyte-A (Baxter, Irvine, California, USA) is used to fill the circuit completely, all gas bubbles are removed. Blood is then added to the circuit, displacing the clear prime: one unit for a neonatal circuit, and two units for paediatric and adult patients. Either whole blood, packed cells or SAG-M re-suspended cells may be used. Following addition of blood the sweep gas is started and a sample is sent for blood gas analysis and biochemistry. The sweep gas should then be adjusted to ensure a normal PCO_2. The potassium concentration in the prime may be grossly elevated, if this is the case calcium 1-2 mMol and sodium bicarbonate 1-2 mMol or THAM should be added to the circuit (doses given for neonatal circuit). Other centres add FFP and platelets to their prime in addition to red cells.

MECHANICAL COMPLICATIONS OF ECMO

It will be clear from the previous discussion that ECMO is a technically involved and complex treatment. In many systems it seems to be self evident that the simpler the system, the lower the potential for complications. ECMO circuits and management protocols are designed to be as simple as possible, but the inherent characteristics of the circuit design and diseases being treated means that mechanical complications are almost

inevitable. If the ECMO specialist at the bedside is properly trained the majority of complications can be either prevented, or dealt with immediately with minimal risk to the patient. If, however the person at the bedside does not have these skills, then complications often result in the patient's demise. The mechanical complications given below are taken from the ELSO registry for the period up to December 1996. Raceway rupture and its prevention will then be discussed in more detail.

Mechanical Complications of ECMO by Age group up to end of 1996

(ELSO Registry)

	Neonatal	(11921 patients)	Paediatric	(1124 patients)	Adult	(355 patients)
	No. Reported	% Survived	No. Reported	% Survived	No. Reported	% Survived
Oxygenator Failure	620	64%	190	75%	73	41%
Raceway/ Tubing Rupture	147	73%	75	45%	20	40%
Pump Malfunction	199	77%	42	43%	15	40%
Heat Exch. Malfunction	129	72%	10	30%	6	50%
Cannula Problems	1419	75%	176	41%	44	39%
Other	5656	74%	309	52%	91	43%

RACEWAY MANAGEMENT PROTOCOLS IN ELSO REGISTERED CENTRES

28/123 (23%)	Tubing walked forward with pump off		
20/123 (16%)	Tubing walked forwards with pump on		
6/123 (5%)	Tubing walked backwards with pump on		
26/123 (21%)	not walked		
43/123 (35%)	walked at specific times:	n	time (days)
		4	0.5-1
		1	1-2
		1	2-3
		5	3-4
		7	4-5
		10	5-7
		14	>7
		20	when looks worn.

GLENFIELD HOSPITAL RACEWAY PROTOCOL

age	rpm	walk at
neonatal		5 days.
Adult/paed	< 80	5 days
	80-90	3 days
	90-100	2 days
	>100	1 day

Raceway rupture is rare, but carries a significant mortality, and is therefore one of the most feared mechanical complications of ECMO. Seventy nine percent of centres registered with ELSO "walk" their raceways to try and reduce the risk of rupture. Raceway management protocols from the 123 ELSO registered centres and Glenfield hospital are given in the tables above.

At Glenfield the raceway is walked by placing the patient on full ventilation on 100% O2 or hand bagging, venous access is flushed and arrest drugs such as adrenaline and atropine are drawn up. The patient is then clamped off from the circuit, and the pump is switched off. The raceway tubing is then removed from the pump boot and advanced to place fresh tubing in the pump. The pump is then restarted and flow is re-established to the patient. This manoeuvre can usually be accomplished in 15 to 45 seconds. There is only enough tubing in the circuit to allow three raceway walks, and after this a new piece must be cut in, this obviously takes longer.

The frequency of raceway walking is directly proportional to the pump speed which in turn is an index of the amount of extracorporeal support that is required. The corollary of these facts is that it is the most unstable patients who can least tolerate the momentary withdrawal of extracorporeal support who must have the most frequent raceway maintenance. Often these unstable patients develop severe hypotension, hypoxia and even cardiac arrest during raceway maintenance. This is especially true when a new piece of tubing is cut in as this usually takes a minimum of 1.5 to 2 minutes off ECMO.

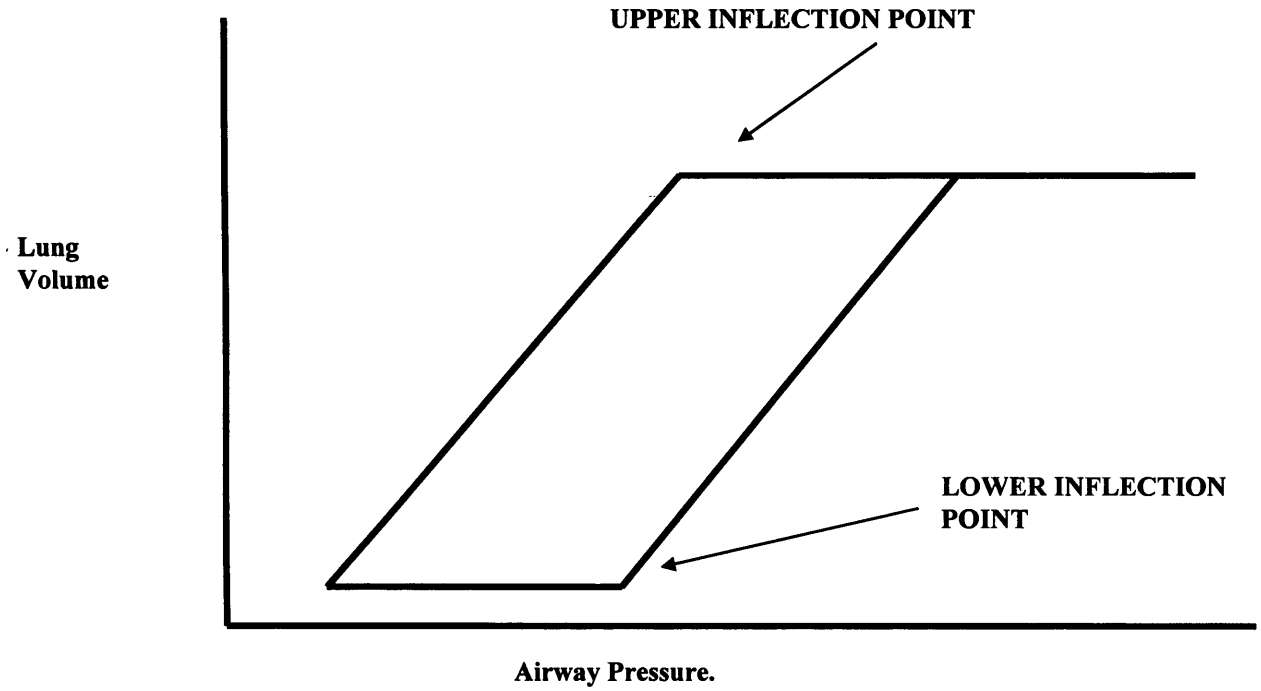
More detailed information on raceway durability, or the advent of a more durable tubing would greatly reduce these problems.

OXYGEN DELIVERY, VENTILATION & LUNG MANAGEMENT ON ECMO

As discussed above one of the central tenants of successful respiratory ECMO is the ability to maintain normal blood gases whilst resting the lungs, to prevent further barotrauma. This principal may be equally applied to patients receiving cardiac ECMO, as they often have respiratory impairment as a result of pulmonary oedema. We use similar ventilator settings to achieve this in all our patients. Firstly, as soon as extra-corporeal flow is established the positive end expiratory pressure (PEEP) is increased to 10cmH2O, this has been shown to prevent alveolar collapse and result in faster weaning from ECMO than lower levels of PEEP (Keszler et al. 1989)), occasionally patients with very stiff lungs will require higher levels of PEEP to achieve alveolar recruitment and prevent collapse, this will be apparent from the chest X-ray and lung mechanics.

The level of PEEP should be just above the lower inflection point (Papadakos and Apostolakos, 1996; Roupie et al. 1995; Mancebo et al. 1994; Gattinoni et al. 1993). Whilst considering PEEP it is worthwhile to discuss the management of patients with severe barotrauma and air leaks as evidenced by the presence of pneumothorax, pneumomediastinum, pneumopericardium, surgical emphysema and pulmonary interstitial emphysema. These patients are best managed on CPAP (continuous positive airway pressure) of approximately 10 cmH2O until 24 hours after the air leak has stopped, during this period the patients should not be hand ventilated, as this risks causing further air leaks. The peak inspiratory pressure (PIP) is quickly reduced to 20 cmH_2O, this is easy to achieve with pressure control ventilators, but when using volume control may take some

adjustment of the tidal volume until a satisfactory PIP is obtained. The respiratory rate is set at 10 breaths per minute, and the FIO_2 is reduced down to 30%.



Relationship of Lung volume to airway pressure.

How are blood gas tensions controlled whilst on ECMO? It is possible to alter the oxygen concentration of the gas flowing through the membrane lung (sweep gas), and this could be used to alter the amount of oxygenation provided. However, nitrogen (i.e. air) is much more dangerous that oxygen in terms of gas embolism as it takes much longer to dissolve, whilst gas embolism is rare occurring in 4% of neonatal ECMO patients with 67% survival (January 1st 1997 ELSO Registry Data) we use 100% oxygen as the sweep gas or rarely Carbogen (95% O2, 5% CO2) to reduce the risk from potential gas embolism. Therefore the only way to alter the amount of oxygenation is to alter the amount of oxygenated blood returned to the patient (since it is all 100% saturated), and this is done by

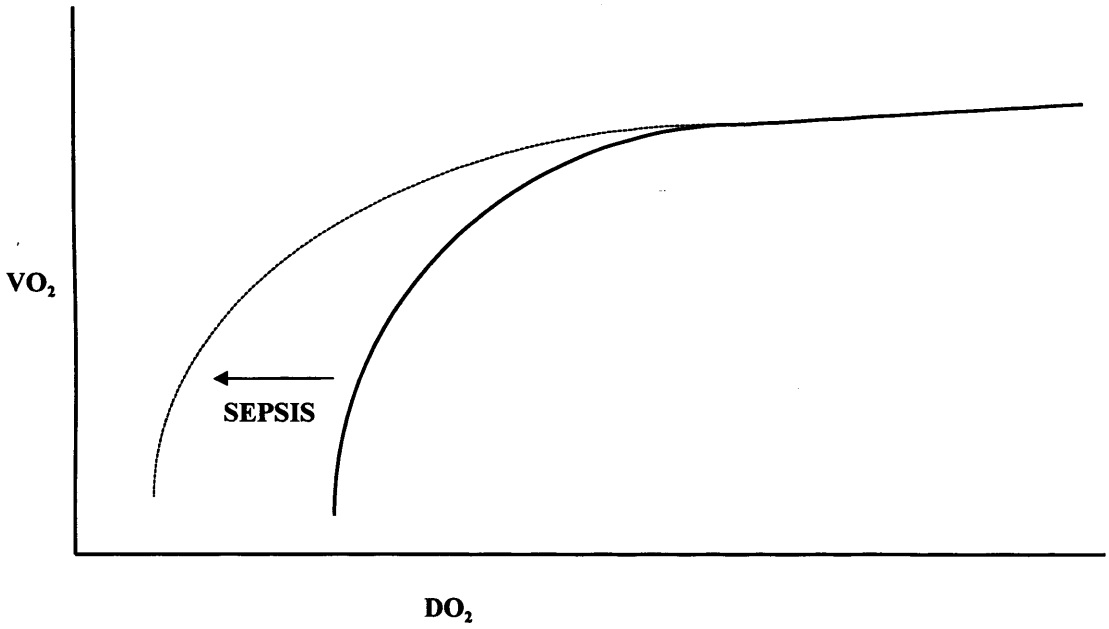
adjusting the pump flow rate. Full flow, i.e. enough blood flow to fully support gas exchange is usually 100ml/kg/min for VA ECMO and 120ml/kg/min for VV ECMO. The flow for VV ECMO is higher because of recirculation. These values may be higher in septic or pyrexial patients. The amount of CO_2 removal can be adjusted by altering the rate of sweep gas flow (cf. Tidal volume during IPPV), the sweep can safely be increased up to double the blood flow through the oxygenator. Higher levels of sweep are sometimes necessary, especially in septic patients, but carry an increased risk of gas embolism.

Different parameters of adequate oxygen delivery are used during VV and VA ECMO for determining the pump flow and sweep gas flow, this is because of recirculation during VV ECMO. The most easily applied measure of adequate global tissue oxygen delivery is the mixed venous oxygen saturation (MVO_2) (Cornish et al. 1989). The MVO_2 allows us to follow the relationship between oxygen delivery (DO_2) and oxygen use (VO_2) without any more equipment than a syringe and a blood gas machine.

During normal physiology we operate in the upper flat part of the curve such that oxygen uptake is independent of oxygen delivery, as oxygen delivery is reduced, by hypoxia, or reduced cardiac output we move down into the lower part of the curve where oxygen uptake is proportional to oxygen delivery i.e. we have become "Supply dependant" in sepsis the curve may become shifted to the left so that supply dependence occurs at high rates of oxygen delivery (Yu et al. 1996; Rady et al. 1996).

We can show that MVO_2 is directly proportional to DO_2 and this value alone can be used to follow oxygen delivery. For practical purposes a mixed venous saturation of >65% represents adequate oxygen delivery. Strictly speaking a mixed venous sample should be taken from the pulmonary artery, but a sample from the right atrium gives a close approximation (Hirschl et al. 1993), thus samples from the venous line can be used to measure the MVO_2 during VA ECMO. However, the presence of re-circulation makes this

unreliable during VV ECMO. If the MVO_2 is < 65% this indicates that DO_2 is insufficient, going back to first principals we can see that there are two parameters that we can alter to improve DO_2, one is cardiac output and the other is the oxygen content of the blood.



Relationship of Oxygen delivery (DO_2) and Oxygen utilisation (VO_2).

The total "cardiac" output of a patient on VA ECMO is the native cardiac output plus the pump flow, thus we can improve DO_2 by turning up the pump flow. The oxygen content of the blood is determined by the following expression:

O_2 Content of 100ml of blood = 1.36ml x Hb (g/dL) x SaO2 (%)

Since the blood is 100% saturated when it leaves the circuit, we must increase the haemoglobin concentration to increase the oxygen content, we transfuse our patients up to a haemoglobin of 14 g/dl to improve oxygen delivery, and do not encounter problems due to hyperviscocity either in the extracorporeal circuit or the patients circulation at this

haemoglobin concentration. It is also possible to use the venous blood gas to determine CO_2 clearance, and an acceptable $PvCO_2$ is usually 2 KPa worse than one would accept for an arterial sample, thus if you want a $PaCO_2$ of 6 KPa then the $PvCO_2$ should be 8 KPa. Unfortunately it is not possible to use the mixed venous oxygen saturation as a measure of DO_2 during VV ECMO because of the presence of re-circulated oxygenated blood in the venous circulation. Therefore arterial blood gases are used to adjust the gas exchange during VV ECMO.

FLUID BALANCE DURING ECMO

It has long been known that pulmonary oedema reduces pulmonary gas exchange, and by analogy that fluid restriction improves both gas exchange and outcome in a number of lung diseases (Takeda et al. 1995; Barie, 1995; Hachenberg et al. 1994), the pulmonary oedema which accumulates in the cardiac ECMO candidate as a result of cardiac failure, high pulmonary blood flow and capillary leak syndrome is no less problematical. Many patients referred for ECMO have received large volumes of colloid and crystalloid infusion during resuscitation, and may be clinically oedematous. This is compounded by the development of a capillary leak syndrome (Takeda et al. 1995) and a low serum albumin concentration due to illness, catabolism and haemodilution. Diuresis to dry weight is therefore an important part of the lung management. If a weigh bed is not being used, or the patients normal weight is unknown then clinical parameters are used to gauge the amount of diuresis. We usually aim for a negative fluid balance that will remove the total amount of positive fluid balance from the referring hospital over 24-48 hours, if this is not known then diuresis is continued until the CVP or PAWP is less than 10 mmHg, the liver is of normal size, the anterior fontanelle (if present) is neutral / sunken and the serum urea and creatinine are at (or slightly above) the upper range of normal. To achieve this patients

are fluid restricted to 60-80 ml/kg/day for neonates and approx. 1 ml/kg/hour for other patients on the first day of admission, and frusemide is given by bolus and infusion. The dose of frusemide may be higher than usually required as it is extensively bound (63-87%) to the ECMO circuit (Scala et al. 1996), but a bolus dose of up to 5mg/kg (max. 250mg) will usually suffice. Frusemide will often not work if the patient is anuric as it acts from within the lumen of the loop of Henle (Wittner et al. 1991) and in this setting a combination of Mannitol 0.25 - 0.5 g/kg and Aminophylline 5-10mg/kg (max. 250mg) can be used to initiate a diuresis, if necessary the Aminophylline can be continued by infusion at a rate of 10 mcg/kg/min, with monitoring of serum levels (Huet et al. 1995; Lochan et al. 1997)). We believe that the maintenance of a normal serum albumin concentration is also important for the resolution of oedema and the reversal of capillary leak (Greenough et al. 1993). This is controversial as some authors believe that albumin given intra-venously will just leak out into the tissues where it will become fixed and then hold water in the tissues osmotically (M Singer, Personal communication). We have not been aware of this problem clinically, but the diuretic strategy in our unit may account for this absence of interstitial oedema. During the course of this forced diuresis patients become intra-vascularly empty whilst peripheral oedema still remains, this problem is easily dealt with as it is usually also necessary to give several units of blood products. Whilst this volume must be subsequently removed it can be used to correct the immediate hypotension whilst the rate of diuresis is reduced slightly. When discussing fluid balance for patients on ECMO it is important to focus on the patients primary problem, namely they are usually dying of pulmonary insufficiency. Correction of this problem is therefore the most important task, the lungs will not recover whilst they remain oedematous. It is occasionally necessary, therefore, to continue to diurese a patient who is manifestly dehydrated in order to salvage the patient. This may result in the development of pre-renal failure requiring

haemofiltration whilst on ECMO, this usually recovers 4-6 weeks later. None of our patients have required long term renal replacement therapy thus far.

Patients who are referred in excellent fluid balance can receive more fluid, but it is still sensible to keep them slightly fluid restricted and in a zero balance, so 100 ml/kg/day for a neonate and 1.5 ml/kg/hr is usually appropriate for these patients. Even patients who are not volume overloaded may need large doses of frusemide to maintain a zero fluid balance when the volume of blood and platelet transfusions that are given in the first few hours to achieve the necessary haematological parameters are taken into account.

If haemofiltration is required whilst on ECMO the haemofiltration circuit can be attached to the ECMO circuit thus obviating the risk of dialysis catheter insertion in these heparinised patients. If only removal of volume is required a passive haemofiltration circuit can be constructed using the pressure generated by the ECMO pump (Heulitt and Pickert, 1995), but this will provide little correction of biochemical parameters such as acidosis, uraemia or hyperkalaemia. If using this type of haemofiltration the pump flow must be increased to account for the effective shunt of blood flowing through the filter, unless flowmeters are being used this requires a considerable degree of expertise to manage. We therefore use a conventional Gambro (Lund, Sweden) pumped continuous veno-venous haemofiltration (CVVH) unit which is connected to the venous side of the ECMO circuit before the bladder, the inclusion of haemofiltration fluid in this system allows the correction of the biochemical abnormalities. The regime used is the conventional CVVH regime used in our hospital, which further simplifies the patients management.

ANTI-COAGULATION & HAEMATOLOGY DURING ECMO

Many groups are currently trying to reduce the degree of surface activation occurring in extra-corporeal circuits in order to make heparinisation unnecessary during ECMO (Palmer et al. 1995; Perchinsky et al. 1995; Kawahito et al. 1994; Lazzara et al. 1993). Unfortunately even the best commercially available heparin coated ECMO circuit still requires significant quantities of heparin administration to the patient and frequent changes of oxygenator (Keh et al. 1995; Rossaint et al. 1992), therefore we have some time yet to wait before heparin free ECMO becomes a reality. Bleeding is an ever present risk during ECMO, especially during cardiac ECMO with a recent sternotomy and possible trans-thoracic cannulation, and every measure is taken to prevent it. This starts with patient selection, and continues with careful, extra-thoracic if possible, cannulation technique. Avoidance of invasive procedures such as intra-muscular injections and central line insertion during ECMO is also crucial in preventing bleeding. Equally, indwelling central lines, umbilical artery catheters, chest drains etc. that are present when the patient is cannulated for ECMO are left in situ during the ECMO run, as severe haemorrhage may accompany their removal. The level of heparinisation during ECMO is much lower than during cardio-pulmonary bypass (CPB) in order to further decrease the risk of bleeding. An activated clotting time (ACT) of 200-250 is adequate for cannulation, and can usually be achieved with a bolus heparin dose of 50 u/Kg. Whilst on ECMO the ACT is kept between 180-200 seconds with hourly or half hourly adjustment of heparin infusion rate, 30-60 u/kg/hr being the normal range. If the patient is at risk of bleeding or requires surgery the ACT can be reduced to 160-180 seconds, but some clots will begin to form in the circuit at these levels, and therefore 160 seconds must be considered the safe lower limit. A number of factors may result in fluctuations in the ACTs: The most common are urine output, haemofiltration, platelet transfusion and Antithrombin III (AT III) levels. Heparin is

cleared in the urine, and therefore the dose requirement will vary according to the urine output (Short, 1993a). The dose of heparin will also have to be increased following a platelet transfusion (Short, 1993a). Since heparin acts by increasing the natural anticoagulant effect of Antithrombin III there must be sufficient AT III in the circulation for heparin to be effective. Some centres measure AT III levels routinely (Glauber et al. 1995) , but usually this is not necessary. ECMO circuits are designed to eliminate areas of stasis, for this reason the venous reservoir of the CPB circuit is reduced to a small (60ml) haemodynamically profiled silicon bladder. This design ensures blood is flowing at all times and that lower levels of heparinisation do not cause undue clotting in the circuit.

There must be close co-operation between the ECMO programme and the Blood Transfusion Service as large amounts of blood products may be required. The bleeding encountered in previous reported studies of adult ECMO (Morris et al. 1994; Zapol et al. 1979; Gattinoni et al. 1986) can be almost eliminated by tight heparin control and modified cannulation technique, this reduces the need for red cell transfusion unless surgery is required. However some red cell transfusion is necessary, since one to two units of whole blood or packed red cells will be required to prime the circuit, and then several units may be required to obtain the required haemoglobin of 14 g/dl. Once this target has been achieved red cell transfusion requirements should be minimal. When calculating the red cell requirement the corrected weight should be used for neonatal and paediatric patients because of the relatively large size of the circuit compared to their circulating volume. The corrected weight is an artificial concept used to aid transfusion calculation at the bedside, and is only used for calculating red cell requirements. Corrected weight is the weight the patient would be if it had a blood volume equal to the patients blood volume plus the circuit volume:

$$\text{Corrected Weight} = \text{Patients Weight} + \frac{(\text{Circuit Volume in ml})}{80}$$

The volume of our neonatal circuit is 500ml and our paediatric circuit 1000ml, so the corrected weight for a neonate is usually 8.25-10kg, this weight can then be used in conventional formulae to calculate the red cell requirement, i.e. 4ml/kg packed cells x desired rise in Hb (g/dl) (Shann et al. 1994).

When the extracorporeal circuit is exposed to the patients blood for the first time a wide range of plasma proteins and cells adhere to the surface and become activated (Plotz et al. 1993), pre-washing the circuit with 20% human albumin solution decreases this tendency, acting to passivate the circuit to some degree (Tsai et al. 1988), although significant adsorption and activation of fibrinogen and platelets still occurs. Serum albumin is also adsorbed. This process occurs in a dynamic manner for 2-3 days until an equilibrium is reached, at which point little further net adsorption or activation takes place. The clinical course of this phenomenon can be followed from the requirement for exogenous platelets and blood products and the radiological appearance. Chest films usually deteriorate for the first one or two days on ECMO with complete white out, which gradually clears. It may be necessary to give some cryoprecipitate to keep the fibrinogen concentration > 1 g/L and Fresh Frozen Plasma to keep the prothrombin time in the normal range. The thrombin time will be prolonged by the presence of heparin, but this does not follow a linear correlation with the ACT. This cycle of surface activation may be repeated to a lesser degree if a new oxygenator has to be inserted.

We aim to keep our patients platelet counts over 100,000/ml, or over 150,000/ml if they are bleeding, this is especially important in the neonate in order to reduce the risk of intra-cranial haemorrhage (Anonymous. 1996). This target may seem relatively high when compared to the level at which haemorrhage occurs in haematological patients, around 50,000/ml (Williford et al. 1989). However it may be justified both empirically (Stallion et

al. 1994) , and also by the presence of a functional platelet deficit as a result of the extra-corporeal circuit (Urlesberger et al. 1996; Hennessy, Jr. et al. 1977)). Other factors may also contribute to thrombocytopenia apart from activation by the circuit, such as Heparin Associated Thrombocytopenia (HAT), anti-platelet antibodies and disseminated intra-vascular coagulation. HAT is a rare but well recognised complication of continuous heparin administration thought to occur in 1/2000 patients (Magnani, 1993). If HAT is confirmed then the heparinoid Org10172 (Magnani, 1993; Greinacher et al. 1993b; Kikta et al. 1993; Greinacher et al. 1993a) can be used instead of heparin to anti-coagulate the patient. The exposure of ECMO patients to platelet transfusions from multiple donors increases the chances of them developing anti-platelet anti-bodies (Kurz et al. 1996; Koksch et al. 1995), these anti-bodies are measured in the regional transfusion laboratory, and then HLA matched platelets prepared for transfusion instead of unmatched platelets . The sudden development of thrombocytopenia several days into an ECMO run in a patient who has been previously stable may be the first herald of sepsis with the development of DIC, an increased CO_2 production may also precede the classical laboratory indices of leucocytosis and raised C-Reactive protein. In this situation a septic focus should be sought and drained if possible, cultures should be taken and the anti-biotics reviewed. The possibility of fungal infection should not be overlooked in these patients. Equally a DIC like picture may result from a failing oxygenator filling with clot, which is more likely if the pressure gradient is increasing or the post oxygenator gases are deteriorating.

Platelet function may be preserved to some degree by infusion of the serine protease inhibitor Aprotinin. Whilst Aprotinin is extremely useful to control and prevent haemorrhage during ECMO, especially if surgery is required it makes the heparin management very difficult (Glauber et al. 1995; Brunet et al. 1992; Wildevuur et al. 1989). The presence of Aprotinin interferes with the ACT, Thrombin Time and Activated Partial

Thromboplastin Time. Indeed, it has been shown that there is no correlation between anti Xa levels and the ACT during Aprotinin infusion. Another confounding factor is the fact that Aprotinin has a mild anticoagulant activity of its own (Despotis et al. 1995; de Smet et al. 1990; van Oeveren et al. 1987b), despite being effective as an adjunct to haemostasis. We use a loading dose of 1 million units of Aprotinin for adults, followed by an infusion of 300-500,000 units per hour until the bleeding has settled, the manufacturers do not give a recommended dose for children but we have used Aprotinin in neonates and children on numerous occasions with a loading dose of 1000 units (1 ml) per kg followed by an infusion of 1ml/kg/hr. During Aprotinin infusion we keep the ACT 20 seconds longer than the previous parameter, thus if ACTs of 160-180 seconds are required we keep the ACT between 180-200 seconds. This strategy seems to work in practice, and is certainly effective in achieving haemostasis, but after 2-3 days of Aprotinin clots may be quite marked in the bladder, oxygenator and other parts of the circuit. It should always be remembered however that very often bleeding can be stopped by physical means, such as pressure, a purse string suture around a cannulation site, or surgical exploration. Aprotinin is merely an adjunct to this. In patients with trans-thoracic cannulation bleeding usually occurs around the cannulae, often the venous cannula is worse than the arterial, as the atrium moves more than the aorta. Although this can be treated with an additional purse string, it is usually preferable to insert cervical cannulae and remove the atrial and aortic access to achieve haemostasis.

The maintenance of normothermia is an essential part of haemorrhage prevention, all coagulation assays , including the ACT are conducted at 37^0 C and thus have little bearing on the function of the coagulation cascade at lower temperatures. Centres advocating hypothermic cardiac ECMO have yet to document acceptable outcome data.

SEDATION AND THE CENTRAL NERVOUS SYSTEM DURING ECMO.

The vast majority of patients referred for ECMO will have been sedated and paralysed on a ventilator for the entire course of their illness, and therefore only very limited assessment of the CNS will be possible. The subject has been adequately discussed by other authors (Gleason, 1993), and so only a few points will be highlighted here. The pre-ECMO condition of the CNS is an important selection criterion, and no neonate should be considered for ECMO until ultrasound screening for intra-cranial haemorrhage has been carried out. Intra-cranial haemorrhage greater than grade 1 is an absolute contra-indication to ECMO (Taylor et al. 1989; Short, 1993b). Head ultrasound should be repeated daily in neonatal patients, as changes in anti-coagulation parameters may have to be considered if intra-cranial haemorrhage occurs. Brain imaging is not so easy once the fontanelle has closed, but intra-cranial bleeding is not such a common response to critical illness and hypoxia in older paediatric patients.

To maintain sedation during ECMO we use continuous infusions of morphine and midazolam. Other combinations such as fentanyl and diazepam can also be used, but are more expensive, provide little advantage in terms of efficacy and, especially fentanyl often require escalating doses (Leuschen et al. 1993; Arnold et al. 1991; Arnold et al. 1990). Binding of drugs to the circuit can also necessitate higher doses, for example morphine is 36% bound (Dagan et al. 1993). We aim to titrate the level of sedation so that patients are settled but respond to endotracheal suction and move when stimulated. However, if the patient is very unstable, and desaturates on suction or movement then an atracurium infusion is used in addition to the sedation. Isoflurane via the oxygenator may be useful for short periods to cover painful procedures (Atkinson et al. 1994), but should not be used for long term sedation as we have noticed that severe withdrawal reactions occur characterised by jittery movements. Propofol should not be used for sedation during

ECMO, as the lipid vehicle tends to accumulate within the oxygenator and impair its function. In any case propofol is contra-indicated in patients under 3 years of age (Zeneca Pharmaceuticals datasheet).

THE CARDIO-VASCULAR SYSTEM ON ECMO

The specific haemodynamic effects of VA ECMO have already been discussed, other than these problems/advantages there is one other issue peculiar to ECMO, namely hypertension. This occurs in 7% of neonatal ECMO patients (January 1st 1997 ELSO registry figures). Patients on VA ECMO, especially on high flows gradually become hypertensive. This is thought to be a result of the vasomotor centre interpreting the non-pulsatile pressure waveform generated by the pump as hypotension, reflex vasoconstriction therefore occurs secreted resulting in hypertension (Taylor, 1995). Treatment with vasodilators such as glyceryl tri-nitrate, hydralazine or phenoxy-benzamine is usually effective, although high doses may be required.

MICROBIOLOGY, SEPSIS AND ANTI-BIOTICS ON ECMO

Many ECMO patients have a known source of sepsis as their primary diagnosis such as pneumococcal pneumonia or menningococcal septicaemia, in these cases it is easy to decide upon appropriate chemotherapy. In addition to drugs aimed at proven or suspected sepsis we also ensure that our patients have adequate anti-staphylococcal cover as prophylaxis for their ECMO cannulae. This prophylactic cover would usually be either Flucloxacillin and Gentamicin, Vancomycin or an appropriate cephalosporin. A full series of screening cultures are taken on admission and on alternate days thereafter. These cultures have a low yield in percentage terms, but are still important as the majority of positive culture results are clinically significant and act as a guide to therapy. Continuous

involvement of the micro-biology department is helpful to correlate laboratory and clinical information.

An interesting phenomenon is encountered after a patient is decannulated from ECMO that clinical resembles a wave of bacteraemia. Patients often become pyrexial and vasodilated with a leucocytosis. This reaction has proved rapidly fatal on one occasion in our hospital. Giving a dose of vancomycin prior to decannulation usually masks this response, although blood cultures are usually negative. We believe that this response represents bacteraemia from colonisation of the cannulation wound, a shower of bacteria are dispersed when the cannulae are withdrawn. Once the patient has been removed from ECMO we re-site all venous and arterial lines, and send the old ones for culture. We also change the patients antibiotics, unless they are aimed at culture proven infection, or a strong clinical suspicion of a particular microbe.

WEANING FROM ECMO, DE-CANNULATION AND POST ECMO CARE

Respiratory patients are ready to be weaned from ECMO when their lung compliance, and chest X-ray have improved, and when they have good blood gases on rest ventilator settings and minimum flow. Minimum flow is defined as 50 ml/kg min for neonates (with a lower limit of 10 rpm, or 130 ml/min), or 1 l/min for adults. At this point the patient can be trialled off ECMO. The ventilator is adjusted for normal ventilation, with an FIO_2 of 40-60%. If the patient is on VV ECMO all that needs to be done is the sweep gas is taken off the oxygenator, the circuit flow is maintained to prevent stasis and clotting, and all infusions of drugs and heparin can continue into the circuit as before. Arterial blood gases are measured every twenty minutes during the trial off, and once acceptable gas exchange (PaO_2 ~ 8-10 KPa with $FIO_2 < 60\%$, and normal $PaCO_2$) without very high minute volume/respiratory rate or airway pressure (< 30 cmH_2O for a neonate and < 35

cmH_2O for an adult) has been demonstrated for 2 hours the patient can be decannulated. If adequate venous access is not already present then this will have to be established prior to cannula removal. If a central line is essential, by reason of inotrope infusion, for example, then a site where pressure can be used to obtain haemostasis in the event of failure is preferable, such as the femoral or left jugular vein, or a peripherally inserted central venous line may be used. Prior to decannulation patients should be paralysed to reduce the risk of air embolism. Removal of percutaneous cannulae is very simple, a mattress suture is placed around the cannula which is then clamped and withdrawn by an assistant whilst the suture is tied, much like removing a chest drain. Pressure is not required to achieve haemostasis. If the semi-Seldinger technique has been used to insert a neonatal double lumen cannula it can be removed in the same way. Open cannulation with vessel ligation necessitates surgical removal.

Patients are ready to be weaned from VA ECMO when adequate tissue oxygen delivery can be demonstrated on minimum flow. Minimum flow is defined as 30 ml/kg/min (with a lower limit of 10 rpm, or 130 ml/min for neonates). At this point the patient can be trialled off ECMO. The ventilator is adjusted for normal ventilation, with an FIO_2 of 40-60%, and the same pressure limits as given for VV ECMO. Trialling off from VA ECMO is complicated, as the patient must be clamped off from the circuit during the trial off. This is because removal of the sweep gas during VA ECMO even on low flow would create an unacceptably large right to left shunt of de-oxygenated blood, therefore the patient must be isolated from the circuit. Venous access is therefore essential prior to a VA trial off, and all infusions must be changed over from the circuit to the patient. It is also necessary to have a heparin infusion on the patient and the circuit during the trial off to ensure adequate anticoagulation in both circulations. These heparin infusions are usually started at half the rate of the previous circuit heparin infusion, and are adjusted

independently on the basis of ACTs taken from the circuit and the patient. During the VA trial off the patient is clamped off from the circuit near to the cannulae, and the bridge is opened to allow the circuit blood to re-circulate. The sweep gas is removed from the oxygenator. The blood in the cannulae and infusion and drainage lines is static, and therefore will clot eventually even in the presence of heparin. To prevent this the cannulae are unclamped and blood is allowed to flow through every ten minutes. We usually trial patients off for a minimum of 2 hours prior to decannulation. De-cannulation from VA ECMO requires a formal exploration of the cannulation site, with repair or ligation of the target vessels. For the standard jugulo-carotid cannulation it is theoretically preferable to repair the vessels, but this is usually either technically impossible because of the state of the vessels after a long ECMO run, or inadvisable because of suspected bacterial colonisation of the wound. For these reasons we usually ligate both jugular and carotid at the time of decannulation. It is safer to remove the arterial cannula first, as the carotid is thicker walled than the vein, and the tissues are therefore less likely to disintegrate, the cannula is removed by an assistant whilst the vessel is either clamped or occluded with forceps, and two liga-clips are applied. If control of the vessel is lost it may retract back into the chest, and a median sternotomy may be required to gain control. Because the vein is much more friable there is a substantial chance that vessel integrity may be lost on decannulation. If the arterial cannula has already been removed this need not be a major problem, as bleeding can be stopped by pressure with a swab for five minutes, and then the wound can be quickly closed. It is not necessary to perform a sternotomy and ligate the right jugular vein in the chest. Jugular venous disruption has occurred 5 times out of 70 (7%) VA decannulations on neonates and paediatric patients, we have successfully used this technique in all cases without encountering cardiac tamponade from venous bleeding.

The most important principle in deciding when to decannulate is that the level of support required to get off ECMO must be moderate only, cardiac patients should be able to come off ECMO on moderate levels of inotropic support such as <10 mcg/kg/min of dopamine or <0.3 mcg/kg/min of adrenaline, exceptions to this rule occur when there is a pressing need to get off ECMO such as severe intractable coagulopathy with bleeding or suspected colonisation or infection of the circuit. In these circumstances higher levels of support may be accepted to allow weaning from ECMO.

Patients who have been sufficiently ill to warrant ECMO usually develop a marked capillary leak syndrome during their illness. The careful, negative or zero, fluid balance required to combat this in the acute phase must not be relaxed on decannulation, as the patients remain at risk of developing pulmonary oedema and impaired gas exchange for up to a week after weaning from ECMO.

FUTURE DEVELOPMENTS

The main limitation of ECMO is the current need for anticoagulation, this prevents the application of ECMO to neonates less than 2 kilos in weight and complicates the use of ECMO in trauma patients and following surgery. The development of more biocompatible materials for the circuit tubing and oxygenator coupled to non-occlusive blood pumps which cause less cellular damage and activation may result in a true heparin free extracorporeal circuit which is entirely inert causing little, or no inflammatory response in the patient. Such a circuit could be used as an artificial placenta to support premature neonates until their lungs have matured enough to allow ventilation, or be used in conjunction with cell salvage and auto-transfusion and possibly hypothermia in the acute resuscitation of surgical emergencies or patients with severe trauma. Once surgical haemostasis has been obtained patients could be slowly rewarmed over several hours and

then supported in the ITU for as long as necessary. Advances in the field of transplantation (Shumway, 1993; Van Meurs et al. 1994; Crombleholme et al. 1990; Schueler et al. 1992; Bando et al. 1989; Bando et al. 1988b; Starnes et al. 1996; Roberts et al. 1996) could result in a greatly enlarged donor pool, making transplantation a realistic possibility for patients with ARDS, congenital heart disease or congenital diaphragmatic hernia. ECMO would be used to bridge these patients to transplant, and provide intra-operative support. The evolution of the artificial heart, implantable left ventricular assist devices and even implantable cardio-pulmonary prostheses (Copeland et al. 1996; Westaby, 1996; Masters et al. 1996; Pierce et al. 1996; Black, 1995; Szefner, 1995; Pavie et al. 1995; Tatsumi et al. 1991) may make adult cardiac ECMO more viable, since even patients with severe coronary artery disease who are unable to wean from bypass could be supported with ECMO, and offered an implantable mechanical device if they failed to improve on ECMO.

CONCLUSION

The efficacy of ECMO for severe neonatal respiratory failure is now proven beyond a reasonable doubt, but there is still widespread scepticism amongst many intensivists about the role of ECMO in children and adults. Despite these misgivings many units around the world continue to offer this service and continue to document acceptable survival figures in a group of moribund patients. It seems likely that a randomised trial or a case control study will eventually determine the place of adult and paediatric respiratory ECMO in intensive care. However, until that time it seems reasonable to continue to offer extra-corporeal support to moribund patients with potentially reversible disease if conventional treatment is failing. It seems unlikely that it will ever be possible to construct an adequately controlled trial of ECMO for cardiac support due to the small numbers and

the rapidity with which decisions must be made when caring for these critically ill patients. Since the survival with conventional treatment of patients in post cardiotomy cardiogenic shock is so appalling it seems reasonable to offer a treatment with a better than 50 % chance of success. Equally the heterogeneity of diagnoses and smaller numbers of adult and paediatric respiratory ECMO patients make a randomised trial difficult, although use of historical controls and cohort studies may allow some guarded conclusions to be reached about the use of ECMO in these patients. It is usually preferable to discuss the patient with an ELSO registered ECMO capable unit as the outcome is likely to be improved, than when ECMO is provided by inexperienced users. Mobile ECMO units are now available which means that almost any patient is capable of being transfered, even if they are unable to wean from cardiopulmonary bypass. If intensivists are not happy with the morbidity or mortality of ECMO they will not refer patients, since referrals are increasing we must assume that a large number of intensivists are convinced of the efficacy of extracorporeal support.

CHAPTER 2

BLOOD-SURFACE INTERACTIONS DURING EXTRACORPOREAL CIRCULATION, THE INFLAMMATORY RESPONSE TO ECMO

INTRODUCTION

It is likely that even pre-historic hunter-gatherers were aware that when blood is removed from its natural conduits that coagulation ensues rapidly. Indeed in more recent times scientists have tried to investigate this phenomenon starting when Hewson in the 18th Century discovered that blood stationary in a vein would remain fluid for hours, but when let out into a bowl clotted immediately (Gulliver, 1846). This property of the blood precluded development of extracorporeal circulation until Jay McLean discovered heparin (McLean, 1959).

Heparin could produce enough inhibition of the coagulation cascade to allow the extracorporeal circulation of the blood (Gibbon, 1937), but it was soon apparent that extracorporeal circulation (ECC) activated a lot more than the coagulation system. Indeed 40% of Kirklin's first 40 patients succumbed to a novel Post Perfusion Syndrome (Kirklin et al. 1956). Initial investigators of this syndrome noted micro-embolic phenomena (Solis et al. 1975; Reed et al. 1974; Connell et al. 1973; Page et al. 1974), and also documented the denaturation of proteins by the perfusion apparatus (Lee et al. 1961). We now know that ECC causes widespread activation of all of the bodies defence mechanisms against non-self, leading to a "pan-endothelial injury" (Moat et al. 1993), and that simply filtering the blood prior to return to the patient is not the panacea it was once thought (Loop et al. 1976).

Two other "new" diseases appeared around the same time as the post perfusion syndrome. Both the Acute Respiratory Distress Syndrome (ARDS) (Ashbaugh et al. 1967) and the sepsis syndrome , more accurately named the Systemic Inflammatory Response

Syndrome (SIRS) (Morton, 1975; Howes, Jr. and McKay, 1975; Pingleton et al. 1975; Shatney et al. 1976) first became noted in the late 1960s and early 70s. There were many similarities in the clinical course of post perfusion syndrome, ARDS and SIRS, and investigators quickly realised that these three syndromes were intimately related. In this chapter the response to ECC will be described, with particular reference to the prolonged perfusion of ECMO. Differences between cardio-pulmonary bypass (CPB) and ECMO will be discussed, and a brief overview of ARDS and SIRS, in so much as they relate to ECMO will be presented.

DIFFERENCES BETWEEN CPB AND ECMO

ECMO is an extension of CPB and has many similarities, however there are sufficient differences between the two that it is not possible to use a standard pump-oxygenator as an ECMO circuit. The majority of the research into ECC has been directed towards CPB, and therefore a clear understanding of the differences between the two techniques is necessary too allow us to determine which inflammatory mechanisms are applicable equally to CPB and ECMO and which are specific to CPB. A more detailed discussion of the ECMO circuit can be found in chapter 1.

The most obvious difference between CPB and ECMO is the duration. An ECMO run can last several weeks, but CPB rarely lasts more than 2-3 hours. Because of the risk of haemorrhage lower range heparinisation is used for ECMO than CPB, initial loading doses of heparin being 50u/Kg for ECMO and 300 u/Kg for CPB. Once ECC is established target ACTs are 160-200 seconds for ECMO and 500-1000 seconds for CPB. At the end of CPB heparin is neutralised by giving Protamine, which can cause further complement activation (Hakim, 1993; Tulunay et al. 1993), and also results in production of Thromboxane and pulmonary vasoconstriction (Fratacci et al. 1991), of course, Protamine

is not used during ECMO. Because of the reduced anti-coagulation used during ECMO circuits are designed to eliminate areas of stasis such as the venous and cardiotomy reservoirs of the CPB circuit. Cardiotomy suction itself is known to cause intense activation of the aspirated blood, which is mixed with air and particulate debris from the surgical field. Re-infusion of this blood, after filtration, is a significant addition to the inflammatory insult (van Oeveren et al. 1987a; Nilsson et al. 1990a). Haemodilution is not used during ECMO, and neither is hypothermia, both of which can affect the concentration and activity of inflammatory mediators (Edmunds, Jr. and Addonizio, 1987) . Re-perfusion injury after removal of the aortic cross clamp is known to cause significant inflammatory activation, myocardial dysfunction, and could also affect the lung (Gu et al. 1991; Das et al. 1992). Although re-perfusion injury shortly after initiation of ECMO is a theoretical consideration, especially in the cerebral circulation after extreme hypoxia, which abolishes cerebral autoregulation of blood flow (Short et al. 1994a; Short et al. 1994b), it is not thought to be a major problem in the majority of cases. Avoidance of rapid re-establishment of full cerebral blood flow with well oxygenated blood in an asphyxiated hypotensive patient is thought to protect against this phenomenon (Walker et al. 1994).

Another important difference between CPB and ECMO is that ECMO usually only provides partial Cardio-respiratory support. In the case of VA ECMO this means that the heart is ejecting and perfusion is pulsatile. CPB is usually non-pulsatile, and for the majority of patients this does not cause major problems. However, there are theoretical advantages of pulsatile perfusion in terms of preserving organ function and preventing vasoconstriction. Vasoconstriction occurs due to increased catecholamine secretion in response to non-pulsatile ECC (Taylor, 1995). Adrenaline is known to influence platelet secretion (Hennessy, Jr. et al. 1977), and could therefore influence inflammatory

activation. The theoretical improvement in organ perfusion may translate into a clinical advantage in certain high risk groups. Of course VV ECMO is always fully pulsatile even on full flow, as the circulation is still functioning normally.

The type of oxygenator used for CPB can also have an impact on laboratory indices of inflammation (Videm et al. 1989), but this is not thought to affect clinical outcome during routine adult CPB (Nilsson et al. 1990b; Nilsson et al. 1990a). However the increased activation seen with the bubble oxygenator precludes its use during ECMO, indeed ECMO is Membrane Oxygenation. Almost all centres use spiral wound silicone oxygenators. In many respects ECMO is a much more uniform procedure around the world than CPB as there are fewer possible permutations for circuit design and components.

These differences between CPB and ECMO eliminate the effects of hypothermia, cardiotomy suction, protamine administration and largely re-perfusion injury from the pathophysiology of the response to ECMO. However, the reduced levels of heparinisation during ECMO are likely to impact on blood surface interactions particularly as the perfusion is more prolonged.

AN OVERVIEW OF THE RESPONSE TO ECC

In this section a brief review of the main processes which are activated during ECC will be presented, a more detailed discussion of each individual pathway will then follow.

Prior to cannulation for ECC heparin is given to anti-coagulate the blood, heparin acts by increasing the activity of ATIII (Souhami and Moxham, 1994) and inhibiting factor Xa (Samama et al. 1996), but it also has a binding site for complement, which inhibits complement activation (Nojiri et al. 1995), and another action to activate platelets (Edmunds, Jr. and Addonizio, 1987). Thus even before ECC is commenced the

inflammatory cascade has been affected. The existence of ARDS (Gadek, 1992; Goldstein and Luce, 1990; Langlois et al. 1989), SIRS (Bone, 1992a; Sessler et al. 1995), Meconium Aspiration Syndrome (Bui et al. 1991), or other diagnoses as the indication for ECMO also results in significant inflammatory activation prior to initiation of ECC.

The ECMO circuit is washed with 20% albumin in order to reduce fibrinogen binding prior to crystalloid priming (Tsai et al. 1990; Tsai et al. 1988; Eberhart et al. 1987). The clear prime is then displaced with bank blood, which has already high levels of inflammatory mediators (Plotz et al. 1993). Initiation of extracorporeal flow exposes the circuit to the patients blood and infuses the prime into the patient, one of the first steps to occur is the surface binding of fibrinogen onto the circuit, this then acts to activate platelets which undergo a release reaction (Edmunds, Jr. and Addonizio, 1987; Salzman and Merrill, 1987). The platelet mediators activate other platelets, and inflammatory cells such as neutrophils. Some platelets remain bound to the surface, but some move back into the blood stream, many of these platelets remain in the circulation, but having undergone a release reaction are effete and do not function properly (Robinson et al. 1993). This results in a major functional platelet deficit, despite seemingly adequate platelet numbers. The complement system is activated mostly by the alternative pathway (Hakim, 1993), liberating the anaphylatoxins C3a and C5a which have direct effects to increase capillary permeability and as vasodilators, and also activate leukocytes (Craddock et al. 1977). The membrane attack complex (C5b-9) is generated which can kill prokaryotic cells, resulting in haemolysis (Roitt, 1994), but can also cause sub-lethal damage to eukaryotic cells (Li et al. 1994) which could result in organ dysfunction.

Activated neutrophils accumulate in the lung, where they de-granulate, releasing elastin, lactoferrin and other enzymes (Edmunds, Jr. and Addonizio, 1987). They also undergo an oxidative burst with generation of free radicals (Ward et al. 1985). The

presence of these active neutrophils in the lung is thought to be an important step in the generation of "pump lung" (Kirklin et al. 1983), and ARDS (Gadek, 1992). Other leukocyte subtypes are also important in the generation of ARDS such as eosinophils (Modig et al. 1986).

The coagulation system is also activated despite the presence of heparin, mostly by the extrinsic pathway, and significant amounts of thrombin are generated, leading to conversion of fibrinogen to fibrin (Edmunds, Jr. and Addonizio, 1987). This usually occurs in areas of turbulence, and white strands of fibrin can be seen at the edges of connectors. If there is an area of lower shear stress, such as in the oxygenator or bladder, red cells and platelets will also become enmeshed in the fibrin framework, forming a classical "red" thrombus (Anonymous1985). The fibrinolytic system is also activated in order to remove these accumulating thrombi. Plasminogen is converted to plasmin (Edmunds, Jr. and Addonizio, 1987), and in the presence of uncontrolled circuit thrombosis a clinical Disseminated Intravascular Coagulation (DIC) like state can develop, with elevation of Fibrinogen Degradation Products (FDPs) and platelet consumption (Urlesberger et al. 1996).

The contact system is activated with liberation of Kallikrein from Pre-Kallikrein (Edmunds, Jr. and Addonizio, 1987). Lipid mediators such as Thromboxane A2 are liberated from platelet/neutrophil interaction (Fletcher et al. 1993), and cause pulmonary vasoconstriction (Li et al. 1995), which is usually partially offset by the pulmonary vasodilator effect of oxygenated blood. Other mediators such as Tumour Necrosis Factor (TNF) also appear, released from neutrophils (Casey et al. 1992). Endotoxin, a product of gram negative bacteria is a potent stimulator of TNF release, and appears in the circulation after initiation of CPB (Casey et al. 1992). It is not clear what the source of endotoxin is, but it could possibly come from the bowel via translocation.

As ECMO proceeds other proteins become adsorbed onto the circuit such as thrombin, Thrombospondin, von Willebrands factor, etc. (Edmunds, Jr. and Addonizio, 1987), these undergo conformational change and eventually become inactive. The circuit is then said to have been passivated, and little further inflammatory activation occurs (Plotz et al. 1993). The time course for this process coincides with the clinical behaviour of patients on ECMO. After initiation of ECC the majority of patients experience a marked worsening of their lung disease, with infiltration seen on chest X-ray, and a decrease in lung compliance. This usually lasts around 24-48 hours, and then resolves. The recurrence of such changes if a new circuit or oxygenator is inserted is compelling evidence that it is the blood activation by the circuit which is responsible for the clinical changes in the lungs.

Whilst the inflammatory activation reaches a nadir with time, the activation of the coagulation system is slowly ongoing (Plotz et al. 1993), this seems to result clinically in a gradual diminution in oxygenator function over several weeks, and slow accumulation of red and white thrombi in the circuit. In most patients this does not have any clinical significance, but if it becomes progressive and uncontrolled, clinical DIC can result (Steinhorn et al. 1989). The physical trauma to blood as a result of shear stress, positive and negative pressure (Blackshear et al. 1965; Bernstein et al. 1967) results in haemolysis, which has inhibitory effects on end organ function, especially on the kidney (Pedersen et al. 1997).

In summary, initiation of ECMO activates the humoral and cellular components of the inflammatory and coagulation systems, this results in a whole body inflammatory reaction and ARDS like changes in the lungs for a period of 1 to 2 days. After this time the circuit becomes passivated and inflammatory activation wanes. The coagulation system, however, is continually active during the entire ECMO run. The individual pathways and

mediators involved in these reactions will now be discussed in detail, together with possible mechanisms by which these processes could be ameliorated.

THE COAGUALATION SYSTEM

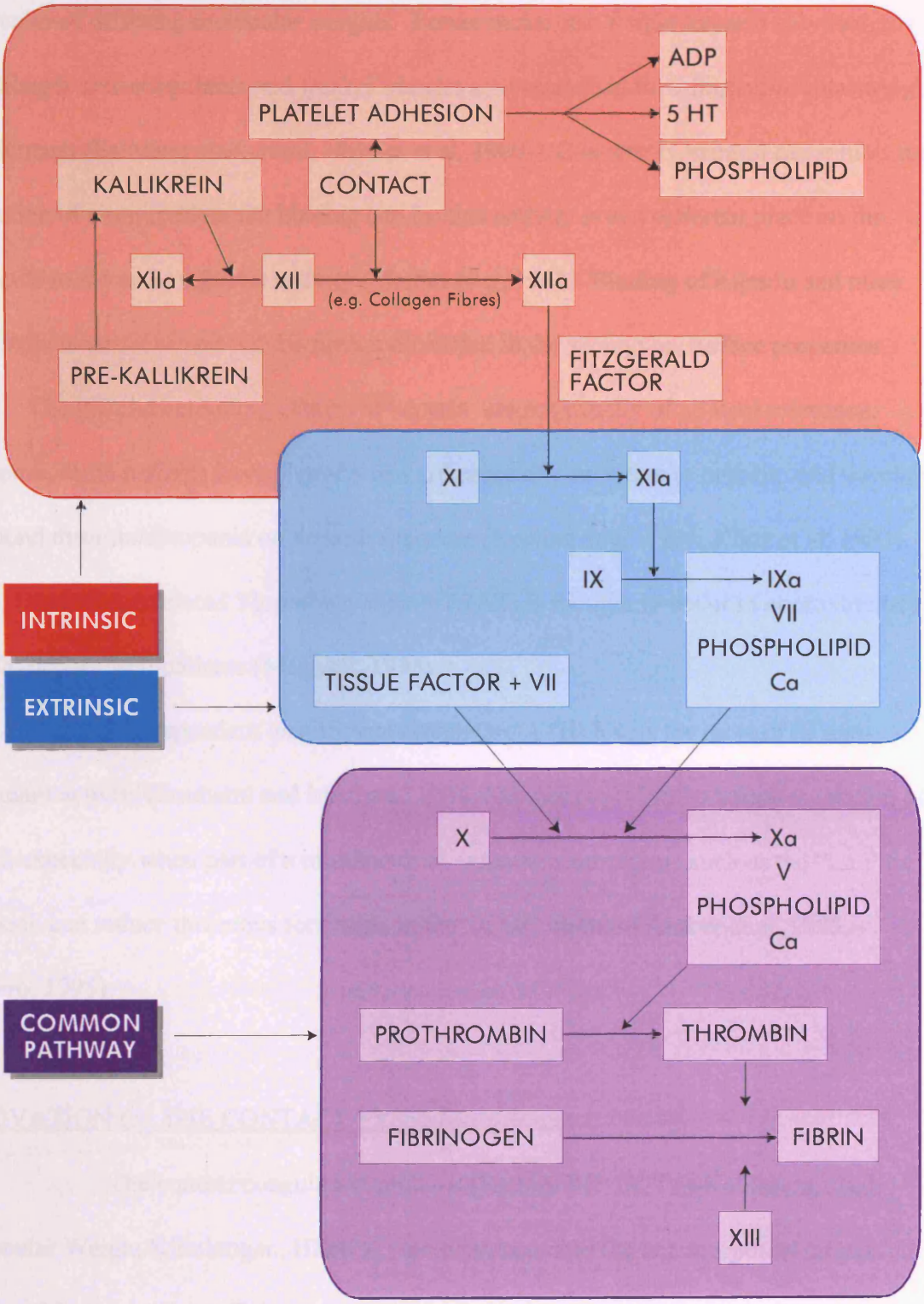
In terms of its physiological function the coagulation and fibrinolytic systems has evolved to seal breaches in blood vessels with clot, and then recanalise those blood vessels by removing the clot as healing occurs. The coagulation cascade can be divided functionally into the intrinsic and extrinsic pathways, and it is mainly the extrinsic (and the contact system) pathway which is implicated in the response to ECC. The activity of both pathways and their interrelationships are outlined (in a simplified form) in figure 2.1, overleaf.

ACTIONS OF SYSTEMIC HEPARIN

Heparin inhibits the clotting cascade in a number of places sufficiently to prevent circuit thrombosis, but it does not completely block all activation of the coagulation pathways, as significant amount of thrombin and FDPs, are generated in 20% of patients during ECC despite seemingly adequate ACTs (Edmunds, Jr. and Addonizio, 1987). Heparin's main action is to increase the activity of anti-thrombin III (ATIII). ATIII is an endogenous antagonist to factors XI, IX, X, XIII and Thrombin (Souhami and Moxham, 1994), which accounts for the prolongation of the Thrombin, and activated partial Thromboplastin times when heparin is used as an anticoagulant, but also explains why the Prothrombin time, initially with most reagents, remains normal (Solomon et al. 1995). Heparin also has activity against factor Xa (Samama et al. 1996), thereby inhibiting one of the steps in the final common pathway of coagulation.

Fig. 2.1 The Coagulation Cascade

(Re-drawn and modified from Muirs Textbook of Pathology 12th Edn. Ed: Anderson, J.R. Pub: Edward Arnold, London, 1985)



Heparin has two other active sites, one for platelets, and one for complement. Heparin actually activates platelets rather than inhibiting their action, but not all heparin sub-fractions are equal platelet activators. "Heparin" is a hetero-disperse mixture of molecules of differing molecular weights. Lower molecular weight heparin sub-fractions are stronger anti-coagulants and weaker platelet activators than their high molecular weight counterparts (Salzman et al. 1980; Holmer et al. 1980). Conversely heparin can inhibit the activation of complement, the binding site for this activity is at a different place on the molecule to the anticoagulant activity (Maillet et al. 1988). Binding of heparin and other mediators to surfaces and will be further discussed in the section on surface properties.

The platelet activating actions of heparin are not usually of clinical relevance. However, some patients have platelets that are especially sensitive to heparin, and develop profound thrombocytopenia on heparin exposure (Keeling et al. 1994; Kikta et al. 1993). Such, Heparin Associated Thrombocytopenia (HAT) is thought to occur in approximately 1/2000 heparinised patients (Magnani, 1993).

Heparin is dependent on adequate circulating ATIII levels for most of its anti-coagulant activity (Souhami and Moxham, 1994; Skinner et al. 1997). Supplementation of ATIII, especially when part of a multifactorial anticoagulant regime such as the "La Pitie" protocol, can reduce thrombus formation in the ECMO circuit (Glauber et al. 1995; Shapiro, 1995).

ACTIVATION OF THE CONTACT SYSTEM

The contact coagulation proteins (Factors XII, IX, Pre-Kallikrein, High Molecular Weight Kinninogen, HMWK) are adsorbed onto the extracorporeal circuit during CPB, but not in sufficient amounts to reduce plasma concentrations (Edmunds, Jr.

and Addonizio, 1987). However, raised levels of kallikrein-C1 inhibitor complexes demonstrates activation of the contact system (Edmunds, Jr. and Addonizio, 1987).

In a detailed study of this phenomenon during ECMO Plotz and colleagues (Plotz, 1994) measured complement and contact activation during clinical ECMO in neonates. The presence of elevated Factor XIIa-C1 esterase inhibitor complexes, decreased kallikrein inhibitory capacity, thrombin-antithrombin III formation and generation of Fibrin degradation products (FDPs), indicated activation of the contact system. There were two temporal activation peaks detected, one was shortly after initiation of ECMO, with peak Factor XIIa concentrations after 1 hour of ECMO. The other activation peak was after 72 hours of ECMO, although only thrombin-ATIII complexes showed a perfect bi-modal distribution. The levels of other indicators seems to indicate a more continuous activation of the contact and fibrinolytic systems as there was a steady increase in FDPs, Tissue Plasminogen Activator (TPA), and kallikrein-inhibiting capacity during the entire ECMO run, which fell to baseline after discontinuation of ECMO. Another similar study confirms these findings (Urlesberger et al. 1996).

In the discussion Plotz correlates these results with experimental findings during CPB and hypothesises that it is the continuous activation of the contact system that is responsible for the haemostatic / platelet deficit during ECMO. This argument is based on the fact that during CPB decreased haemostasis can be correlated with a reduction in platelet adhesive function (Zilla et al. 1989; van Oeveren et al. 1990a), which can, in turn, be related to activation of the contact, kinnin, coagulation and fibrinolytic systems (van Oeveren et al. 1990b; Wildevuur, 1991) . Multiple platelet transfusion makes this impossible to measure during ECMO, although we can be sure that it is circuit which is the source of the continued contact and fibrinolytic activity, for two reasons. Firstly the return to baseline of all levels in Plotz's study after ECMO decannulation, and secondly the return

of FDPs to normal after elective circuit change (Steinhorn et al. 1989). We also know from CPB experiments that inadequate heparinisation can result in increased coagulation and fibrinolysis(Young, 1982; Edmunds, Jr. and Addonizio, 1987). Other authors have shown a coagulation factor deficit on initiation of ECMO (Urlesberger et al. 1996), which could be due to consumption or dilution or both. This could explain the bleeding diathesis commonly seen immediately after cannulation, but does not explain the ongoing functional platelet deficit and impaired haemostasis during ECMO. It would be reasonable, therefore to conclude that the ECMO circuit, with only limited heparinisation, acts as a continued thrombotic stimulus which in turn results in continued fibrinolytic activity, but it is the effect of the contact and fibrinolytic activity on platelet function which actually results in a haemostatic defect. The effect of Aprotinin on these reactions will be discussed in the section on platelet function, below.

ACTIVATION AND INHIBITION OF PLATELETS, INCLUDING THE USE OF APROTININ

Platelets can be detected adhering to the circuit within one minute of ECC initiation (Dutton and Edmunds, Jr. 1973; Baier and Dutton, 1969). The rate of platelet adherence is directly proportional to the amount of fibrinogen adsorbed (Lyman et al. 1974) , but can also occur in the absence of fibrinogen (Bartlett and Andersen, 1982). The fluid dynamic characteristics, Blood Volume : Surface Area ratio (Hennessy, Jr. et al. 1977) and surface profile (Baier, 1977) of the circuit are as important as the materials fibrinogen affinity in determining platelet adhesion. Whilst it is possible to design smoother circuits and more gently profiled connectors, the necessity for gas exchange to occur in the membrane lung obligates thin blood films and turbulence, thus initiating platelet/surface interaction.

Once adhered to surface fibrinogen via any one of its 55,000 fibrinogen receptors (Musial et al. 1985) the platelet becomes activated. Platelets undergo conformational change becoming spiculated spheres rather than their usual discoid shape (Salzman and Merrill, 1987). They also release their granule contents, and serum levels of Platelet Factor 4 (PF4), Thrombospondin and Thromboxane B2 (TxB2, the stable metabolite of TxA2) increase (Davies et al. 1980; Edmunds, Jr. et al. 1982; Harker et al. 1983), ADP, ATP, Serotonin and Calcium ions are also released (Salzman and Merrill, 1987). During ECMO (Robinson et al. 1993) platelet numbers decrease 26% from control values within 15 minutes, then falls a further 16 % over the next hour, platelet transfusions in this study were only transiently effective in improving platelet counts, and did not improve platelet function. This is contrary to our own and others' experiences (Stallion et al. 1994). Circulating platelets show reduced activity in response to in vitro agonists such as adrenaline and ADP (Edmunds, Jr. and Addonizio, 1987). Remaining platelets are left shifted, an increased average size demonstrating mobilisation of younger platelets (Laufer et al. 1975).

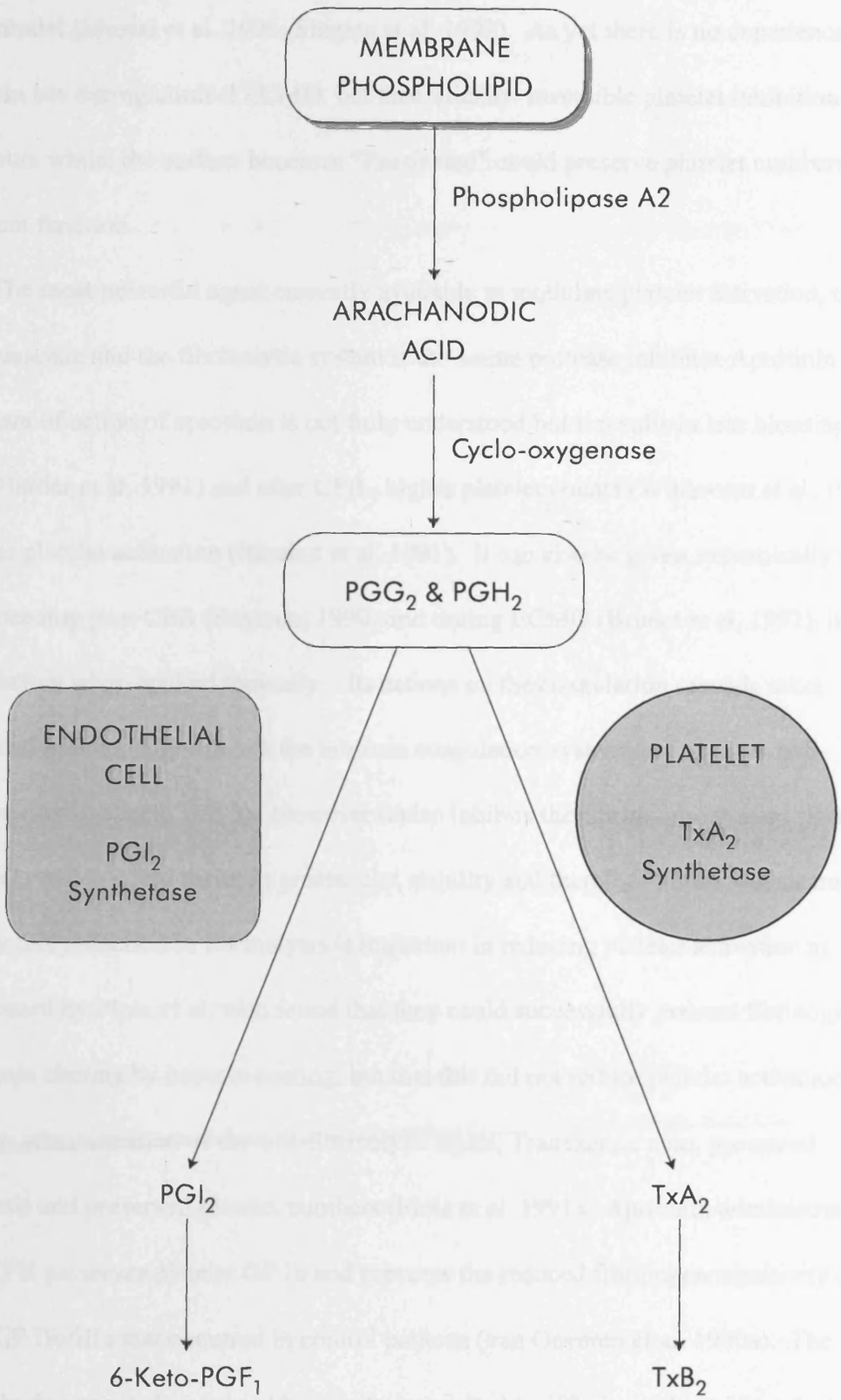
Platelets may also be activated by other systems such as the contact system (Plotz et al. 1993), neutrophils and cytokines (Caplan et al. 1990). Equally activated platelets can activate those same systems in a complex array of positive and negative feedback loops. Other important physiological modulators of platelet function which could be pharmacologically manipulated during ECMO are the Nitric Oxide and Cyclo-oxygenase pathways. Nitric Oxide (NO) is the Endothelial Derived Relaxing Factor (EDRF) (Ignarro et al. 1987) and its actions are legion (Billiar, 1995). One of these many actions is the prevention of thrombin induced platelet adhesion to normal vascular endothelium (Royston et al. 1992). This action can be mimicked pharmacologically during

ECMO. Addition of 15-77 ppm NO to the sweep gas across the membrane oxygenator can significantly reduce platelet loss and activation, as measured by β-Thromboglobulin levels, during in-vitro recirculation (Mellgren et al. 1996). The Cyclo-oxygenase pathway is involved in the synthesis of prostaglandins from membrane phospholipid, differences in enzymes between platelets and endothelial cells result in production of prostaglandins with platelet agonist and antagonist properties respectively (see figure 2.2). During ECMO physiological production of Prostacyclin from vascular endothelium actually increases for the entire duration of the ECMO run (Leuschen et al. 1991). Whilst this is probably advantageous in preventing thrombosis in the patient and the circuit, Prostacyclin administration is not likely to be helpful to enhance or replace heparin as an anticoagulant during ECMO. Prostacyclin use during CPB results in higher platelet numbers (Walker et al. 1981) and lower heparin requirements during CPB (Longmore et al. 1981), and lower blood loss and bleeding time after bypass (Fish et al. 1986), since it inhibits platelet activation by the circuit during bypass(Walker et al. 1981), unfortunately it also blocks physiological platelet activation as well. Its use during the prolonged ECC of ECMO would therefore increase circulating platelet numbers (Cottrell et al. 1988), but would actually reduce platelet function still further. Theoretically surface binding of Prostacyclin would locally inhibit platelet activation, as would regional inhibition using hypothetical ultra-short acting Prostacyclin analogues. Local or regional Prostacyclin use would also circumvent its systemic vasodilator effects which can cause profound hypotension at higher doses (Aren et al. 1983). Prostacyclin cannot prevent coagulation of the blood in response to ECC, even when platelet inactivation is complete (Addonizio et al. 1981), and therefore is not a viable alternative to heparin.

Manipulation of platelet surface adhesion and activation receptors is another mechanism that can be used to protect platelets during ECMO.

Fig. 2.2 Prostaglandin Pathways in Platelets and Endothelial Cells

(Modified and re-drawn from Bartlett et al, Blood Surface Interactions in: Biologic & Synthetic Vascular Prostheses, Ed: Stanley, J.C. Pub: Grune & Stratton 1982)



Viper venom contains low molecular weight peptides called "Disintegrins" which reversibly bind to platelet Glycoprotein (GP) IIb/IIIa receptors and preserve platelet numbers and function during in vitro recirculation experiments, and 16 hours of ECMO in a sheep model (Musial et al. 1990; Shigeta et al. 1992). As yet there is no experience of disintegrin use during clinical ECMO, but theoretically reversible platelet inhibition for 24-48 hours whilst the surface becomes "Passivated" could preserve platelet numbers and subsequent function.

The most powerful agent currently available to modulate platelet activation, the clotting cascade and the fibrinolytic system is the serine protease inhibitor Aprotinin. The mechanism of action of aprotinin is not fully understood but it results in less bleeding both during (Harder et al. 1991) and after CPB , higher platelet counts (Wildevuur et al. 1989) and lower platelet activation (Blauhut et al. 1991). It can also be given systemically to reduce bleeding post CPB (Royston, 1990) and during ECMO (Brunet et al. 1992), it is even effective when applied topically . Its actions on the coagulation cascade seem paradoxical as it actually inhibits the intrinsic coagulation system, acting as an anti-coagulant (de Smet et al. 1990). However it also inhibits the fibrinolytic system (Blauhut et al. 1991) which could result in greater clot stability and therefore improved haemostasis. Certainly this reduction in fibrinolysis is important in reducing platelet activation as demonstrated by Plotz et al, who found that they could successfully prevent fibrinogen uptake onto circuits by heparin coating, but that this did not reduce platelet activation. However, administration of the anti-fibrinolytic agent, Tranexamic acid, prevented fibrinolysis and preserved platelet numbers (Plotz et al. 1991). Aprotinin administration during CPB preserves platelet GP Ib and prevents the reduced fibrinogen sensitivity of platelet GP IIb/IIIa that occurred in control patients (van Oeveren et al. 1990a). The previously documented improved haemostasis resulted in 40% lower blood loss during

CPB, despite complete inhibition of the clotting and Kinnin systems by aprotinin. The interaction of aprotinin with heparin is equally poorly understood. Some studies show prolongation of ACT and APTT and a synergistic effect with heparin (de Smet et al. 1990), whilst others show no correlation of ACT and heparin levels(Despotis et al. 1995), heparin levels do correlate with anti-Xa activity, and therefore the automated protamine titration assay can be used to measure heparin levels. In the presence of low heparin concentrations, in vitro, coagulation is accelerated via the extrinsic pathway. The finding that aprotinin also blocks the inhibitory activity of protein C on Factors Va and VIIIa which may promote coagulation via the extrinsic pathway could explain this phenomenon(van Oeveren et al. 1992). In order to prevent this accelerated coagulation during CPB most perfusionists increase the amount of heparin given by an arbitrary amount to ensure ACTs of well over 1000 sec's, this seems to be effective in preventing circuit thrombosis and does not inhibit the beneficial haemostatic effects of aprotinin. With the finer tolerances of heparin control needed during ECMO the confounding effects of aprotinin on the ACT becomes more important. There is little agreement between centres as to the optimal heparin protocol during aprotinin use, very few centres in the USA, have much experience of aprotinin use during ECMO. The originators of one of the first descriptions of Aprotinin use during ECMO (Brunet et al. 1992), also have limited experience, but do no alter their heparin management during aprotinin use (F. Brunet, personal communication). In our practice we routinely use aprotinin during surgical procedures on ECMO, and also when uncontrolled haemorrhage occurs, and it has proven very effective. During aprotinin infusion (see chapter 1 for doses) we increase the target ACT by 20 seconds. Although we do not usually encounter problems acutely there is often a slow build up of thrombus in the circuit, confirming the in-vitro observation made by van Oeveren et al (van Oeveren et al. 1992). On two occasions we have run circuits in patients

with uncontrollable pulmonary haemorrhage with aprotinin and no heparin, ACTs fell initially to around 140-160, but after 48 hours increased to 300-400 sec's, despite effective haemostasis. We have also encountered this phenomenon during veno-venous haemofiltration without heparin, but with prostacyclin and aprotinin.

Maintenance of an adequate platelet count during ECMO is the single most important factor in haemorrhage prevention (Stallion et al. 1994), and fluctuations in platelet counts, platelet transfusion requirements and heparin requirement seem to occur prior to intracranial haemorrhage (Hirthler et al. 1992). Obviously preservation of platelet function is as important as maintenance of platelet numbers in haemorrhage prevention, and to this end aprotinin is an invaluable adjunct, although its exact mode of action remains a mystery. It is not clear what the optimum heparin management protocol should be during ECMO when aprotinin is used.

THE COMPLEMENT SYSTEM

The complement system is a cascade of at least 20 different plasma proteins (Schreiber and Muller-Eberhard, 1979) much like the coagulation system. Complement has a central function within the body's defences against non-self, is involved in regulation of other effector systems such as neutrophils, and also has direct cytotoxic activity of its own. Like the coagulation system it has 2 pathways activated by different stimuli, but which converge to a common effector system. The classical pathway is stimulated by antibody (Roitt, 1994), whilst the alternative pathway is the main system involved in the response to ECC (Hakim, 1993). An overview of the complement cascade is presented in Figure 2.3, and this will be followed by a more detailed discussion which will include the complement response to ECC and ECMO.

COMPLMENT ACTIVATION AND ACTIONS

As can be seen in figure 2.3 both the classical and alternative pathways are triggered to activate a sequence of events leading to the generation of an enzyme which can catalyse the activation of C3. This "C3 Convertase" is a different molecule in each pathway (Roitt, 1994), the next step is generation of C5 Convertase, again these are different molecules with the same action in each pathway. C5 convertase activates C5 to produce C5a and C5b. The pathways are now fully convergent and result in formation of the membrane attack complex (MAC).

CLASSICAL PATHWAY

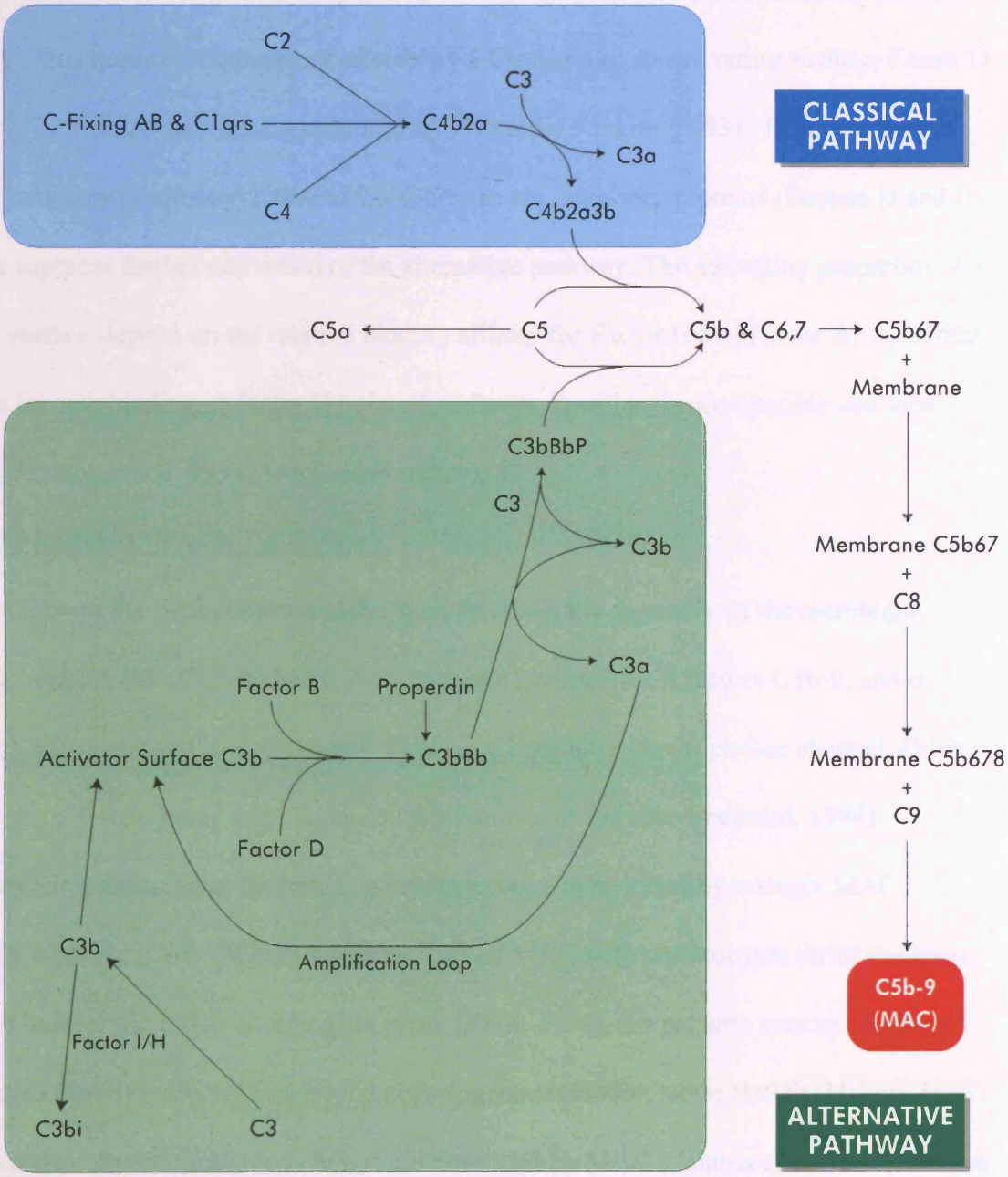
The classical pathway is triggered by antigen-antibody complexes, either IgG or IgM, and some viral particles. Like the alternative pathway it requires magnesium as a cofactor, but takes little part in the complement response to ECC (Hakim, 1993).

ALTERNATIVE PATHWAY

The alternative pathway may be triggered by "foreign" molecules such as bacterial, fungal and plant cell walls. It is said to be phylogenetically older than the classical pathway. It is independent of antibody, and is the main pathway implicated in the response to ECC (Chenoweth, 1991; Chenoweth, 1987; Chenoweth, 1986). The initial step in the alternative pathway is the splitting of C3 to produce C3a and C3b.

Fig. 2.3 The Complement System

(Modified and re-drawn from Moat, N.E. et al Eur J Cardio-thorac Surg. 1993;7:563-573)



This reaction occurs at a continuous "tick-over" level by spontaneous hydrolysis of C3, the resultant $C3.H_2O$ can bind and activate Factor B and cleave another C3 molecule forming C3bBb, which is the C3 Convertase of the alternative pathway (Pangburn et al. 1981). In order for the alternative pathway to be propagated an amplification loop must be set up. This requires the presence of stable C3 Convertase, an activating surface, Factor D and Mg^{++}. C3bBb can also be stabilised by Properdin (Hakim, 1993). Because there is continuous low grade production of C3bBb there are inhibitory proteins (Factors H and I) which suppress further activation of the alternative pathway. The activating properties of a given surface depend on the relative binding affinity for Factor H and Factor B. A surface which favours binding of factor H rather than Factor B will be biocompatible and vice versa (Janatova et al. 1991; Meri and Pangburn, 1990).

MEMBRANE ATTACK PATHWAY

This is the final common path which results in the assembly of the membrane attack complex (MAC). The MAC is composed of complement factors C5b-9, and is assembled on cell surfaces, eventually forming a lipophillic, hydrophobic channel which spans the cell membrane and causes cell lysis and death (Muller-Eberhard, 1984). Prokaryotic cells, such as bacteria and erythrocytes, can be killed by a single MAC molecule (Roitt, 1994). Whilst the interaction of MAC with erythrocytes forms the basis for the haemolytic CH50 complement assay (Roitt, 1994), the patients own red cells are protected from lysis by surface bound homologous restriction factor (HRF) (Hakim, 1993). Eukaryotic cells are more resilient and are not killed by MAC (Salama et al. 1988; Morgan, 1989), however it is thought that multiple MAC strikes may be responsible for the impaired cellular and organ function which results from complement activation (Moat et al. 1993).

THE ANAPHYLATOXINS

C3a, C4a and C5a are known as the anaphylatoxins, with the MAC they form the main effectors of the complement system. C4a is much less potent than C3a and C5a, and is produced only by the classical pathway, whilst C3a and C5a are produced by both systems. All the anaphylatoxins can cause histamine release from mast cells and basophils, cause smooth muscle contraction and an increase in capillary vascular permeability (Hakim, 1993). They also act as opsonins, preparing bacteria for ingestion by macrophages (Roitt, 1994). C3b, although not an anaphylotoxin, is also an important opsonin (Moat et al. 1993). C5a has additional properties in the regulation of leukocyte activity, it is a potent chemotactic factor for neutrophils, and an activator for neutrophils, and monocytes (Hakim, 1993). C5a is rapidly taken up onto neutrophil cell membranes, and serum levels therefore give an underestimation of its activity (Chenoweth et al. 1981). Once surface bound it stimulates neutrophil degranulation and the oxidative burst (Till et al. 1982), it also acts to mobilise neutrophils from the bone marrow (Kajita and Hugli, 1990).

The anaphylatoxins are rapidly destroyed in plasma by conversion to their desArg metabolites. C4adesArg is inactive, but is a marker of classical pathway activity. C3adesArg is mostly inactive, but retains an ability to release IL-1 from monocytes (Haeffner-Cavaillon et al. 1987), and C5adesArg retains much of the activity of the parent molecule.

BIOLOGICAL CONSEQUENCES OF COMPLEMENT ACTIVATION

Aside from the well known effects of the anaphylatoxins described above, what evidence is there that complement activation causes clinical morbidity ? There are numerous studies documenting complement activation during routine adult CPB (Kirklin et

al. 1983; Nilsson et al. 1990b; Videm et al. 1990), but the full blown post perfusion syndrome is extremely rare following CPB.

Descriptions of dialysis patients known to experience the "first use syndrome" have postulated a correlation between severity of symptoms such as fever and pruritis and levels of inflammatory mediators, particularly complement (Ing et al. 1995; Kes, 1994). In animal models injections of complement subunits, or plasma which has been activated by exposure to dialysis membranes results in pulmonary hypertension, hypoxaemia, depression of cardiac output, coronary vasoconstriction (Cheung et al. 1986; Hakim et al. 1984; Heideman and Hugli, 1984). In paediatric CPB the level of complement activation (C3a) is correlated with post CPB pulmonary morbidity (Kirklin et al. 1983). Also, C3a and C4a levels can be correlated with injury severity in trauma patients who have not undergone CPB (Heideman and Hugli, 1984). We thus have reasonable grounds to associate complement activation with undesirable sequelae, but no explanation as to why most adults can undergo CPB causing marked complement activation with few ill effects.

COMPLEMENT ACTIVATION AND ECMO

As previously discussed one of the important differences in terms of blood activation between CPB and ECMO is the fact that the disease which is the indication for ECMO often results in significant inflammatory activation itself (Gadek, 1992; Goldstein and Luce, 1990; Langlois et al. 1989; Bone, 1992a; Sessler et al. 1995; Bui et al. 1991). This is also true of complement activation, two studies have shown elevated C3adesArg (Hocker et al. 1991) and C3a (Plotz et al. 1993) compared to normal cord blood in neonates before initiation of ECMO for a wide range of indications (MAS,PPHN, Sepsis and CDH).

Complement is further activated once ECMO is initiated (Hocker et al. 1991; Plotz et al. 1993) with peak C3a at around 8 hours (Plotz et al. 1993). Complement activation

then declines nearly to baseline (although baseline levels are significantly higher than normal) where they remain for the duration of ECMO. Plotz notes that the pattern of C3a activation is parallel to changes in plasma Elastase and TNF levels, and mirrors a reduction in leukocyte count (Plotz et al. 1993). The transient leukopenia on initiation of ECMO was also documented by Hocker et al (Hocker et al. 1991). Plotz's explanation for these changes is that C5a stimulates neutrophils, which become sequestered in the lung and release their granule contents (Hocker et al. 1991) (Zach et al. 1990) (Craddock et al. 1977), including Elastase. The main sources of TNF release are monocytes and macrophages stimulated by endotoxin or C5a (Tracey et al. 1989; Beutler and Cerami, 1987; Rock and Lowry, 1991). The predominant lymphocyte subtype involved in ECMO related leukopenia in Hocker's study was the monocyte (Hocker et al. 1991). The time course of the complement and subsequent leukocyte activation corresponds closely with the impairment of lung function and X-ray appearance after initiation of ECMO (Bartlett et al. 1985; Taylor et al. 1986; Pangburn et al. 1981). Although this is good evidence for the involvement of complement in a series of adverse blood-surface interactions resulting in end organ (lung) impairment there is one study with conflicting findings.

Westfall et al (Westfall et al. 1991) randomised neonatal ECMO patients to receive either 30mg/kg IV methyl-prednisolone before ECMO or no medication. They found an increase in C3 and C5 levels in the steroid group, but a reduction in ECMO duration and ventilator days compared to controls. This does not agree with classical teaching regarding the action of steroids towards the anaphylatoxins. Steroid administration during CPB has been shown to reduce reperfusion injury (Jansen et al. 1991), and this effect is thought to be most marked with methyl-prednisolone (Moat et al. 1993). Differences between CPB and ECMO could be a result of pre-existing inflammatory activation in ECMO patients, temperature effects or protamine administration.

It is likely that complement activation is an important step in the inflammatory reaction to ECMO, and that biomaterials should be developed with a regard to reducing complement activation. However, it would be naïve to think that this will result in a completely inert material, as the inflammatory response is known to be multi-factorial and multi-faceted with double or triple redundancy at every step. Any anti-inflammatory strategy must therefore be multi-pronged in order to be effective, much like the La Pitie anti-coagulation protocol (Glauber et al. 1995; Shapiro, 1995).

NEUTROPHILS, CYTOKINES, FREE RADICALS & THE INFLAMMATORY RESPONSE SYNDROMES.

The inflammatory response to ECC is remarkably similar to the activation which occurs during the Systemic Inflammatory Response Syndrome (SIRS) (Bone, 1991). SIRS is usually a result of sepsis and can result in Multi-Organ Dysfunction Syndrome (MODS) (Goris et al. 1986; Goris et al. 1985), the pulmonary manifestation of which is the Acute Respiratory Distress Syndrome (ARDS) (Goldstein and Luce, 1990). "Pump lung" is the ARDS caused by CPB and is indistinguishable from other forms of ARDS (Treasure, 1994). Patients who require ECMO usually have established ARDS, Sepsis or both. Even neonatal patients whose hypoxia and shunting is a result of persistent fetal circulation (PFC) rather than intra-pulmonary shunting, all have raised levels of cytokines and inflammatory mediators prior to the initiation of ECMO (Plotz et al. 1993). Numerous cytokines, chemokines and other mediators have been implicated in these syndromes, and the literature is diverse and confusing if viewed from the standpoint of the mediators. Equally, attempts to modulate SIRS, MODS and ARDS by the use of specific "anti

mediators" have been disappointing (Bone, 1992b; Bone, 1992a; Goldstein and Luce, 1990) because of the redundancy discussed in the previous section.

If, however, we examine inflammatory activation from the different perspective of the neutrophil, we come closer to understanding the process. The neutrophil is the final effector in terms of cell and tissue damage (Fujishima and Aikawa, 1995). Neutrophils are the focus of many mediators, and the major source of the remainder (Fujishima and Aikawa, 1995). Finally, and most importantly, neutrophil depletion almost eliminates the lung injury associated with the inflammatory response (Till et al. 1982). In this section the activators, actions and products of the neutrophil will be discussed with respect to ECC and SIRS. The possible modulation of neutrophil action will also be discussed.

NEUTROPHIL ACTIVATING STIMULI

Neutrophils may be activated by a number of stimuli, once activated they undergo a stereotypical release reaction, with degranulation, production of cytokines, arachadonic acid metabolites, free radicals, and an increased adhesive capacity (see Figure 2.4 below). Neutrophil activating stimuli include cytokines, bacteria and their products, complement, bio-active lipids and cell adhesion. Almost all of these stimuli are equally relevant during ECC and SIRS.

The most important Cytokines are Interleukin 1 (IL-1), Tumour Necrosis Factor Alpha (TNF-α), IL-8, and Interferon Gamma (IFN-γ). IL-1 and TNF-α can induce degranulation and a respiratory burst (Ferrante et al. 1988), both IL-1 (Hirthler et al. 1992) and TNF- α (Casey et al. 1992; Plotz et al. 1993) are known to be generated during ECC. IL-8 is a very powerful neutrophil chemotactic factor (Matsushima et al. 1988; Pevri et al. 1988) , and is generated during ECC (Underwood et al. 1995; Finn et al. 1993). IL-8 can also activate neutrophils causing degranulation and an oxidative burst (Matsushima et al.

1988; Pevri et al. 1988). IFN-γ (Shalaby et al. 1985) together with the colony stimulating factors (CSFs) Granulocyte-Macrophage CSF (GM-CSF) (Gasson et al. 1984), and Granulocyte CSF (G-CSF) (Ichinose et al. 1990) can induce weak activation, but also prime neutrophils for secondary activation by other stimuli.

Complement is obviously an important stimulator of neutrophil activation, as opsonisation of bacteria and amplification of the inflammatory response are two of its main functions (Roitt, 1994). Animals congenitally deficient in the C3 component of complement are unable to mount a neutrophil response to CPB (Gillinov et al. 1994a). The anaphylatoxin C5a is one of the most powerful neutrophil chemotactic and stimulating factors (Webster et al. 1980). C5a is extensively bound to neutrophils during ECC, and serum levels may not reflect true levels of activation (Chenoweth et al. 1981). Neutrophils also have cell surface receptors for C3b (Fujishima and Aikawa, 1995).

The bioactive lipids are metabolites of arachadonic acid via the lipo-oxygenase and cyclo-oxygenase pathways (Bartlett and Andersen, 1982). Leucotriene B4 (LTB4) (Ford-Hutchinson et al. 1980) and Platelet Activating Factor (PAF) (Gay and Stitt, 1988) are two such lipids which are potent neutrophil stimulators, both are generated during ECC (Dobyns et al. 1994; Zehr et al. 1995).

Lipopolysaccheride (LPS) is the endotoxin produced from gram negative bacteria, it is an important mediator of gram negative sepsis, but LPS can also be detected during CPB (Casey et al. 1992), and ECMO (Hirthler et al. 1992). The neutrophil cell surface receptor CD-18 is specific for LPS-LPS binding protein complexes (Wright et al. 1990), and can induce activation. Bacterial n-formylmethionyl peptide (fMLP) is also a chemotactic and activating stimulus (Schiffman et al. 1975). Phagocytosis itself can also stimulate neutrophils (Fallma et al. 1989), as does adhesion to an endothelial surface via binding of the neutrophils surface receptors Membrane Adhesion Complex 1 (MAC-1) and

Lymphocyte Function-associated Antigen 1 (LFA-1) to endothelial Intracellular Adhesion Molecule 1 (ICAM-1), or Sialyl Lewis^x to Endothelial Leukocyte Adhesion Molecule (ELAM-1) (Pardi et al. 1992; Nathan et al. 1989). Most of the cytokines listed above as neutrophil stimuli, are also neutrophil products, as can be seen from Figure 2.5 (below), and therefore can act as autocrine stimuli.

MEDIATORS AND EFFECTORS PRODUCED BY NEUTROPHILS

The main classes of substances produced by activated neutrophils are outlined in Figures 2.4 and 2.5 below. These substances can be broadly divided into mediators (the cytokines and bioactive lipids) which mostly act as intra-cellular messengers to amplify the inflammatory response, and effectors. The effector mechanisms include neutrophil granule contents, reactive oxygen metabolites and the adhesion molecules (which allow margination, diapedesis, migration and phagocytosis).

MEDIATORS

BIO-ACTIVE LIPIDS: Leucotriene B4 (LTB4) and Platelet Activating Factor (PAF) are the main arachadonic acid metabolites produced by activated neutrophils. LTB4 is strongly chemotactic for other neutrophils, but only a weak neutrophil activator (Prescott et al. 1984). PAF, as the name suggests, was originally described as a stimulator of platelets (Koller et al. 1996), but is now known to be directly toxic to endothelial cells (Henson, 1981). PAF is therefore more of an effector than a mediator, and could therefore contribute to the capillary leak syndrome and pulmonary oedema seen during ECC and SIRS.

PRO-INFLAMMATORY CYTOKINES: The cytokines produced by activated neutrophils are listed in Figure 2.5, the majority are pro-inflammatory. IL-1 α & β stimulate other neutrophils (Lord et al. 1991). IL-6 is a pyrogen and induces a hepatic acute phase response

Fig. 2.4 Neutrophil Derived Mediators

(Re-drawn and modified from: Fujishima et al. Int Care Med. 1995. 21:277-285)

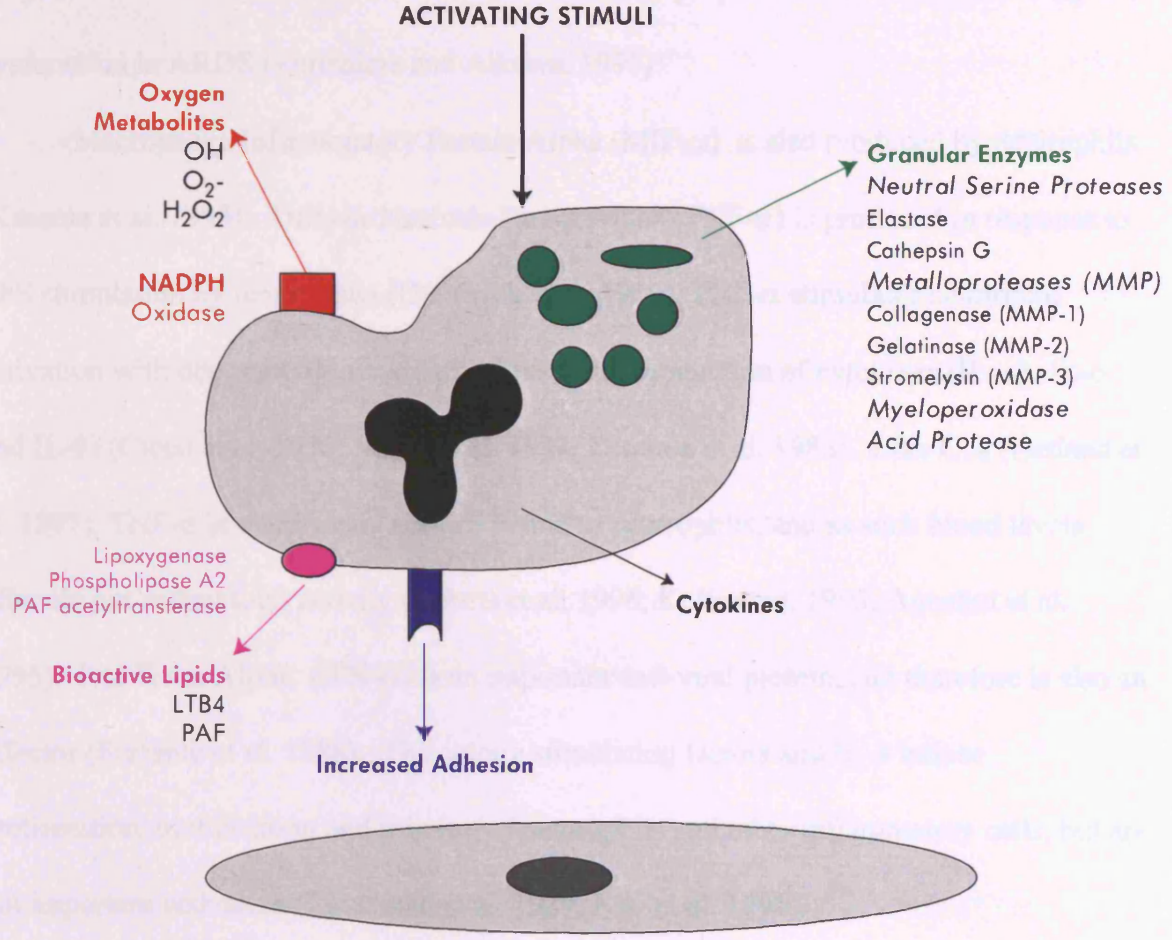


Fig. 2.5 Neutrophil Derived Cytokines

(Abridged from: Fujishima et al. Int Care Med. 1995. 21:277-285)

Cytokines	Stimuli						
	LPS	TNF-α	IL-1β	GM-CSF	PMA	Others	
IL-1α	Yes			Yes			
IL-1β	Yes	Yes	Yes	Yes		IL-1α, Zymosan	
IL-6		Yes		Yes	Yes		
IL-8	Yes	Yes	Yes	Yes	Yes	Phagocytosis	Pro-inflammatory
MIP-1α	Yes			Yes			
TNF-α	Yes			Yes		*C.albicans*	
IFN-α	No					G-CSF	
M-CSF, G-CSF				Yes			
GM-CSF, IL-3						Ionomycin	
TGF-β	No					Spontaneous Release	Anti-inflammatory
IL-1ra	No	Yes	No	Yes			

(Cicco et al. 1990). IL-8 is produced by monocytes and neutrophils and is chemotactic for neutrophils, basophils and T-lymphocytes. IL-8 is also a potent neutrophil activator (Fujishima et al. 1993). Serum IL-8 levels are directly proportional to the level of lung dysfunction in ARDS (Fujishima and Aikawa, 1995).

Macrophage Inflammatory Protein Alpha (MIP-α) is also produced by neutrophils (Kasama et al. 1993). Tumour Necrosis Factor Alpha (TNF-α) is produced in response to LPS stimulation by neutrophils (Dubravek et al. 1990). TNF-α stimulates neutrophil activation with degranulation, oxidative bursts and production of cytokines (IL-1β, IL-6 and IL-8) (Cicco et al. 1990; Marty et al. 1994; Ferrante et al. 1988). Like C5a (Hetland et al. 1997), TNF-α is extensively surface bound to neutrophils, and as such blood levels often do not reflect total activity (Debets et al. 1996; Kolls et al. 1995; Agostini et al. 1995). Interferon Alpha (IFN-α) is an important anti-viral protein, and therefore is also an effector (Ferrante et al. 1988). The colony stimulating factors and IL-3 induce proliferation, mobilisation and priming of neutrophils and other inflammatory cells, but are not important activators (Lindeman et al. 1989; Kita et al. 1991).

ANTI-INFLAMMATORY CYTOKINES: Interleukin 1 receptor antagonist (IL-1ra) competes with IL-1 for receptor binding, thereby inhibiting its pro-inflammatory effects (Kita et al. 1991; Dinarello, 1992). Recombinant soluble IL-1ra has been used successfully as an anti-inflammatory agent during CPB (Gillinov et al. 1993). Transforming Growth Factor beta (TGF-β) also has inhibitory properties (Grotendorst et al. 1989).

EFFECTORS

REACTIVE OXYGEN METABOLITES: When stimulated neutrophils undergo an intense respiratory burst which results in the generation of free radicals or reactive oxygen metabolites (Fortenberry et al. 1996; Wachtfogel et al. 1987; Underwood et al. 1995). The

enzymes NADPH oxidase and cytochrome b558 are necessary for this reaction (Li and Guillory, 1997) which generates the superoxide anion (O_2^-) from NADPH and oxygen. Superoxide Dismutase (SOD) then converts the superoxide to hydrogen peroxide and subsequently to hydroxyl radicals (Fukahara et al. 1997). All of these species are reactive and highly cytotoxic. Myeloperoxidase can generate highly microbicidal hypochlorous acid (cf. Bleach) from physiological concentrations of chloride ions and water (Furlaneto and Campa, 1997). The action of SOD in consuming hydrogen ions is important in raising the pH within phagocytic vacuoles to allow the defensins and neutral proteases such as Elastase and Cathepsin G to become microbicidal (Gabay and Almeida, 1993). In addition to its microbicidal properties Cathepsin G is an important inflammatory effector causing collagen breakdown, increased capillary permeability, platelet activation and lung injury (Tervahartiala et al. 1996; Steinmeyer and Kalbhen, 1996; Rabhi-Sabile et al. 1996; Allen and Tracy, 1995; Iacoviello et al. 1995; Sukura et al. 1995; LaRosa et al. 1994). The free radicals generated by these processes are important anti-microbial agents, but also damage eukaryotic cells, and can therefore cause significant organ dysfunction and capillary leak. Free radical generation occurs during CPB, especially during re-perfusion, where they are thought to be major mediators of re-perfusion injury (Das et al. 1992; Bando et al. 1988a; Forman et al. 1991). Inhibition of free radical production reduces the severity of re-perfusion injury . Free radicals are produced during ECMO, in the absence of the ischaemia/re-perfusion which accompanies aortic cross-clamping on CPB (Underwood et al. 1995). Tissue oedema increases (Anonymous1991) along with free radical activation on CPB (Komai et al. 1994) resulting in increased vascular permeability. The decrease in total body water during ECMO described by Underwood et al is probably a result of our diuretic strategy (see chapter 1) rather than an absence of tissue injury from free radical production.

NEUTROPHIL GRANULE CONTENTS: There are two types of granules present in the cytoplasm of the neutrophil, based on different staining characteristics. The primary azurophilic granule contains myeloperoxidase, defensins, bactericidal/permeability increasing factor (BPI) and Cathepsin G. The secondary specific granules are peroxidase negative and contain lactoferrin, lysozyme, alkaline phosphatase and cytochrome b558. The neutral serine protease Elastase is the most abundant of these enzymes (Janoff and Scherer, 1968). Apart from elastin, elastase can also degrade collagen, fibrinogen and fibronectin (Gadek et al. 1980; McDonald and Kelley, 1980). Other important enzymes include the metalloproteinases; collagenase (Macartney and Tschesche, 1983), gelatinase (Dewald et al. 1982) and stromelysin (Matzner et al. 1985), which can digest collagen, gelatin and proteoglycan respectively and are therefore important enzymes in causing tissue damage. Myeloperoxidase is a cytotoxic enzyme which catalyses the formation of hypochlorous acid from hydrogen peroxide and chloride ions (Morishita et al. 1987), it also acts as a useful marker for neutrophils both in blood, and tissue (Nilsson et al. 1988; Schierwagen et al. 1990; Goldblum et al. 1995). Lactoferrin is an iron binding protein found in the secondary specific granules of neutrophils, blood levels of lactoferrin can also be used as a marker of neutrophil activation (Modig et al. 1986).

THE NEUTROPHIL IN SIRS

The assertion given at the beginning of this section that ARDS and SIRS could be understood far more clearly from the perspective of the neutrophil may seem unlikely after this brief review of neutrophil stimuli and products. However, I will now restore the confidence of the reader by correlating this information to show that the neutrophil is the main effector in the genesis of SIRS/ARDS after appropriate stimulation by ECC, sepsis etc. If we use the same principals used by Koch in his postulates concerning the infective

aetiology of Tuberculosis (Lyons and Petrucelli, 1978), and adapted to inflammatory mediators by Henson and Murphy (Anonymous1989b)we begin to see that the neutrophil is the central agent in the genesis of SIRS. However, the breadth and complexity of information regarding associated mediators, cytokines and other pathways shows that the neutrophil is merely the "star" of the show, which has a "cast of thousands":

I) ARDS IS ASSOCIATED WITH PRESENCE OF ACTIVATED NEUTROPHILS IN THE LUNG: High pressure ventilation is a well known cause of ARDS (Ventilator Lung Injury), and the infiltration of neutrophils into the damaged lung is coincident with the development of ARDS (Tsuno et al. 1991). Neutrophil sequestration has been documented in descriptions of ARDS (Tsuno et al. 1991; Sugiura et al. 1994), although only occurs in 20% of adults during CPB (Zimmerman and Amory, 1982). Hypovolaemic shock promotes lung neutrophil infiltration (Anderson et al. 1991). Broncho-alveolar lavage (BAL) fluid from patients with ARDS has grossly elevated elastase levels, indicating the presence of activated neutrophils (Gadek, 1992).

II) LEVELS OF NEUTROPHIL ACTIVATORS OR PRODUCTS CORRELATE WITH THE SEVERITY OF ARDS / SIRS: The levels of lactoferrin and myeloperoxidase in blood during CPB correlate closely with the degree of post CPB cerebral and renal dysfunction, complement activity did not show this association (Nilsson et al. 1990c). The correlation of neutrophil aggregating ability of complement with the development of ARDS shows that complement activation can result in ARDS, but it does so via neutrophil activation (Hammerschmidt et al. 1980). This dependence on leukocyte, and especially neutrophil activation is confirmed by correlation of ARDS with lactoferrin and Eosinophil Cationic Protein (ECP) levels, but not with complement activation (Hallgren et al. 1984).

In this study the additional role of the eosinophil in causing ARDS is also shown by the correlation of ECP and ARDS. Levels of IL-6 correlate with development of septic and non-septic MODS, and IL-8 levels correlate with death in septic MODS patients (Marty et al. 1994). Levels of IL-8 correlate with the development of ARDS and the degree of pulmonary dysfunction (Donnelly et al. 1993), (Fujishima and Aikawa, 1995). P-selectin is a platelet and endothelial neutrophil adhesion molecule, blood levels of P-selectin correlate with lung injury scores during ARDS (Sakamaki et al. 1995).

III) REMOVAL OF NEUTROPHILS OR INHIBITION OF THEIR ACTIONS ELIMINATES OR AMELIORATES ARDS / SIRS: Leukocyte depletion by filtration results in better lung function compared to controls during experimental CPB (Bando et al. 1990), and also during the ischaemia/re-perfusion injury of experimental lung transplantation (Schueler et al. 1992). Leukocyte depletion also attenuates the increased microvascular permeability seen in experimental ischaemia/re-perfusion of the gut (Hernandez et al. 1987). Inhibition of neutrophil chemotaxis and degranulation by intravenous perflurocarbon reduced infarct size, neutrophil infiltration and improved myocardial function in a canine model of myocardial infarction (Forman et al. 1992). Inhibition of free radical damage either by iron chelation with deferoximine, or scavenging with dimethyl sulfoxide, can eliminate the lung injury caused by Cobra Venom Factor (CVF) (Ward et al. 1983). CVF is a potent complement activator, and this effect was still present in the treated animals despite a complete absence of lung injury indicating that complement is a messenger but only a weak effector in the genesis of ARDS. This is further confirmed by the reduced CD18 expression, MPO activity and pulmonary oedema seen in complement deficient dogs undergoing CPB compared to normal controls (Gillinov et al. 1994a). These indices of reduced neutrophil activation equated with improved lung

function in the C3 deficient animals. Inhibition of neutrophil adhesion with NPC 15669 (which blocks up-regulation of MAC-1) during experimental CPB reduces MPO activity in the lung, and results in improved lung function post CPB (Gillinov et al. 1994b).

IV) ARDS DOES NOT OCCUR IN THE ABSENCE OF NEUTROPHILS: Experimental neutrophil depletion renders animals immune from the complement/neutrophil mediated lung injury caused by cobra venom factor (Till et al. 1982). Inflammation of the lung can occur in neutropenic states, but the incidence and severity of ARDS is diminished (Schilero et al. 1995; Terashima et al. 1995; Henwick et al. 1993).

NEUTROPHILS AND ECMO

Total leukocyte counts fall soon after the initiation of ECMO, with reductions in neutrophil counts but proportionally greater reductions in monocyte numbers (Plotz et al. 1993) (Hocker et al. 1991). Neutrophils are activated even before ECMO is started, as shown by elevated levels of TNF-α and elastase in both the prime and the patients blood prior to cannulation (Plotz et al. 1993). There is further activation once ECMO flow is started with a peak of elastase and TNF-α release which subsides by 24 hours (Plotz et al. 1993; Underwood et al. 1995) . Plasma levels of neutrophil Complement Receptor 3 (CR3) follow a similar time course, shadowing the peak of complement activation (Plotz et al. 1993) (Hocker et al. 1991). Free radical activity is also increased during ECMO, but levels stay elevated for the duration of perfusion (Underwood et al. 1995) (Hocker et al. 1991). Hirthler documented a second peak of complement and neutrophil activation at 36 hours of ECMO associated with the presence of LPS (Hirthler et al. 1992), this response was not seen in the patients of Plotz or in our patients (Underwood et al. 1995; Plotz et al. 1993). This difference could be due to the exclusive use of VA ECMO in Hirthler's study,

compared to the predominant use of VV cannulation in the others. Hirthler describes a coincident MODS and "septic" state in her patients around the time of this second activation peak (36 hours), this could be due to sub-acute intestinal ischaemia and translocation of endotoxin. Further much larger studies would be needed to clarify this issue. The initial peak of neutrophil activation described by all authors on initiation of ECMO coincides well with the consolidation and opacification of the lungs which occurs shortly after ECMO is started, and usually lasts 1-2 days (Taylor et al. 1986).

In conclusion it seems likely that leukocyte depletion of the prime, or indeed of the patient for a short period, could ameliorate or eliminate the lung consolidation seen after ECMO is initiated. This approach seems more likely to be successful that pharmacological manipulation with steroids or pentoxifyline (Goldstein and Luce, 1990). ECMO gives us access to almost the entire cardiac output, and thus the opportunity to use physical techniques such as leukocyte filtration or plasma-pherisis to treat ARDS and SIRS. Early reports of such technology are promising (Karl Hultqvist, personal communication).

CONCLUSION

The similarity between the mediation of the inflammatory response syndromes (SIRS/ARDS/MODS) and the response to ECC points to a common patho-physiology. Almost all of the cellular and humoral elements of the blood are involved in the response to ECC, including the contact and coagulation systems, platelets, the fibrinolytic system, complement, cytokines, neutrophils and other leukocytes. It seems that neutrophils are the main effectors of cell damage and organ dysfunction. Experiments with inhibitors of single mediators have been disappointing as treatments for sepsis and ARDS, as have attempts to modify the coagulation response by heparin coating of circuits. Both these failures highlight the complexity of these cellular and humoral cascades and direct us towards a

multi-factorial approach. Recent work coating tubing with a nitric oxide donor indicates a powerful local anti-platelet effect in a rabbit model (R Bartlett personal communication). We should look upon the unprecedented access to the circulation afforded by the ECMO circuit as an opportunity to modify the inflammatory process, rather than a cause of ARDS, as once the initial activation is over the ECMO circuit becomes "passive". We should also remember the lesson from CPB, that release of mediators does not necessarily equate with organ damage and outcome, and we should focus our researches towards inflammatory effectors, rather than mediators.

CHAPTER 3

RESULTS OF ECMO TREATMENT AT GLENFIELD HOSPITAL

INTRODUCTION

In this chapter the clinical results of patients treated with ECMO at Glenfield Hospital between the start of the programme in 1989 and the end of December 1996 will be presented and discussed. Results will be subdivided by age group and whether the indication for ECMO was primarily respiratory or cardiac support. A more detailed study of the first 50 adult respiratory patients treated will also be presented. The discussion will focus on the evidence for the use of ECMO in these patient populations, the use of scoring systems for patient selection, and the outcomes with conventional treatment.

SETTING

The ECMO unit at Glenfield Hospital operates within the Department of Cardiothoracic Surgery. It is recognised by the European Extracorporeal Life Support Organisation (EESO) and the international Extracorporeal Life Support Organisation (ELSO). The unit treats patients of all age groups, and accepts patients from other hospitals when clinically indicated. This means that, with the exception of the cardiac patients, all patients have been managed by other clinicians during the initial phases of their illness. The unit is staffed by a multidisciplinary team of Cardiothoracic surgeons, Anaesthetists, Paediatricians, Physiotherapists, Perfusionists and specially trained nurses (ECMO specialists).

METHODS

Data was collected retrospectively from the Glenfield ECMO patient database. This database is compiled from the ELSO registry forms which are filled in shortly after each patient is discharged.

The primary end point was survival to hospital discharge. In the neonatal patients the Oxygenation Index (OI) was taken as the measurement of severity of respiratory failure, whilst in the adult and paediatric patients the ratio between the PaO_2 and FIO_2 was used. Other data recorded was diagnosis, type of perfusion, duration of ECMO and age.

The detailed study of the first 50 adult respiratory patients was also retrospective. Data was collected using a pro-forma from the case notes, ECMO specialists chart, ECMO database, blood transfusion laboratory database and ELSO form. Admission chest radiographs were also reviewed by the same observer (GJP). The pre-ECMO data was collected by postal survey of the referring hospitals. The Murray lung injury score (Murray et al. 1988) was used as the severity index, and the primary end point was survival to hospital discharge.

The survival with ECMO was compared with two published series of conventionally ventilated patients. Study data was compared with multi-institutional survival data for conventional treatment taken from the paper of Vasilyev et al (Vasilyev et al. 1995) using logistic regression, and analysing for the effects of hypoxia (PaO_2/FIO_2 < 60 mmHg). Logistic regression was also used to estimate the odds ratios of survival with ECMO compared to the survival with conventional treatment in Vasilyevs patients (Vasilyev et al. 1995), both with and without adjustment for hypoxia. Survival was also compared to the control group receiving Pressure Controlled Inverse Ratio Ventilation (PCIRV) in the paper of Morris et al (Morris et al. 1994) . As these patients were not sub-divided on the basis of hypoxia a Chi-squared test was used to compare this group with our patients. A 2 tailed unpaired t-test was used to compare continuous variables which were normally distributed, the Mann-Whitney U test was used for ordinal variables, and also for

continuous variables which were not normally distributed as detected by Levenes' test. Non-normally distributed variables were: Blood use, Cryoprecipitate use, Pulmonary artery wedge pressure, PaO_2/FIO_2 pre ECMO, Platelet use and Duration of perfusion. A p value of < 0.05 was taken to indicate statistical significance.

This study, including the comparison with historical controls, has been published in "Chest" (Peek et al. 1997).

RESULTS

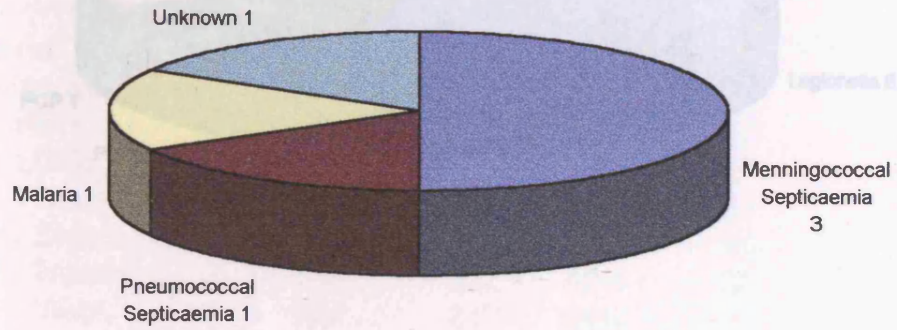
(Data given in tables are means unless otherwise stated)

RESPIRATORY ECMO

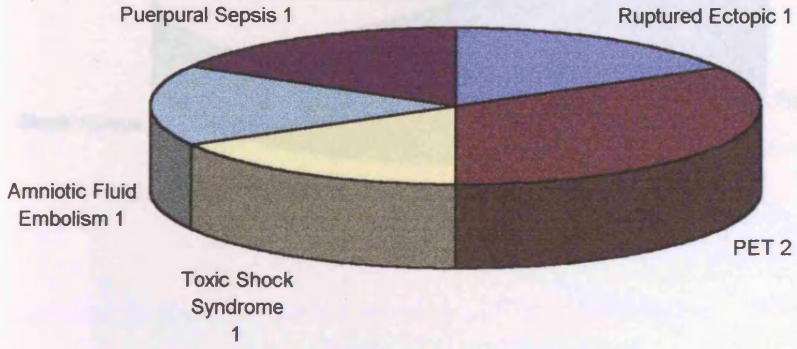
Adult Respiratory ECMO

Diagnosis	n	Age (yrs)	Run (hours)	%VV	PaO2/FIO2 (mmHg)	% Survivors
ARDS Trauma	14	26.3	111	79%	53	79%
ARDS Sepsis	6	28.3	102	100%	47	83%
ARDS Pancreatitis	3	26.3	108	100%	55	100%
ARDS Obstetric	6	27.3	223	100%	71	33%
ARDS Misc.	5	30	201	80%	76	40%
Viral Pneumonia	21	34.2	170	86%	60	76%
Atypical Pneumonia	15	44.2	170	100%	93	60%
Aspiration Pneumonia	14	33.1	152	100%	64	71%
Bacterial Pneumonia	13	39.9	259	100%	52	62%
Wegeners	2	38	139	50%	49	0%
Total	**99**	**34.1**	**169**	**93%**	**65**	**63%**

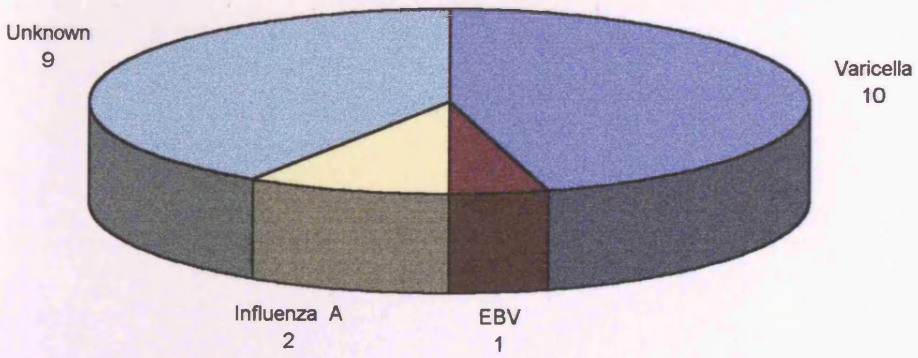
Septic causes of ARDS in adults



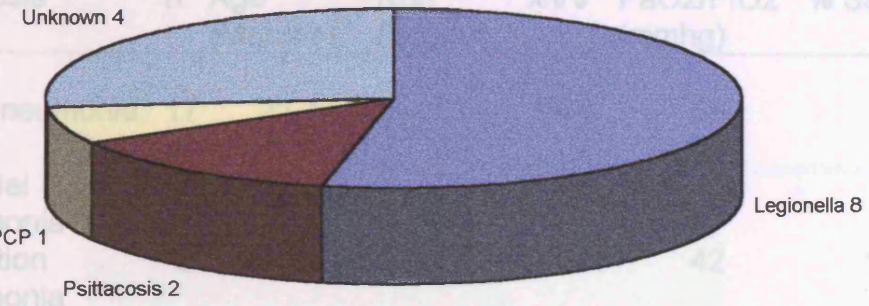
Obstetric causes of ARDS in adults



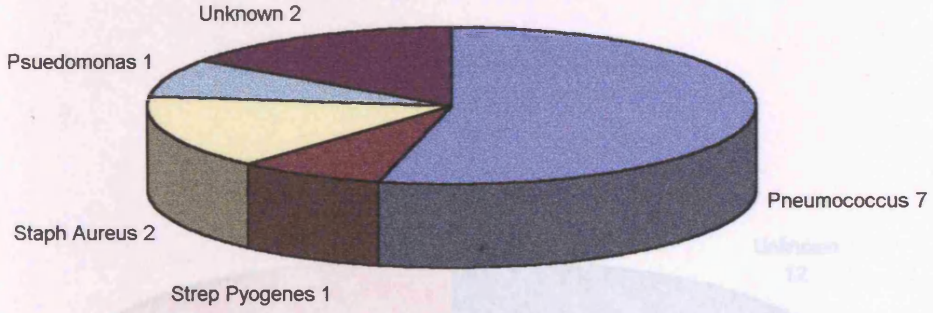
Causes of viral pneumonia in adults



Causes of atypical pneumonia in adults



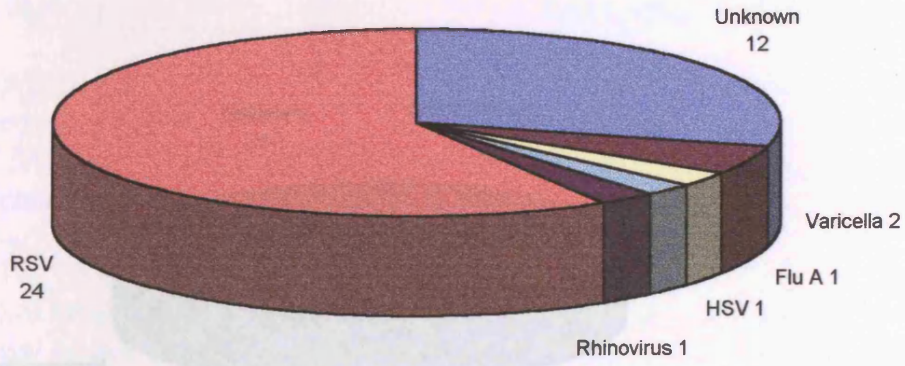
Causes of bacterial pneumonia in adults



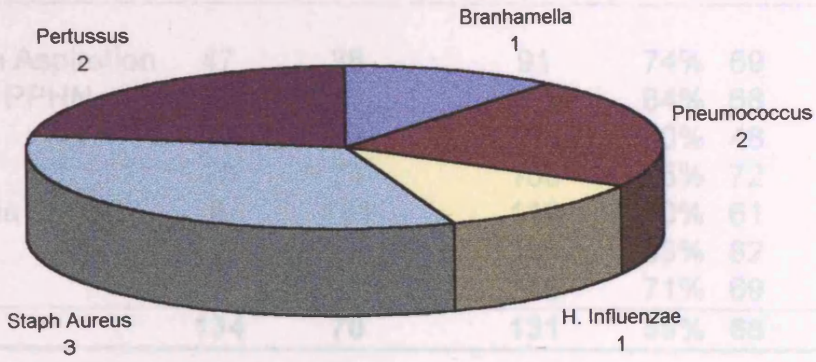
Paediatric Respiratory ECMO

Diagnosis	n	Age (Months)	Run (hours)	%VV	PaO2/FIO2 (mmhg)	% Survivors
Viral Pneumonia	17	22.4	261	59%	58	76%
RSV	24	5.9	169	71%	53	92%
Bacterial Pneumonia	9	71.7	196	89%	49	89%
Aspiration Pneumonia	3	12	274	100%	42	100%
Misc. Pneumonia	2	98.3	58	100%	61	50%
ARDS Sepsis	8	53.4	197	75%	54	63%
ARDS Trauma	2	106	222	50%	68	0%
ARDS Misc.	7	60.1	223	88%	61	75%
Near Drowning	4	54	107	25%	141	50%
Misc.	5	44	51	40%	64	60%
Total	**81**	**34.8**	**191**	**68%**	**61**	**77%**

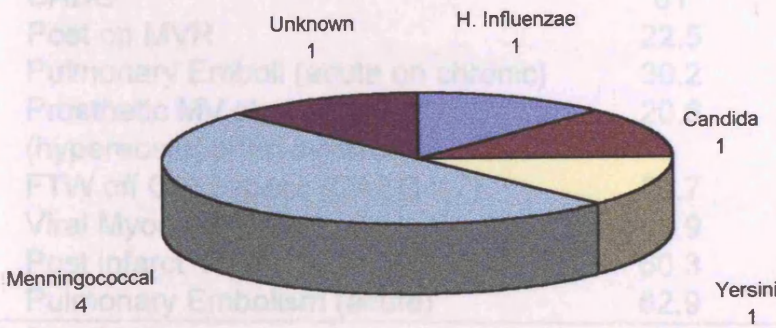
Causes of paediatric viral pneumonia



Causes of paediatric bacterial pneumonia



Septic causes of paediatric ARDS



Neonatal Respiratory ECMO

Diagnosis	n	Age (hours)	Run (hours)	%VV	OI	% Survivors
Meconium Aspiration	47	38	91	74%	69	96%
Idiopathic PPHN	22	73	125	64%	68	64%
IRDS	15	85	115	60%	48	93%
CDH	26	71	189	35%	72	58%
Pneumonia	8	161	112	50%	61	75%
Sepsis	9	102	134	33%	82	44%
Misc.	7	102	228	71%	69	43%
Total	**134**	**70**	**131**	**59%**	**68**	**75%**

CARDIAC ECMO

Adult Cardiac ECMO

(Data given for individual patients except for means denoted *)

	Diagnosis	Age (years)	Mode	Duration	Outcome
	CABG	61	VA	144	Died
	Post op MVR	22.5	VA	49	Surv
	Pulmonary Emboli (acute on chronic)	30.2	VA	96	Surv
	Prosthetic MV obstruction (hypereosinophilic syndrome)	20.6	VA	214	Died
	FTW off C/P bypass (CABG x 7)	53.7	VA	69	Died
	Viral Myocarditis	18.9	VA	715	Died
	Post infarct VSD	60.3	VA	326	Died
	Pulmonary Embolism (acute)	62.9	VA	72	Surv
Total	**8 patients**	**41***	**All VA**	**211***	**38%***

Paediatric Cardiac ECMO

Diagnosis	N	% Survival
Transplant	-	-
Left to Right Shunt (ASD/VSD/PDA/AV Canal)	6	17%
Left Sided Obstructive Lesions (Aortic Stenosis/Mitral Stenosis/Coarctation)	1	100%
Hypoplastic Left Heart	-	-
Right Sided Obstructive Lesions (Pulmonary Stenosis or Atresia/Tricuspid Atresia)	1	100%
Cyanotic Increased Pulmonary Flow (Truncus Arteriosus/TGA/TGV)	4	75%
Cyanotic Increased Pulmonary Congestion (TAPVR/PAPVR)	2	100%
Cyanotic Decreased Pulmonary Flow (TOF/DORV/Ebstein)	8	63%
Anomalous Left Coronary Artery	1	0%
Post Operative (Fontan)	2	100%
Other	3	67%
TOTAL	**28**	**61%**

Detailed Study of Adult Respiratory ECMO Patients

A total of 50 patients records were examined. Of these 50 patients 33 survived (66%). Not all patients had all data points available in their records, either by reason of omission, or because a pulmonary artery catheter was not present, therefore the number of observations on which each datum is based is expressed (n). Survival by diagnosis is given in Table 1, status at ECMO referral is listed in Table 2, advanced treatment before ECMO in Table 3 and blood product use in Table 4. Other results are given in the text below

STABILITY DURING TRANSPORT: Assessed as change in PaO_2/FIO_2 ratio between referral and pre-ECMO values. Mean change was -0.3 mmHg (SD=48.572).

INOTROPE USE: Data was available for all 50 patients. 32 patients had inotrope use (greater than 5mcg/Kg/min Dopamine) recorded, the remainder either received no inotropes or low dose Dopamine only.

CHEST X-RAY APPEARANCE: Number of quadrants involved with infiltration was assessed on 36 available admission chest films, mean number of quadrants involved was 3.8 (SD 0.4).

PERFUSION DATA: Veno-venous access was used in 48 out of 50 cases. Mean duration of perfusion (n=50) was 207.4 hours (SD=177.8).

POST MORTEM FINDINGS: Of the 17 patients who died, post mortem findings were available in 14. Cause of death was recorded as ARDS in 9, pneumonia in 1, multiple organ failure in 2, sub-arachnoid haemorrhage in one and myocardial infarction with a ruptured papillary muscle in one patient.

TABLE 1: Survival by Diagnosis.

Diagnosis	n	Survivors	% Survival
Aspiration Pneumonia	8	7	88%
Atypical Pneumonia	8	5	63%
ARDS Trauma	7	6	86%
Viral Pneumonia	6	5	83%
ARDS Other	5	3	60%
ARDS Sepsis	4	3	75%
Bacterial Pneumonia	4	2	50%
ARDS Obstetric	4	1	25%
Other	4	1	25%
Total	**50**	**33**	**66%**

TABLE 2: STATUS AT ECMO REFERRAL.

Parameter (units)	n	mean	SD
PaO_2/FIO_2 (mmHg)	47	65	36.9
Murray Score	47	3.4	0.5
Time Ventilated (hours)	46	76.5	83.7
Time with $FIO_2 = 1.0$ (hours)	32	14	19.1
Peak Airway Pressure (cmH_2O)	26	39.6	7.4
End Expiratory Pressure (cmH_2O)	30	10	3.3
Minute Volume (L/Min)	29	12.6	3.32
Mean Arterial Pressure (mmHg)	30	82	16.4
Mean Pulmonary Artery Pressure (mmHg)	10	29	3.6
Central Venous Pressure (mmHg)	27	12	3.8
Pulmonary Artery Wedge Pressure (mmHg)	10	12	2.2
Cardiac Output (ml/Kg/min)	8	127	32.7
Urine Output last 4 hours (ml/Kg/hr)	25	1.4	1
Age (years)	50	30.1	10.8
Weight (Kg)	42	71.9	15.5
Haemoglobin (g/dl)	37	10.8	1.9

TABLE 3: ADVANCED TREATMENT BEFORE ECMO.

Treatment	n
Pressure Control Ventilation	6
High Frequency Jet Ventilation	4
Inhaled Nitric Oxide	1
Single Lung Ventilation	1
Prone Ventilation	1
Inhaled Prostacyclin	1

TABLE 4: BLOOD PRODUCT USE DURING ECMO.

Product	n	mean	SD
Blood/Packed Cells (units)48	19	17.3	
Cryoprecipitate (units)	47	1.8	7.7
Fresh Frozen Plasma (units)	47	2.9	5.1
20% Albumin (100ml)	47	2.9	5.1
4.5% Albumin (500ml)	47	8.1	11.9
Platelets (units)	48	47.1	62.4

SURVIVAL.

The survival of patients in our ECMO treated population was compared to the survival of the population of ventilated patients described by Vasilyev et al (Vasilyev et al. 1995) using logistic regression analysis. This group of 1426 patients was collected from 25 international centres between 1991 and 1992. Selection criteria was ventilation with an FIO_2 of >50% for longer than 24 hours, 1426 patients were included in the study with overall hospital survival of 55.6%. Patients were subdivided into 2 groups. Group A patients were hypoxic (PaO_2/FIO_2 < 60mmHg) or hypercarbic, and contained 375 patients with 33.3% survival. Group B patients were not hypoxic (PaO_2/FIO_2 > 60mmHg) or hypercarbic, and contained 1051 patients with 63.6% survival.

Logistic regression shows that the survival of our ECMO treated patients was better that the survival of the conventionally treated patients in Vasilyevs' study, (Chi squared for 1 degree of freedom = 4.399, p=0.036). There was marginal evidence of an interaction between treatment type and hypoxia as defined by Vasilyev (p=0.050), (PaO_2/FIO_2 ratios below 60mmHg).

Odds ratio for improved survival with ECMO was 0.46 (95% CI 0.22-0.97, p=0.036), with an interaction being demonstrated with hypoxia ($p<0.05$).

Our patients survival was also compared to the Control Therapy patients (Pressure Control Inverse Ratio Ventilation, PCIRV, with a computer regulated protocol) in Morris's randomised comparison of PCIRV with Extracorporeal Carbon Dioxide Removal (Morris et al. 1994). There were 19 patients in this group of which 8 survived (42%), their mean PaO_2/FIO_2 ratio was 63.8 mmHg, this is not significantly different from the mean PaO_2/FIO_2 ratio in our patients of 65 mmHg (unpaired t-test, p=0.741). The Murray score calculated from the mean PaO_2/FIO_2 ratio, PEEP and Compliance was 3.7 for Morris's patients, compared to 3.4 for our patients; the Murray scores were not compared statistically as they are calculated from mean values in one group and from individual patient data in the other, also we have no lung compliance data for our patients and Morris gives no Chest X-ray data. The survival in our patients is significantly improved compared to the PCIRV ventilated group (Chi Sq., 1 degree of freedom = 11.71, p=0.0006).

PREDICTORS OF MORTALITY.

All of the variables listed above were compared for survivors and non survivors to identify parameters which may identify patients with a potentially poor outcome. Four variables showed a significant difference between survivors and non-survivors (see table 5). The remaining variables are listed for survivors and non-survivors in table 6.

TABLE 5: VARIABLES CORRELATING WITH NON-SURVIVAL.

Variable	Mean + SD (Survivors)	Mean + SD (Non-Survivors)	p
Age	27.8 yrs (9.8)	34.5 yrs (11.7)	0.036
Blood use	14.2 units (10.9)	28.5 units (23.4)	0.0221
Platelet use	27.6 units (29)	86.3 units (89.4)	0.018
Duration of Perfusion	163.5 hrs (140.5)	292.5 hrs (213.7)	0.0256

TABLE 6: VARIABLES HAVING NO CORRELATION WITH SURVIVAL.

Variable	Mean + SD (Survivors)	Mean + SD (Non-Survivors)
Cardiac Output (ml/kg/min)	117 (44.2)	137 (17.5)
Cryoprecipitate Use (units)	0.4 (1.6)	4.4 (12.9)
Central Venous Pressure (mmHg)	12 (4.1)	13 (3.2)
Change in PaO2/FIO2 after transfer-4 (30.2)		9 (79.7)
FFP use (units)	2.8 (4.5)	3.1 (6.4)
20% Albumin use (x 100ml)	2.6 (5.1)	3.4 (5.1)
4.5% Albumin use (x 500 ml)	6.7 (11.4)	10.7 (12.8)
Haemoglobin pre ECMO (g/dl)	10.7 (1.8)	11.3 (2.1)
Hours on 100% O2	15.3 (21.5)	10.3 (9.2)
Hours Ventilated	62 (77.5)	109.6 (90.6)
Mean Arterial Pressure (mmHg)	84 (17.4)	77 (13.6)
Mean Pulmonary Artery Pressure (mmHg)	29 (17.4)	30 (2.4)
Murray Score	3.3 (0.48)	3.6 (0.47)
Minute Volume (Litres)	11.9 (2.9)	14.1 (3.8)
Wedge Pressure (mmHg)	13 (3.2)	12 (0.8)
End Expiratory Pressure (cmH2O)	10 (3.3)	11 (3.3)
PaO2/FIO2 at Referral (mmHg)	54.9 (19.7)	72.3 (58.3)
Peak Inspiratory Pressure (cmH2O)39 (7.2)		42 (7.9)
Urine Output (ml/Kg/Hr)	1.4 (1.1)	1.3 (0.8)
Weight (Kg)	71.8 (17)	72.1 (11.6)

DISCUSSION

The use of ECMO for respiratory failure will be discussed individually for each age group. Cardiac ECMO will be discussed separately.

NEONATAL RESPIRATORY ECMO

The indications for ECMO use in severe neonatal respiratory failure are now well defined following the publication of the UK collaborative ECMO trial (Anonymous. 1996). In this study babies with respiratory failure refractory to conventional treatment as defined by an Oxygenation Index (OI) >40 (see chapter 1) were randomised to receive either continuing conventional intensive care or to be transported to one of the UK's 5 neonatal ECMO centres. Fifty four of 92 patients randomised to conventional treatment died, compared with 30 out of 94 who received ECMO. Relative reduction in risk was 0.55 (95% CI 0.39-0.77; p=0.0005). This is equivalent to one extra survivor per 3 patients treated. Developmental outcomes at 1 year and costs per survivor were not statistically different when survivors were compared from ECMO and non-ECMO groups. The UK study confirms the tentative earlier findings of Bartlett(Bartlett et al. 1985) and O'Rourke (O'Rourke et al. 1989) who used modified design randomised trials to asses the impact of ECMO compared to conventional treatment, both showed improved outcome with ECMO. Thus clinical decision making in the neonatal patient is straightforward, once the OI exceeds 40 outcome is improved with ECMO. The situation for babies with congenital diaphragmatic hernia (CDH) is slightly different, no CDH patients in the conventional group survived, and none were operated on. It was a strong clinical impression that for patients with CDH a lower OI should be taken as the indication

for ECMO, perhaps 25-30 rather than 40, but another trial would be needed to clarify this point.

Of the 134 patients aged between birth and 4 weeks treated at Glenfield between 1989 and the end of 1996 the overall survival of 75% is close to the 80% survival recorded in the ELSO registry (see chapter 1). Proportions of presenting diagnoses are similar as are survivals within groups. The proportion of patients treated with Veno-venous perfusion (59%) is lower in the neonates than the paediatric and adult respiratory patients. This is due to the patients smaller than ~ 3 Kg who often require veno-arterial cannulation as their jugular veins are frequently too small to accept the smallest double lumen cannula (12F). The lower survival in the pneumonia/septic groups is similar to the trend seen in the ELSO registry, but a significant difference in survival has not been shown between septic and non-septic patients by detailed analysis of the ELSO registry (Meyer and Jessen, 1995). Although septic patients did exhibit a higher incidence of complications, which probably account for the higher mortality in our relatively small series. The 58% survival of infants with Congenital Diaphragmatic Hernia (CDH) gives a false impression of the outcome in this group. Whilst none of the CDH patients in the conventional arm of the UK trial (Anonymous. 1996) underwent diaphragmatic repair, and ECMO survival was therefore infinitely superior it is noticeable that these babies often have associated abnormalities such as malrotation of the bowel. Consequently they may have an unacceptably high level of long term morbidity from Gastro-oesophageal reflux or neurological impairment (Bernbaum et al. 1995). Whether continued treatment of these babies is justified will become clearer when the longer term follow up of the patients in the UK ECMO trial is completed.

PAEDIATRIC RESPIRATORY ECMO

As already discussed in chapter 1, the criteria for the use of ECMO in the paediatric (1month to 18 years) age range are not so clear cut as in the neonatal population. We believe it is appropriate to offer ECMO when conventional treatment has failed, or has little chance of success.

In the absence of a randomised study we must look to the literature to find indices predictive of a poor outcome. The Arterial-alveolar (A-a) oxygen gradient is an accurate predictor of death in the paediatric age group. An A-aDO_2 of >450mmhg for >16 hours predicts death with 86% sensitivity and 100% specificity. An A-aDO_2 of >400mmhg for >20 hours also predicts death with a similar degree of accuracy (Tamburro et al. 1991). Since the $PAO_2 = [(P_{atm} - PH_2O) \times FIO_2]-PaCO_2$ (Guzman et al. 1989) we can calculate that an A-aDO2 of > 450 mmhg is approximately equivalent to a PaO_2/FIO_2 ratio of less than 200mmhg (assuming normal atmospheric pressure and PaCO2). An A-aDO_2 of > 400 mmhg is equivalent to a PAO_2/FIO_2 ratio of 250 mmhg. The mean PaO_2/FIO_2 of 61mmhg in the paediatric respiratory ECMO patients would predict a 100% mortality risk by these criteria. It would seem, therefore, that these patients are an appropriate population to consider for ECMO. Obviously a direct comparison is difficult, but the overall survival of 77% would seem acceptable in this high risk group (Moler et al. 1994). The introduction of permissive hypercapnia (Gentilello et al. 1995) into respiratory management must be borne in mind when interpreting A-aDO_2 values, as a higher $PaCO_2$ will result in a lower A-aDO_2 for a given level of lung disease. Another study showed that a combination of a Peak Inspiratory Pressure (PIP) > 40 cmH_2O and an A-aDO_2 > 580 mmhg equated with an 81% risk of death, predicting with 74% sensitivity and 79% specificity (Rivera et al. 1990).

Other authors have found the A-aDO_2 to be less strongly predictive (Caballero et al. 1996), and attempts have been made to construct scoring systems predictive of mortality. The Pediatric Risk of Mortality Score (PRISM) (Pollack et al. 1988) is widely accepted. A case control study of patients receiving conventional and ECMO treatment showed a significant reduction in mortality from 71.4% to 28.6% with ECMO in the 50-75% mortality risk category defined by PRISM (Green et al. 1996), the PRISM score accurately defining the mortality risk in the conventionally treated patients. The most complex, and most accurate system for predicting mortality is the Pediatric Respiratory Failure score (PeRF). The PeRF is a computer based system combining age, operative status, PRISM score, FIO_2, respiratory rate, PIP, PEEP, PaO_2 & FIO_2 to accurately predict mortality (Timmons et al. 1995). Unfortunately the system is too unwieldy for routine clinical use. We therefore return to individual clinical assessment, PIP and A-aDO_2 or PaO_2/FIO_2 as being the most clinically useful parameters to select patients who are failing conventional treatment and may benefit from ECMO. Most clinicians would agree that it is appropriate to use ECMO in this fashion, but the exact size of the presumed survival advantage will need a randomised study to quantify.

The overall survival for the 81 paediatric respiratory patients is 77%, for a mean PaO2/FIO2 ratio of 61mmhg. The mean age was 34.8 months, duration of ECMO 191 hours, compared to 70 hours for the neonates, and 68% of patients were managed with veno-venous (VV) perfusion. Sixty eight percent of patients had pneumonia, and 21% ARDS. Excluding patients with miscellaneous diagnoses, or patient groups with small numbers, survivals and age between groups seem similar, irrespective of diagnosis. Exceptions are the large group (n=24) of young

(5.9 months) patients with Respiratory Syncitial Virus (RSV) who seem to do well, with 92% survival. Another exception is the older group (71.7 months) of patients with bacterial pneumonia who also have good overall survival at 89%.

The most important conclusion to be drawn from these results is that paediatric respiratory ECMO is much more complicated than neonatal respiratory support. The diagnoses are a heterogenous group, and patients require support for much longer than neonatal patients.

ADULT RESPIRATORY ECMO

The use of ECMO in adults with respiratory failure is also more complicated and less clear cut than the situation in neonates. There have been two randomised trials of ECMO in adult patients, neither of which have shown any difference in outcome between ECMO and control treatments. The first trial was the NIH sponsored study performed in the USA in the 1970's (Zapol et al. 1979). Only 4/48 conventional and 4/42 ECMO patients survived. Important lessons have been learnt from this study such as the importance of veno-venous, rather than VA perfusion, the use of lung rest, and selection of patients who have not been ventilated too long and too hard (see chapter 1). Modern ECMO practice is sufficiently different from the perfusion practised in this study to justify continuing, despite the study outcome.

Following the publication of Gattinoni's series of patients receiving $ECCO_2R$ (Gattinoni et al. 1986) with overall survival of 44.8%, it seemed that low flow $ECCO_2R$ coupled with low frequency positive pressure ventilation to provide apnoeic oxygenation may be an effective treatment in adult respiratory failure. We and other centres (RH Bartlett, personal communication) have tried to duplicate Gattinoni's technique, but have always been unable to provide sufficient oxygenation by the

apnoeic method, requiring higher extracorporeal flows to more fully support gas exchange. We believe this failure to be due to the advanced state of our patient's lung disease.

In order to more fully evaluate low flow $ECCO_2R$ Morris and colleagues conducted a randomised study of $ECCO_2R$ vs. Pressure Control Inverse Ratio Ventilation (PCIRV) in patients fulfilling the NIH ECMO criteria (Morris et al. 1994). Survival was 42% and 33% in the PCIRV and $ECCO_2R$ groups respectively ($p=0.8$). Morris noted that the survival in the PCIRV group was significantly better than the control patients in the NIH ECMO study. The inability of $ECCO_2R$ to adequately support gas exchange was also demonstrated by this study as the airway pressures were significantly higher in the $ECCO_2R$ group, and moreover the maximum accepted airway pressure was increased halfway through the study. Technical problems with haemorrhage and circuit thrombosis were also reported. The finding that low flow $ECCO_2R$, even when conducted by an inexperienced team, with many technical problems can give equal survival to the very best PCIRV is notable. We believe, as already discussed in chapter 1 that with circuits designed to allow almost full support of gas exchange, elimination of bleeding around cannulation sites and most importantly, lung rest, that ECMO can result in improved survival in adult patients who are failing conventional therapy.

In total we have treated 99 adult (>18 years) respiratory patients with ECMO up to the end of 1996. Overall survival is 63% for a mean PaO_2/FIO_2 ratio of 65 mmhg. Approximately half the patients presented with ARDS of diverse causes and half with pneumonia. Excluding very small groups, patients with aspiration pneumonia, trauma ARDS and septic ARDS seem to do well, with 71%, 79% and 83% survivals respectively. The worst survival is in the Obstetric ARDS group at

33%. In order to characterise our patient population more fully, the detailed study of the first 50 adult respiratory ECMO patients treated at our institution was conducted. To determine when ECMO was indicated in preference to continued conventional treatment we compared the outcome in these 50 patients with the PCIRV patients in Morris's study (Morris et al. 1994), and a large study of conventionally ventilated patients (Vasilyev et al. 1995). This comparison cannot take the place of a randomised trial, but may give some indication that the expected outcome in these patients is very poor, and that the survival with ECMO is acceptable.

The overall survival in the 50 patients was 66%. The mean change in PaO_2/FIO_2 ratio during transport was only -0.3 mmhg but the standard deviation of 48.6 mmhg shows that many patients were unstable, although none died. Thirty two out of 50 patients required inotropes, although the mean arterial pressure and cardiac outputs recorded were within the normal ranges. The cardiac outputs are expressed as ml/kg/min rather than L/SqM as body surface area was not recorded for most patients. The level of respiratory support received was assessed by recording the mode of ventilation, ventilator settings, and the use of advanced treatments such as Pressure control ventilation and inhaled nitric oxide. Mean minute ventilation of 12.6 l/min (mean patient weight 71.9 kg) indicates high volume ventilation, the mean Peak Airway Pressure of 39.6 cmH_2O and mean PEEP of 10 cmH_2O also confirm the maximal level of conventional respiratory support. Mean CVP and PAWP values of 12 mmhg (SD 3.8 & 2.2 respectively) confirm the non-cardiogenic nature of the pulmonary infiltrates affecting 3.8 (SD 0.4) quadrants of patients admission chest films. Patients respiratory failure was quickly progressive, mean duration of ventilation prior to ECMO referral was only 76.5 hours, with only 14 hours on 100% oxygen. The degree of respiratory failure was also assessed using the

Murray score (Murray et al. 1988), this score has a maximum value of 4.0 and scores above 2.5 indicate severe lung injury. Mean Murray score of 3.4 (SD 0.5) indicates advanced respiratory failure. Survival by group was similar in this cohort to the rest of our patients, with good survival in the Aspiration pneumonia patients (88%) and poor (25%) in patients with obstetric ARDS. Blood use was still heavy, even when cannulation site bleeding is eliminated. Mean blood usage was 19 units (SD 17.3), for a mean run time of 207.4 hours, equivalent to 2.2 units per day. This blood use also includes the 4-6 units of blood used to transfuse patients up to the target haemoglobin of 14g/dl from the admission haemoglobin of 10.8g/dl. Blood use in survivors was only 14.2 units, but as duration of ECMO was shorter at 163.5 hours this equates to 2.1 units per day. Use of platelets was also high at 47.1 units, as already discussed in chapter 1 platelets are transfused to maintain a count of 100,000/ml in order to prevent haemorrhage. The difference between platelet use in survivors and non-survivors is more marked at 27.6 and 86.3 units respectively ($p=0.018$). This equates to a platelet use of 4.1 units per day for survivors, and 7.1 units/day for non-survivors. The use of other blood products does not seem excessive. Despite these relatively large amounts of blood transfused, blood use was still lower than recorded by other authors (Gattinoni et al. 1986; Morris et al. 1994; Zapol et al. 1979).

An attempt was made to discover variables that could be used to predict non-survival. Four variables had statistically significant differences between survivors and non-survivors; Age, Blood use, Platelet Use and Duration of perfusion. Unfortunately it can be seen from the size of the standard deviations and in the case of age, closeness of the means, that these variables could not be used clinically to base decisions for withdrawal of treatment. Other authors have also found that

amount blood products used does not invariably predict non-survival (Butch et al. 1996).

The comparison of survival data from our population of ECMO treated patients with the two historical controls (Vasilyev et al. 1995; Morris et al. 1994) is not an ideal situation. Such a comparison could result in a Type 1 error as a result of treatment and historical control group being incomparable. Such an error is particularly likely when groups are not contemporaneous, as technology may change. For this reason the NIH study patients (Zapol et al. 1979) were not selected as the control group, despite the fact that survival for severe ARDS has changed little between 1979 (Zapol et al. 1979), and 1992 (Vasilyev et al. 1995). Differences in severity of illness may also lead to erroneous conclusions, we hope to have reduced this risk by selecting one control group with much less severe respiratory failure than our ECMO patients (Vasilyev et al. 1995). The entry criteria for Vasilyevs study was positive pressure ventilation with an FIO2 > 0.5 for > 24 hours, this essentially includes almost all ventilated patients in an Intensive Care Unit, rather than our group of patients all of whom have failed treatment in a conventional ITU. To show better survival ($p=0.036$) in the ECMO patients than this less severely ill group of patients largely over-rules reservations about comparability of groups. Indeed the use of these groups for comparison has been peer reviewed by the American College of Chest Physicians, and accepted for publication in "Chest"(Peek et al. 1997). The PCIRV group from Morris's study more closely matches our own patient population, as seen by the equivalence of the two groups PaO_2/FIO_2 ratios ($p=0.741$), as would be expected the survival advantage with ECMO is more marked in this sicker group of patients ($p=0.0006$). The fact that our ECMO population are relatively young at 30.1 years when compared to the general ITU population has been advanced as the

sole reason for the good outcome in our patients. This criticism is difficult to answer without age matched controls or a formerly randomised study. However, it is notable that in the neonatal, paediatric and adult populations hypoxia in the order of a PaO_2/FIO_2 ratio of 65mmhg is strongly predictive of death (Vasilyev et al. 1995; Tamburro et al. 1991; Bartlett et al. 1975).

Differences in intensity of care may also account for differences in outcome, however both studies were conducted in University intensive care units, and Morris's PCIRV protocol was computer controlled, so it is difficult to envisage more intensive management than this. We believe, therefore, that ECMO is an appropriate treatment for adults with severe, but potentially reversible respiratory failure who are deteriorating despite maximal conventional treatment. This comparison strengthens that assertion, but is not completely satisfactory. A fully randomised study of ECMO vs. conventional treatment in adult respiratory failure is being planned under the auspices of ELSO, to provide a better comparison than our use of historical controls. Organisation of such a trial would have to echo the neonatal ECMO trial (Anonymous. 1996), with centres referring all suitable patients for randomisation. Patients randomised to ECMO are then transported to the ECMO centre. This weights any comparison against ECMO as the morbidity/mortality of transport is then analysed as part of the ECMO group, finding a survival advantage is then definitely significant. Another benefit of this type of trial construction is that it eliminates the chance of two patients receiving different treatments in the same hospital, as it is very difficult for clinicians to withhold a treatment they believe to be effective if the other treatment is not. Another way round this problem is to have a cross-over design, but in a study with such small numbers this is ill-advised. Cross-over of patients between treatment groups also introduces an element of doubt

regarding their outcome, whether it is as a result of, or in spite of, a particular treatment.

CARDIAC ECMO

The outcome with conventional treatment in patients suffering severe cardiac failure is not well documented. It can be assumed that all patients who fail to wean from cardiopulmonary bypass would die if not treated with extracorporeal support. However, in patients developing post operative low cardiac output syndrome that is refractory to inotropes and afterload reduction, whilst we assume that most of these patients would die, we cannot be certain of the exact numbers. The University of Michigan Criteria (see chapter 1) for cardiac ECMO case selection are based on a study comparing survival in paediatric cardiac patients before and after the inception of an ECMO programme (Klein et al. 1990). Of 312 patients having open cardiac operations in the pre-ECMO era 27 died. Age, weight, circulatory arrest time, crossclamp time, heart rate, systolic BP, left atrial pressure, right atrial pressure, urine output, dopamine dose, isoprenaline dose, fluid administration, FIO_2, PaO_2, ventilator rate and pH were all useful in predicting mortality. Combining variables was more predictive, and showed that 91% of non-survivors had a urine output of < 1.2 ml/kg/hr and dopamine infusion of > 5 mcg/kg/min at 1 hour post operatively, whereas only 3.1% of survivors achieved these parameters. After ECMO became available 36 post operative cardiac patients were supported, 61% survived. The ECMO treated population were essentially identical to the pre-ECMO population in terms of the predictive indices given above, survival of 61% in this group of moribund patients was interpreted as a significant survival advantage.

The Glenfield series of paediatric cardiac patients includes 28 children with 61% survival. Numbers within diagnostic groups are small, and so few conclusions can be drawn, but subjectively we believe that patients with isolated right ventricular failure have the best outcome from ECMO support. Further clinical and subjective impressions are discussed in chapter 1.

Our experience with ECMO support in adult cardiac patients is less successful with only 3 survivors from 8 patients. However both patients with pulmonary embolism did well and appeared to be in end stage right heart failure prior to the initiation of ECMO, which was performed with patients conscious, and self ventilating throughout the entire ECMO run.

In summary we believe that it is appropriate to offer cardiac ECMO support to paediatric patients who fulfil the University of Michigan criteria, and good outcomes are possible. Adults, however, should only be treated if they have a surgically correctable lesion (Nishina et al. 1995; Hochman et al. 1995; Murakami et al. 1994), as a bridge to transplant, or in cases of massive pulmonary embolism (Davies et al. 1995).

CONCLUSION

The use of ECMO for the treatment of neonates with severe but potentially reversible respiratory failure who have an OI > 40 despite maximal conventional treatment is now accepted following the conclusion of the UK trial. Mortality is reduced by approximately one half, with no increase in morbidity. The use of ECMO in adults and children with respiratory failure is more controversial, although case control studies and comparison with historical controls indicate that this form of treatment is

promising. Properly constructed randomised trials are needed to further clarify the situation, however, due to the smaller numbers and heterogeneity of adult and paediatric ECMO patients the organisation of such a trial will be difficult.

The use of ECMO for cardiac support is more effective in children than adults. This could be due to the higher prevalence of right ventricular failure in children as patients with predominately right ventricular failure seeming to respond better than those with left or bi-ventricular failure. It is likely that this difference is a result of the haemodynamic effects of high afterload and myocardial ischaemia on the left ventricle. Predicting the outcome with conventional treatment in the cardiac patients is even more uncertain than those with respiratory failure, but in the children at least treatment seems to result in increased survival. Construction of a randomised trial in cardiac patients would be almost impossible due the very small numbers, and differing diagnoses. It is probably not appropriate to consider ECMO for cardiac support in adults except in selected cases of massive pulmonary embolism, where further surgical treatment is possible, or as a bridge to transplant.

CHAPTER 4

MECHANICAL ASPECTS OF TUBING WEAR DURING ECMO

INTRODUCTION

The De-Bakey type occlusive roller pump (DeBakey, 1934) is an elegant solution to the problem of propelling blood in the extra-corporeal circuit. They are efficient and the blood path is an entirely disposable piece of tubing which is relatively easy to manufacture. Unfortunately roller pumps can cause significant blood damage and haemolysis (Bernstein and Gleason, 1967) if improperly calibrated (over-occluded). Tubing wear resulting in liberation of particles from the tube wall into the blood (Spallation) also occurs, and is one factor in the micro-embolism observed during ECC (Page et al. 1974; Solis et al. 1975). During the prolonged roller pump use of ECMO raceway tubing may actually rupture leading to air embolism, haemorrhage, and 50% mortality (ELSO Registry). In this chapter spallation and raceway rupture will be investigated in a series of 3 experiments. Each experiment will be presented and discussed separately, but the conclusions will be presented together at the end of the chapter.

EXPERIMENT 1: DESTRUCTION TESTING

INTRODUCTION

The raceway is the piece of tubing within the actual pump boot, and swept by the rollers. The gap between the rollers and the pump boot (occlusion) can be adjusted according to the wall thickness of the tubing used. Maximum forward flow occurs at full occlusion i.e. when the tubing is squashed completely flat by the rollers, and retrograde regurgitation of blood past the roller cannot occur.

Unfortunately full occlusion also results in significant blood damage(Bernstein and Gleason, 1967; Bernstein et al. 1967). The ideal occlusion settings for ECMO are therefore said to be slightly under occlusive. This is defined as settings which just allow reflux of air past the rollers prior to circuit priming. Such under occlusion has been shown to result in less haemolysis (Bernstein and Gleason, 1967) than full occlusion, and is also thought to prolong tubing life. The exact duration that a tubing can be used in a roller pump before it ruptures is dependant on many factors which are not fully understood, but are thought to include the occlusion setting, revs per minute, pressure and temperature (Mortensen et al. 1979). Manufacturers of extracorporeal tubing are unwilling to specify safe maximum time limits for tubing use due to fear of litigation. This fear is well founded and has resulted in a significant out of court settlement following a raceway rupture during paediatric ECMO in Phoenix, Arizona, USA. In this experiment the longevity of two potential new ECMO tubings, LVA and SRT are compared to that of the currently used ECMO tubing, Tygon, as a control.

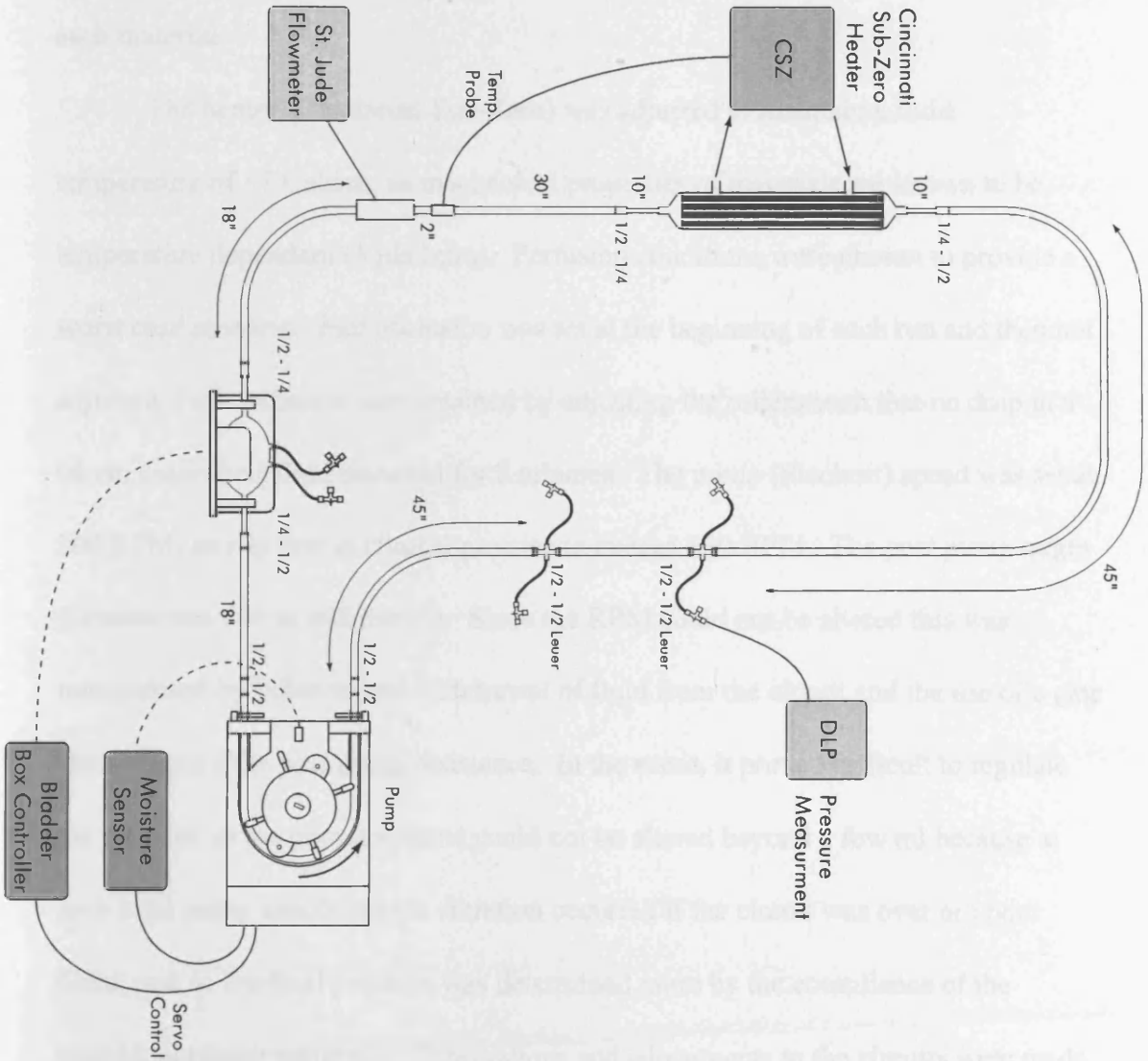
AIMS

To measure time to tubing rupture of Tygon, LVA and SRT under conditions exceeding those seen during clinical perfusion.

MATERIALS & METHODS

A closed loop circuit was constructed and primed with Hartman's Solution. The same circuit was used for all experiments with the raceway being replaced each time. The pump was servo regulated by the conventional bladder box and controller (Seabrook), and also by a moisture sensor (manufactured in house) which was placed

Fig. 4.1 Destruction Testing Circuit



in the well of the pump. In the event of raceway rupture fluid on the sensor caused the pump to stop and also stopped an integral timer (see Figure 4.1)

The two potential new ECMO tubings: LVA (Portex 800-500-575, Portex Industries, Hythe, Kent, UK) and SRT 620 (Rehau UK, Langley, Slough, UK) were compared to Tygon S-65-HL (Norton Performance Plastics, Akron, Ohio, USA), as a control. The end point was time to tubing rupture. Five runs were performed for each material.

The heater (Cincinnati Sub-Zero) was adjusted to maintain a fluid temperature of 37 Celsius, as mechanical properties of materials are known to be temperature dependant (Vide Infra). Perfusion conditions were chosen to provide a worst case scenario. Full occlusion was set at the beginning of each run and then not adjusted. Full occlusion was obtained by adjusting the rollers such that no drop in a 60 cm column of fluid occurred for 2 minutes. The pump (Stockert) speed was set at 200 RPM, as it is rare in clinical practice to exceed 130 RPM. The post pump target pressure was 400 to 600 mmHg. Since the RPM could not be altered this was manipulated by infusion and withdrawal of fluid from the circuit and the use of a gate clamp to vary the post pump resistance. In the event, it proved difficult to regulate the pressure as the circuit volume could not be altered beyond a few ml because at such high pump speeds severe vibration occurred if the circuit was over or under filled, and so the final pressure was determined more by the compliance of the individual tubing materials. Observations and adjustments to the circuits were made on a daily basis. It was not possible to conduct experiments in a random order due to the availability of tubing materials and concurrent in vivo experiments. An electro-magnetic flow meter (St.Jude) was used for the second half of the experiment.

STATISTICAL METHODS

Failure times for LVA and SRT were compared to the control, Tygon, using the un-paired students t-test. A p value of < 0.05 was taken to indicate significance.

RESULTS

FAILURE TIME: Times to tubing rupture, mean and (SD), for the three materials were as follows: Tygon (Control) 243.7 hours (175.4), LVA 121 hours (14.3) and SRT 6.6 hours (2.1). Failure times for both LVA and SRT were significantly different from the control (unpaired t-test, $p<0.001$). The minimum failure times for Tygon and LVA were 99 hours and 101 hours respectively. Failure times are displayed graphically in figure 4.2.

TYPE OF FAILURE: Tubing failed in three ways (Fig 4.3); through the longitudinal crease built up by the repeated folding of the tubing in the pump, through transverse tears, presumably caused by shearing, and also through scallop shaped defects in the inlet side of the raceway, see Figure 4.3. The relative frequency of each mode of failure is given in the table below. Note that one piece of LVA failed simultaneously in two places.

TABLE TO SHOW TYPE OF TUBING FAILURE

TUBE TYPE	N	TYPE OF FAILURE		
		SCALLOP	CREASE	TEAR
SRT	5	5	0	0
LVA	5	0	5	1
TYGON	5	0	3	2

Fig. 4.2
TIME TO TUBING RUPTURE

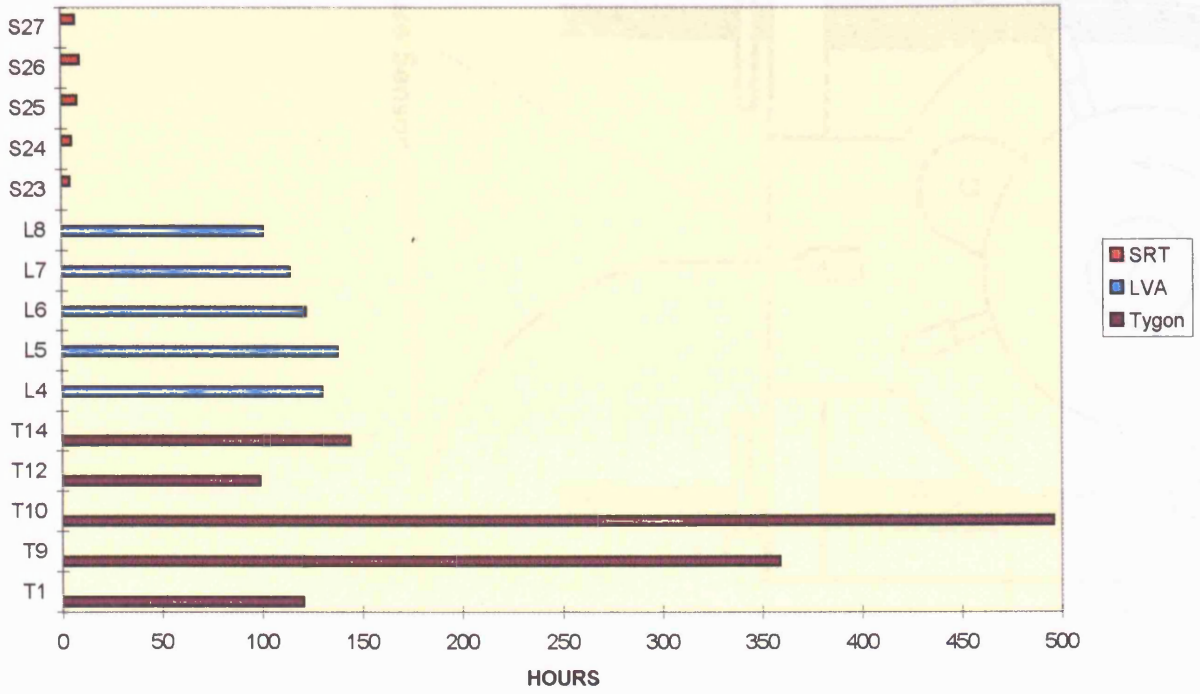
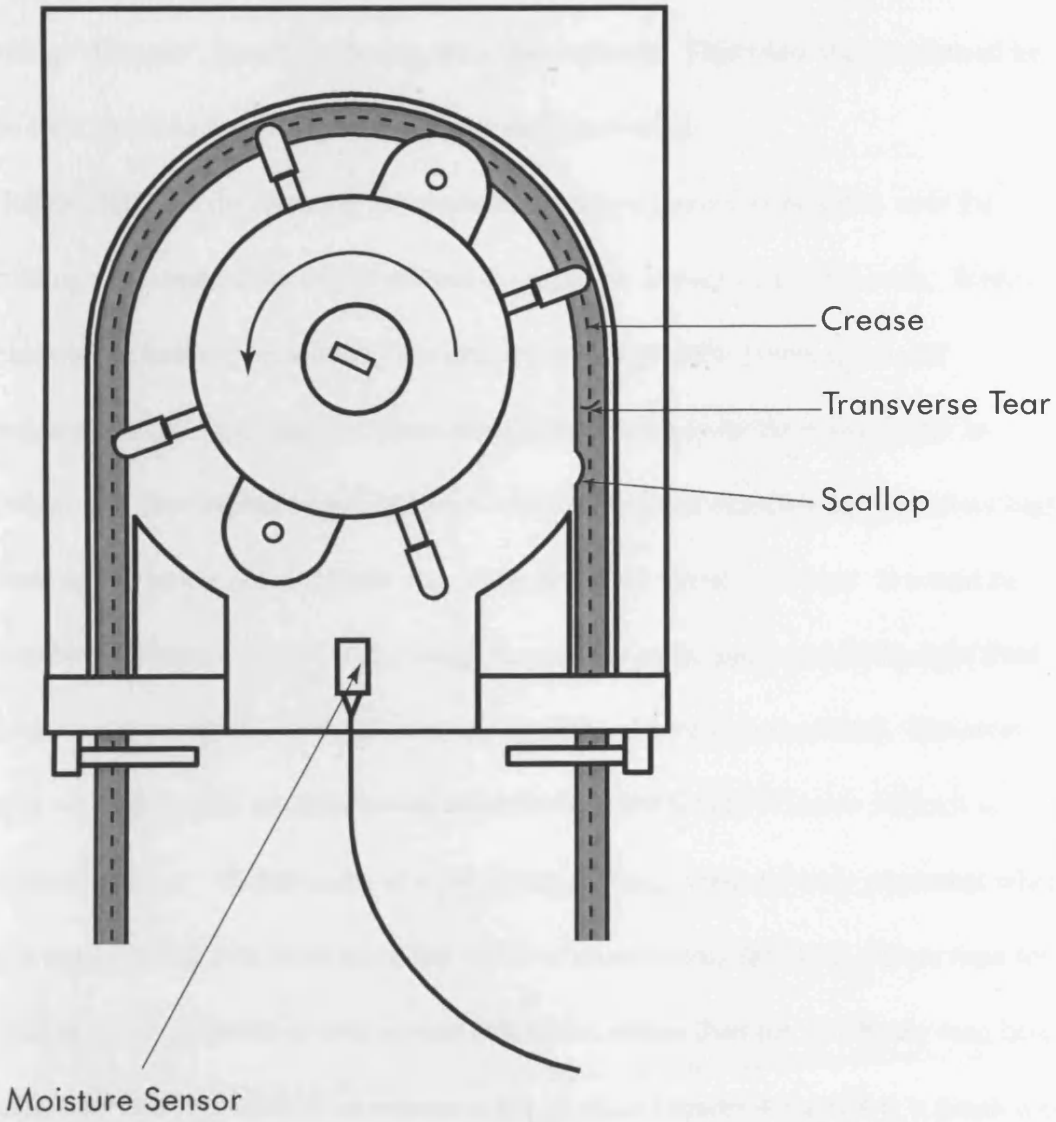


Fig. 4.3 Types of Failure



REDUCTION IN FLOW WITH TIME: The flow meter was only available from half way through the experiments only 3 runs with Tygon and the five pieces of SRT were examined. Since all the SRT failed between set-up and the first time point, meaningful results were only obtained with the Tygon. It can be seen from Figure 4.4 that flow gradually decreases over time despite a constant RPM. This was presumed to be due to a progressive loss in the tubing's ability to resume a circular shape during "diastole", thereby reducing the stroke volume. This view was confirmed by the oval shape of the tubing at the end of the experiment.

PRESSURE: As discussed in the methods section it proved impossible with the existing experimental circuit to control the pressure at such high flow rates. Whilst this was not entirely satisfactory the importance of constant pump speed and temperature took precedence. Since shear stress seems to be the major factor in tubing wear (see experiment 2 below) it was decided that maintaining a constant high pump speed was more important than controlling the circuit pressure. It would be possible to design a circuit with a large venous reservoir, and a variable height fluid column post pump in which the pressure could be accurately controlled. However such a circuit would require custom manufacture, and would be more difficult to thermo-regulate. Maintenance of a physiological temperature is very important when measuring tubing life, as in an earlier series of experiments the mean failure time for LVA at room temperature was around 300 hours, rather than the 121 hours seen here. Circuit pressures during all experiments are given in Figures 4.5 and 4.6, a graph was not drawn for SRT since only the starting pressures were recorded as the failure times were so short. Mean Starting pressure for SRT was 600.2 mmHg with a Standard Deviation of 20.36.

Fig. 4.5
Tygon: Pressure vs Time

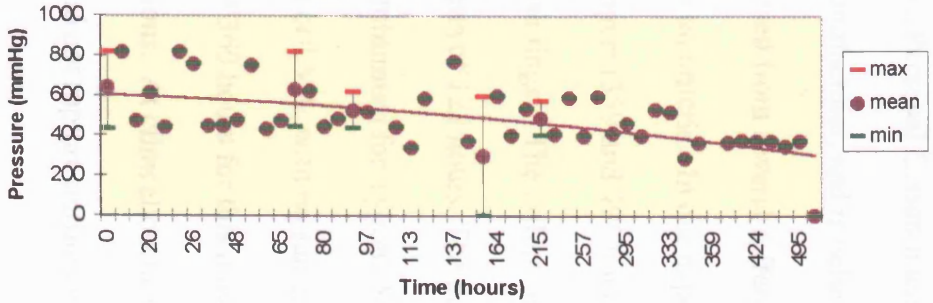


Fig 4.6
LVA: Pressure vs Time

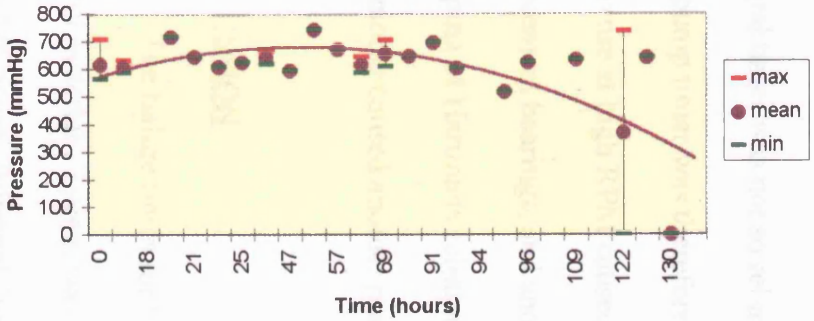


Fig. 4.4
Tygon: Flow vs Time

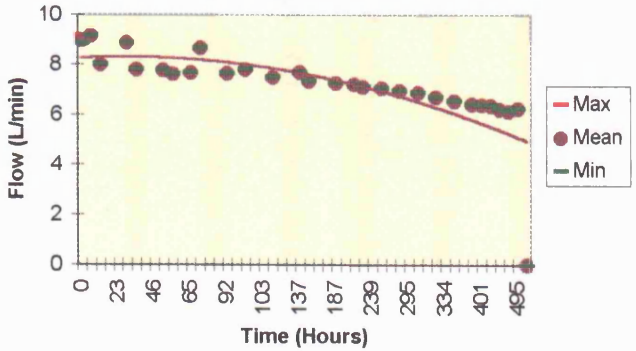
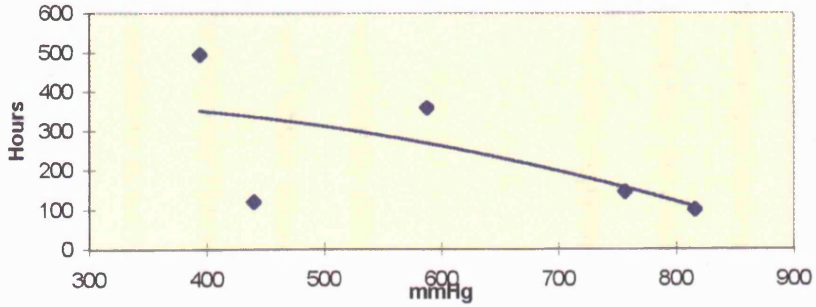


Fig. 4.7
Tygon: Failure time vs Pressure



EQUIPMENT FAILURES: During the course of this experiment the moisture sensor functioned reliably in terms of servo-regulating the pump. Unfortunately the integral timer was not so reliable, and often failed to stop when the tubing ruptured. The pump timer was therefore used for all values given herein. The continuous pump use at high RPM caused the bearings on one pump to fail, requiring replacement bearings, and another pump was so badly damaged as to be irreparable. Dripping of Hartmans solution from the well of the pump into the casing and onto the spindle caused another pump to seize, this pump was also irreparable.

DISCUSSION

The failure times for the three materials were significantly different. The control material , Tygon, was the most unpredictable with failure times ranging from 99 to 496 hours. Informal discussions with Norton Performance Plastics (Sue Hinson, Personal Communication) revealed that this variability is well recognised by the manufacturer, and is believed to stem from the fact that the base polymer is purchased from several different suppliers. Tubing is passed if it passes certain minimum criteria. In our experiments the two pieces of Tygon tubing which lasted the longest (359 and 496 hours) were from a new shipment, and had a slightly greenish tinge. The failure time for the LVA was more predictable, being close to the mean of 122 hours. The minimum failure time being 101 hours, similar to the 99 hours minimum for Tygon. SRT is an order of magnitude less durable than either of Tygon or LVA, with no run exceeding 9 hours. Rehau quote an unofficial durability time of 360 hours for this material, but are unwilling to release the data and testing conditions. At clinical occlusion settings in the in vivo experiments (Chapters 5 & 6) SRT did not rupture during up to 48 hours of perfusion. All pieces of SRT had

scallops on the inlet portion of the raceway, one piece ruptured through the scallop after the animal was clamped off prior to decannulation, presumably failing at this point when the inlet pressure became positive as the circuit was re-circulated. A further series of destruction runs was started with the SRT with gradually decreasing occlusion, but still at 200 rpm. Unfortunately the pump seized up following two runs, and further experiments were cancelled. Under-occlusion to give flow rates of -5% and -13% of fully occluded flow resulted in failure times of 7 and 5 hours respectively. These times are similar to those seen with full occlusion. Comparison of materials at lower pump speeds and different levels of occlusion may have resulted in more acceptable durability for the SRT, however this would not be clinically relevant to adult ECMO where extreme durability is a pre-requisite.

There appeared to be two failure mechanisms resulting in three different types of tubing failure. Firstly folding of the tube across its axis resulting in cycles of compression and tension causes the tube to fail along a longitudinal crease, this was the predominant type of failure for the LVA, but was also evident in 3/5 pieces of Tygon. This mechanism is also responsible for the loss of restitution shown by the gradual diminution in flow with time, and the eventual oval shape of the tubing at the end of each run. This type of failure is the mode 1 failure discussed in experiment 2. The other types of tubing failure through transverse tears or "scallops" on the inlet side of the tubing are most likely to be due to shearing forces set up by the rotation of the rollers over the tubing which is held still by the shoes and clamps of the pump boot and also by friction between the outer wall of the tubing and the pump boot. This mode 2 failure seems to be an important mechanism in the failure of Tygon occurring in 2/5 runs, but also evident on pieces of Tygon tubing used clinically (see experiment 2).

The circuit pressure seemed to be controlled by the compliance of the tubing materials. This can be surmised from the similarity of pressures between materials, SRT around 600mmHg, LVA 600-700 mmHg and Tygon again showing the most variability 400-800 mmHg. It could be argued that the Tygon durability was variable because the pressures were variable, or that pressures and durabilities were both related to the mechanical properties of each piece of tubing, which are known to be variable. This question would be difficult to answer due to the very variability of the tubing, but an experiment with servo-control of the circuit pressure and large numbers would be required. The inverse correlation between median circuit pressure and failure time for Tygon (Figure 4.7, above) could be a result of the confounding effects discussed above, or be a result of variability in tubing compliance, in that more compliant tubing will have a lower working pressure and will require less energy to deform it during each compression-relaxation cycle, and will therefore last longer. Again complex experiments in the engineering laboratory are needed to answer this question.

EXPERIMENT 2: MECHANICAL TESTING

INTRODUCTION

There seems to be very little published work concerning the forces generated in raceway tubing during roller pump use. A fuller understanding of the exact mechanisms of tubing failure would seem to be essential if tubing rupture is to be prevented. Accordingly help was sought from the Department of Engineering, University of Leicester, to characterise the mechanisms at work during tubing wear.

This investigation was part of a final year Engineering BSc project for KM Wong (Degradation of flexible piping in a peristaltic pump), and was supervised by CJ Morrison. The authors involvement in the project was in the design of experiments, provision of tubing materials, and in collaboration with Mr Morrison, review of results and interim reports. The investigation was originally conceived as a thorough analysis of all the forces involved in tubing failure with computer modelling, finite element analysis (FEA) and development of test rigs to duplicate the forces seen clinically . The final goal was to be a computer model of the ideal tubing material. Unfortunately tubing manufacturers were reticent about the mechanical properties of their materials, which therefore had to be measured. During the delay so engendered another student damaged the load cell on the FEA machine, so the computer modelling was no longer possible. The eventual work that was completed by Mr Wong will be presented below.

AIMS

i) To measure the number of load cycles required to cause tubing failure due to compression alone, in the absence of shear.

ii) To examine tubing used clinically by scanning electron microscopy (SEM) in order to determine which forces have been acting on the material.

MATERIALS & METHODS

i) COMPRESSION TESTING: A test rig was constructed to generate pure compressive forces in a piece of ½ inch Tygon S-65-HL tubing (see Figure 4.8 & 4.9). The apparatus consisted of a circuit filled with Hartman's solution. Fluid was pumped through the piece of Tygon under test by a magnetically coupled

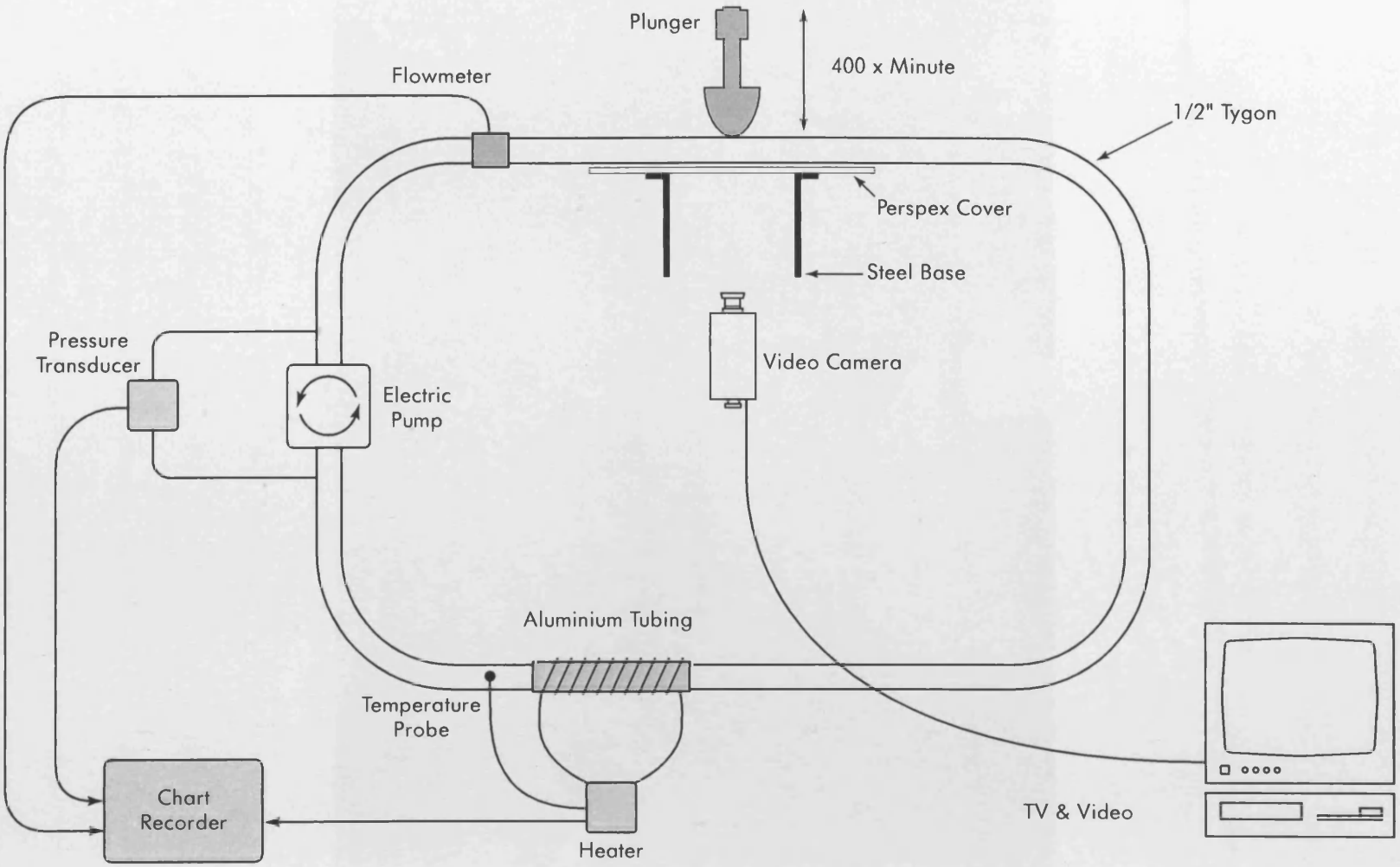
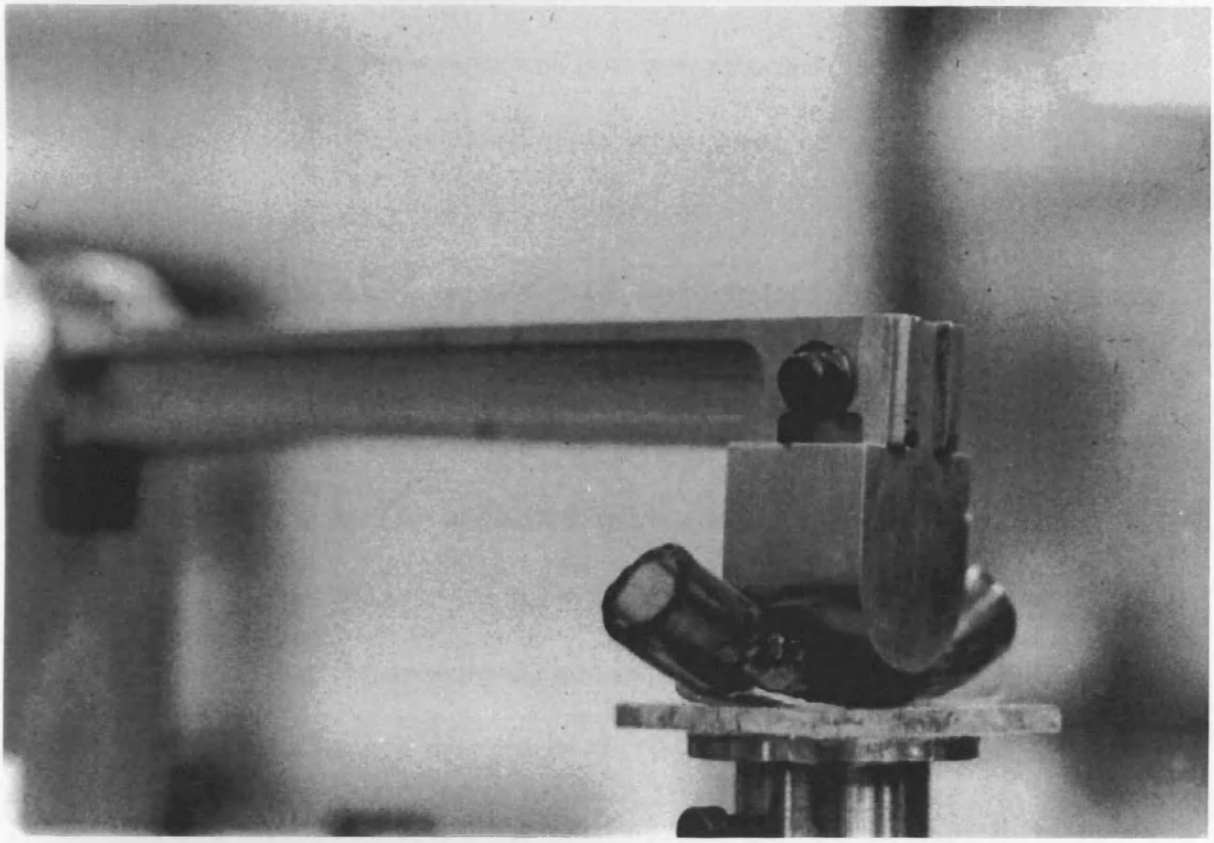


Fig. 4.8 Apparatus for Compression Testing

Fig. 4.9 Arm of Compression Test Rig



pump (RS) at a flow rate of 5.6 L/min, confirmed by a flow sensor. Fluid was maintained at 37^0 Celsius by a servo-regulated heating coil placed around part of the circuit made from aluminium tubing. Inlet pressure (to the RS pump) was maintained at 50 mmHg and outlet pressure at 200 mmHg. The tubing under test was compressed by a plunger on a moving arm at a rate of 400 x per minute (equivalent to 200 rpm with 2 rollers). Occlusion was set at full, by calculation of the required occlusion from measurements of the tubing wall thickness, and confirmation by pressure transduction and the closed circuit television system (CCTV). Pressure, temperature and flow were recorded continuously by means of a chart recorder, whilst evolution of cracks was recorded by time lapse CCTV. The tubing compliance was also recorded daily.

ii) SEM: A piece of ½ inch Tygon S-65-HL was examined that had been used during a clinical ECMO run for approximately 48 hours at 75 rpm. The tubing was taken and examined with the naked eye. The portion with the most visible wear marks was cut out as a specimen and a mark was made parallel to the fatigue cracks (i.e. the crease). The specimen was frozen in liquid nitrogen and then struck on the mark with a hammer, breaking the tube in two along the crease. The tube was thawed to room temperature, coated with Gold film and then examined under the Scanning Electron Microscope (SEM).

RESULTS

i) COMPRESSION TESTING: Compression testing was continued until the compliance curve (Figure 4.10) reached a plateau. This was after 3.67 million stress cycles, equivalent to 305.8 hours at 200rpm. Tubing was then cut and examined macro and microscopically, no cracks were visible, the tubing had not

Fig. 4.10 Compliance Testing

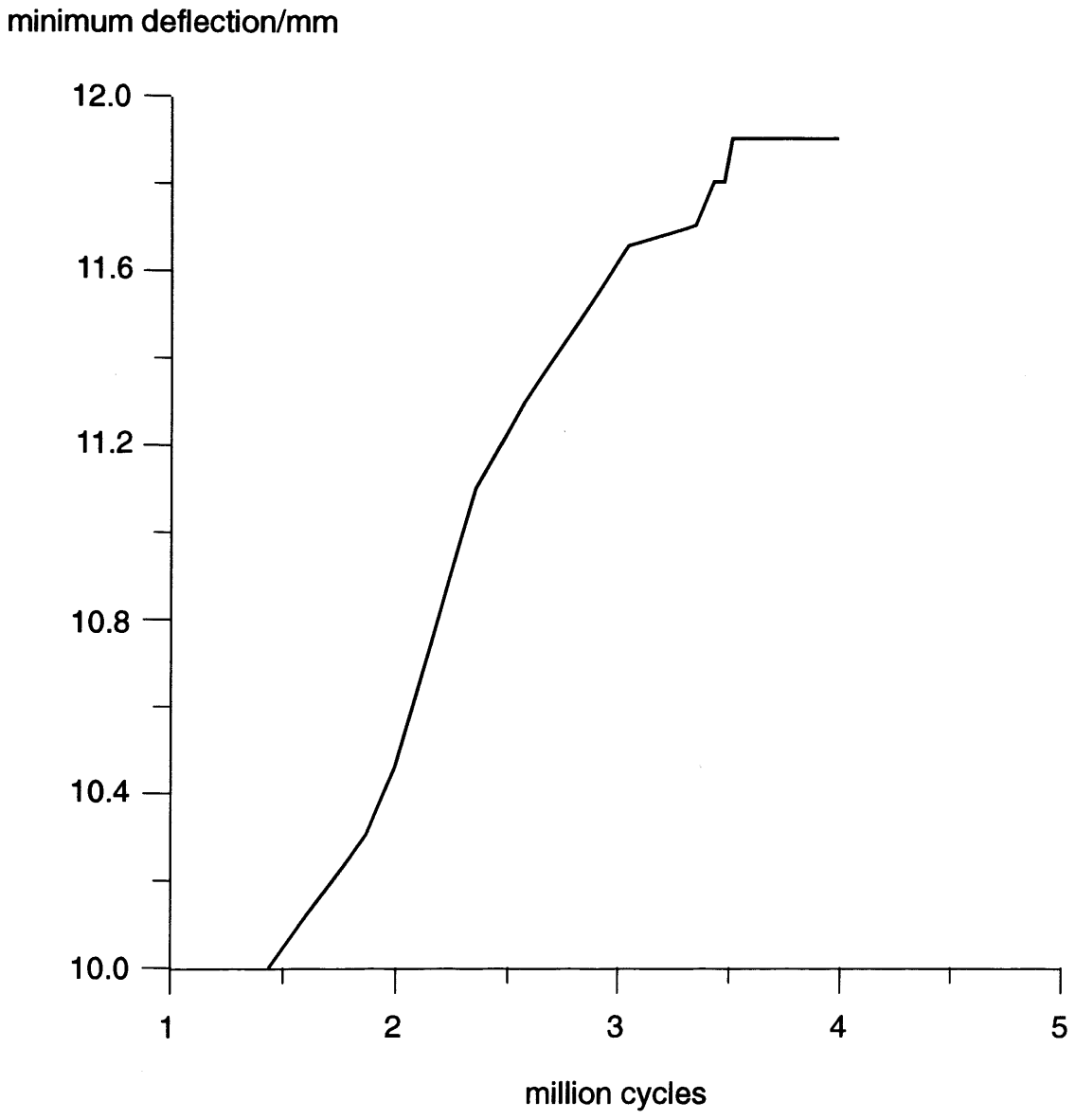
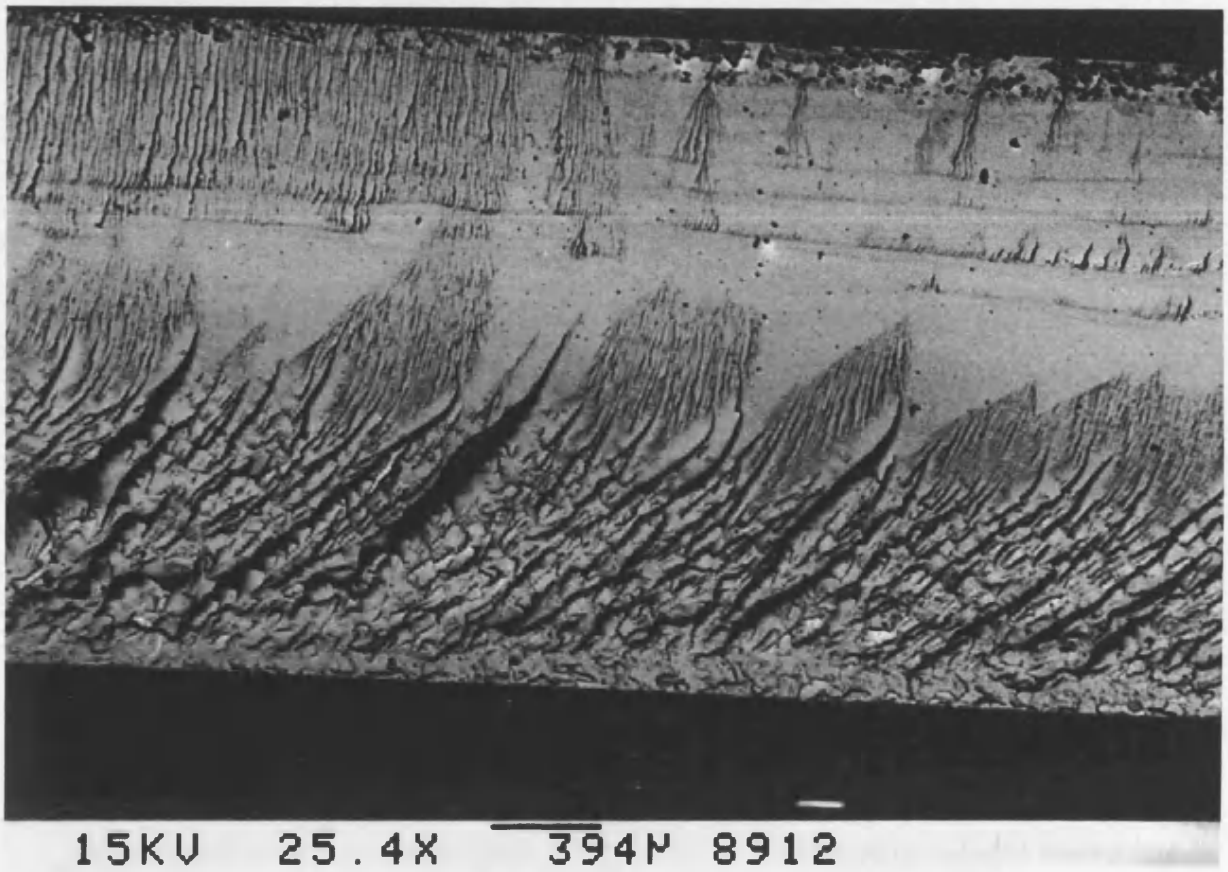


Fig. 4.11 Scanning Electron Micrograph of crack fractured Tygon S-65-HL



failed. A further experiment was performed with the tubing over occluded, fatigue cracks developing within 24 hours.

ii) SEM: A representative electron micrograph is shown above (Figure 4.11). The bottom of the picture is the luminal surface of the tube, and the roller was moving left to right. The dominant features in this specimen are the striations extending from the luminal surface of the tube, and swaying towards the right. These marks are evidence that shearing force is acting upon the tubing in addition to the compressive forces already discussed.

DISCUSSION

i) COMPRESSION TESTING: Engineers use the word "failure" in the context of fatigue testing to indicate a loss of the original mechanical properties of a material. Clinicians and Perfusionists, however would not consider the tubing to have failed until rupture occurred, or looked inevitable. We know from clinical tubing use and the SEMs that fatigue cracks are present in Tygon after 24 to 48 hours, and strictly therefore the tubing has "failed". However, in experiment 1, the tubing continued to function for a minimum of 99 hours before eventual rupture occurred. The fact that the tubing can be compressed and released 3.67 million times without developing fatigue cracks indicates that if shear forces could be eliminated or reduced that tubing life is likely to be greatly prolonged. Other groups have shown that shear is also the force responsible for much of the blood damage in both roller pumps and centrifugal pumps (S Ichiba, personal communication) (Bernstein et al. 1967; Pedersen et al. 1997), and have been working to develop non-occlusive roller pumps like the Michigan / Affinity pump (Avecor) and the Collin-Cardio (Healthcare Materials SA) pump. These pumps reduce shear by eliminating the pump boot, the raceway being

stretched over the rollers, tube life would theoretically be much longer in these devices. However there are limitations with non-occlusive peristaltic pumps as to which types of tubing they can use, as the raceway must be distensible. The Michigan pump use a custom made poly-urethane raceway constructed from two flat sheets, like a "popcicle" packet, whilst the Collin-Cardio pump uses oval silicone tubing. Tube life of 121 hours has been recorded with the Collin-Cardio pump in our laboratory for a standard silicone material that would be expected to last 6-12 hours in an occlusive roller pump. So although the life of the material is enhanced the limitations on which materials can be used may mean that there is little advantage in terms of patient safety and raceway maintenance intervals.

ii) SEM: The fact that the tubing which was loaded by pure compressive (mode 1) forces did not fail indicates that this mechanism does not initiate cracks. The presence of skewed cracks in the clinical tubing specimen indicates that longitudinal forces (Shear or mode 2) must play an important part on crack initiation and propagation.

EXPERIMENT 3: SPALLATION

INTRODUCTION

Spallation is the liberation of tubing fragments from the luminal surface of the tube into the blood path. These fragments act as micro-emboli as they are perfused into the patient (Uretzky et al. 1987; Boretos and Wagner, 1971; Orenstein et al. 1982). Arterial line filtration can greatly reduce embolism from tubing spalls and other particulate debris in the circuit and infusion fluids (Page et al. 1974; Reed et al. 1974; Solis et al. 1975; Connell et al. 1973), and is used routinely during CPB

in many centres. Unfortunately arterial line filtration is not a viable option during prolonged ECMO perfusion as filters tend to clot rapidly under low range heparinisation, and can act as a source of emboli (R Reeves, personal communication), thereby negating their function. Little is known about the spallation characteristics of ECMO tubing, we assume that the amount of spallation which occurs with Tygon S-65-HL is clinically acceptable as this is the tubing in widespread use. The prospective new ECMO tubing's LVA and SRT must have similar amounts of spallation to Tygon to be acceptable.

AIMS

To measure the amount of spallation with Tygon and compare it to LVA and SRT. Also to determine the relationship between spallation and occlusion, pump speed, and time for all three materials.

MATERIALS AND METHODS

The two potential new ECMO tubings: LVA (Portex 800-500-575, Portex Industries, Hythe, Kent, UK) and SRT 620 (Rehau UK, Langley, Slough, UK) were compared to Tygon S-65-HL (Norton Performance Plastics, Akron, Ohio, USA), as a control. All raceways were ½ inch diameter and 3/32 inch wall thickness, or nearest metric equivalent. The test circuit was the same for all runs, with the raceway being changed each time (see Figures 4.12 & 4.13).

The circuit design was intended to allow variation in inlet and outlet pressures by varying the height of the venous reservoir and heat exchanger respectively. However the ceiling of the laboratory was not high enough to allow this to be done, and therefore the reservoir and heat exchanger heights were left constant for all runs.

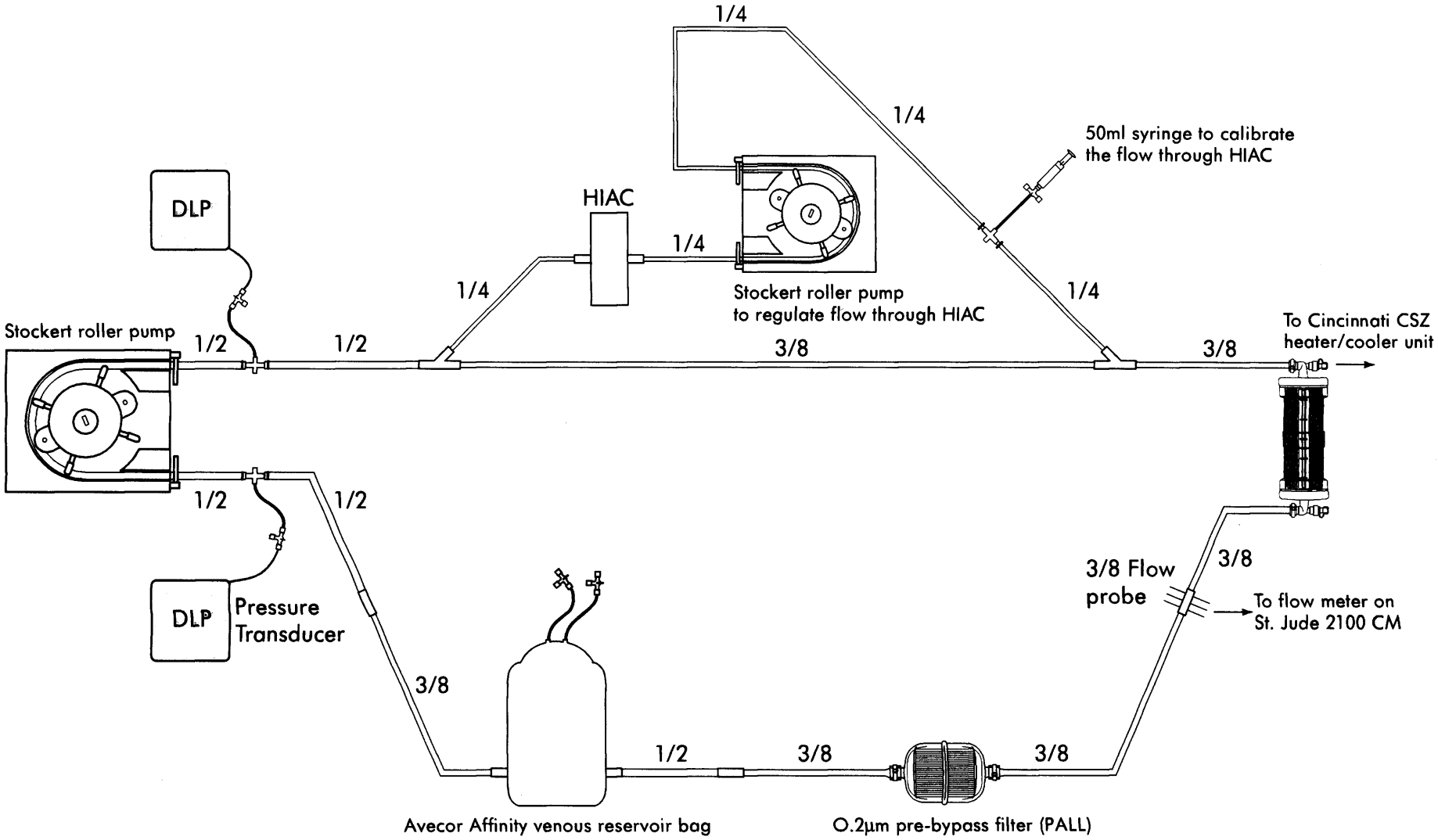
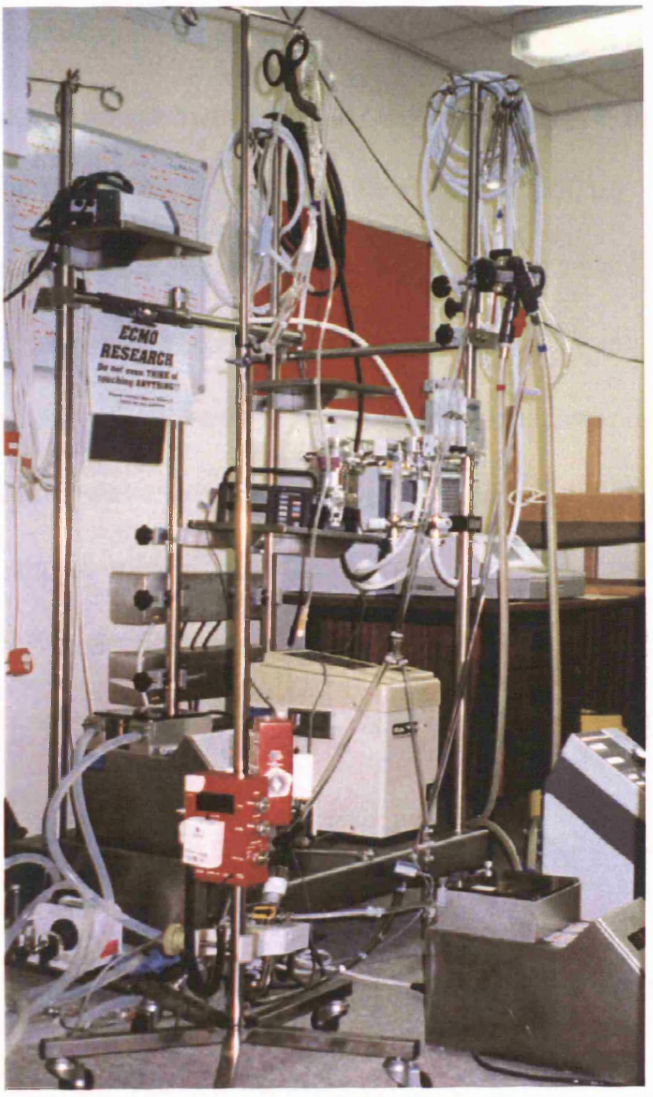


Fig. 4.12 Spallation Testing Circuit

Fig. 4.13 Spallation Testing Circuit



Inlet and outlet pressure was therefore a function of flow. Normothermia (36-37^0 Celsius) was maintained using the Cincinnati Sub-Zero heater. Occlusion was set dynamically, adjusted against the flow recorded by the St.Jude electromagnetic flow meter. Spallation was measured using the HIAC particle counter (Pacific Scientific International, High Wycombe, UK.), flow rate through the laser diode counting chamber was controlled at a constant 60 ml/min, using a second Stockert pump, placed post counter. Flow through the sensor was calibrated by a modified "bucket test" using a syringe, stopwatch and clamping the tubing distal to the syringe. Sampling time was 15 minutes giving a sample volume of 900 ml. Particle counts were expressed as counts per litre, and an average of 5 counts were taken for each set of experimental conditions. For the prolonged experiments (24 and 72 hours) the HIAC counter was set with the same flow rate of 60ml/min, sampling time was 60 minutes, and sampling was continuous. Thus particle counts are given as counts per litre for the entire duration of the experiment. All counts were then multiplied by the pump flow rate to give the final result in particles per minute. For each tube material the following experiments were performed, using a fresh piece of tubing each time the conditions were altered:

Experiment	Occlusion	RPM	Duration
Occlusion	10% to Full	100	75 Mins each
RPM	Full	0 to 200	75 Mins each
24 Hours	Full	100	24 Hours

A further experiment was also conducted where a piece of Tygon was examined at full occlusion and 100 rpm for 72 hours.

STATISTICAL METHODS

Data for the 24 hour runs were compared using the Wilcoxon Signed Ranks Test as only one run was performed per material. The two test materials (LVA and SRT) were compared to the control (Tygon). Data for the occlusion and RPM experiments was examined in a similar way as there was only one piece of tubing per occlusion or RPM setting. a P value of < 0.05 was taken to indicate significance.

RESULTS

Data are summarised below, and graphically in Figures 4.14 - 4.27.

Experiment	Tygon vs. LVA	Tygon vs. SRT	Comment
RPM	NS	P=0.043	Tygon<SRT
24 Hours	P < 0.001	P < 0.001	Tygon<Both
Occlusion	P=0.043	NS	Tygon>>Both

SPALLATION vs. PUMP SPEED (RPM)

Fig. 4.14
Tygon: Spallation vs RPM

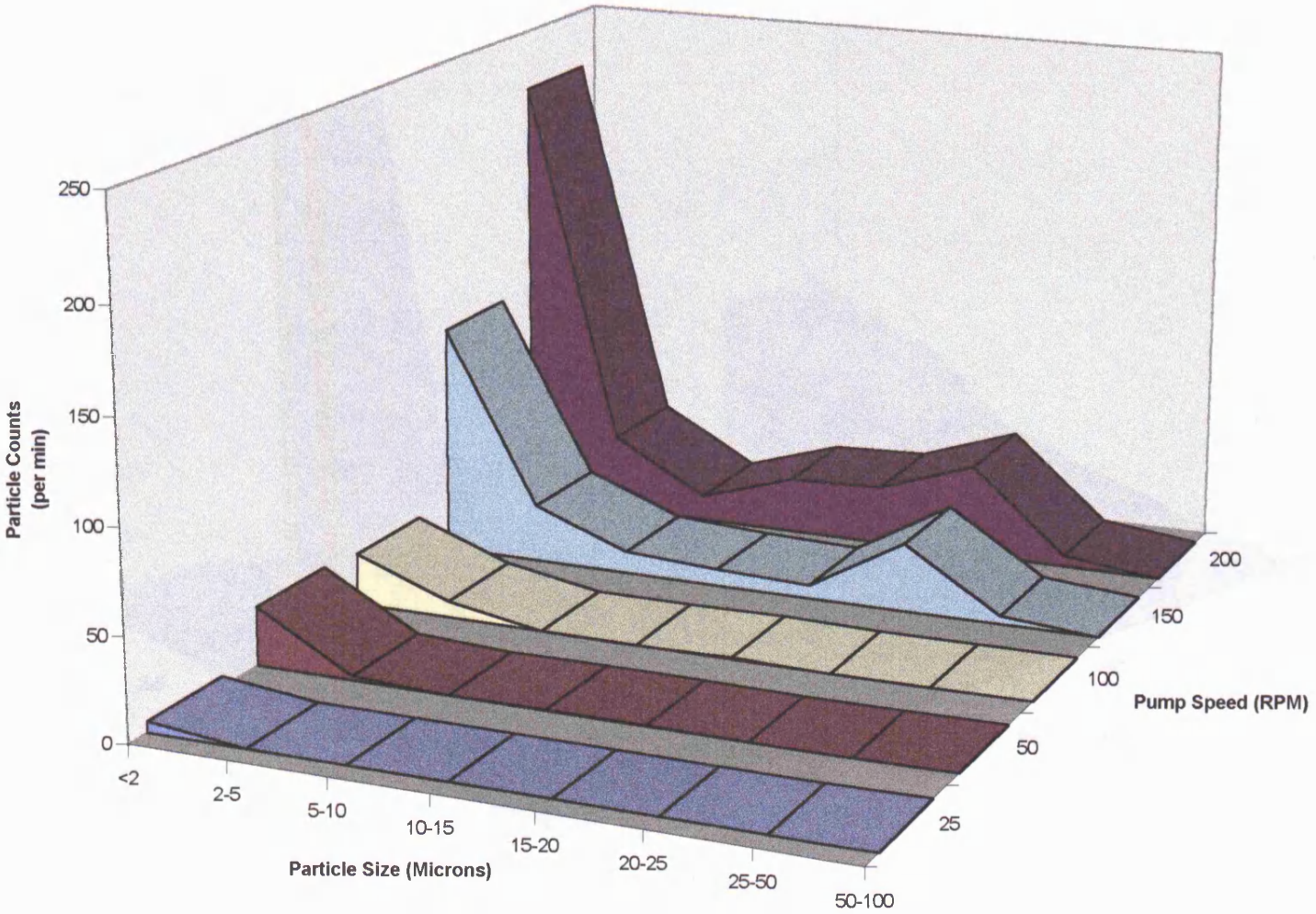


Fig 4.15
LVA: Spallation vs RPM

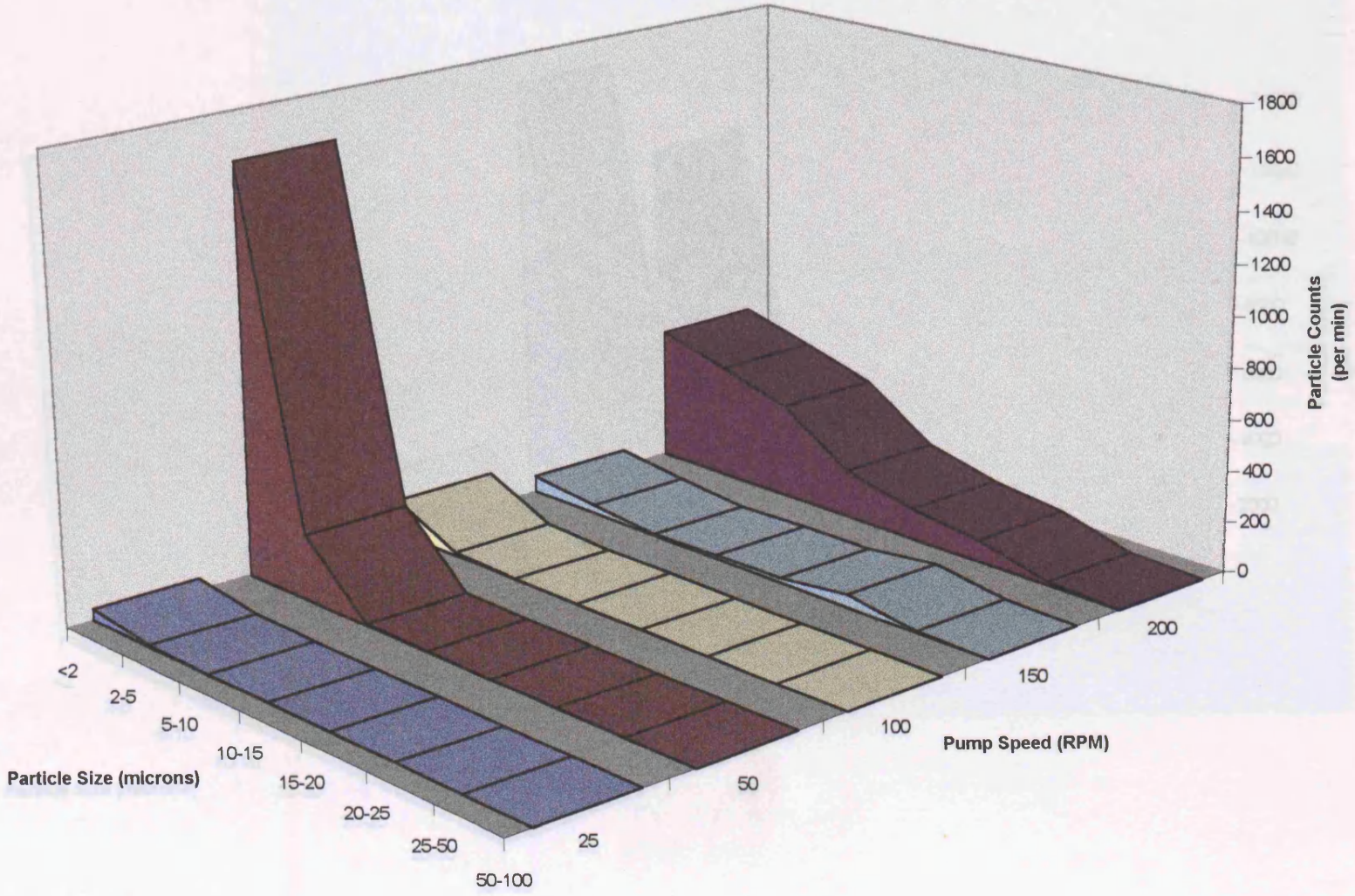
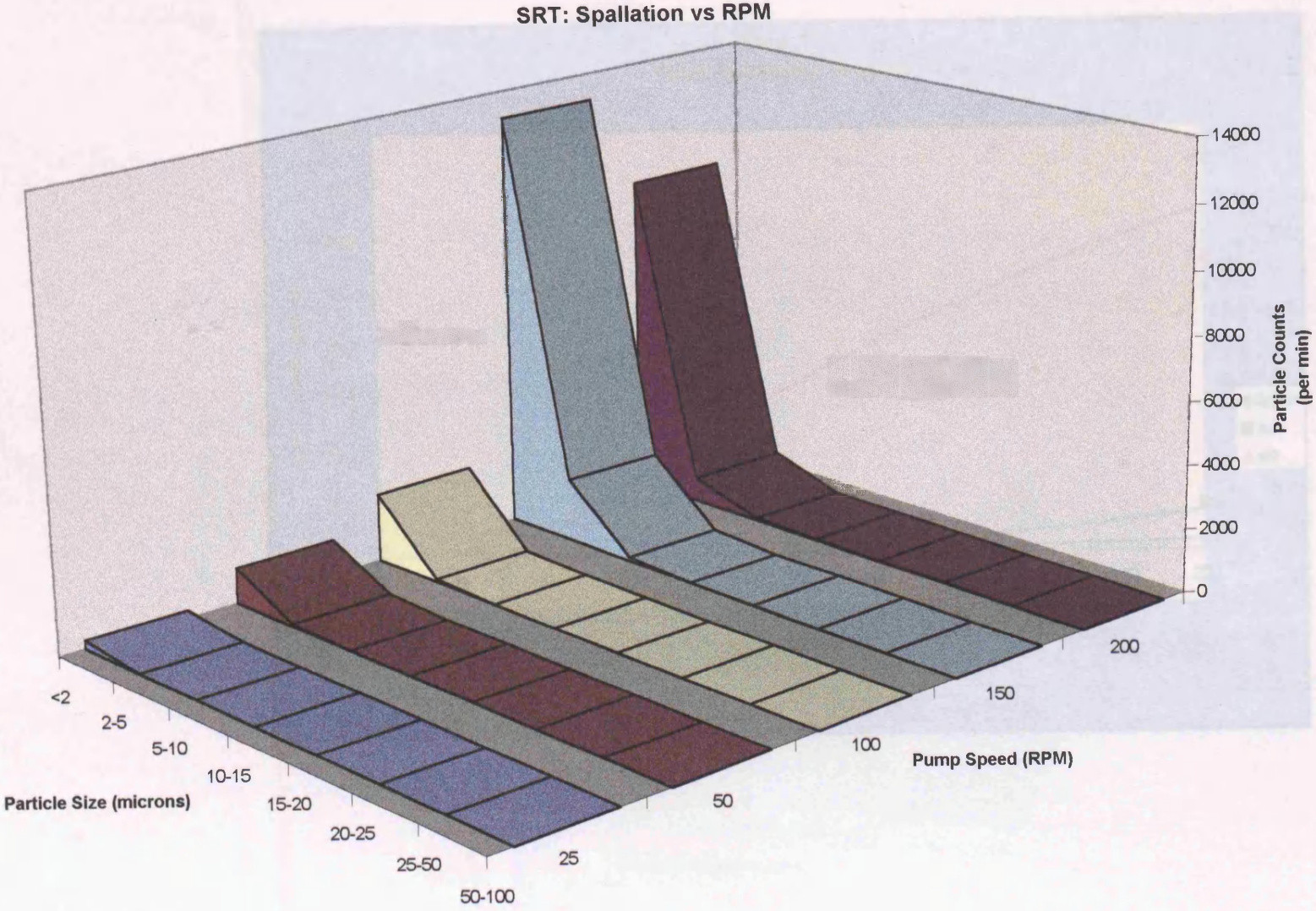


Fig. 4.16
SRT: Spallation vs RPM



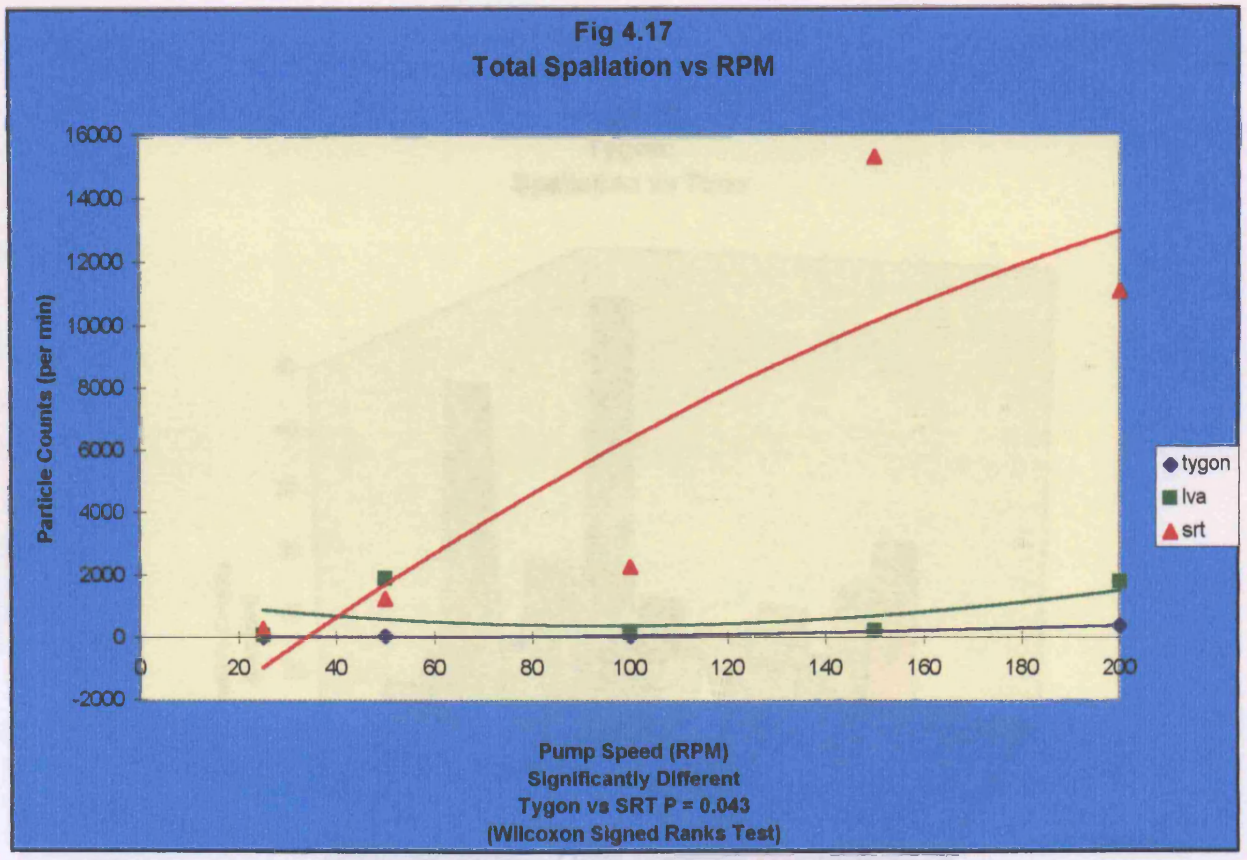


Fig 4.17
Total Spallation vs RPM
Particle Counts (per min)
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
0
20
40
60
80
100
120
140
160
180
200
tygon
lva
srt
Pump Speed (RPM)
Significantly Different
Tygon vs SRT P = 0.043
(Wilcoxon Signed Ranks Test)

SPALLATION vs. TIME

Fig 4.18
Tygon:
Spallation vs Time

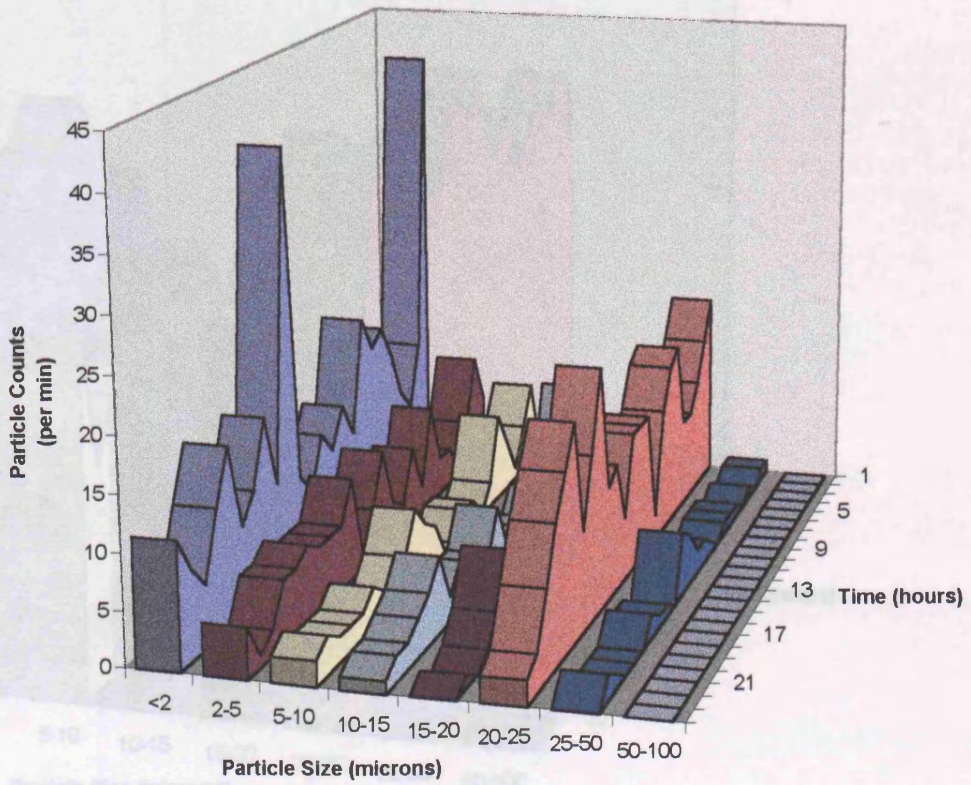


Fig 4.19
LVA:
Spallation vs Time

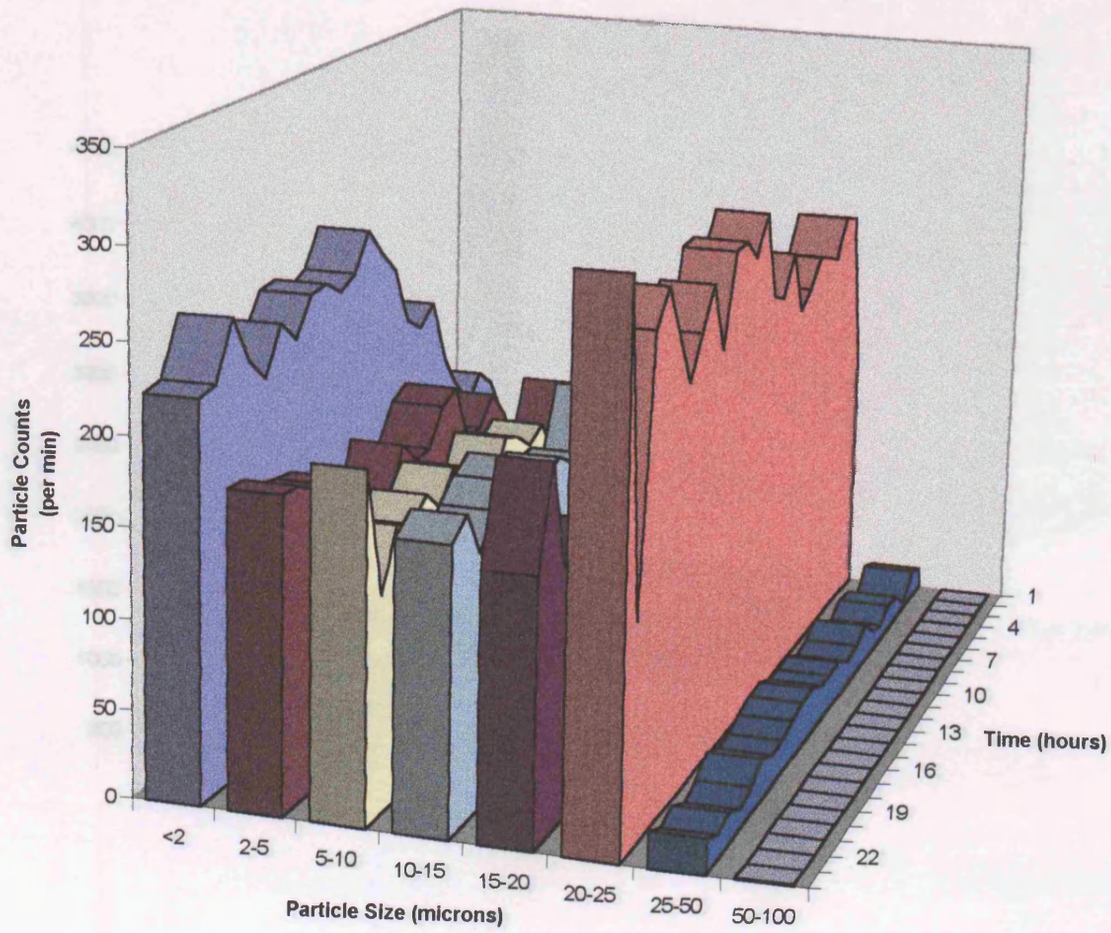


Fig. 4.20
SRT:
Spallation vs Time

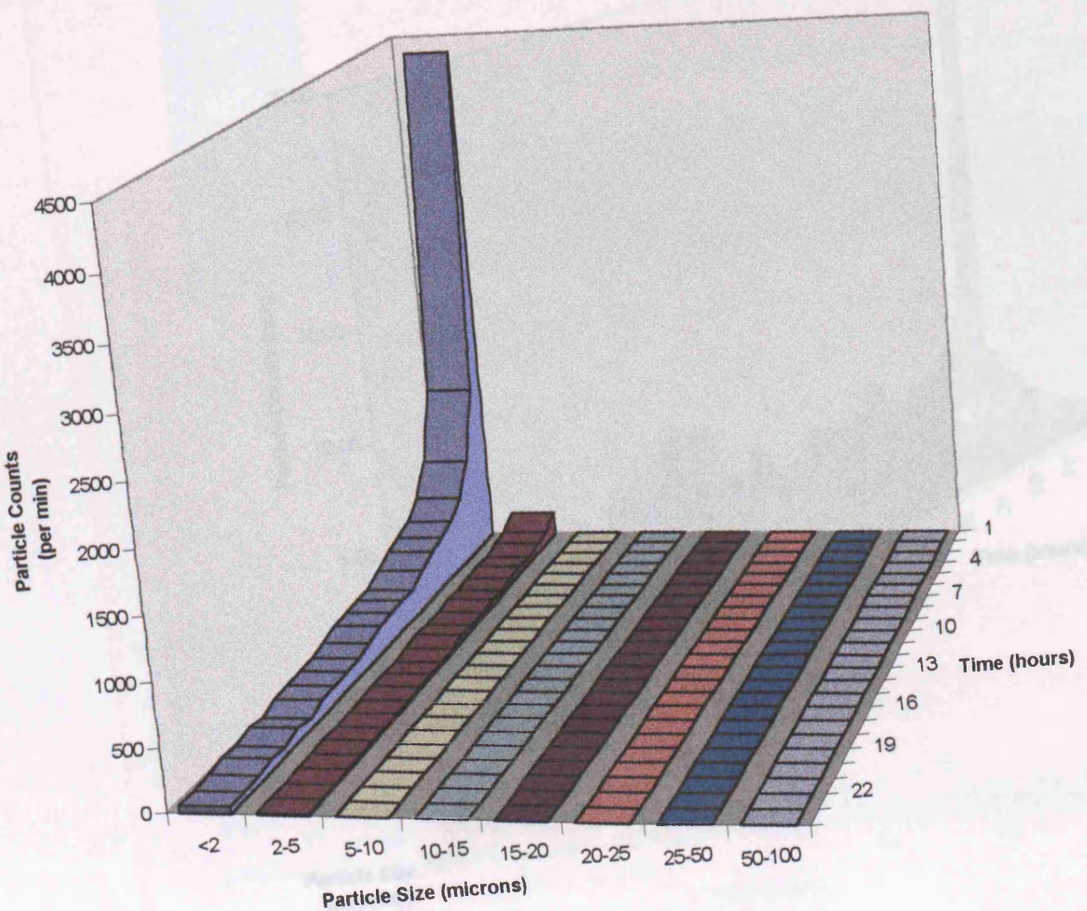


Fig 4.21
Tygon: Spallation vs Time (72 hours)

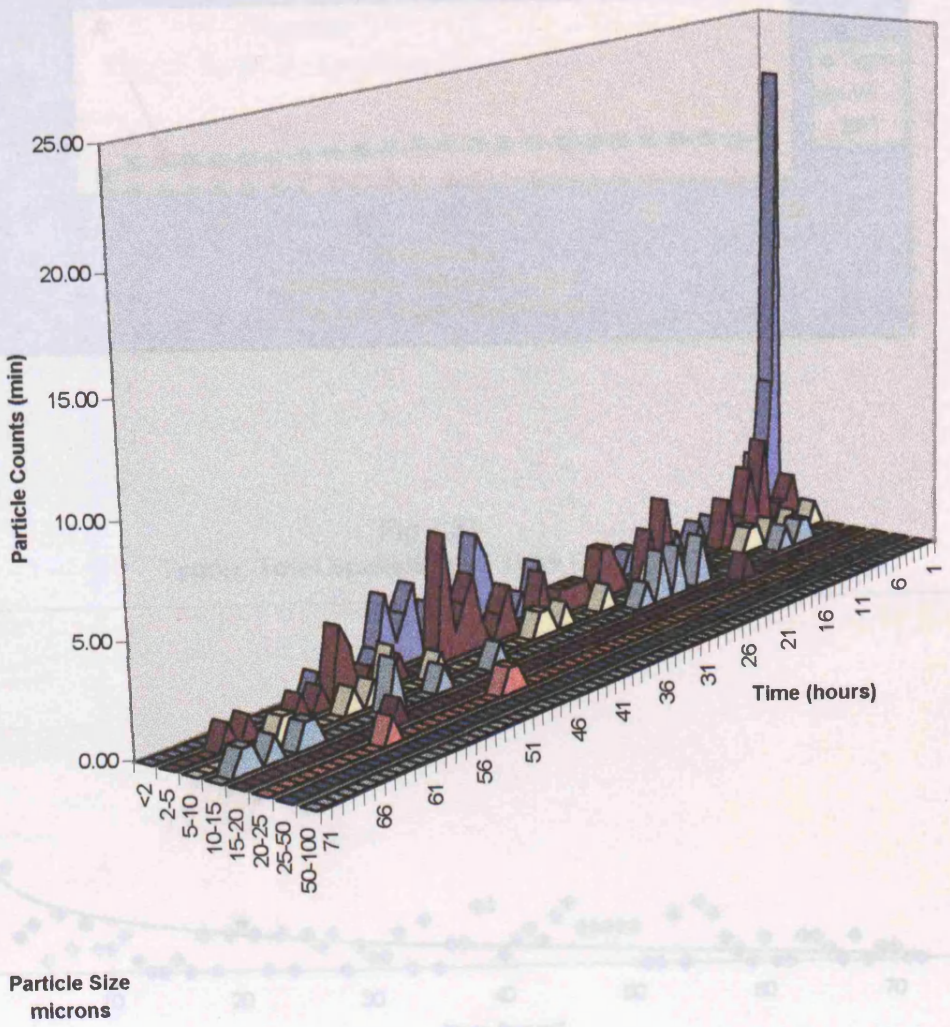


Fig. 4.22
Total Particle Counts vs Time

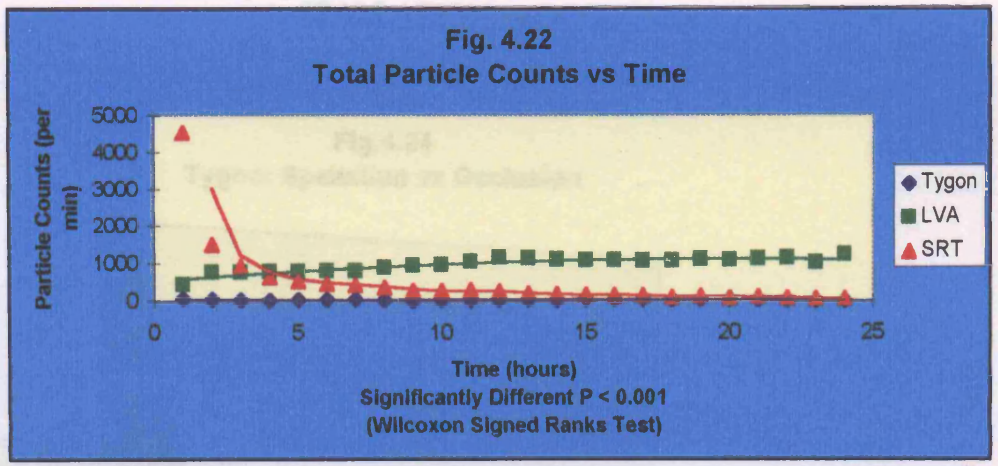
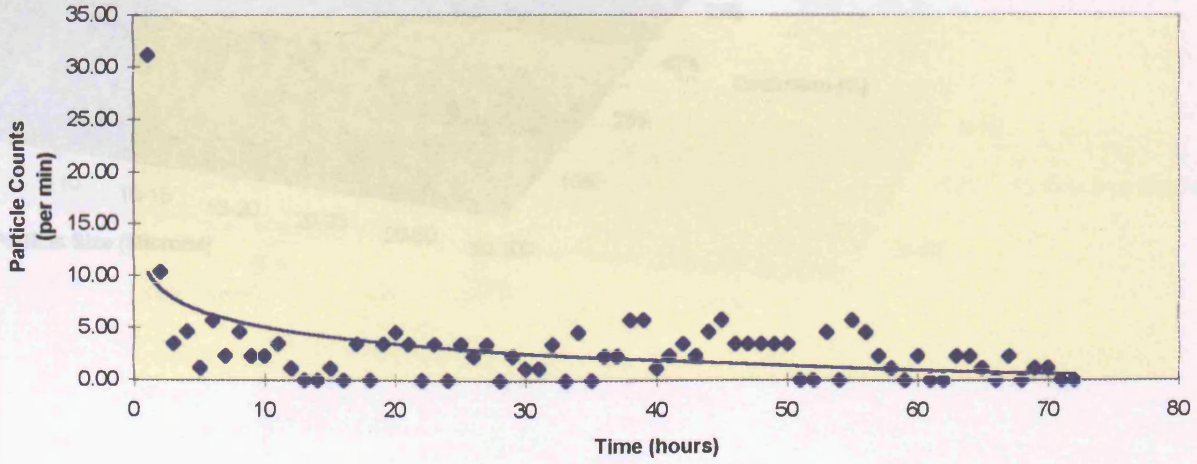


Fig 4.23
Tygon: Total Spallation vs Time (72 Hours)



SPALLATION vs OCCLUSION

Fig.4.24
Tygon: Spallation vs Occlusion

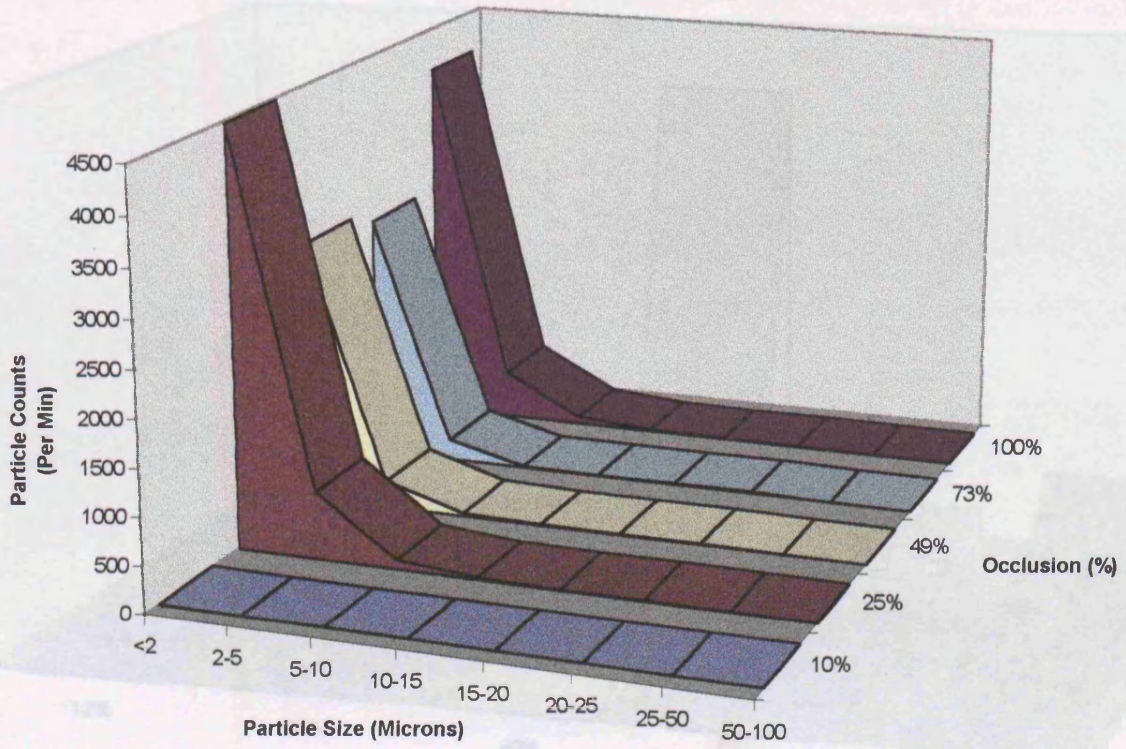


Fig 4.25
LVA: Spallation vs Occlusion

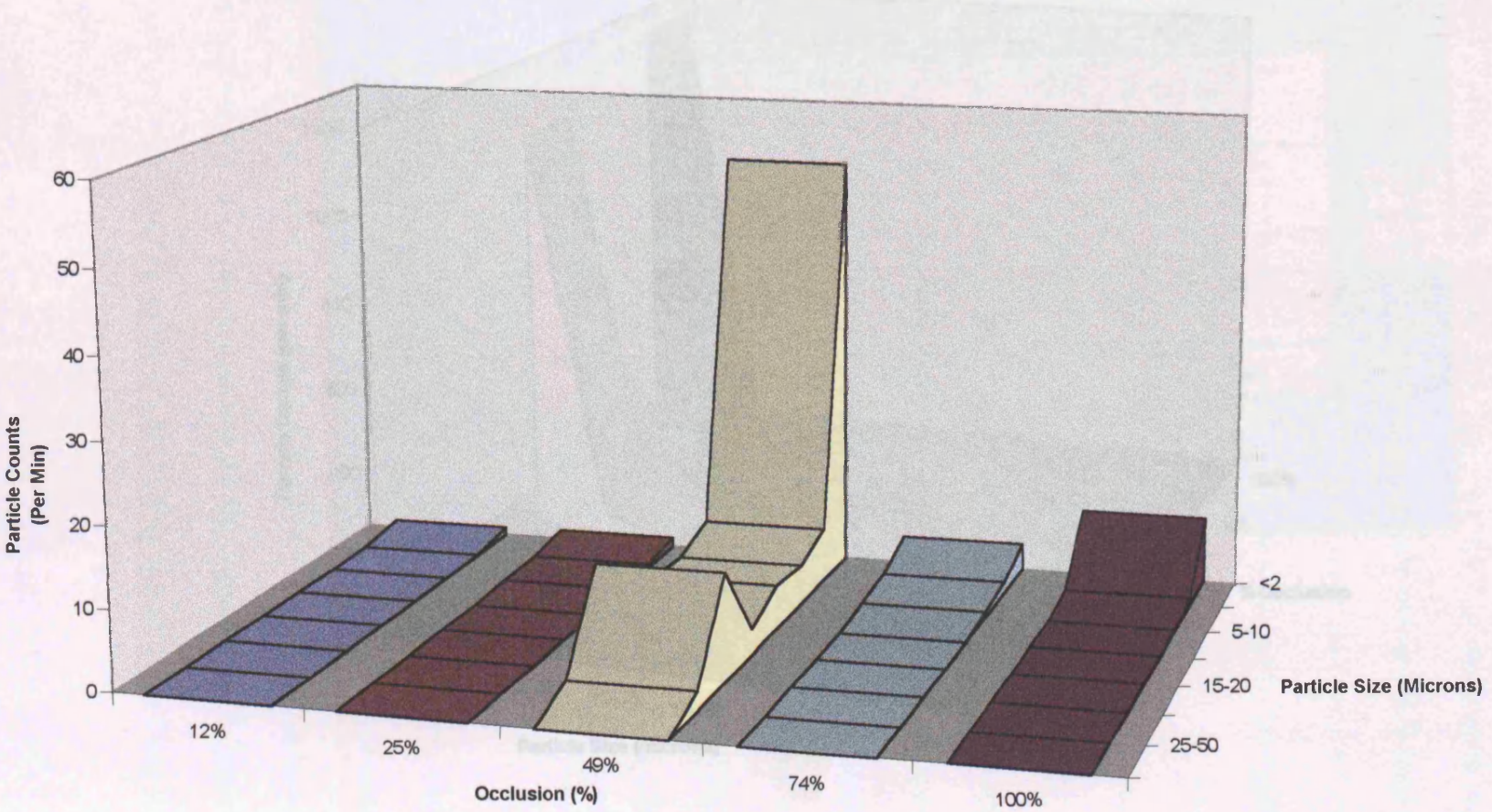
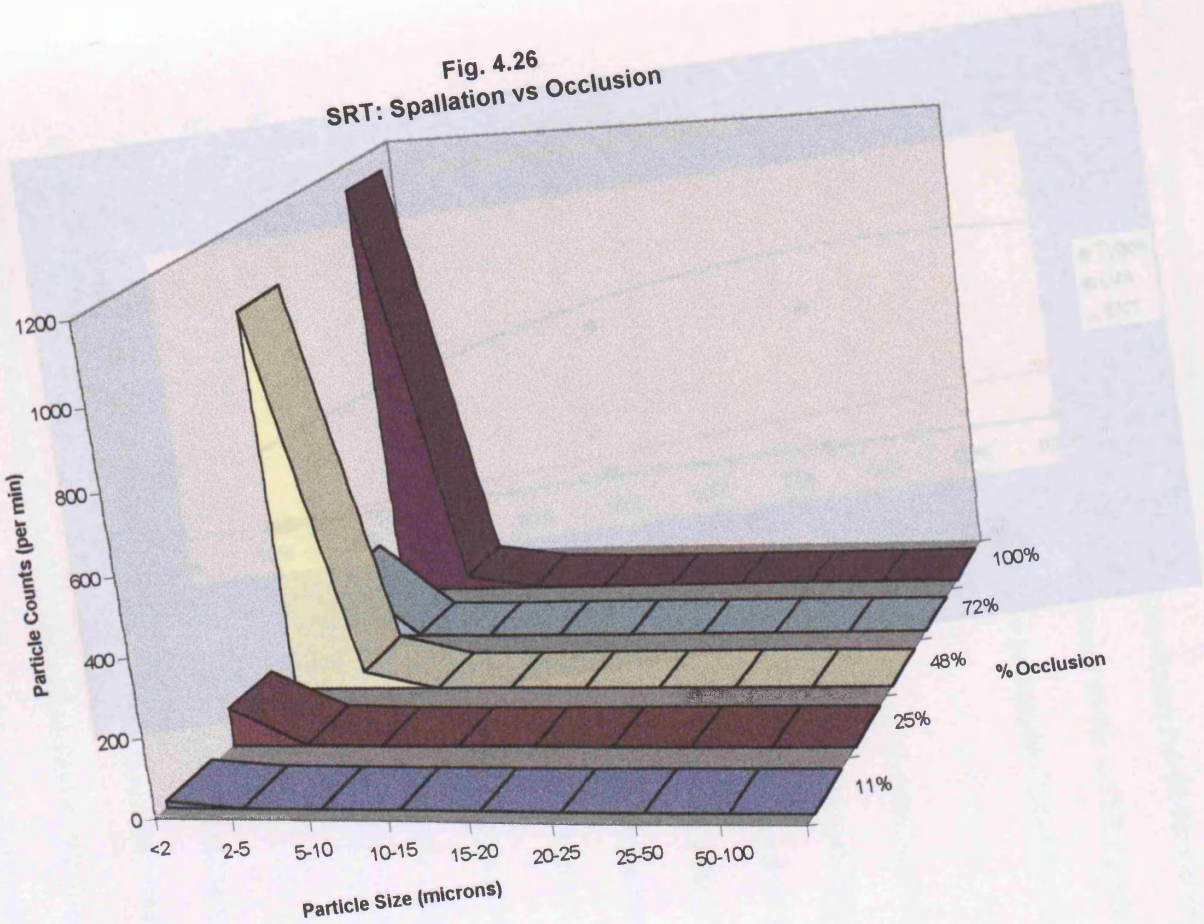


Fig. 4.26
SRT: Spallation vs Occlusion



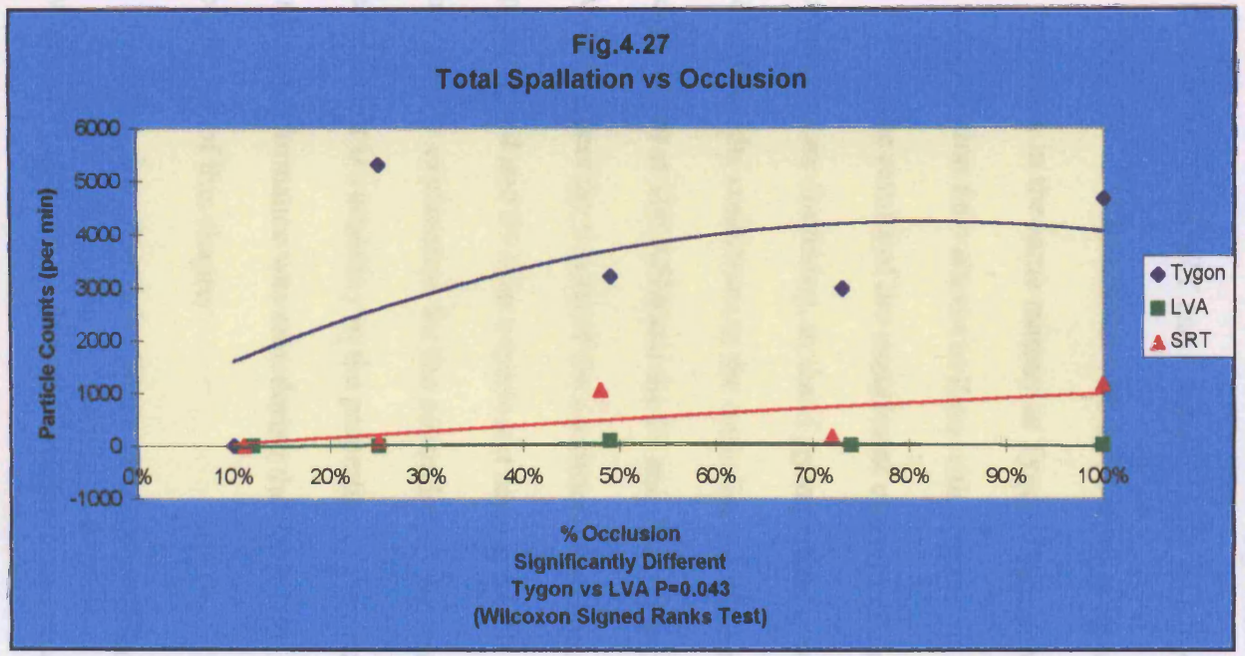


Fig.4.27
Total Spallation vs Occlusion
Particle Counts (per min)
6000
5000
4000
3000
2000
1000
0
-1000
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
% Occlusion
Tygon
LVA
SRT
Significantly Different
Tygon vs LVA P=0.043
(Wilcoxon Signed Ranks Test)

DISCUSSION

Overall the spallation performance of Tygon is superior to both of the other materials. LVA seems to have acceptable spallation at all pump speeds for short term use, but is significantly worse than Tygon during prolonged perfusion. SRT is an order of magnitude worse than both Tygon and LVA as pump speed is increased ($P=0.043$). Both SRT and LVA are worse than Tygon during the 24 hour experiment ($P<0.001$). The Spallation with LVA steadily increases, whilst the SRT gradually decreases, in the same manner as Tygon. During the prolonged, 72 hour, experiment the spallation falls almost to Zero with Tygon.

The results of the experiment examining the effects of differing occlusion on spallation are confusing, in that Tygon performs worse than LVA and SRT. At full occlusion the conditions in the occlusion experiment are identical to the Pump speed experiment at 100 RPM and the 24 and 72 hour experiments. Accordingly one would expect the results of the occlusion experiment at these settings to resemble the pump speed and 24 hour experiment results, which do coincide with each other. The most likely explanation for the contradictory performance of Tygon in the occlusion experiment is variability in the properties of the material. Similar variability in tubing performance was seen during the destruction testing in Experiment 1 at the beginning of this chapter.

The size of particles liberated was also different with the three materials. The two harder materials, Tygon and SRT, produced predominately small particles, most less than 10 microns during the pump speed and occlusion experiments. The majority of particles liberated from the softer LVA were also small, but at higher RPM many more large particles were released, although most were less than 25 microns.

During the 24 hour experiments small particles predominate with Tygon, but there is also an even spread of larger sizes up to 50 microns, however total particle counts are extremely low, and can mislead us as to the significance of these larger particles. With the SRT most of the particles are less than 2 microns and are liberated in the first few hours of the 24 hour experiment, whilst LVA produces a wide range of particles of all sizes up to 25 microns for the entire 24 hours. Since a red blood cell is approximately 7 microns in diameter we could expect particles less than 5 microns to pass through the circulation, although this is merely conjecture. Production of a large number of particles larger than 5 microns is certainly cause for concern, and should probably rule out a tubing material from use during prolonged ECC.

In summary, both SRT and LVA have significantly worse spallation performance than Tygon. However the spallation performance of Tygon seems unpredictable.

CONCLUSIONS

The overall conclusion of this mechanical comparison of Tygon, LVA and SRT is that Tygon is the superior material for ECMO tubing. However, its mechanical properties are unpredictable and variable. Standardisation of the base polymer by the manufacturer might result in more uniform mechanical properties. LVA seems to have sufficient durability to be considered a viable ECMO tubing, although not as good as Tygon at its best, it is as good as the worst Tygon, and its failure time seems predictable. Unfortunately the spallation seen with LVA is too

excessive to recommend clinical use. SRT has poor durability and severe spallation, and is not a viable material for this application.

There appear to be two types of forces acting on the raceway tubing resulting in failure, shear stress and compression. Compression alone was not able to initiate crack formation in Tygon. Scanning electron microscopy demonstrates the co-existance of both mechanisms during clinical tubing use, evidence further supported by the failure of Tygon by both mechanisms. Improvements in pump design to reduce shear stress, and use of under-occlusion to reduce compressive forces might result in improved tubing durability.

CHAPTER 5

In Vivo I: OVERVIEW OF THE PORCINE MODEL, EXPERIMENTAL TECHNIQUE & METHODS

INTRODUCTION

Prior to the introduction of any new technique, drug or biomaterial into clinical practice it is mandatory that a full evaluation of potential benefits and side effects is carried out. Much of this testing can be in vitro, examining haemolysis caused by extracts in the test tube (DIN 58362 Parts 1 and 2). But in Vivo testing is also necessary to ensure the lowest risk to our patients. This necessity is recognised by the United States Pharmacopoeia, the USP VI regulations are currently the most widely recognised standards for biomaterials. Unfortunately these regulations are geared more towards implantation of biomaterials rather than their use for extracorporeal circulation. Materials are tested in Mice and Rabbits for toxicity to injections of extracts of the materials, and also to subcutaneous implantation of materials.

Since the exposure to a new biomaterial during its use as an ECMO tubing would be prolonged, and patients so exposed are critically ill, it was decided that evaluation of such materials should include a model more closely resembling clinical ECMO. An animal model of the physiological, inflammatory and coagulative response to veno-venous extracorporeal circulation was therefore developed, and this model was used to compare the response of Tygon S-65-HL to the two novel materials LVA (Silicone) and SRT (Poly-Oelefin). In this chapter the development and experimental conduct of the porcine model will be discussed.

METHODS

DURATION OF PERFUSION

As already discussed in chapter 2 much is known about the inflammatory response to short term cardiopulmonary bypass (CPB), but the understanding of the response to more prolonged perfusion is less complete. The response to CPB lasts for many days following the end of perfusion, although the clinical correlates of the inflammatory response are unclear. The work of Plotz and other authors (Plotz et al. 1993; Haeffner-Cavaillon et al. 1989; Weerwind et al. 1995; Tulunay et al. 1993; Gillinov et al. 1994a) seems to indicate that the response to the prolonged perfusion of ECMO is characterised by an initial peak during the first 24-48 hours, and then a gradual diminution of response as the circuit becomes passivated, presumably by albumin coating (Roohk et al. 1976; Eberhart et al. 1987). This would certainly correspond to what is seen in clinical ECMO practice. Accordingly any animal model of this response should reflect this time course. Most published animal ECMO experiments last from 6-24 hours (Zobel et al. 1992; Walker et al. 1996; Rais-Bahrami et al. 1995) hours , and perhaps, therefore, do not measure the full range of the response to perfusion. A perfusion duration of 48 hours was selected as a compromise between being long enough to ensure sufficient exposure of the animal to extracorporeal circulation and achieve end organ changes (Bui et al. 1992; Kolobow et al. 1988), and short enough to be conducted without excessive fatigue by the author.

CHOICE OF EXPERIMENTAL ANIMAL

Having decided upon a prolonged experiment this excluded the use of small animals. The New Zealand White rabbit is an established model for neonatal ECMO

(Yanagi et al. 1996; Dorrington and Radcliffe, 1991; Dorrington et al. 1989; Nowlen et al. 1989; Kundu et al. 1989; Whittlesey et al. 1988) but would not have sufficient blood volume to allow sampling over such a prolonged experiment. Transfusion of donor blood was considered to circumvent this problem, but blood transfusion results in significant activation of cytokines and other inflammatory processes (Davenport, 1996; Gafter et al. 1996; Snyder et al. 1996; Federowicz et al. 1996) which would mask the responses being measured. Larger animals were therefore required, that had sufficient blood volume to withstand the haemodilution upon initiation of extracorporeal circulation, and the numerous blood samples without transfusion.

The sheep is the standard animal used for ECMO experiments in the majority of centres (Walker et al. 1996; Murata et al. 1996; Palmer et al. 1995; Moller et al. 1993; Zwischenberger et al. 1993), it is also used in the USA as an animal model for training ECMO technicians. The advantages of the ovine model are ease of cannulation via the large jugular and femoral veins and the extreme hardiness and docility of the sheep. Unfortunately the coagulation system of the sheep is significantly different from the human system, particularly the very high platelet count (R Bartlett, Personal Communication). This means that ACTs must be kept much higher than the usual 160-200 sec's during ovine ECMO, and therefore little useful information regarding the relative activation of the coagulation system by the three different materials would be obtained. Other potential experimental animals such as primates and cattle were not ethically, financially or practically realistic. The similarity between the pig and man was noted by George Orwell in "Animal Farm", but it is the similarity of the porcine and human coagulation systems (Karges et al. 1994) which makes the pig a good choice of animal for this model. In addition pigs tolerate extracorporeal circulation extremely badly (Mickelson et al. 1990)

developing capillary leak syndrome and haemolysis. This makes the pig ideal for comparing responses to extracorporeal circulation (ECC) as any minor differences between materials should be magnified. A number of investigators have used a porcine model for the study of ECMO (Liem et al. 1996; Liem et al. 1995; Vardi et al. 1995; Purohit et al. 1993; Koul et al. 1992; Zobel et al. 1990).

ANATOMICAL ASPECTS OF INTUBATION, AND CANNULATION

As pigs are not docile animals like sheep it was essential that they were kept sedated throughout the procedure. Intermittent positive pressure ventilation (IPPV) was to be used in order to duplicate the clinical situation and also to provide a measure of lung compliance. In order to ensure that it would be possible to establish a secure airway, sufficient vascular access, in addition to ECC cannulation a "dry run" was performed on an animal anaesthetised, intubated and then killed by overdose of pentobarbitone under Schedule I of the Animals (Scientific Procedures) Act 1986. Several important anatomical facts were learned during this procedure.

Firstly pigs should be intubated prone, as when they are turned supine respiratory excursions become limited and desaturation ensues. Another reason for prone intubation is the mandible is very heavy, and the larynx a long way from the tip of the jaw, making laryngoscopy very arduous when working against gravity, especially with the 12 inch straight blade laryngoscope used for the purpose. When the pig is turned prone it continues to breathe and an assistant can aid during laryngoscopy by lifting the maxilla and head with the aid of a bandage arranged as a sling around the maxilla. Since the "patient" is inverted with respect to the usual clinical human position the laryngoscope must be held in the right hand, not the left. The epiglottis extends superior to the soft palate and must be displaced before the

cords are visible, topical local anaesthesia with lignocaine spray helps prevent laryngospasm. Because of the caudal position of the larynx, large size and floppiness of the epiglottis a bougie can aid intubation. Having intubated the trachea the oesophagus can be visualised posterior and caudal to the larynx using the laryngoscope, to allow the passage of an oro-gastric tube. The large turbinates in the pig make passage of a nasogastric tube impossible, although there is sufficient space to allow insertion of a naso-pharyngeal temperature probe.

As discussed in chapter 1 in humans the optimal route for insertion of the drainage cannula is via the right internal jugular vein. Dissection of the neck revealed that the internal jugular vein in the pig is extremely deep seated, lying in the tracheo-oesophageal groove, and also is very small (see Fig. 5.1). However the external jugular vein is a larger structure and can be used to access the right atrium. In the 55Kg "dry run" animal the right external jugular vein would accept a 21 F cannula, which could be threaded down into the right atrium, confirmed by palpation of the heart. The surface marking for the external jugular is a line from 1 finger breadth lateral to the angle of the mandible, running postero-inferiorly to the mid-clavicular point. The vessel was exposed via a transverse incision, about ½ way down the neck. The subcutaneous fat and platysma were divided, platysma is approximately 3cm thick in the pig, exposing another layer of fat, the vein lies in this plane, superficial to sternomastoid. Dissection was extended through the deeper layer of fat to reveal the vein, which usually has a "sentinel" lymph node just superficial to it, removing the lymph node exposes the vein (see Fig 5.2 & 5.3).

The femoral vessels were also dissected to allow insertion of an arterial line, 8.5F venous sheath and Swann-Ganz catheter, on the left, and the other ECMO cannula on the right.

Fig. 5.1 Showing Right Internal Jugular Vein

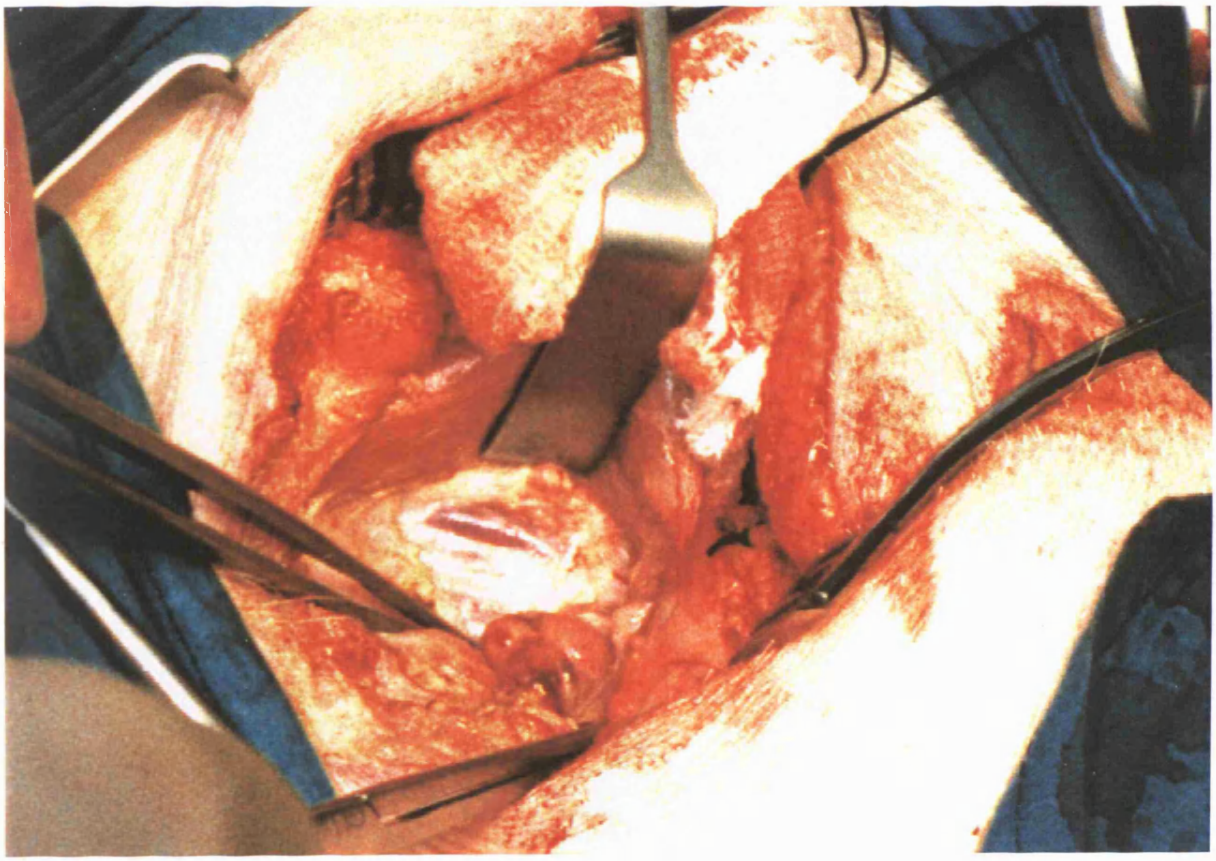


Fig. 5.2 Lymph node marking position of Right External Jugular Vein

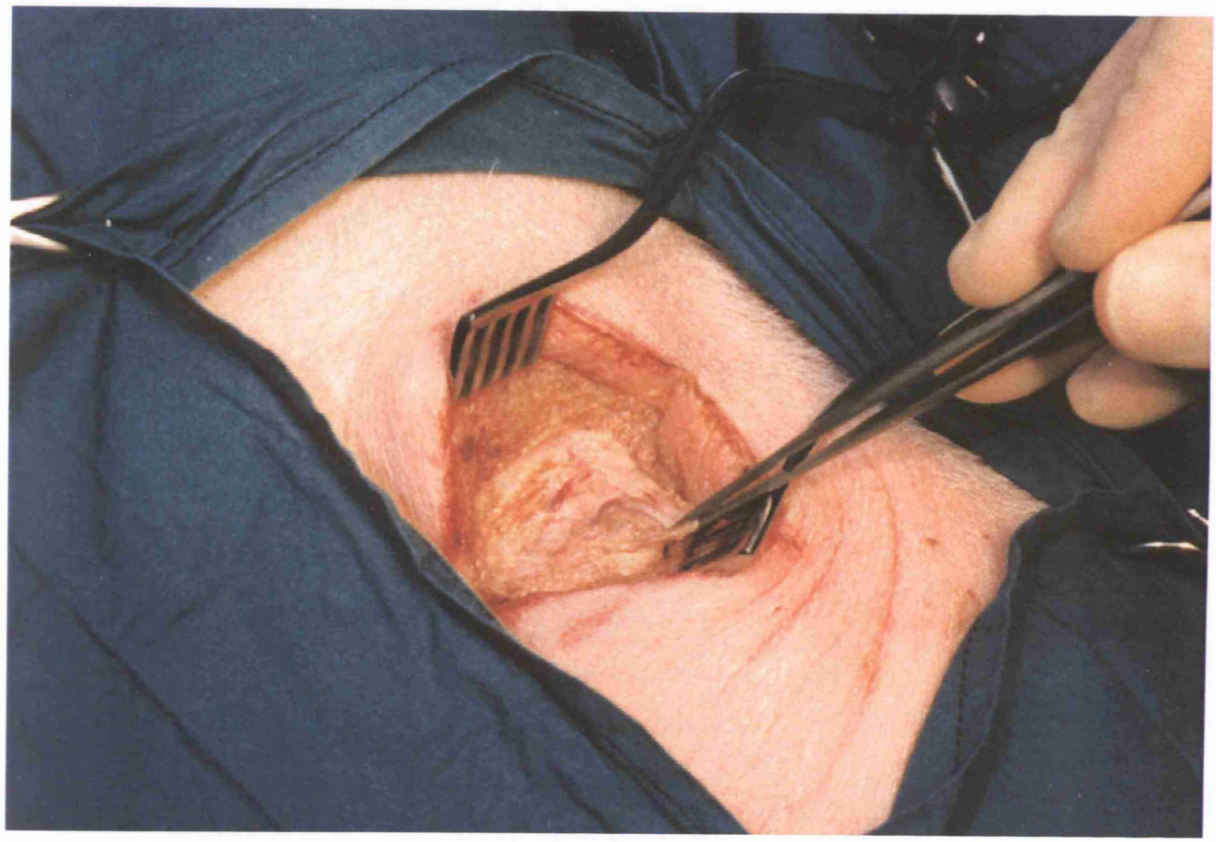
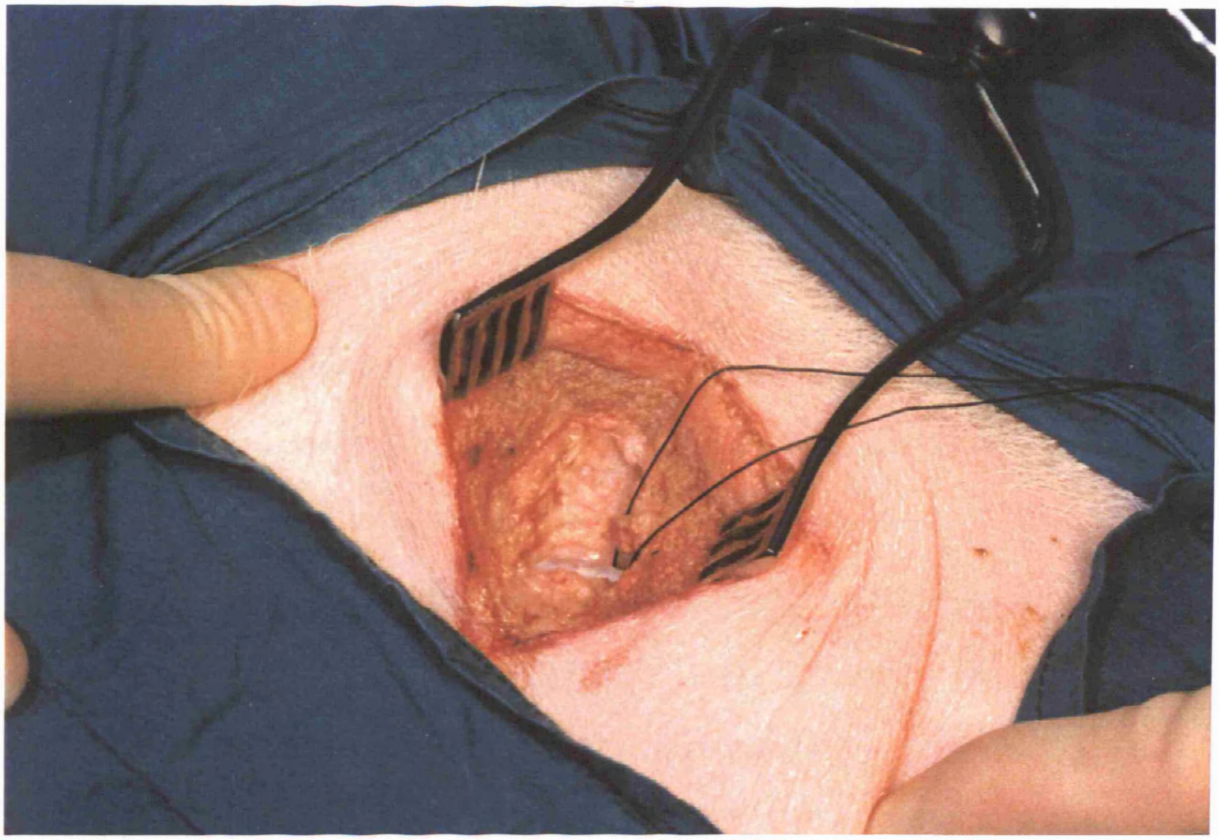


Fig. 5.3 Right External Jugular Vein



The femoral vessels lie medial to the mid inguinal point. Since the pig does not walk upright there is no femoral triangle, but the position of the femoral vessels is marked by a small fat pad, just below the inguinal ligament (see Fig 5.4 & 5.5). The femoral vein was larger than the external jugular, and would easily accept a 21F cannula in the 55Kg animal.

The male pig has a tortuous urethra, making catheterisation of the bladder by this route impossible. Female pigs were therefore selected to facilitate catheterisation. Unfortunately in the juvenile nulliparous animal dissected the urethral meatus was not visible and supra-pubic cystotomy via a small midline laparotomy was used to establish drainage of the bladder. Probing the urethra from inside the bladder revealed the meatus to lie inaccessible, high within the vagina.

EXPERIMENTAL PROTOCOL

As this was a pilot study of materials which have never been used for experimental or clinical ECC it was not possible to use a power calculation to calculate sample size. This was because we had no indication of whether a difference would be present between groups, or what size such a difference would be. A sample size of 5 animals per group was selected as being a manageable, and affordable number. There were three groups, a control group perfused with Tygon S-65-HL, and two trial groups perfused with circuits made from LVA (Silicone) and SRT (Poly-Oeleifin).

Procedures were conducted in accordance with the Animals (Scientific Procedures) Act 1986, (Project Licence No PPL 80/1065). Female large white pigs 33-75Kg were obtained from the same supplier. Pigs were delivered to the university at least 1 week prior to procedure to allow acclimatisation. Feed was withheld for 12 hours

pre-procedure, but water was allowed ad libitum. Each procedure started at 6am, after induction of anaesthesia and intubation there was a six hour stabilisation period prior to ECC cannulation. ECC lasted 48 hours, and animals were then decannulated and sacrificed 1 hour later. All surgery was carried out by the author, who remained present throughout the entire duration of all procedures. At the end of the procedure animals were sacrificed by a combination of exsanguination via the abdominal aorta and overdose of pentobarbitone. A post mortem examination was then performed. An approximate time frame is given in the table below.

TIME	DAY ONE	DAY TWO	DAY THREE
0600	Induction of anaesthesia		
0700			
0800	Insertion of monitoring lines		
0900	Baseline Samples	Samples	Samples
1000			
1100			6 hr creat clearance
1200	6 hr creat clearance	6 hr creat clearance	Samples
1300	ECC cannulation		ECC Decannulation
1400	Samples at ECC +10 & 60 mins		Sacrifice
1500		Samples	Post Mortem
1600			
1700			
1800	Samples		
1900			
2000			
2100			
2200	Samples	Samples	
2300			
2400			
0100			
0200			
0300			
0400			
0500			

Fig. 5.4 The Inguinal approach to the Left Femoral Vessels
(arrow showing the fat pad overlying the femoral vessels)

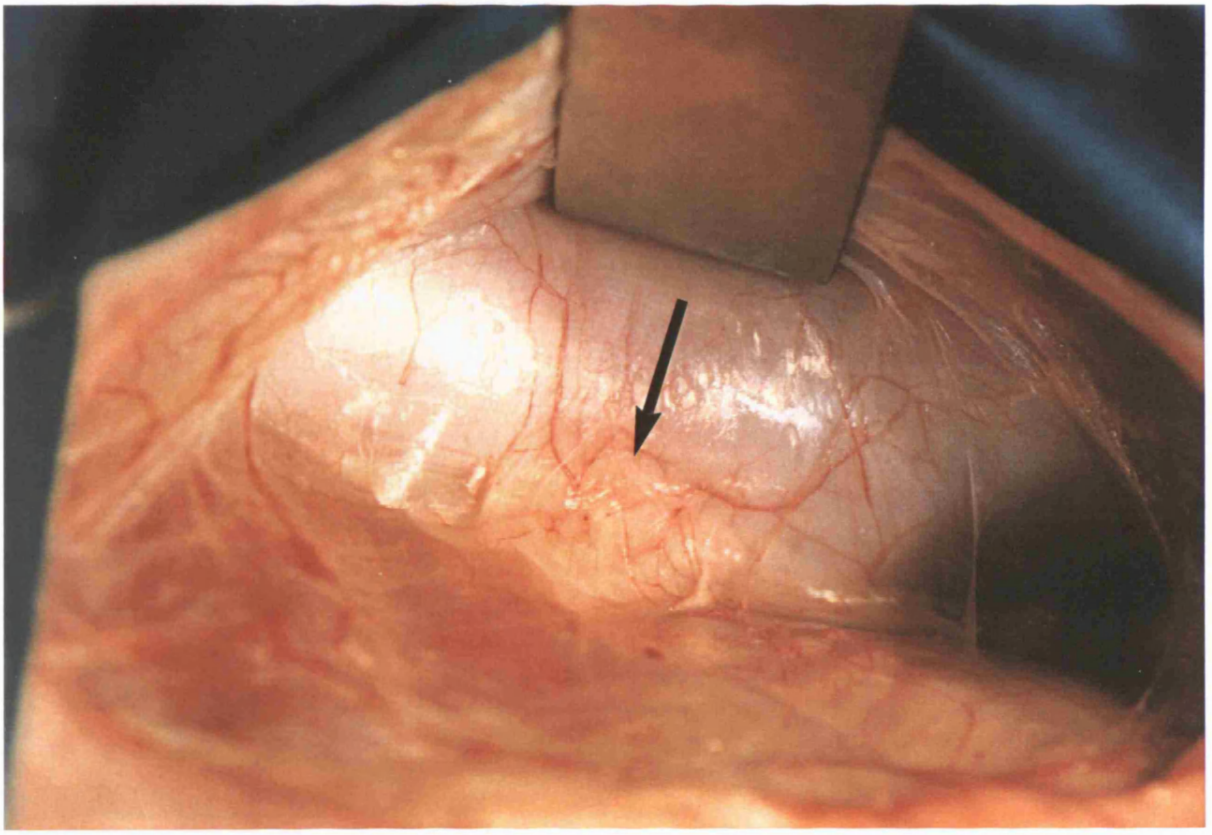
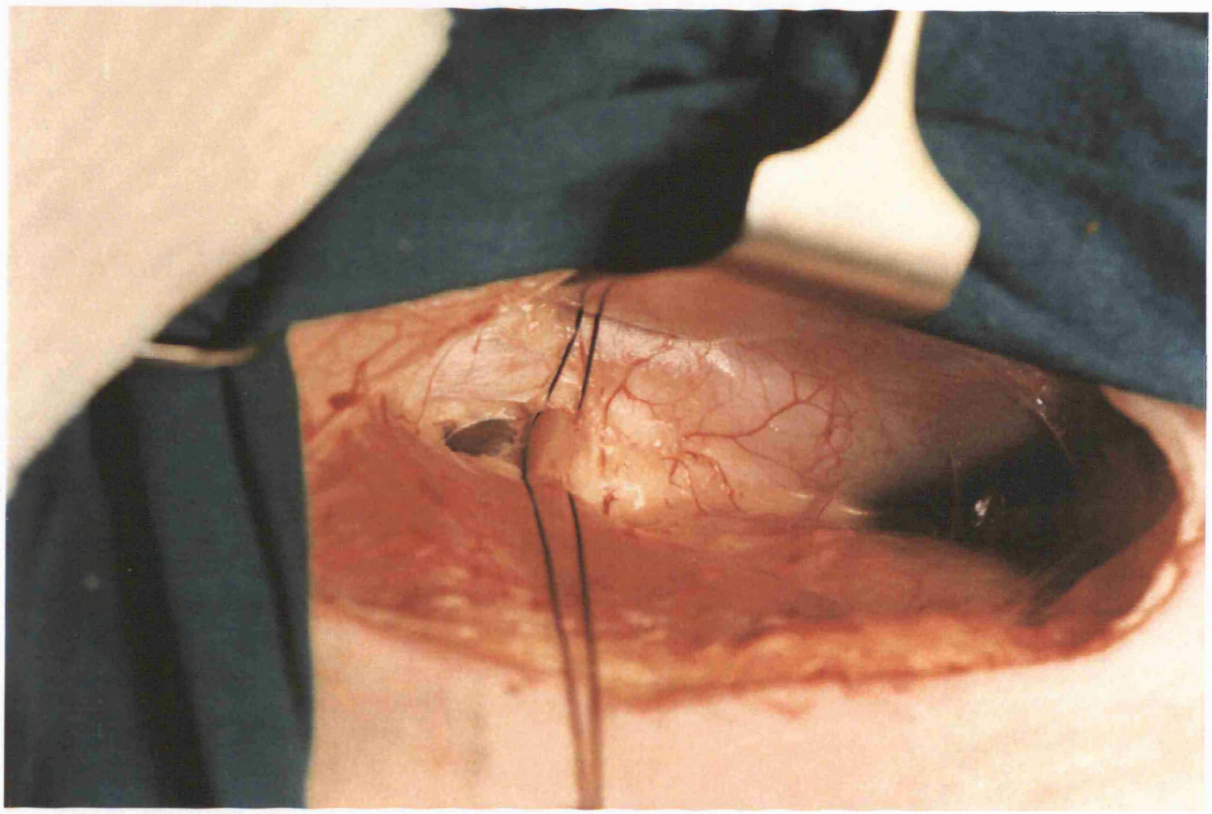


Fig. 5.5 Left Femoral Vessels



Timings were variable as the duration of surgery was not constant. However ECC always lasted 48 hours, except when animals died during perfusion.

ANAESTHESIA & SEDATION

The original anaesthetic protocol followed that developed at the University of Sheffield. In their experience Stresnil (Azaperone) 10mg/Kg IM is sufficient pre-medication to allow cannulation of an ear vein and induction of anaesthesia with Propofol. This protocol was used for the first 2 animals, but did not result in sufficient anxiolysis to facilitate peripheral venous cannulation. Anaesthesia was therefore induced with intramuscular Ketamine, initially 10mg/Kg with supplemental doses if necessary. In subsequent animals the Stresnil was omitted, and Ketamine was administered from the outset. By the fifth animal it was realised that 10mg/kg was an insufficient dose of Ketamine, as supplemental doses usually had to be administered, the induction dose of Ketamine was thereafter increased to 20 mg/kg. Following Ketamine the pigs were usually in plane 1 to 2 of General Anaesthesia, a peripheral venous cannula (22G Jelco) was then inserted into an ear vein, or limb vein and incremental doses of Propofol (2-20ml) were given to produce deeper anaesthesia. Animals were then moved from the holding pen to the operating theatre. Three lead ECG monitoring and pulse oximetry was then started, and oxygen administered prior to intubation. Morphine and Propofol were administered to effect, to allow laryngoscopy, the cords were sprayed with Lignocaine and an endotracheal tube (7.5-9.5 Cuffed) was passed as described above. An orogastric tube was also passed.

It was the initial intention to provide sedation during the procedure with Morphine and Midazolam infusions as used in clinical ECMO practice, unfortunately

the doses required to maintain satisfactory and humane levels of sedation were not practical or affordable, and a pentobarbitone infusion was substituted halfway through the first procedure. In the second animal a pentobarbitone infusion was started via a peripheral line immediately after intubation, severe thrombophlebitis developed rapidly, and the infusion was changed for Propofol. Thereafter Propofol infusion with boluses of Morphine was used to cover the insertion of the arterial line, venous sheath, Swann-Ganz and Urinary Catheter. Atracurium 1mg/Kg was added to this regime to prevent muscular twitching when diathermy was being used for haemostasis and dissection near to the femoral vessels. Once central venous access was established the pentobarbitone infusion was commenced and the Propofol stopped. Sedation was maintained with a continuous infusion of pentobarbitone throughout the procedure, the rate of infusion was titrated to ensure the optimal level of sedation. This was defined as the minimum rate of infusion that would result in suppression of the pedal withdrawal reflex to pain, and the blink reflex, and an absence of haemodynamic evidence of awareness (tachycardia and hypertension). Surgery for ECC cannulation was covered by further administration of Propofol and Morphine intra-venously (see results), neuromuscular blockade with Atracurium (1mg/kg) to allow diathermy use without muscular twitching and also to reduce the risk of air embolism during cannula insertion.

Propofol was also used to quickly deepen the level of sedation on occasions when the animals suddenly "woke up" having been previously well sedated. In this way sedation was immediately re-established, the alternative of administering a bolus of pentobarbitone was judged to be more dangerous due to its longer half life and cardio-vascular suppressant effects. The background infusion of pentobarbitone was also increased.

MONITORING & SAMPLING LINES

Following endotracheal intubation and oro-gastric tube insertion the left femoral vessels were exposed at cut-down for the insertion of monitoring, sampling and infusion lines. Full aseptic technique and prophylactic anti-biotics (Ampicillin 1g IV) were used for all surgical procedures. Diathermy was used for dissection to ensure good haemostasis during prolonged heparinisation. Unfortunately even with neuromuscular blockade significant muscular twitching occurred resulting in diathermy injury to the femoral artery on 3/16 occasions (see table). Blunt dissection and haemostasis via diathermy forceps was therefore substituted. Having dissected out the left femoral artery and vein a sling was passed around each, an 8.5F Sheath (Arrow) was then inserted into the left femoral vein. The arterial line was then inserted into the femoral artery, this proved to be the most technically demanding procedure during the experiment as the artery was very liable to dissect, either at the puncture site, or more proximally (see table). The only death occurred as a result of exsanguination from a puncture of the left common Illiac artery by a soft tipped 0.025" guidewire. A 7.5F 4 channel Swann-Ganz catheter (831HF75, Baxter, Irvine, California) was then floated into the pulmonary artery via the venous sheath. The groin wound was then closed. A Foley catheter was then inserted into the bladder via a supra-pubic cystomy through a mid-line mini-laparotomy. Details of monitoring line sites, types and complications are given in table 5.6 below.

ECC CANNULATION, CIRCUIT DESIGN & PERFUSION

ECC CANNULATION: The "Semi-Seldinger" technique (Peek et al. 1996a) was used for cannulation, this allows cannulation without vessel ligation, and de-cannulation without re-exploration of the wound. The target vessels (right femoral

and external jugular veins) were exposed at cut-down and then entered with a 16SWG Angiocath via a track through one lip of the wound. A 0.038" guidewire is then inserted into the vessel down the Angiocath, and Heparin is given. The initial Heparin bolus was 50u/Kg, this was allowed to circulate for 2 minutes and then an ACT was taken, if the ACT was less than 200 seconds further Heparin was administered. After 3 procedures had been performed it was clear that further Heparin was always required, and therefore the bolus was increased to 100u/kg, which was sufficient to obtain the target ACT of 200 seconds. Having assured adequate anticoagulation the cannulae were introduced over the guidewire in the conventional fashion. DLP (Grand Rapids, Michigan, USA) cannulae were used in all cases except one, when a Research Medical (RMI) (Research Medical Inc., Midvale, Utah, USA) device was used. Once the cannulae were inserted and connected to the circuit extracorporeal flow was initiated. The space around the vein was then packed with swabs, in lieu of the Surgicel and glue used clinically, and the wound closed. Cannula sizes, types and complications are tabulated in Fig.5.7.

The RCFV was used for venous drainage in most cases because the REJV was smaller, and would not accommodate the 21F cannula, the minimum size required to ensure adequate venous drainage. In two cases severe venous haemorrhage developed as a result of the RCFV tearing, packing the wound with swabs and closing it achieved haemostasis, but in one case the cannula had to be removed to obtain enough tamponade to stop the bleeding. In this animal (G) the venous drainage cannula was inserted via an extra-peritoneal approach (Moulton et al. 1993) to the RCIV. In all cases slight oozing developed around the cannulae towards the end of the procedure (after about 36-40 hours) this could always be controlled by pressure, but was exacerbated by moving the animal, therefore the

Fig. 5.6: Table to show sites of monitoring line insertion and complications.

ANIMAL	WEIGHT (Kg)	ARTERIAL Site	Size & Type	Complications	VENOUS/PA Complications	COMMENTS
A	65	LCFA	16SWG Angiocath	Nil	Nil	
B	75	LCFA	16SWG Angiocath	Nil	Nil	
C	43	LCFA	18SWG Leadercath	Nil	Nil	
D	45	LCFA	18SWG Leadercath	Nil	Sheath fell out, vein ligated re-inserted higher up.	Unable to repair femoral vein
E	38	LCFA	18SWG Leadercath	LSFA damaged during dissection, ligated. line to LCFA, guidewire injury to LCIA.	Nil	Animal Died, not included in analysis
F	39.5	LCFA	18SWG Leadercath	LSFA damaged during dissection, ligated. line inserted into LCFA.	Nil	Diathermy Injury
G	37	LCFA	8.5F Arrow	Nil	Nil	
H	33	LCFA	18SWG Leadercath	LSFA damaged during dissection, ligated. IA line inserted into LCFA.	Nil	Diathermy Injury
I	37	LCFA	6F Cordis	Nil	Nil	
J	40	LCFA	6F Cordis	see comments	see comments	Vessels tore, ligated around cannulae.
K	43	LCFA	6F Cordis	Nil	Nil	
L	35	LCFA	6F Cordis	Nil	Nil	
M	44	LCFA	6F Cordis	Nil	Nil	
N	41	LCFA	6F Cordis	Nil	Nil	
O	34	LCFA	6F Cordis	Difficult insertion, line would not sample, artery ligated around cannula	Nil	18SWG Leadercath percutaneously via right post tibial artery
P	36	LCFA	6F Cordis	Nil	Nil	

KEY: LCFA: Left Common Femoral Artery, LSFA: Left Superficial Femoral Artery, LCIA: Left Common Illiac Artery

Fig.5.7: Table showing ECC cannulation sites, types and complications

ANIMAL	WEIGHT (Kg)	DRAINAGE		RETURN		COMPLICATIONS
		Site	Size & Type	Site	Size & Type	
A	65	REJV	21 Mod DLP	RCFV	17 DLP	Nil
B	75	RCFV	21 Short DLP	REJV	17 DLP	Inadequate drainage, 21 long DLP substituted.
C	43	RCFV	21 Long DLP	REJV	17 DLP	Nil
D	45	RCFV	24 RMI	REJV	17 DLP	Nil
F	39.5	RCFV	21 Long DLP	REJV	17 DLP	RCFV tore during cannulation controlled by packing.
G	37	RCIV	21 Long DLP	REJV	17 DLP	RCFV transected during cannulation, unable to control with cannula in situ, cannula removed, wound packed & closed. Illiac vein cannulated via McEverdy approach.
H	33	RCFV	21 Long DLP	REJV	17 DLP	Nil
I	37	RCFV	21 Long DLP	REJV	17 DLP	Nil
J	40	RCFV	21 Long DLP	REJV	17 DLP	REJV dissected during guidewire insertion, cannulated open with vessel ligation.
K	43	RCFV	21 Long DLP	REJV	17 DLP	Nil
L	35	RCFV	21 Long DLP	LEJV	17 DLP	REJV dissected during guidewire insertion, RIJV in severe spasm, therefore LEJV used.
M	44	REJV	21 Mod DLP	RCFV	17 DLP	Nil
N	41	RCFV	21 Long DLP	REJV	17 DLP	Nil
O	34	RCFV	21 Long DLP	REJV	17 DLP	Nil
P	36	RCFV	21 Long DLP	REJV	17 DLP	Nil

KEY: REJV Right External Jugular Vein

LEJV Left External Jugular Vein

RIJV Right Internal Jugular Vein

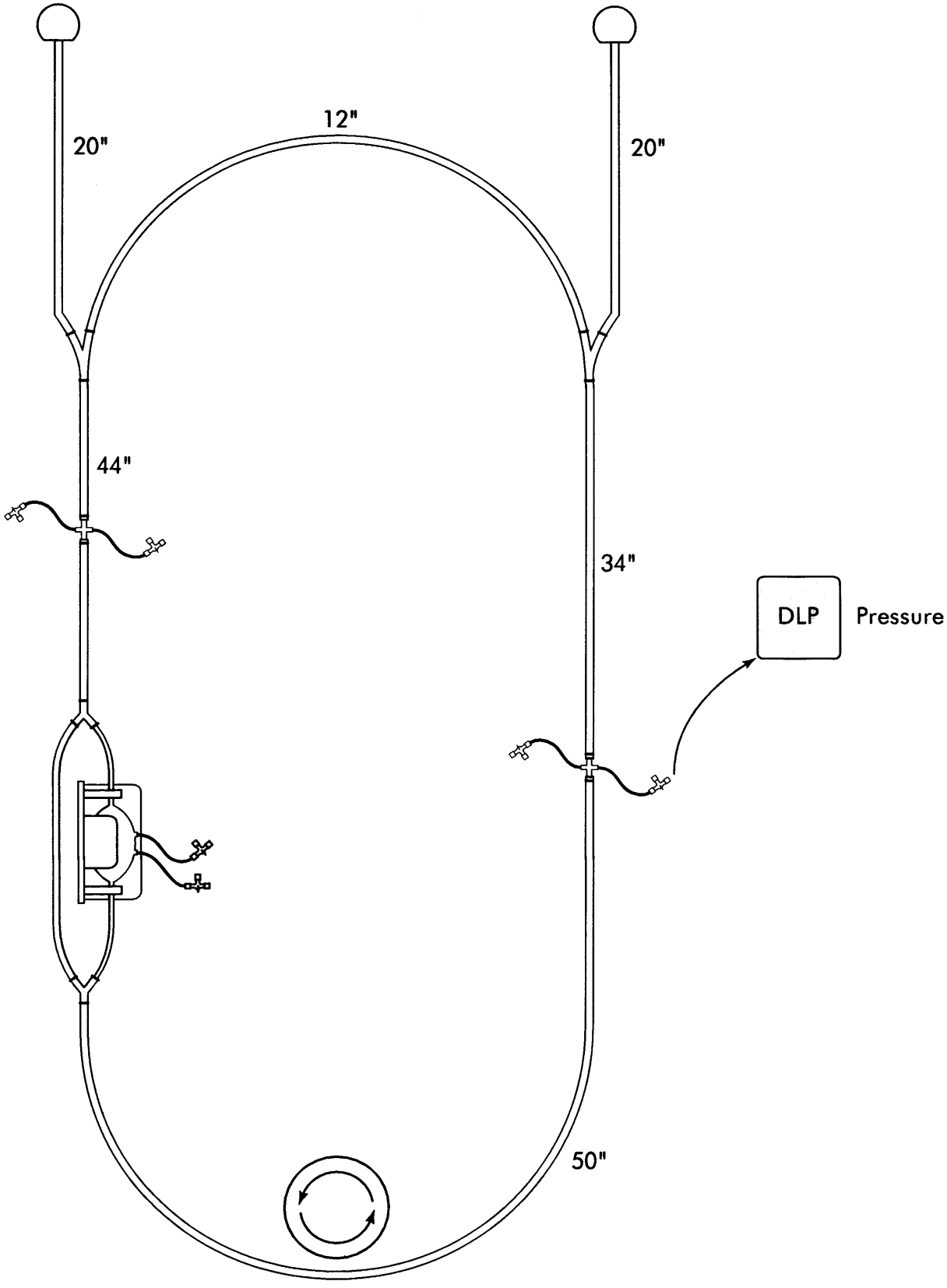
RCFV Right Common Femoral Vein

RCIV Right Common Illiac Vein

animals were not moved. It is likely that ligation of the veins around the cannulae would have prevented this oozing, but venous ligation may have resulted in congestion of the structures drained by the vein. Another reason for choosing the non-ligation technique was that it was envisaged that this model may be used in the future for experiments with de-cannulation and recovery of animals, therefore the practicality of the non-ligation cannulation technique had to be assessed. If a recovery experiment were performed the space around the vein should be packed with Surgicel (Johnson & Johnson) and Tisseel (Immuno AG, Austria) glue as used in clinical practice (Peek et al. 1996a), rather than the swabs used in this experiment.

CIRCUIT DESIGN, PRIMING & PERFUSION: Circuits were constructed of ½" diameter 3/32" wall thickness tubing. Tubing materials were Tygon S-65-HL (Norton Performance Plastics, Akron, Ohio, USA) for the control circuits, and LVA (actually Portex Silicone Tubing 800-500-575, but originally obtained through a subsidiary) and SRT 620 (Rehau GMBH, Germany) for the experimental circuits. Circuits were sterilised by the Central Sterile Supplies Department of Glenfield Hospital by exposure to Ethylene Oxide. Because of problems with availability of tubing from the manufacturers it was not possible to construct all the circuits at the debut of the experiment and then randomly allocate animals. Instead circuits were manufactured and used as supply of materials allowed until 5 animals had been perfused with each material (see results in Chapter 6). Circuits were identical (see Fig 5.8), and had a priming volume of 800ml. Circuits were primed with Hetastarch (Hespan, Geistlich GMBH, Germany) rather than the usual prime used during clinical ECMO. The reasons for this were that we did not want to modify the surface properties of the three materials by washing with albumin (Tsai et al. 1990). Secondly we wanted an asanginous prime as blood transfusion (i.e. blood prime) is known to result in

Fig. 5.8 In Vivo Circuit



significant cytokine production (Davenport, 1996; Gafter et al. 1996; Snyder et al. 1996; Federowicz et al. 1996) which would potentially mask or alter the response to ECC. A colloid rather than crystalloid prime was chosen as pigs are known to develop severe capillary leak syndrome during ECC, particularly if the colloid oncotic pressure is allowed to fall (Mickelson et al. 1990). Hetastarch was chosen as the best available synthetic colloid, as the gelatine solutions do not remain in the intra-vascular space for long (Ostgaard and Onarheim, 1996), the Dextrans have significant anti-coagulant activity (Matthiasson et al. 1995), and both dextran and gelatine solutions cause complement activation (Videm and Mollnes, 1994). Hetastarch also seemed to offer the highest osmolality of the available colloids (Tonnessen et al. 1993). At the time of design of the experimental protocol the post Hetastarch pruritis syndrome (Gall et al. 1996) was not widely recognised, it is possible that this process occurs in the pig, and that it could interact with cytokine release during ECC. However, as the same prime is used for each group of animals any confounding effect should occur equally in each group. In spite of the potential problem with pruritis, the lower osmotic pressure and anti-coagulant effects of the other preparations make Hetastarch the best synthetic colloid for the prime. In addition 100u of Heparin was added to the prime.

The aim of ECC was to expose the animals blood to the material during veno-venous perfusion using blood flow rates of at least 60ml/kg/min, in order to duplicate the shear rates found during clinical ECMO. The oxygenator and heat exchanger were omitted from the circuit in order to isolate the blood surface interaction due to the tubing alone. The pump used was the standard Stockert roller pump used during clinical ECMO. Occlusion was set by the same method as used during clinical ECMO, i.e. just under occlusive for air. All procedures were performed by the same perfusionist (R Scott ACP), to whom I am greatly indebted. The pump was servo controlled with the Seabrook bladder

box and controller, as used during clinical ECMO. Prior to ECC cannulation 50-100 u/Kg Heparin was given, and an ACT of >200 sec's was assured. After full mixing had been achieved a Heparin infusion into the circuit was started to maintain ACTs between 160-200 sec's. Post pump pressure was monitored throughout to act as a warning of return cannula occlusion. The bridge was flashed ¼ hourly to prevent clot formation. After 48 hours perfusion the animals were decannulated by placing a mattress suture around the entry point of the cannula through the skin, the cannulae were then withdrawn by an assistant whilst the suture was tied.

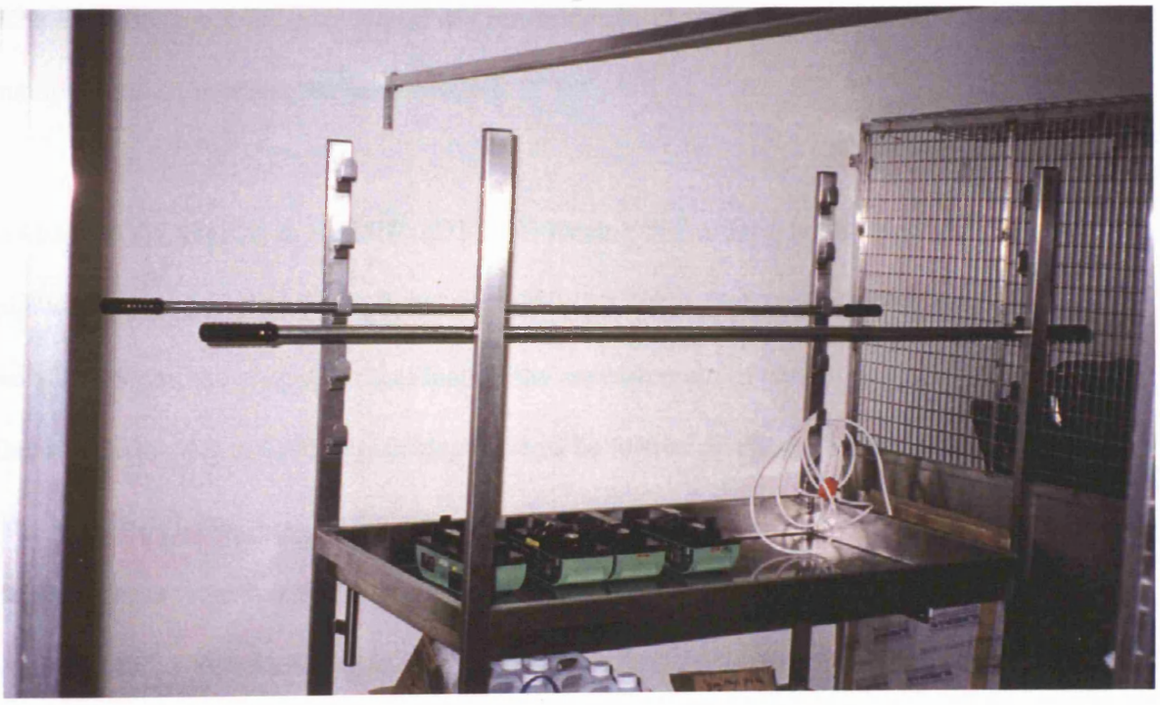
CLINICAL MANAGEMENT

The author was present in the department for the entire duration of each procedure, and made all clinical management decisions. These decisions should therefore have been consistent.

GENERAL: Intubation and insertion of monitoring lines was performed with the animals on a standard operating table. They were then moved to a specially constructed bed (see Fig. 5.9) which supported the animals on a sling between the two poles, thus distributing the animals weight evenly over a wide area, and also to allow the animals to be lifted up to augment venous return to the pump. The canvas of the sling was covered with synthetic "sheepskin" (Vet-Bed, National Veterinary Supplies, Stoke-on-Trent). Normothermia was maintained by adjustment of the room temperature and electric heating blankets (International Market Supplies, Congleton), further Vet-Bed over the pigs, and lagging of the extra-corporeal circuit with aluminium foil if necessary.

IV FLUIDS & ANTIBIOTICS: Intravenous Dextrose 4% / Saline 0.18% with KCL 20mMol per 500ml was administered at a rate of 1ml/kg/hour as maintenance fluid.

Fig. 5.9 Frame for suspension of animals
(canvas hammock not shown)



Supplemental KCL was added to this if the serum Potassium dropped below 4 mMol/L. Serum Potassium was measured hourly using a Ciba-Corning 416 Na/K analyser. Ampicillin 1g IV was given every 6 hours as antibiotic cover for the multiple instrumentation. Ampicillin was chosen after taking Veterinary advice as to the sensitivities of pigs usual skin flora (D Forbes, Personal communication). If volume administration was indicated as evidenced by a reduction in venous return to the pump, low CVP or PAWP, or hypotension accompanied by tachycardia and visible pulsus paradoxus on the arterial line trace, then Hespan was given. Sodium Bicarbonate 8.4% (Dose (mls) = 0.3 x Base Excess x Weight) was given for treatment of metabolic acidosis unresponsive to manipulation of haemodynamics (below).

HAEMODYNAMICS & URINE OUTPUT: During the initial 6 hour "run-in" period each pig was assessed to find filling pressures (CVP/PAWP) which would result in a satisfactory cardiac output. Unfortunately the measurement of cardiac output by thermodilution did not prove reliable, this will be further discussed in the measurements section (below), and so other indices were used to gauge the adequacy of the cardiac output. These were warmness of the peripheries, peripheral (Posterior Tibial) pulse volume, Mixed Venous Oxygen Saturation, Mean Arterial Pressure (MAP) and urine output >1ml/kg/hour. In most animals an MAP of 60mmhg was adequate. If the urine output dropped below 1ml/kg/hour in the presence of clinical correlates of cardiac output which had been previously sufficient then frusemide 1-2mg/kg was administered. If cardiac output fell despite a filling pressure which had been previously adequate then a Dopamine infusion was started. If urine output was still inadequate despite administration of Frusemide and Dopamine then Aminophyline 5-10mg/kg loading dose was given, followed by an infusion at 10mcg/kg/min (Huet et al. 1995; Lochan et al. 1997). If

>15mcg/kg/min of Dopamine were required to maintain blood pressure then an Adrenaline infusion was commenced at 0.05mcg/kg/min, and titrated to effect. Two animals (N & P) required administration of Amiodarone for 24 hours to control tachy-dysrhythmias which were causing hypotension, this was effective in both cases.

VENTILATION: Animals were maintained on Intermittent Positive Pressure Ventilation (IPPV) throughout the procedure. An Engstrom Respiratory System 300 ventilator (Junger Medical, Sweden) was used to deliver warmed and humidified gases via single use closed circuits (Intersurgical 1.6M Breathing System #2009, Intersurgical, Wokingham, Berkshire, UK). Fraction of Inspired Oxygen (FIO_2) was adjusted to the minimum level needed to maintain a peripheral oxygen saturation (S_pO2) of >95%. Positive End Expiratory Pressure (PEEP) was used to prevent atelectasis, and the minute volume was adjusted to maintain end tidal CO_2 ($ETCO_2$) around 6KPa. If the Peak Airway Pressure (PIP) exceeded 35 cmH_2O the respiratory rate was increased to reduce the tidal volume and keep the PIP < 35cmH_2O if possible. Endo-tracheal suction was used to clear secretions as required. It is not possible to adjust the I:E ratio on the Engstrom 300, and the expiratory restriction and negative end expiratory pressure were left off. On line monitors ($ETCO_2$ and S_pO2) were used to adjust ventilation rather than blood gas determinations as it did not prove possible to perform sufficiently frequent blood gas analyses. This was because the portable blood gas analyser, despite costing over £5000 (Stat-Pal II, SenDx Medical Inc. Carlsbad, California), proved unreliable, and the nearest clinical blood gas machine was in the Leicester Royal Infirmary 1 mile away. This was used to perform blood gases intermittently during the hours of 0900-2200, but could not be used during the night as there was no portering available.

OBSERVATIONS: Each hour the technician on duty would fill in a chart recording haemodynamic and other data (below), blood gases were not measured hourly as

TIME **DATE** **NAME**

FLUIDS

IN: Hespan: OUT: Urine:

Dex 4% / Sal 0.18%: Gastric:
(KCL 20mMol/500ml)
Other: Other:

DRUGS

INFUSIONS: Heparin 25000 u / 50 ml: BOLUS: Propofol:

Pentobarbitone 60mg / ml: Ampicillin:

Morphine (dose) : Other 1:

Midazolam 5mg/ml: Other 2:

Other 1 : Other 3:

Other 2 : Other 4:

VENTILATION

Rate: Air: O2: FIO2: PIP: PEEP:

SpO2: ETCO2:

ABG:

ECMO

FLOW: LINE PRESSURE: SYPHON: ACT:

OBSERVATIONS

HR: MAP: PAP: PAWP: CVP:

RYTHYM: NSR / OTHER: NA: K: BM STIX:

CORE TEMP: PERIPH TEMP: HEATER : 1 / 2 / 3

POSITION: SUPINE / L DOWN / R DOWN.

COMMENTS

previously discussed. In the event of any deviation from the parameters given above the author would be called to re-assess and adjust the management accordingly. Note that the heat pad settings of 1-3 correspond to temperatures of 26.8, 30.1 and 40.6 Celsius respectively.

LABORATORY METHODS

Samples were taken according to the sampling protocol and analysed as follows:

i) FULL BLOOD COUNTS: Paired samples were taken from the arterial line and the venous drainage line of the extracorporeal circuit (prior to cannulation the venous sample was taken from the pulmonary artery catheter). Blood was placed in 2.7ml EDTA tubes (EDTA KE, Sarstedt, Germany) and transported to the Leicester Royal Infirmary (LRI) Haematology Department for automated analysis (Coulter STKR FBC analyser). Manual differential white cell counts were also done at LRI (HEMA TEK, Bayer). Trans-pulmonary neutrophil gradient was calculated from the differential white cell counts of the paired samples.

ii) COAGULATION: Three ml of blood was taken from the arterial line into a Citrated tube (Coagulation 9 NC, Sarstedt, Germany) and transported to the LRI Haematology Department for analysis. Because of the similarities between the porcine and human coagulation systems (Karges et al. 1994) it is possible to use routine human haematological techniques. Samples were analysed on an automated coagulometer (Sarstedt Biomatic B10) using Dade reagents (Baxter Diagnostics, Deerfield, Illinois). However, porcine fibrinogen is sufficiently different to human fibrinogen that it did not register on the Claus method used clinically (Clauss, 1957; Vermylen et al. 1963). Fibrinogen estimation was therefore omitted.

iii)FREE HAEMOGLOBIN: Three ml of blood was taken from the arterial line into a citrated tube as for (ii) and transported to the LRI Department of Haematology where it was centrifuged and serum separated and frozen at -20 Celsius for later analysis. Analysis was performed by colorimetric determination (Sigma Diagnostics, Catalog No. 527). This assay is based on the catalytic activity of haemoglobin on the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) by H_2O_2. The rate of colour formation at 600nm is proportional to the haemoglobin concentration (Lijana and Williams, 1979; Standefeder and Vanderjagt, 1977) .

iv) PLATELET ACTIVATION: Plasma Thromboxane B2 (T_XB2) was taken as an index of platelet activation (Li et al. 1995). Blood was taken from the arterial line and collected into cold eppendorf tubes containing 15mcg indomethacin (Sigma) and 1.5mg EDTA (Sigma), to a final volume of 1.5 ml. These were centrifuged at 1000rpm at 4 Celsius for 10 minutes and then two 0.5ml aliquots of Plasma were removed. Plasma samples were acidified with two drops of 1N Hydrochloric acid, and then T_XB2 was extracted with Ethyl-Acetate. Samples were then evaporated under nitrogen to dryness and stored at -70 Celsius for later Enzyme Immunoassay. All assays were carried out by Mr Paul Whitaker of the Department of Biochemistry, Leicester Royal Infirmary to whom I am most indebted. ELISA was performed using commercially available assay kit (BIOTRAK Thromboxane B_2, Amersham, UK). Samples were analysed in duplicate and the mean activity calculated.

v) COMPLEMENT ACTIVATION: One 2.7ml EDTA tube (EDTA KE, Sarstedt, Germany) was filled with blood from the arterial line, plasma was separated and then frozen at -20 Celsius for later analysis. Plasma was analysed for activity of the des arginine metabolite of C3a (C3adesarg) (Roitt, 1994). This was measured using a commercially available assay kit which is based on the competition between unlabelled C3a, the C3adesarg in the sample, and a fixed quantity of ^{125}I labelled C3adesarg for a limited

number of binding sites on a specific antibody (Human complement C3adesard [^{125}I] assay system, Amersham International PLC). If the amounts of antibody and radioactive ligand are fixed the amount of radioactive ligand bound will be inversely proportional to the concentration of the non-radioactive ligand in the sample. All complement assays were performed by Dr Jonathan North, Dept. Immunology, Leicester Royal Infirmary.

vi) LACTOFERRIN: Serum was collected in plain tubes (Z9 Sarstedt, Germany), separated and frozen at -20 Celsius for later analysis. All assays were carried out by Mr Paul Whitaker of the Department of Biochemistry, Leicester Royal Infirmary to whom I am most indebted. Samples were analysed by ELISA after the method of Hegnoj et al (Hegnhoj and Schaffalitzky de Muckadell, 1985). The lactoferrin for the standard was obtained from Sigma (Poole, Dorset, UK), and the antibodies from Dako (Copenhagen). All analyses were duplicated and the mean value taken.

vii) CREATININE CLEARANCE: Six hour creatinine clearance estimations were performed three times during the study, except in cases where animals died before 48 hours of ECC had elapsed. Six hour urine collections were made before ECC cannulation during the "Run In" period, and from 0600-1200 hours on days 2 and 3. Urine volume was measured and an aliquot collected into a universal container. Blood was taken from the arterial line at around 0900 hours and collected into a serum tube (Serum Gel S, Sarstedt, Germany), these samples were taken to the LRI Department of Chemical Pathology for measurement of creatinine concentration. Urine Creatinine was assayed spectrophotometrically after the method of Jaffe et al (Jaffe, 1886) (Technicon Axon, Miles Diagnostics, Tarrytown, New York, USA). Serum creatinine was assayed using the same method but a different kit (Synermed Cat No VI360, Burgess Hill, West Sussex, UK).

Creatinine clearance was then calculated as follows (Marshall, 1988):

$$\text{Creatinine Clearance} = \frac{\text{Creatinine Concentration (Urine) x Urine Volume (ml)}}{\text{Creatinine Concentration (Blood) x Time (Minutes) x Body Weight (Kg)}}$$

viii) HISTOLOGY SAMPLES: At the end of the experiment animals were sacrificed by overdose of pentobarbitone and exsanguination, a post mortem examination was then performed including the excision, weighing and macroscopic examination of the heart, lungs, liver, spleen and kidneys. Samples of these organs, stomach and duodenum were taken and preserved in formalin for later histological analysis. All organs were examined after standard haemaotoxylin and eosin staining, sections were examined and reported by the same histopathologist (Dr A Fletcher, Consultant, Department of Histopathology, University of Leicester) who was blinded as to which group each animal belonged.

The lung was also examined by immunohistochemical staining for neutrophil myeloperoxidase. 4μm sections of lung tissue were cut onto slides coated with aminopropyltriethoxysilane (Sigma UK) and incubated with Streptavidin Biotin Complex / Horse Radish Peroxidase (Duet, K0492, Dako, High Wycombe, UK) for 30 minutes each at room temperature. Endogenous peroxidase was blocked by incubation with 6% hydrogen peroxide for 10 minutes. Following a 10 minute incubation with 1:20 normal goats serum (X0907, Dako, High Wycombe, UK) sections were incubated overnight at 4^0C with rabbit α human myeloperoxidase (A0398, Dako, High Wycombe, UK) diluted 1:50 with Tris Buffered Saline. Sections were then stained with 0.05% di-amino benzidine (BDH, Lutterworth, UK) to highlight the peroxidase positive cells, whilst nuclei were counterstained with haematoxylin. Semi-quantative analysis of myeloperoxidase stained sections was then conducted to yield cell counts per unit area, five random areas of each section were counted and the mean neutrophil counts per unit area calculated.

ix) LUNG WATER: Following sacrifice of the animals at the end of the experiment samples of both lungs were excised and frozen at -20^0 Celsius for later estimation of lung water. Each lung sample was weighed (wet), and then dried at 95^0 Celsius for 72 hours before re-weighing (dry).

Lung water was expressed as a percentage of the wet weight as follows:

$$\% \text{ Lung Water} = \frac{\text{Wet-Dry}}{\text{Wet}} \times 100$$

STATISTICAL METHODS.

Baseline values for each material (SRT & LVA) were compared with the control values (Tygon) using an un-paired t-test. Levenes test is applied automatically by SPSS to ensure that data is normally distributed. This approach was also used to compare data that had only one time point such as lung water and lung neutrophil infiltration.

Data was collected throughout the experiments and is displayed on the graphs as variation with time. The line of best fit is drawn through the mean values, calculated by Microsoft Excel from the results obtained at each time point. Maxima and minima are also displayed. Variables that had a significant difference from the control group (see below) are highlighted with a blue background.

The difference between values at the beginning of the porcine experiments and the end was found by subtracting the baseline value from the final value. A negative sign indicates a decrease from baseline and vice versa. In order to determine if values increased or decreased significantly during the experiment changes from baseline for each group were analysed using an unpaired t-test and control value of zero. The differences were also compared with the mean difference found in the controls (Tygon) using the unpaired t-test.

The survival of animals was measured by tabulating the number of animals in each group alive and dead at the end of the 48 hour experiment. These were then cross tabulated and compared with controls using Fishers exact test.

A P value of < 0.01 was taken to indicate significance. The higher confidence level was chosen to make conclusions more robust. This was important as the assignment of animals was not formally randomised.

CONCLUSION

Although this is only a preliminary study, much has been learnt about the strengths and weaknesses of this porcine model of prolonged ECC. Induction of anaesthesia with 20mg/Kg IM Ketamine seems safe and effective, although additional IV Propofol and topical local anaesthesia are necessary to allow laryngoscopy and intubation. Propofol infusion provides good short term maintenance of anaesthesia without tachyphylaxis for 1 to 2 hours. Continued sedation could be easily achieved with the pentobarbitone infusion without any of the fluctuations in conscious level, exponentially escalating doses and massive expense of Midazolam and Morphine infusions. Peripheral Pentobarbitone caused severe thrombophlebitis, and it was given centrally thereafter.

ECC cannulation was best achieved with a long 21 DLP drainage cannula via the right femoral vein, and a short 17 DLP return cannula in the right external jugular vein. The left external jugular could also be used in the event of complications. Both the arteries and vein exhibited great fragility and were particularly prone to dissection.

Further discussion of the response of the animals to extracorporeal circulation can be found at the end of chapter 7, after presentation of the results.

CHAPTER 6

In Vivo II: RESULTS, DISCUSSION & CONCLUSIONS

INTRODUCTION

In this chapter the results of the porcine perfusion experiments described in chapter 5 will be given. To briefly re-cap, these experiments compared the whole organism response to 48 hours of veno-venous perfusion using circuits made from three different materials. Extracorporeal circuits consisted of a simple loop of tubing, without oxygenator or heat exchanger. There were three groups of five animals each, a control group perfused with Tygon tubing and two experimental groups perfused with LVA and SRT circuits respectively. During perfusion physiological data was recorded hourly and blood samples were taken periodically. Blood was analysed for a wide range of inflammatory markers and indices of activation of the coagulation cascade. After 48 hours of perfusion animals were sacrificed and a full post mortem examination was performed. The statistical comparison of the three groups will be presented alongside the results, and will be followed by a discussion of the findings.

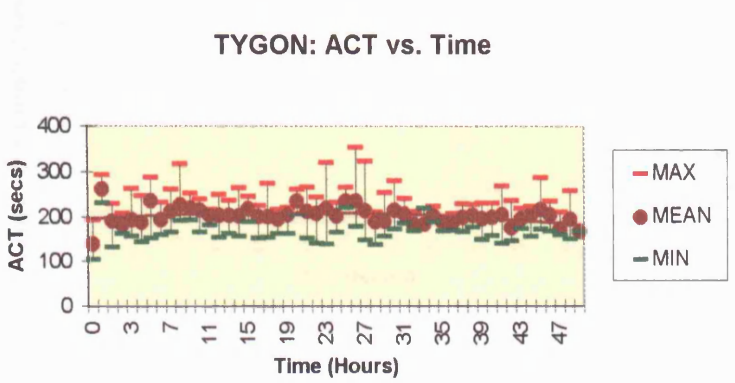
RESULTS

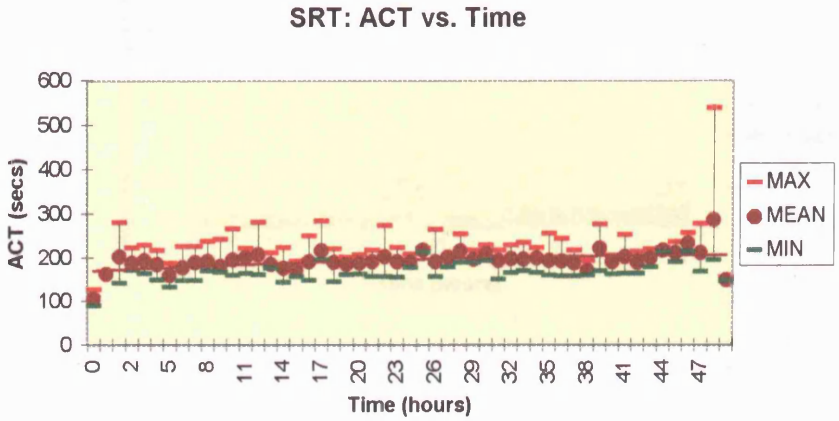
There were a number of significant differences between variables at baseline (see fig 6.33). Variables included ACT, C3adesarg, Creatinine clearance, extracorporeal flow, pulmonary artery pressure, pulmonary to systemic arterial pressure ratio and weight. These are all discussed subsequently. The majority of the results are presented graphically in figures 6.1 to 6.32, a list of contents for these graphs is also given. Each figure comprises four graphs of the same variable, three displaying maximum, mean and minimum vs. time for each tubing type, and one displaying the mean values for all three materials. Graphs are displayed four to a page for ease of comparison. Variables where the final value minus

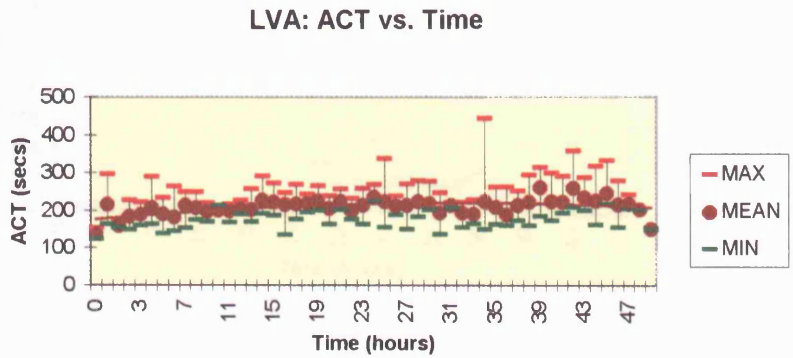
the baseline value differs significantly from the control (Tygon) are highlighted by a blue background. A number of variables have discreet values, which do not lend themselves to graphical display, these are tabulated after the graphs (figure 6.34 c). All results are summarised at the end of the graphs in figures 6.34 a & b. Figures 6.35-6.47 are photomicrographs detailing representative histological specimens. The abnormal histology is summarised in figure 6.48. A table of contents for the photomicrographs is given in the discussion.

TABLE OF CONTENTS FOR GRAPHS

Figure 6.1: ACT vs. TIME







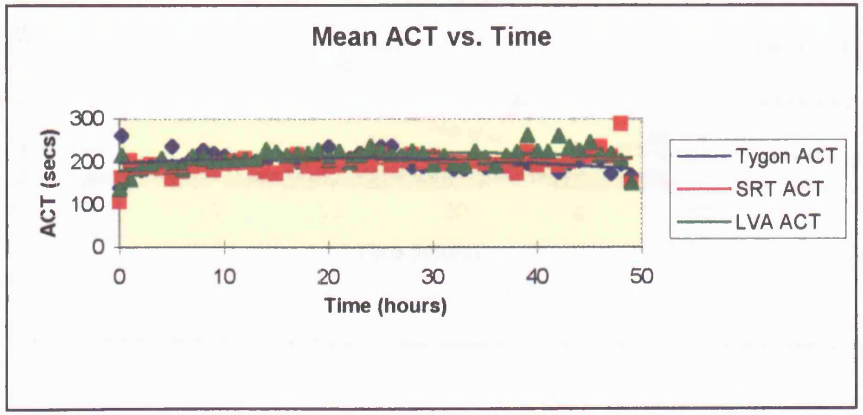
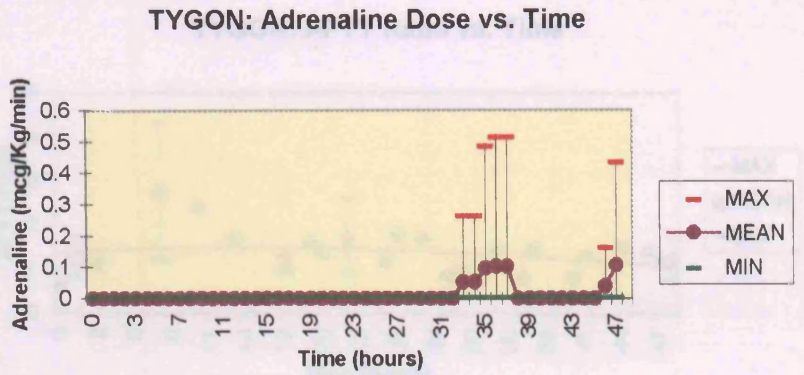
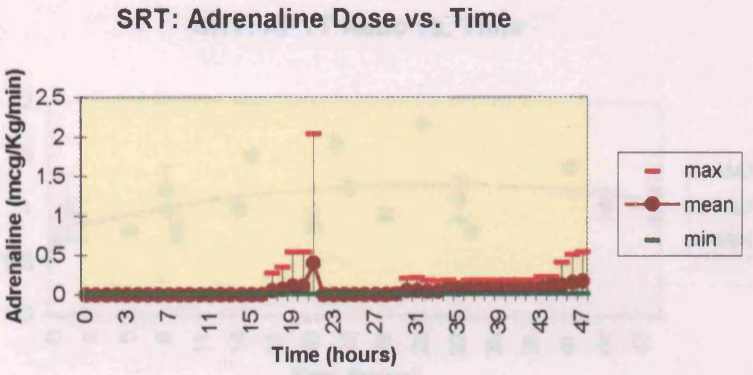
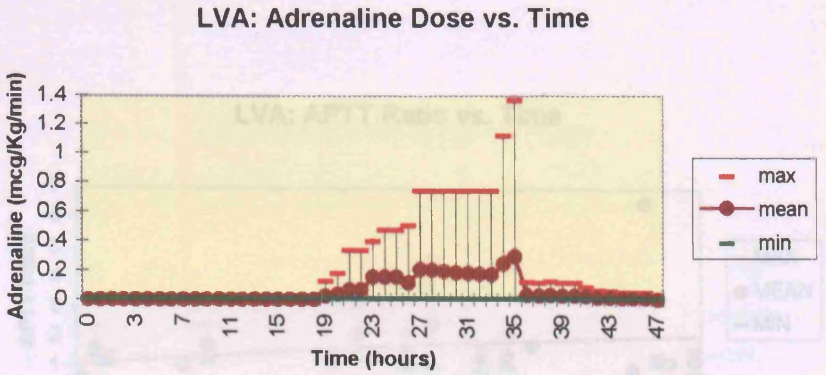


Figure 6.2: ADRENALINE DOSE vs. TIME







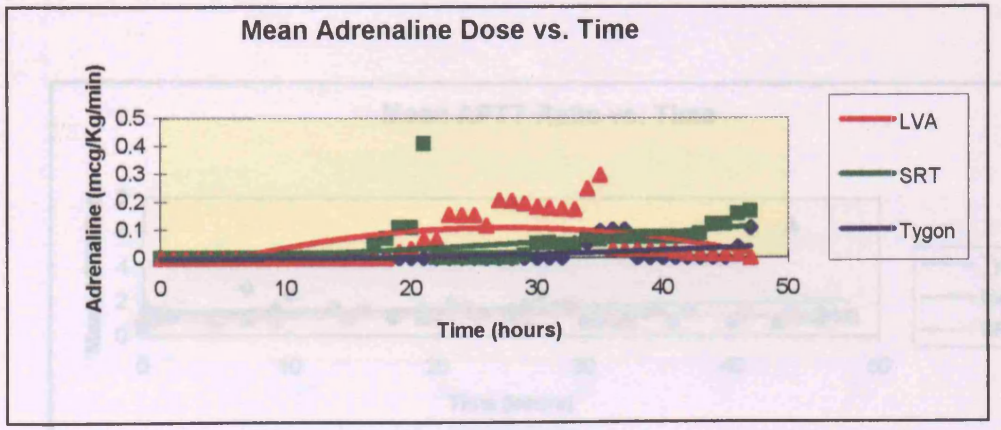
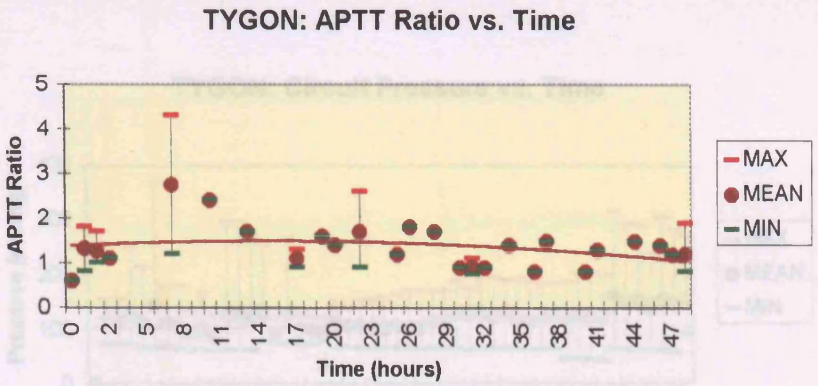
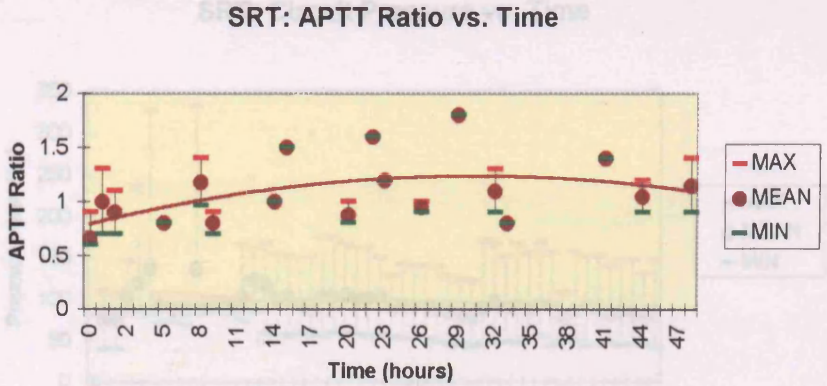
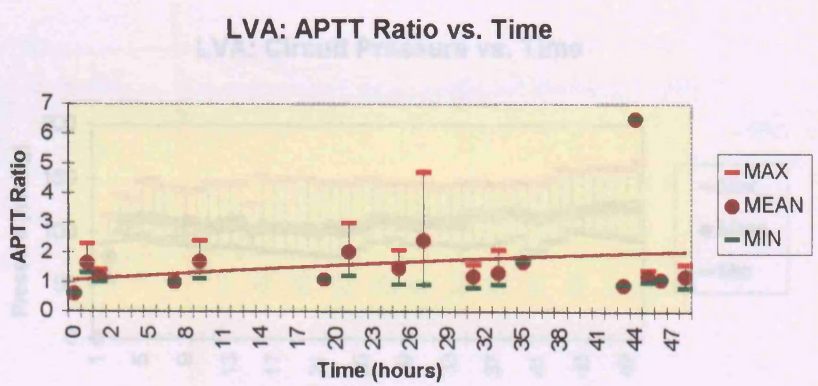


Figure 6.3: APTT RATIO vs. TIME







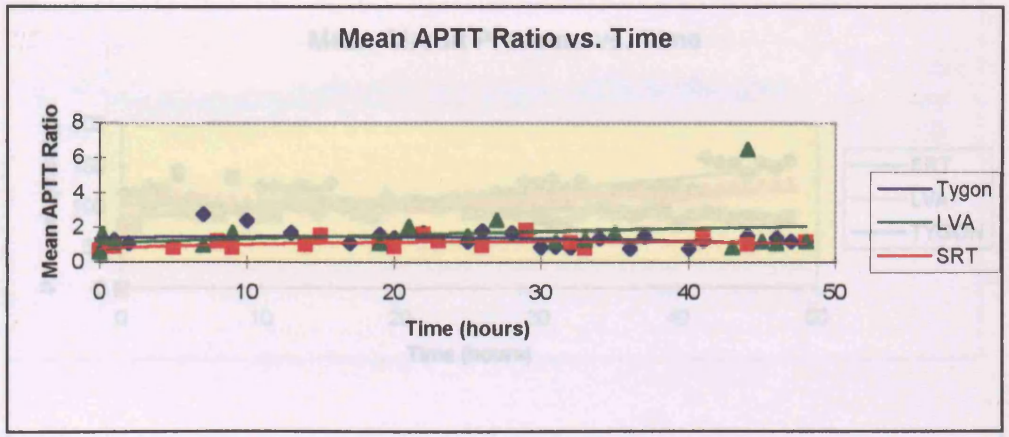
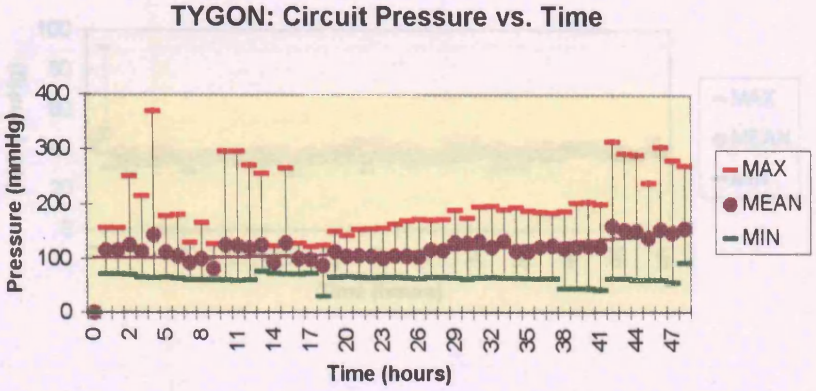
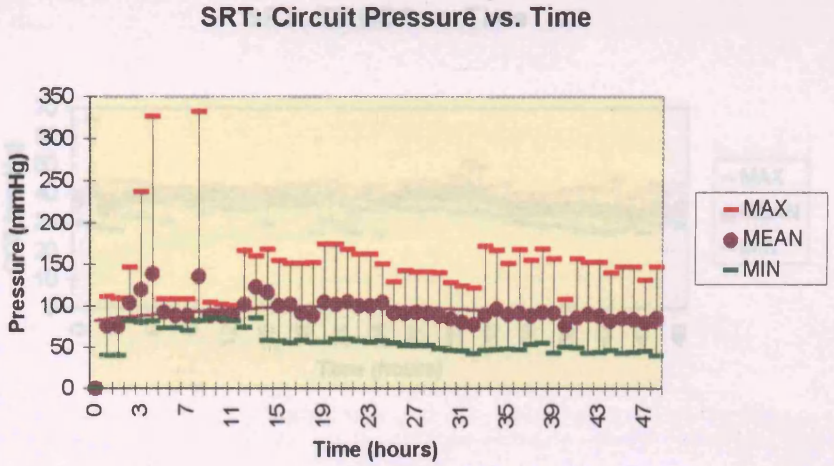
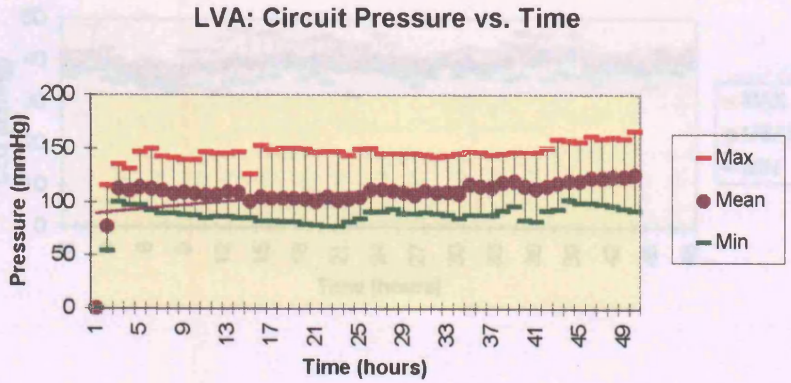


Figure 6.4: CIRCUIT PRESSURE vs. TIME







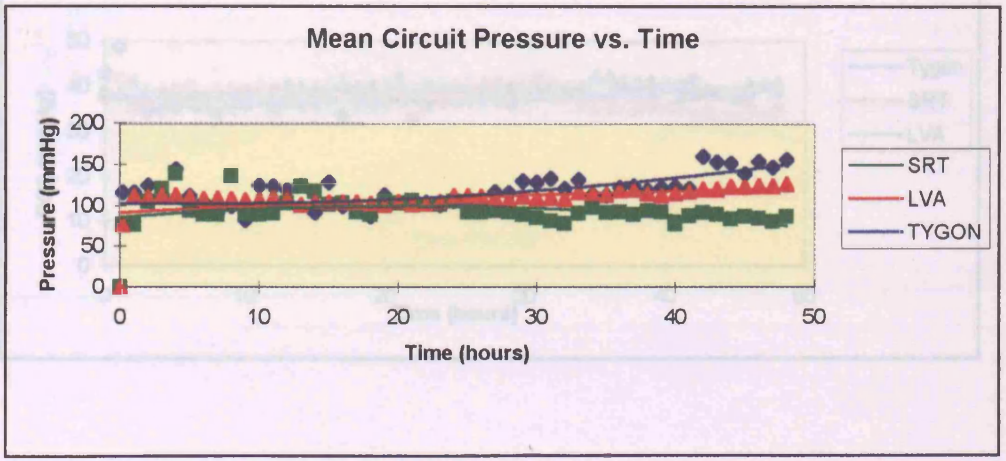
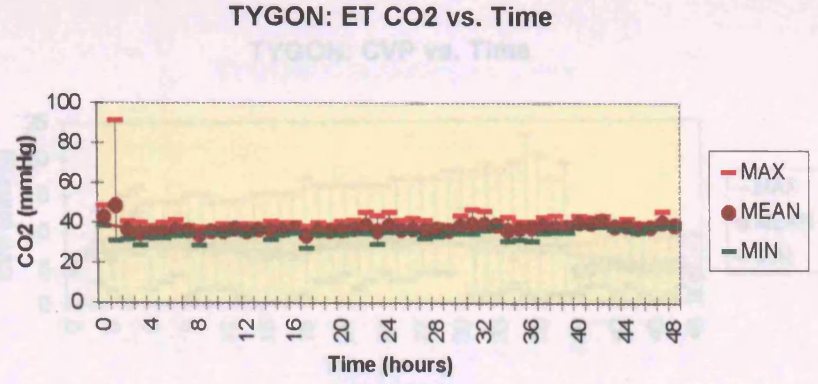
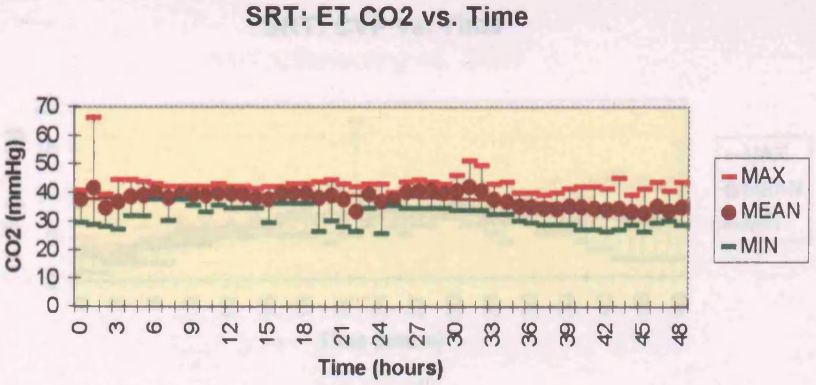
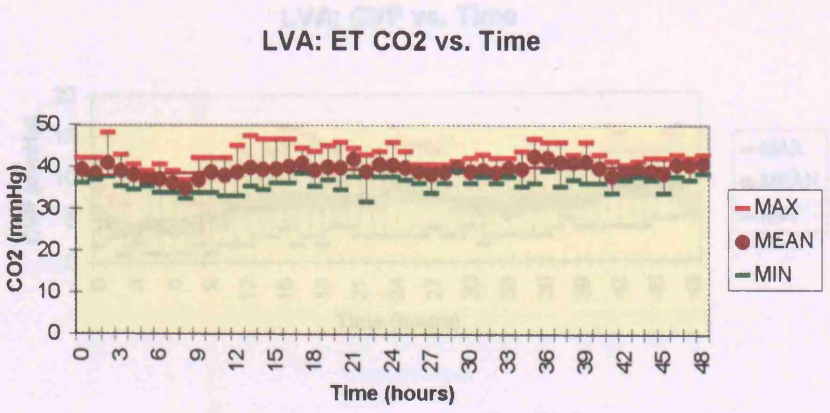


Figure 6.5: CARBON DIOXIDE TENSIONS vs. TIME







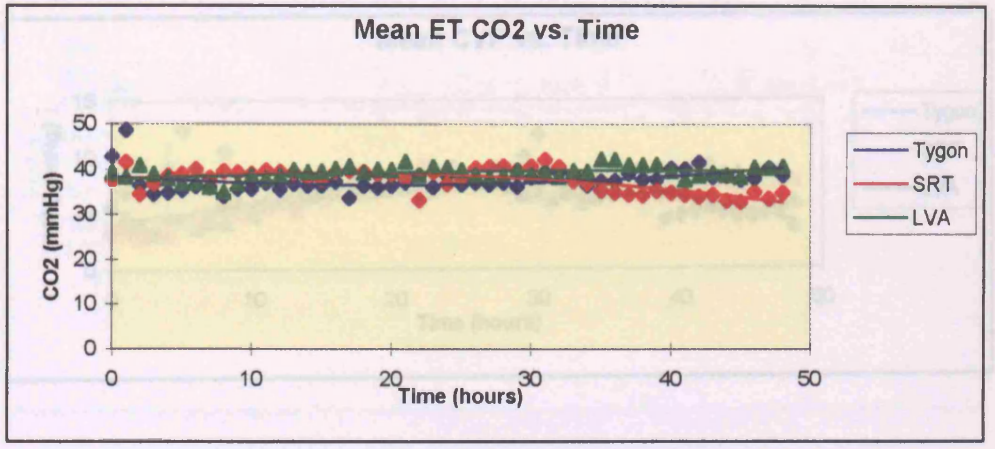
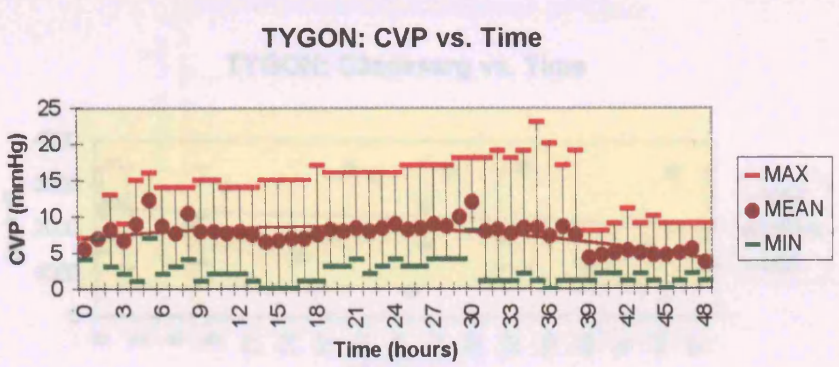
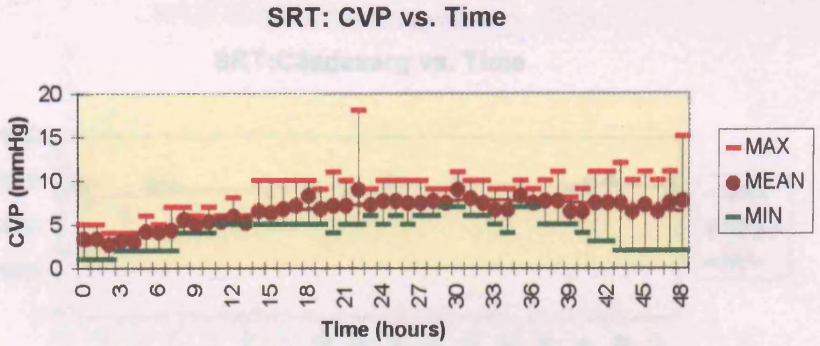
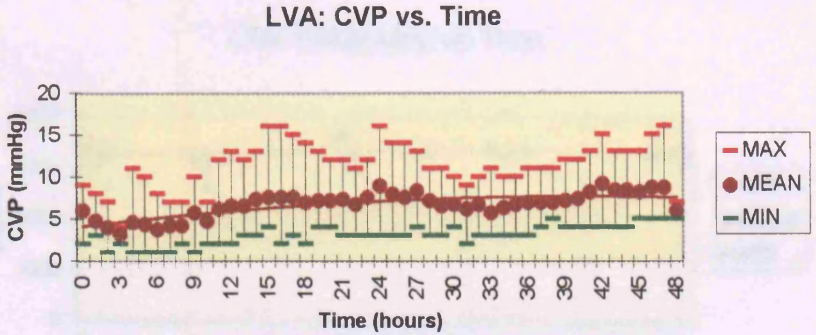


Figure 6.6: CENTRAL VENOUS PRESSURE vs. TIME







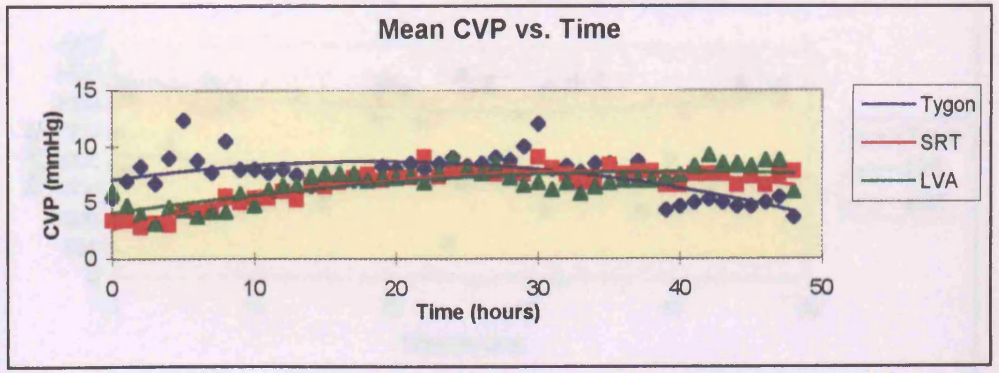
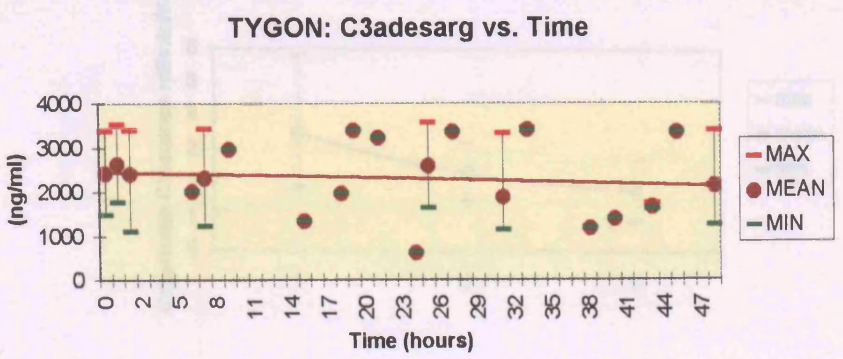
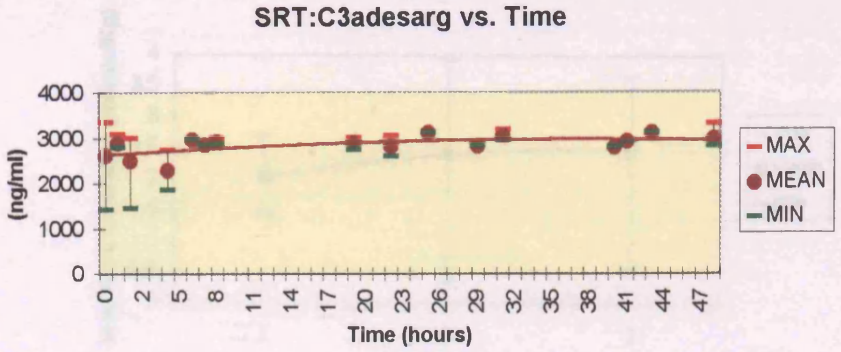
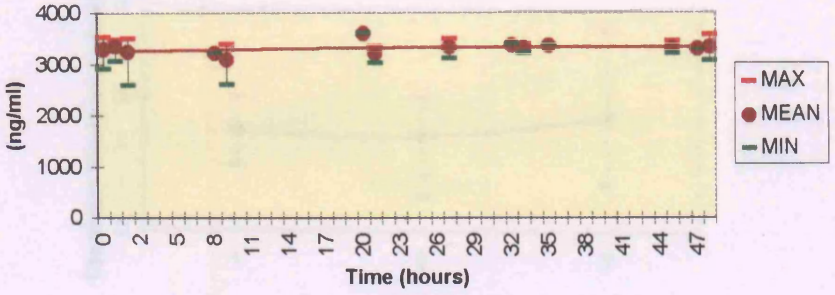


Figure 6.7: C3adesarg vs. Time







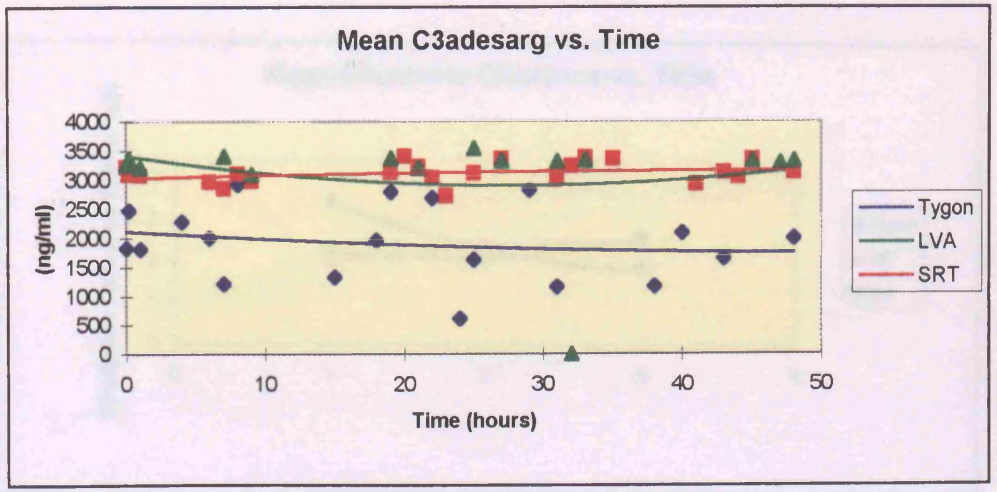
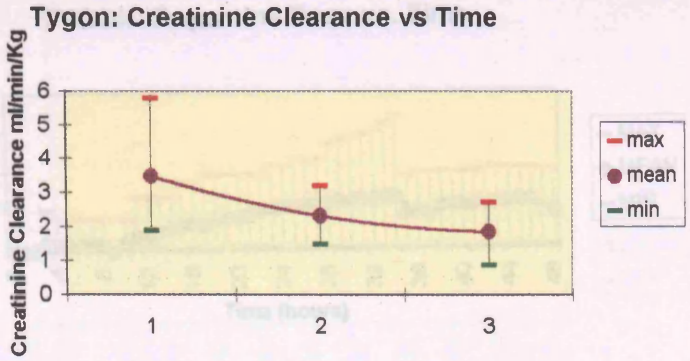
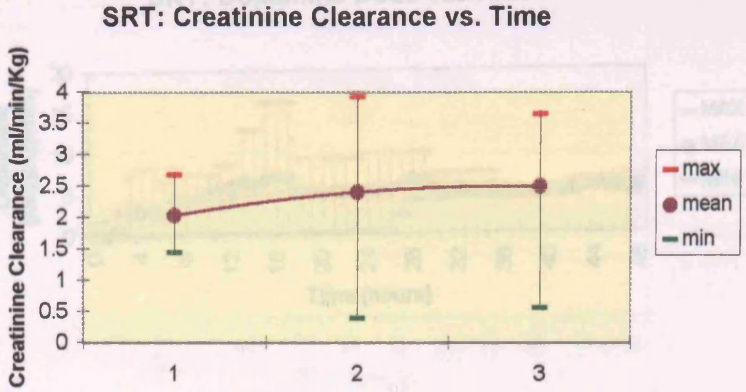
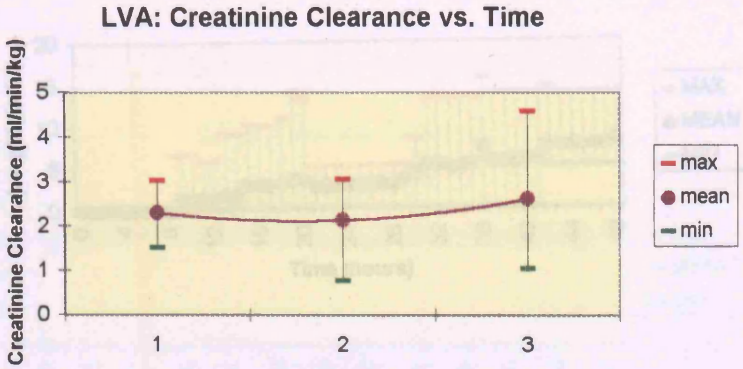


Figure 6.8: CREATININE CLEARANCE vs. TIME







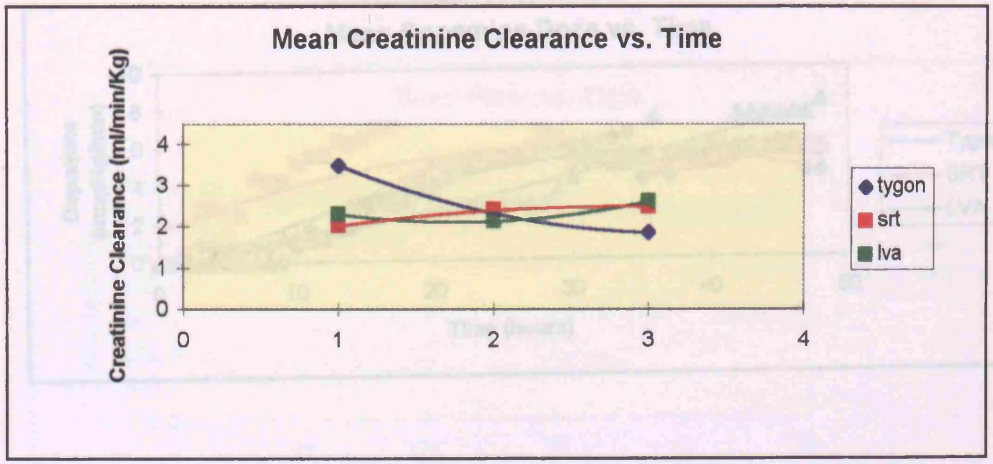
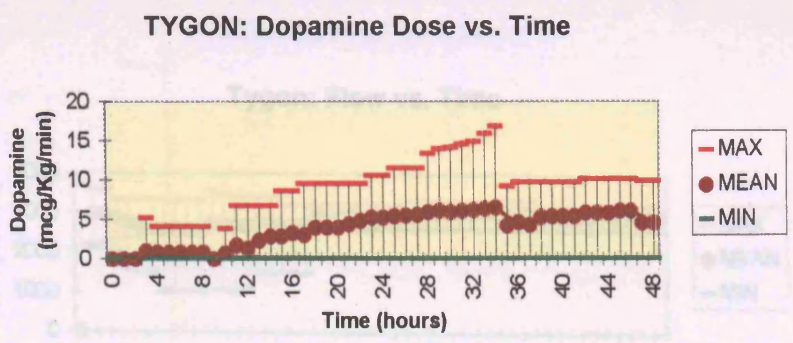
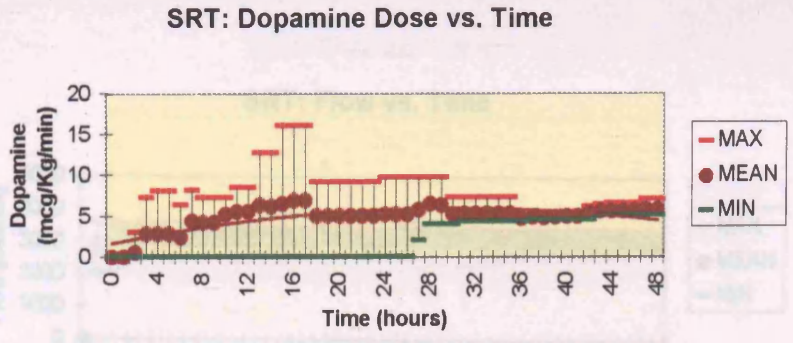
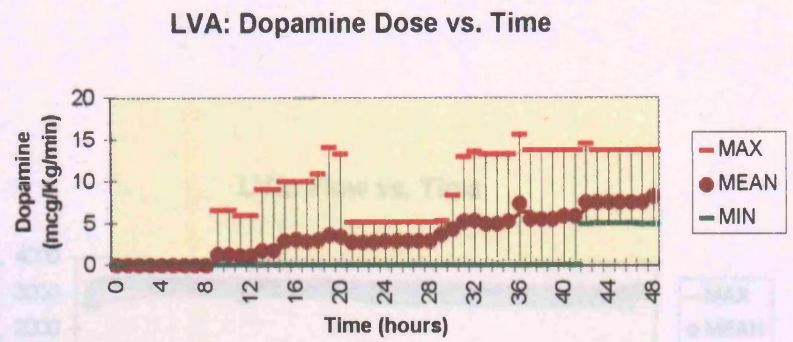


Figure 6.9: DOPAMINE DOSE vs. TIME







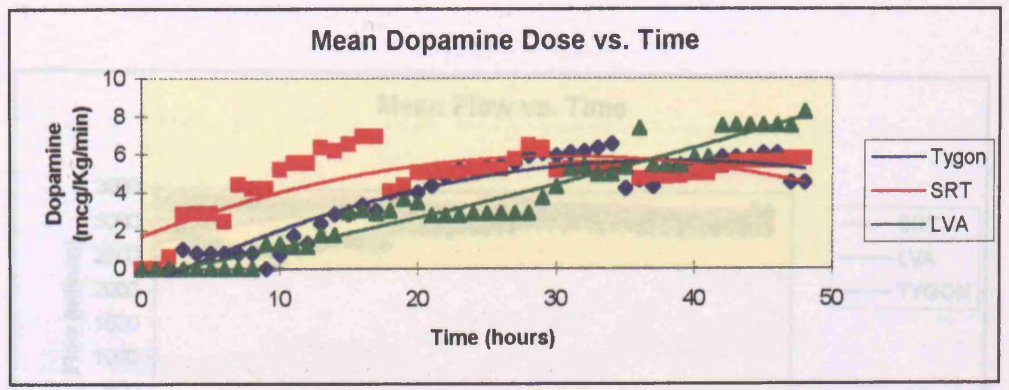
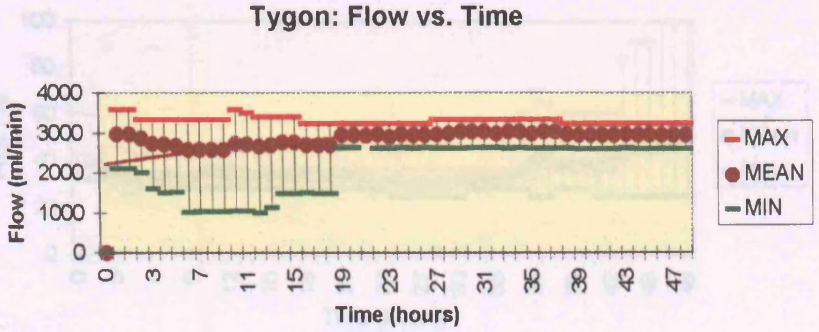
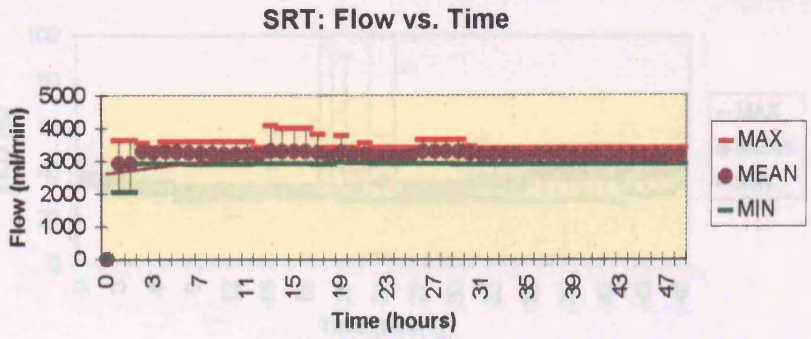
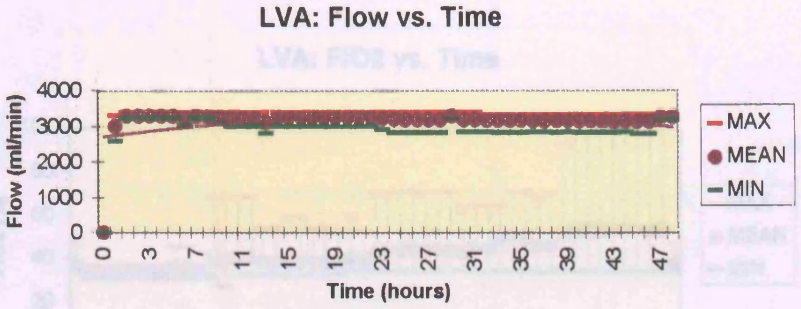


Figure 6.10: EXTRACORPOREAL FLOW vs. TIME







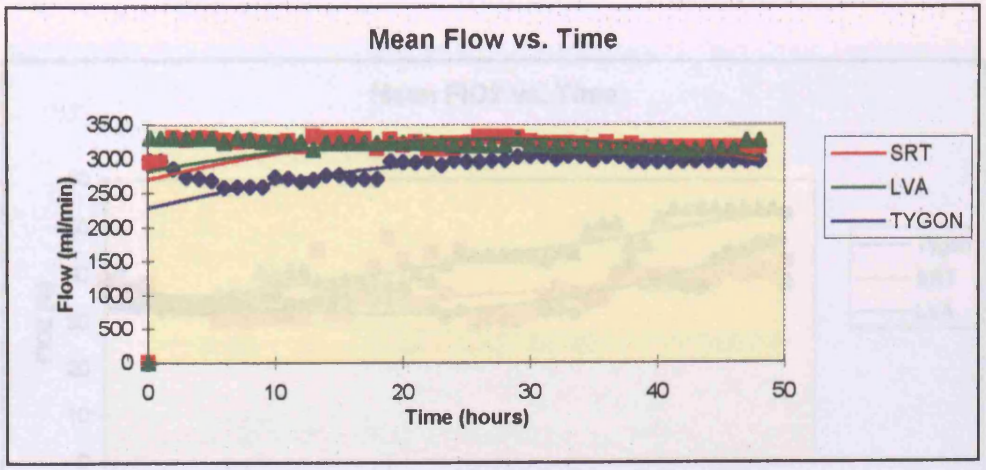
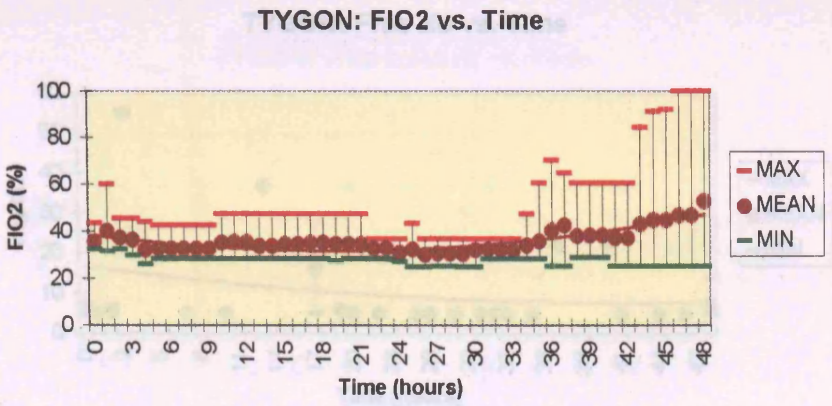
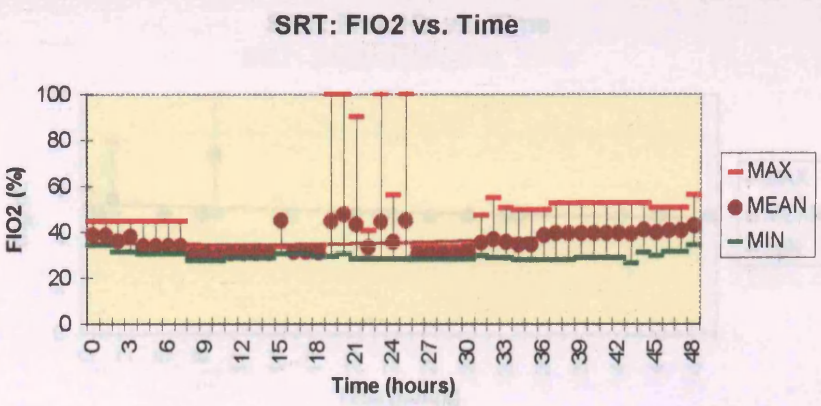
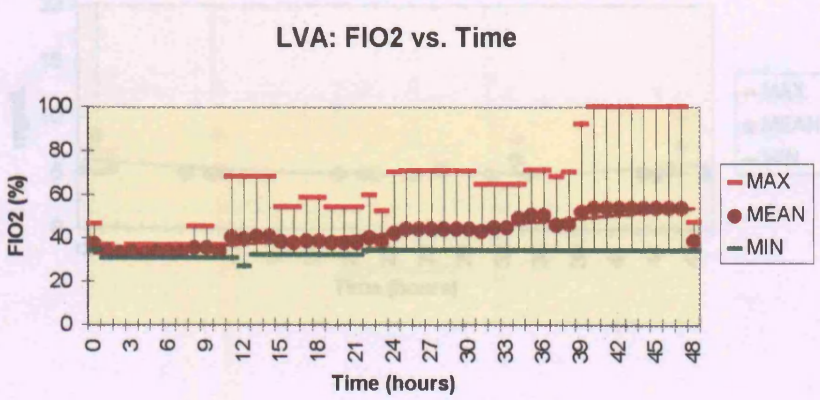


Figure 6.11: FRACTION OF INSPIRED OXYGEN vs. TIME







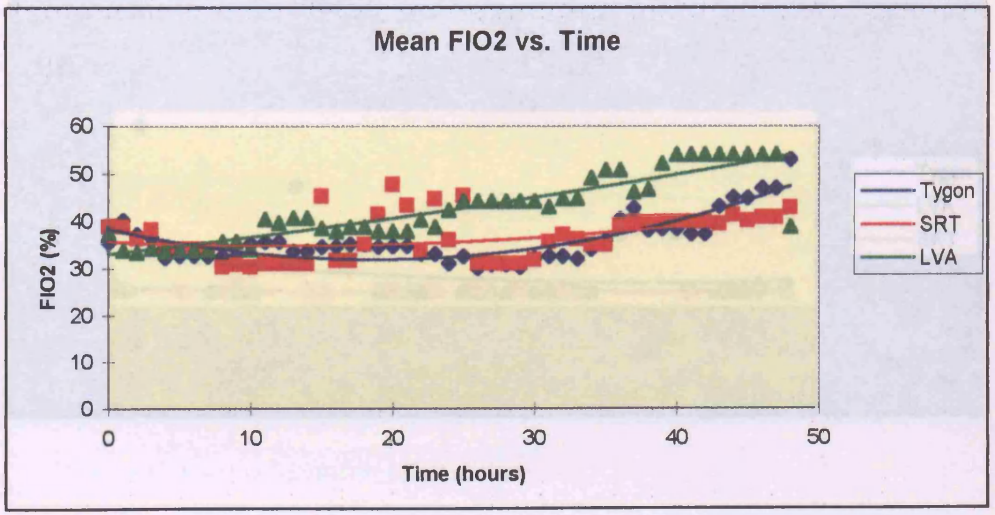
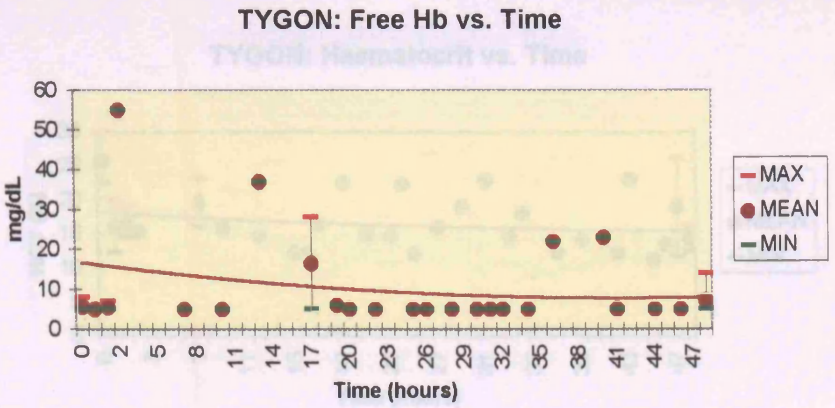
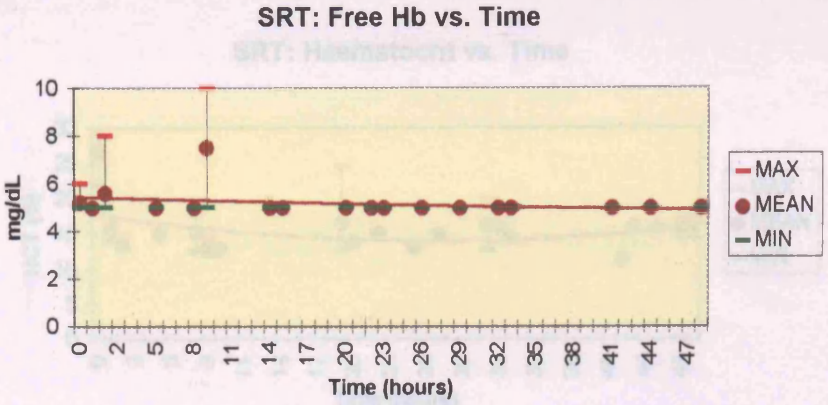
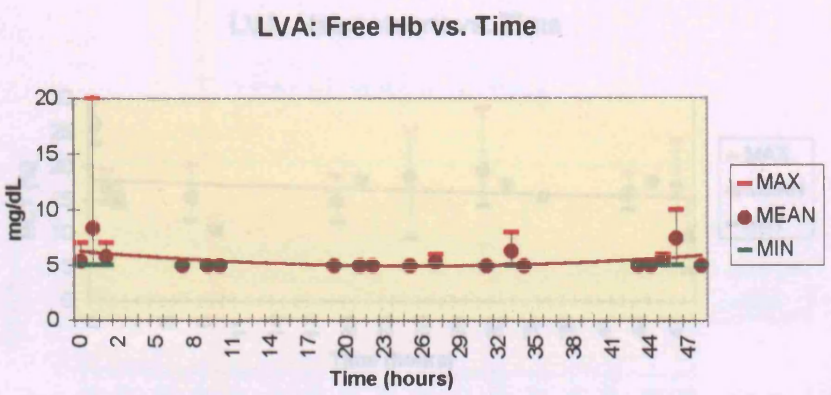


Figure 6.12: FREE HAEMOGLOBIN vs. TIME







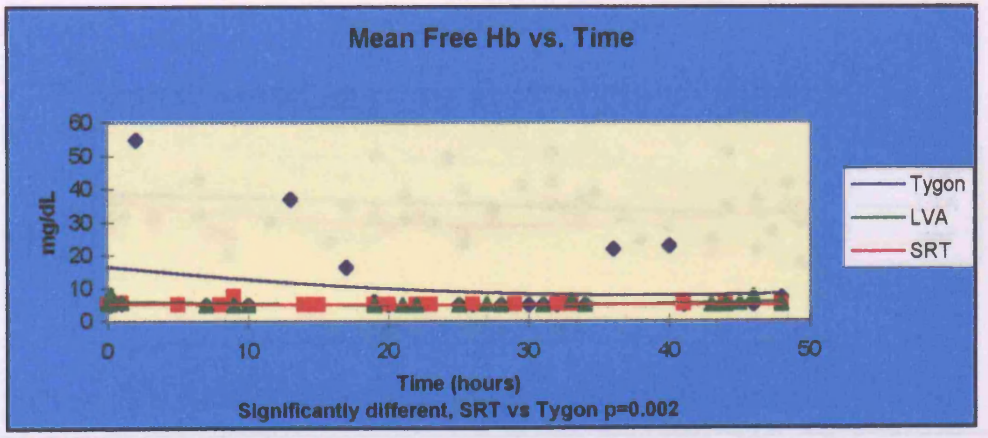
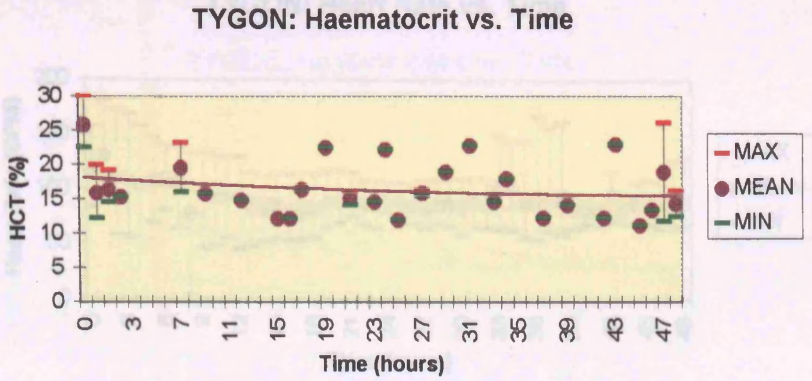
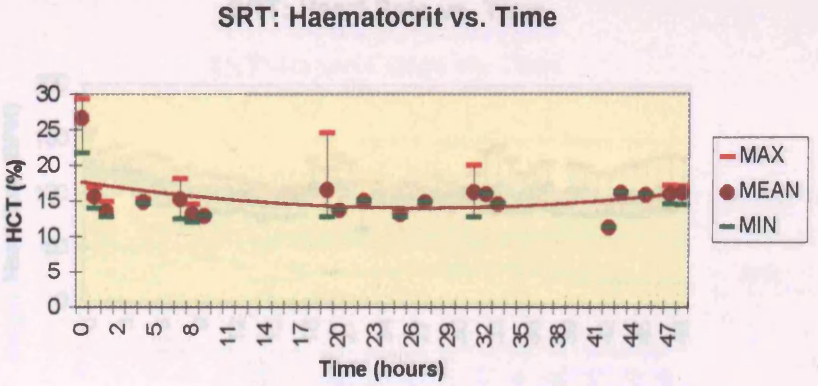
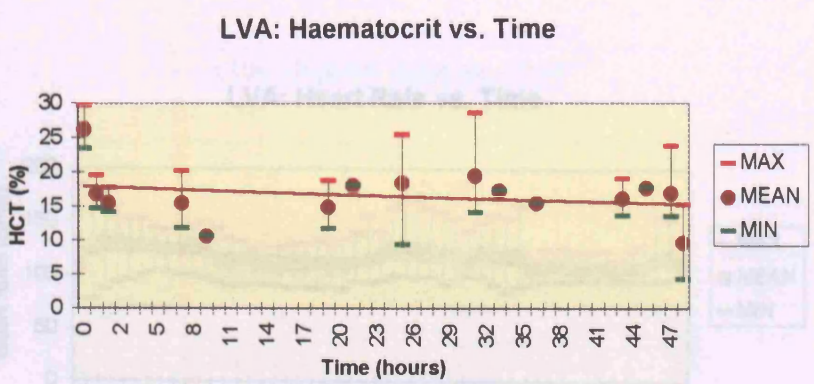


Figure 6.13: HAEMATOCRIT vs. TIME







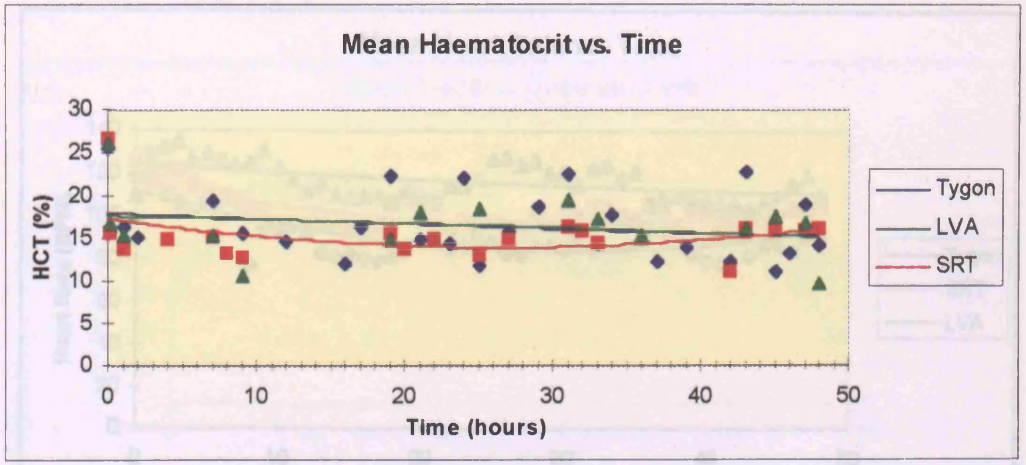
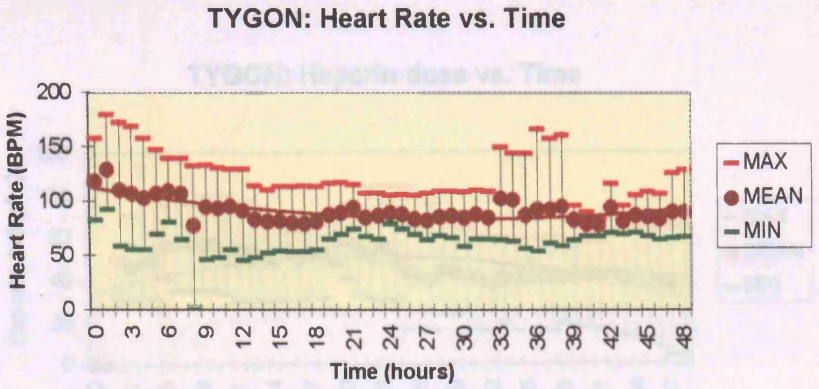
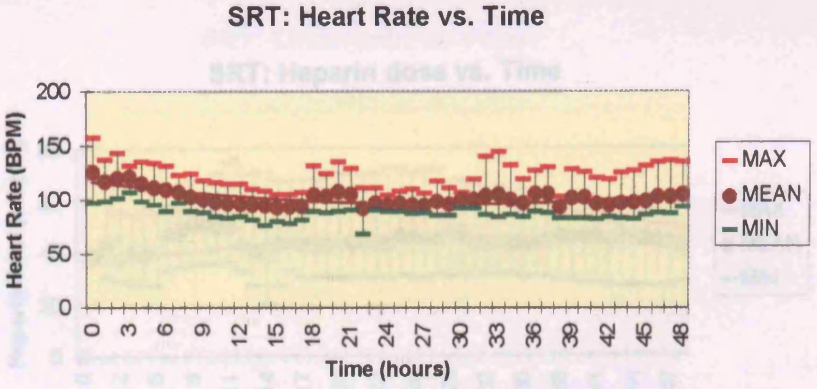
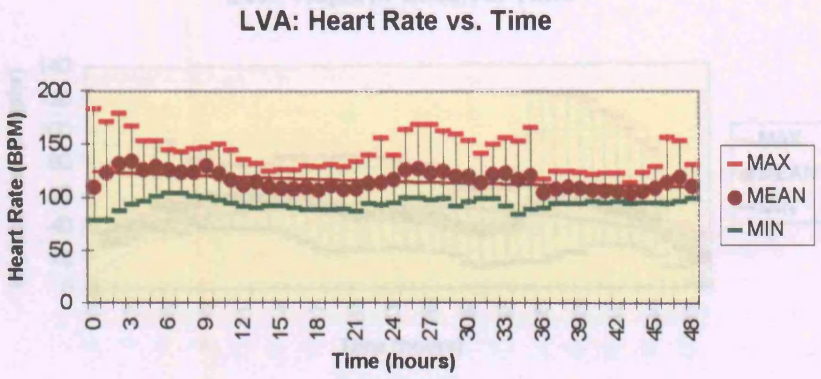


Figure 6.14: HEART RATE vs. TIME







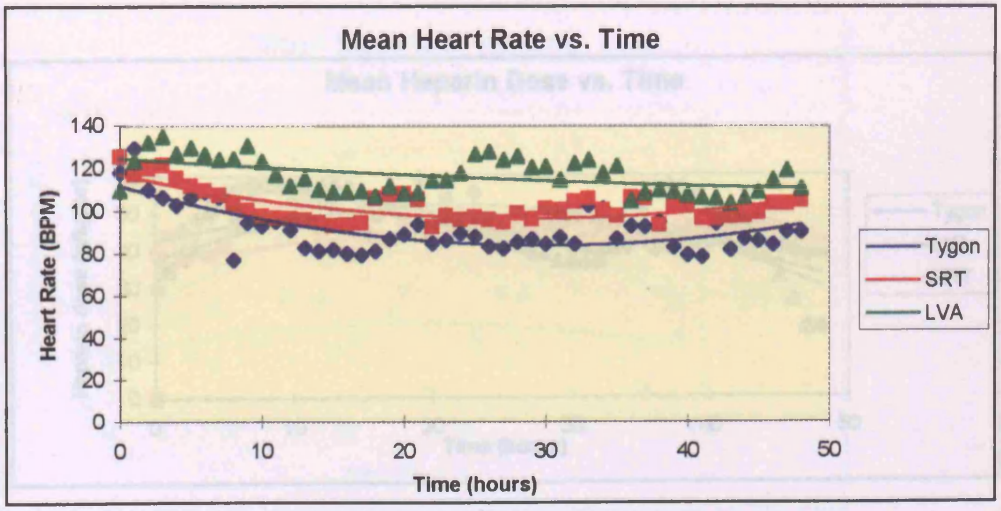
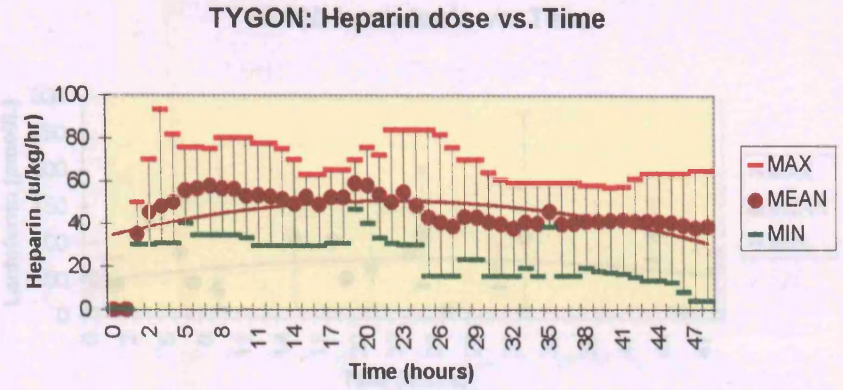
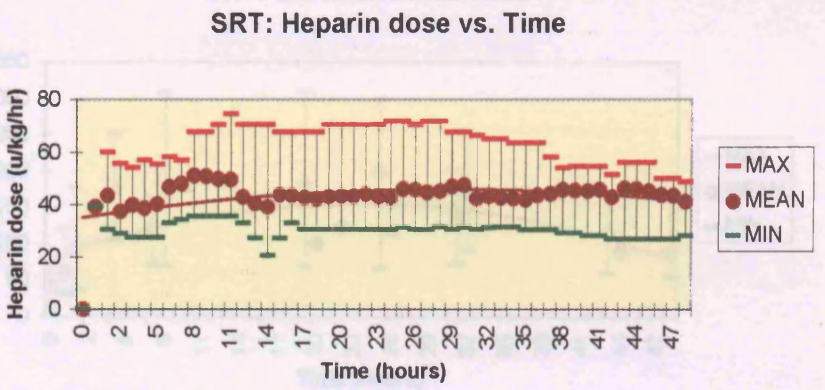
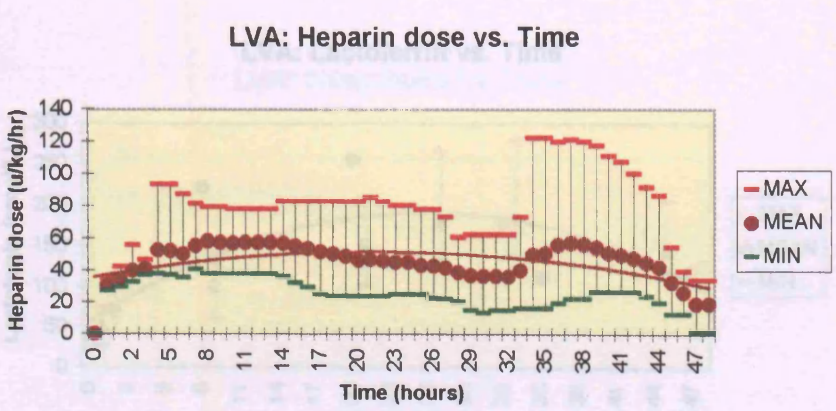


Figure 6.15: HEPARIN DOSE vs. TIME







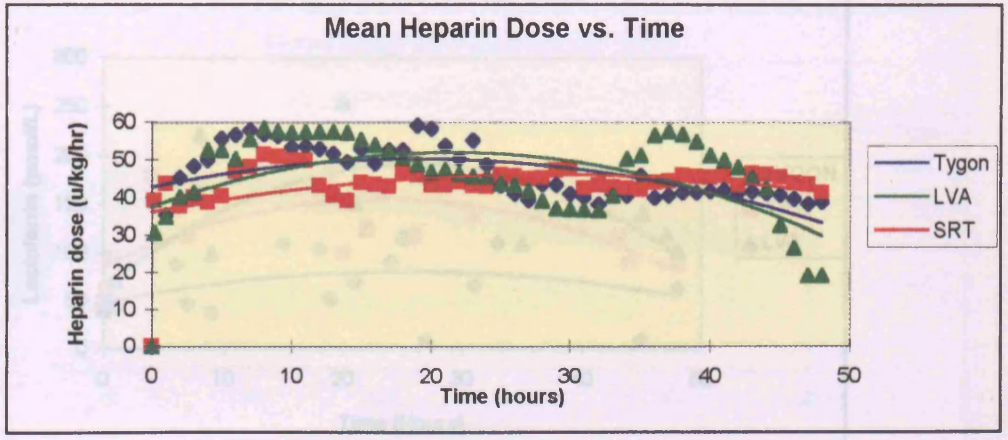
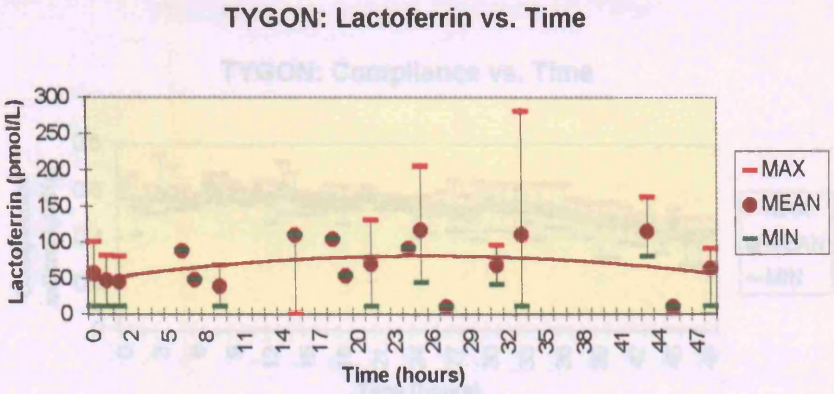
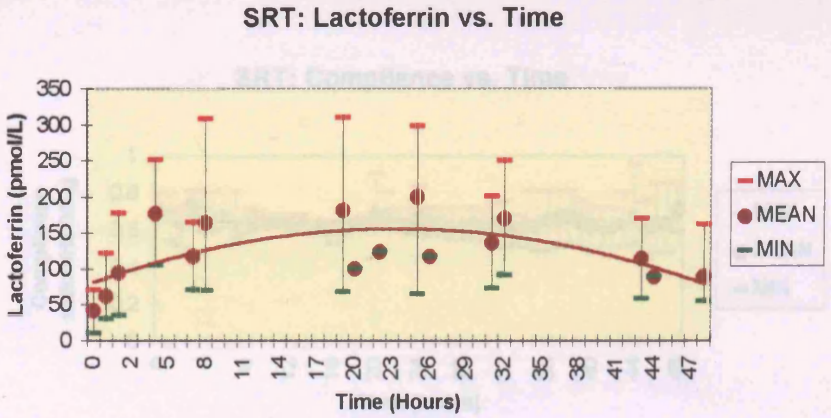
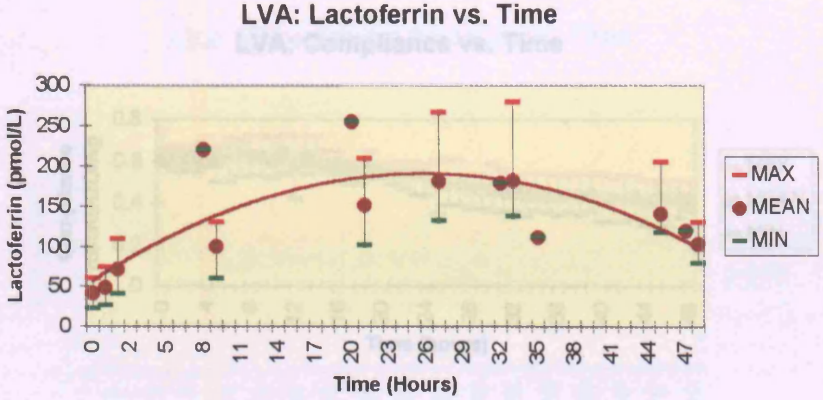


Figure 6.16: SERUM LACTOFERRIN vs. TIME







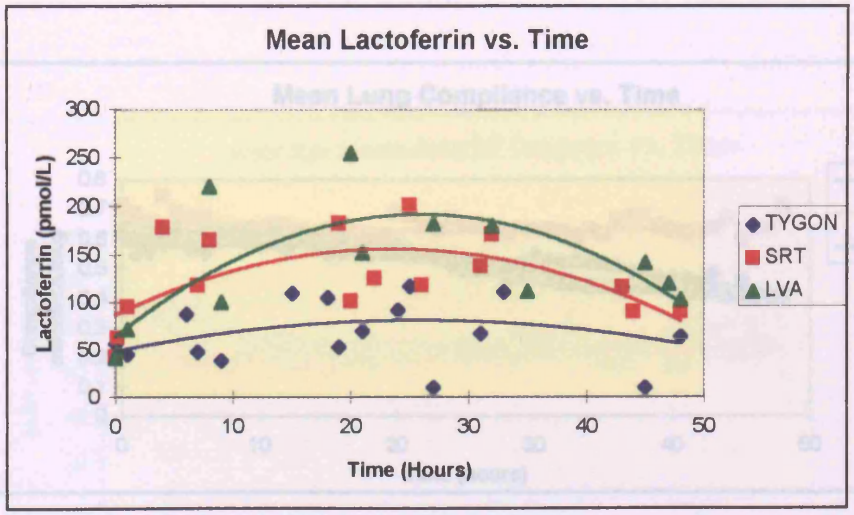
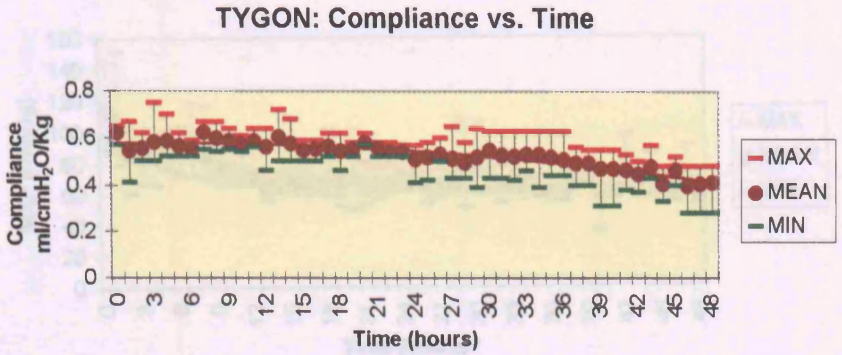
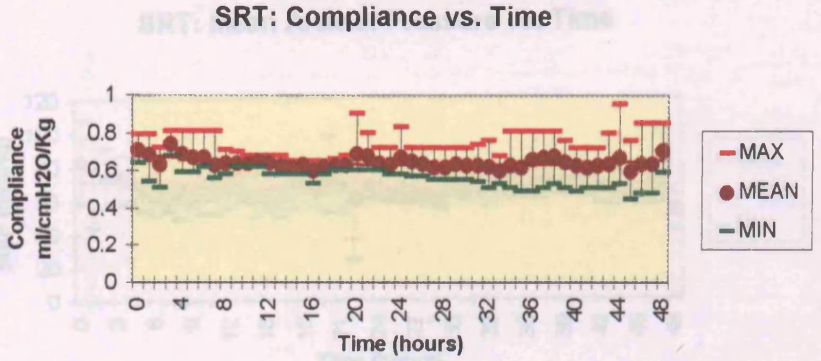
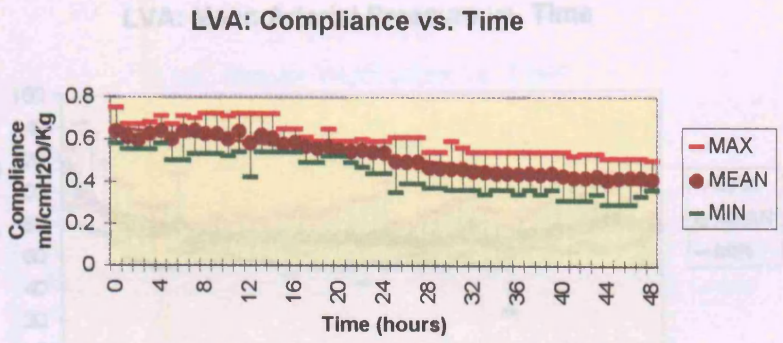


Figure 6.17: LUNG COMPLIANCE vs. TIME







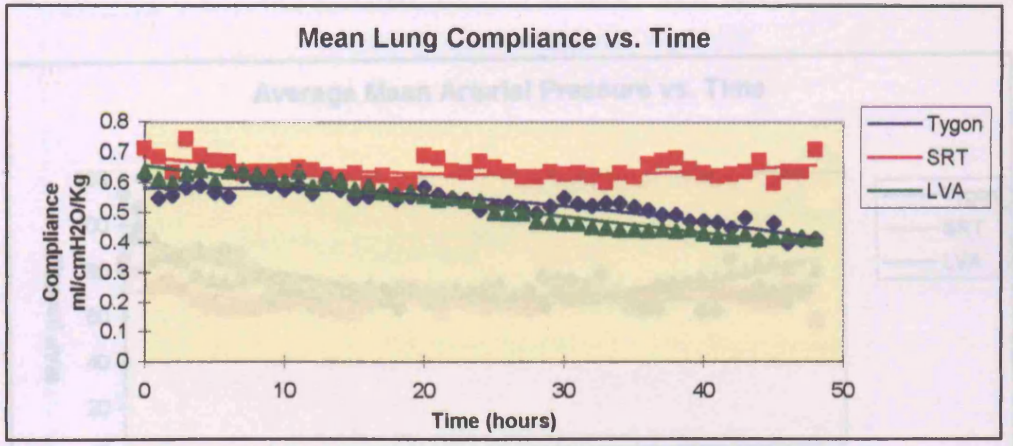
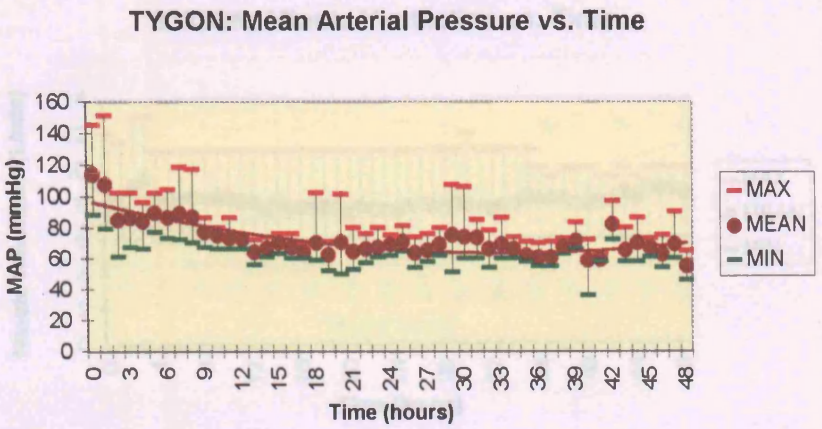
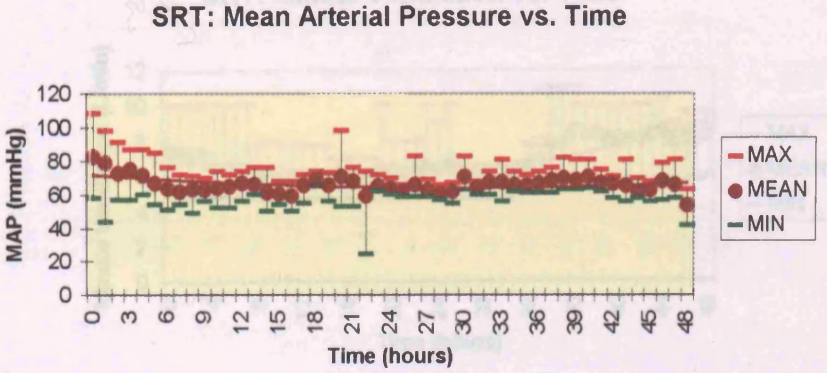
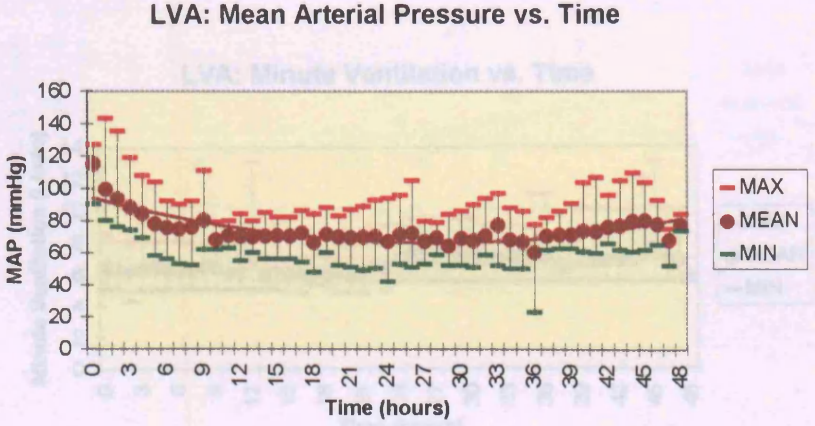


Figure 6.18: MEAN ARTERIAL PRESSURE vs. TIME







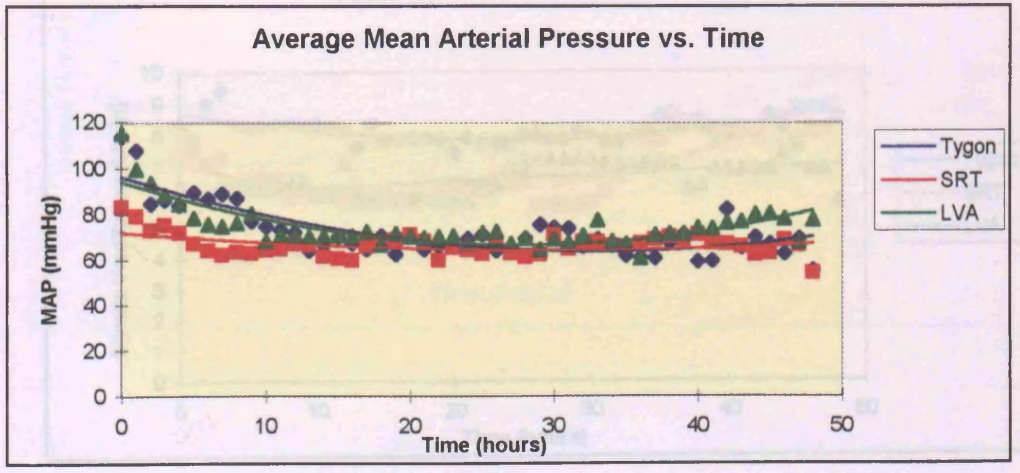
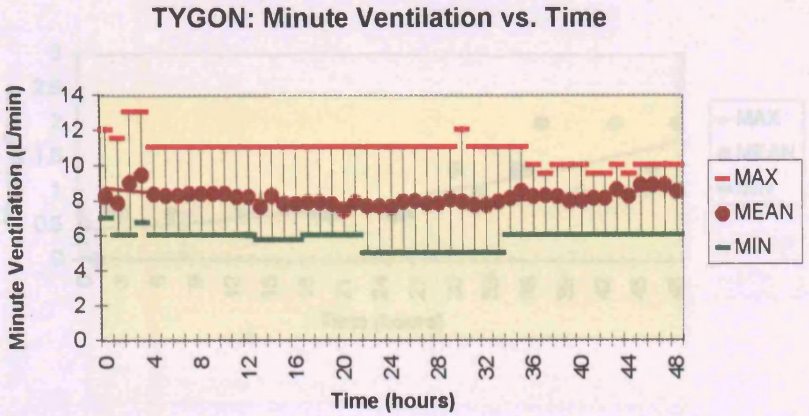
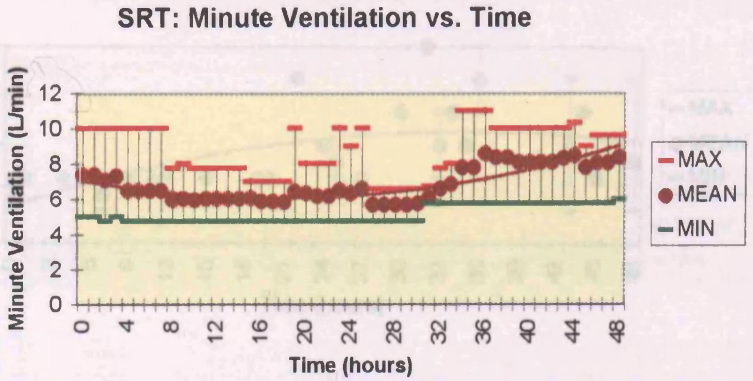
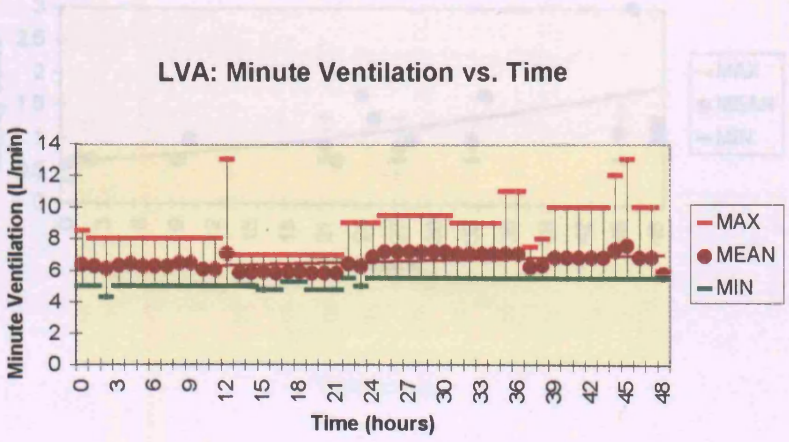


Figure 6.19: MINUTE VENTILATION vs. TIME







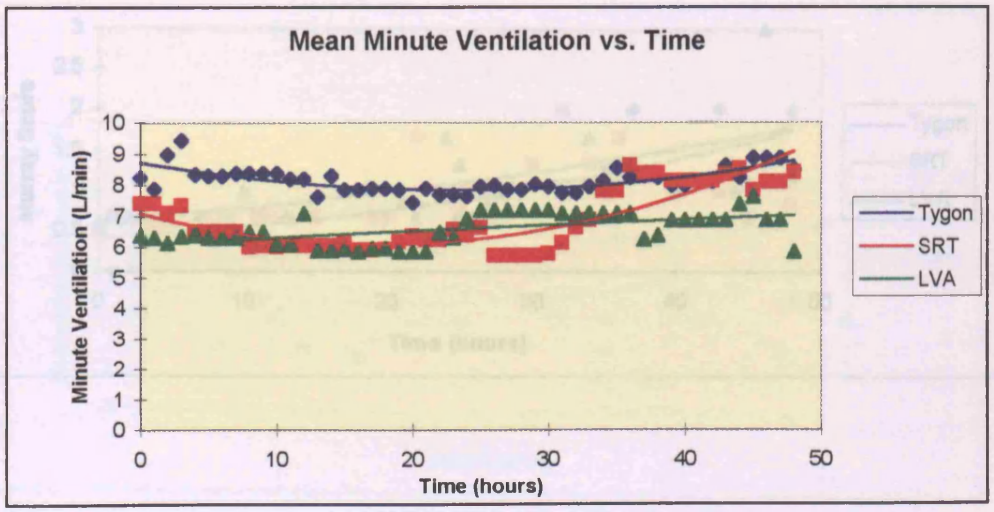
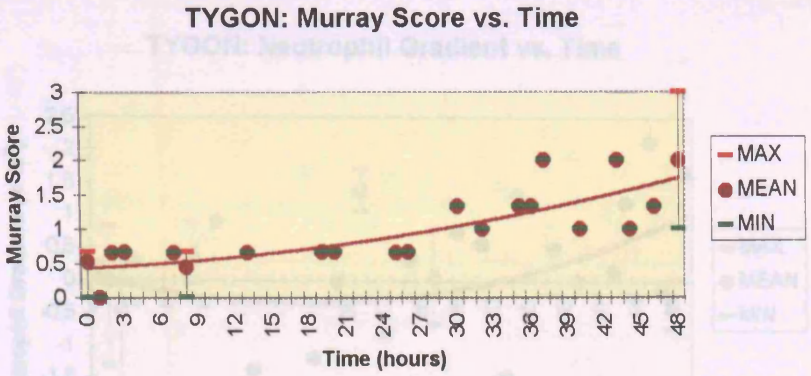
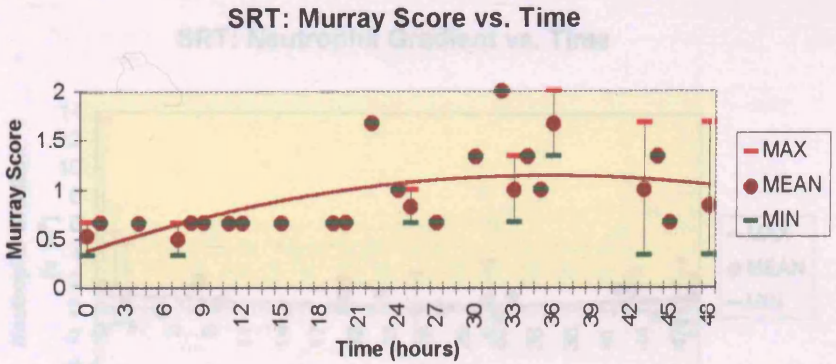
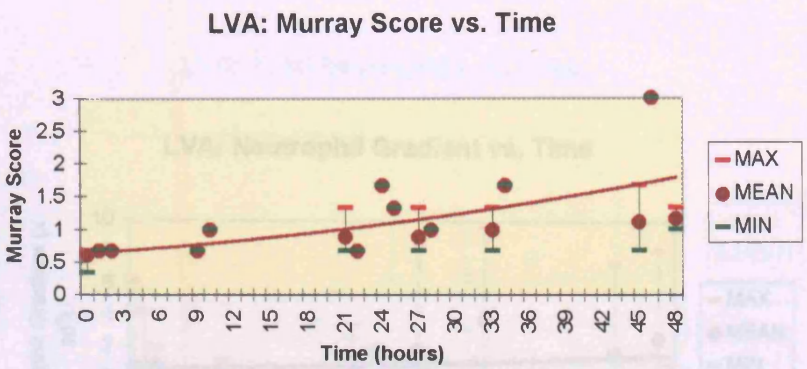


Figure 6.20: MURRAY LUNG INJURY SCORE vs. TIME







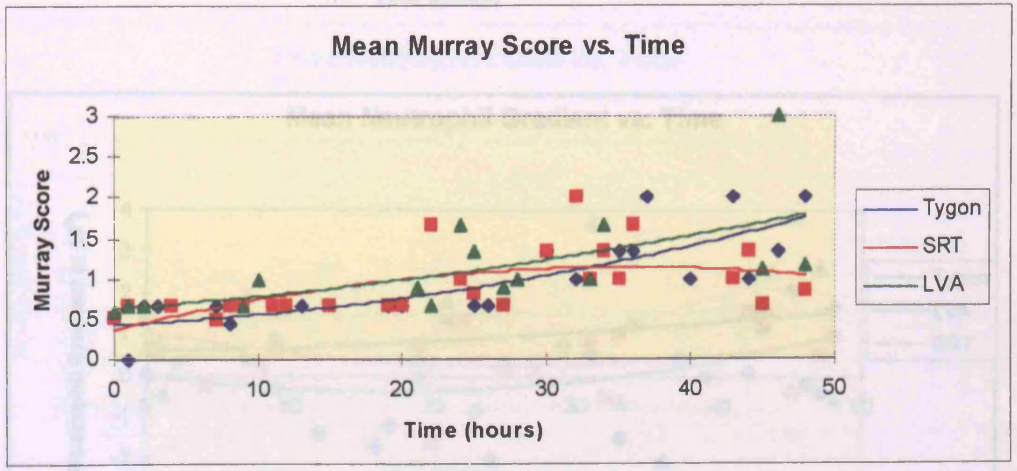
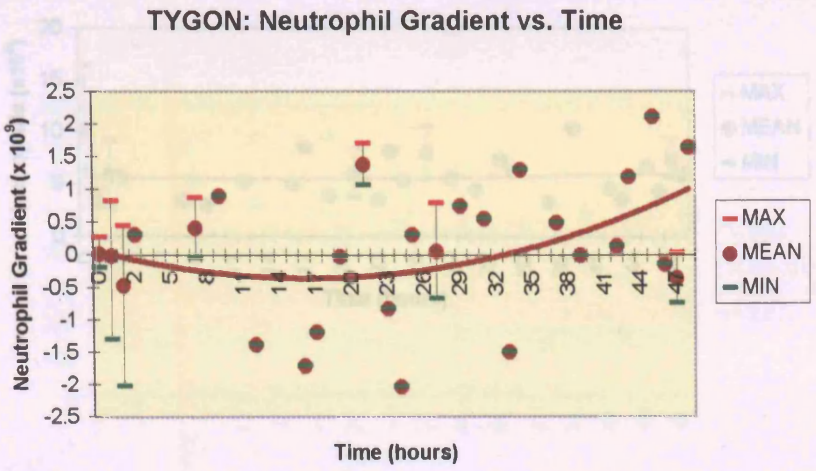
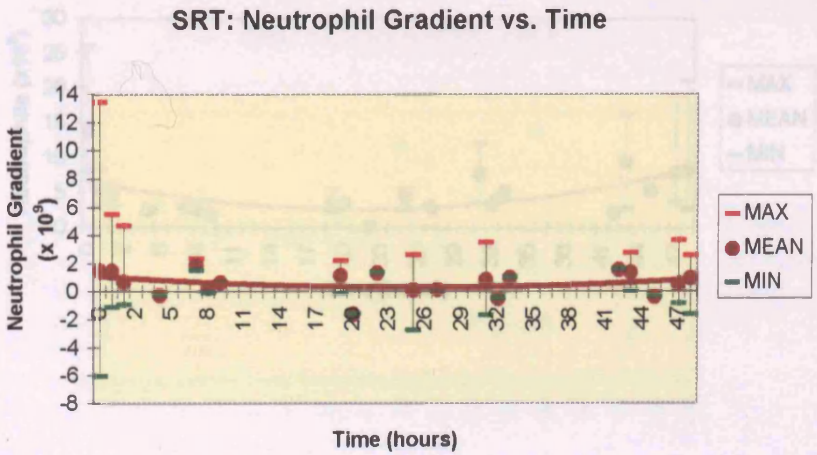
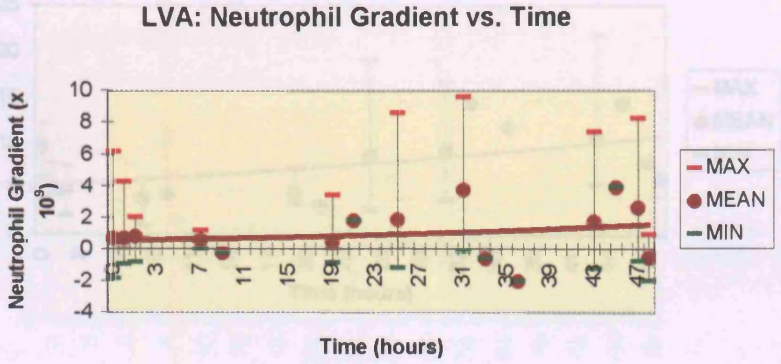


Figure 6.21: NEUTROPHIL GRADIENT (TRANS-PULMONARY) vs. TIME







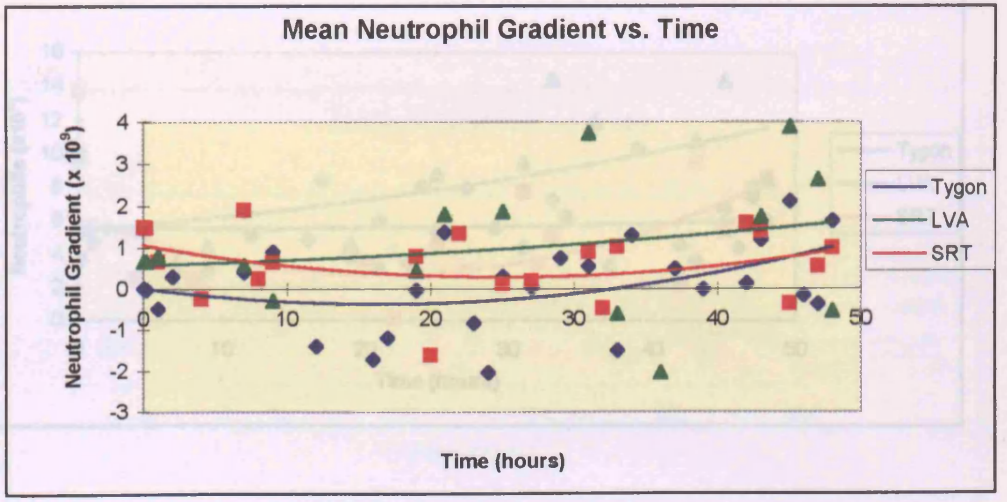
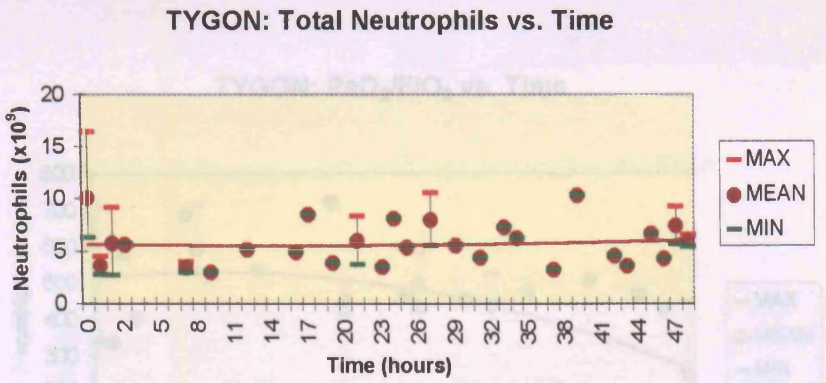
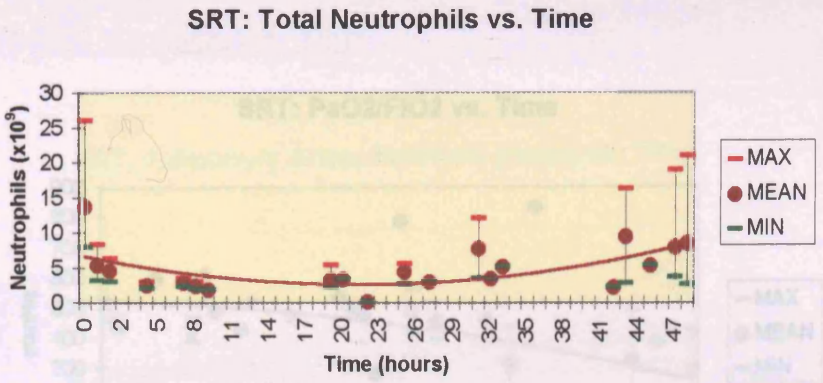
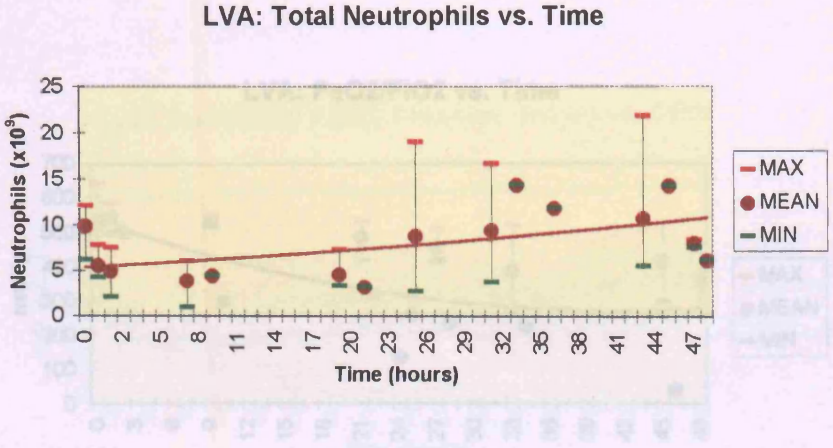


Figure 6.22: NEUTROPHIL COUNT vs. TIME







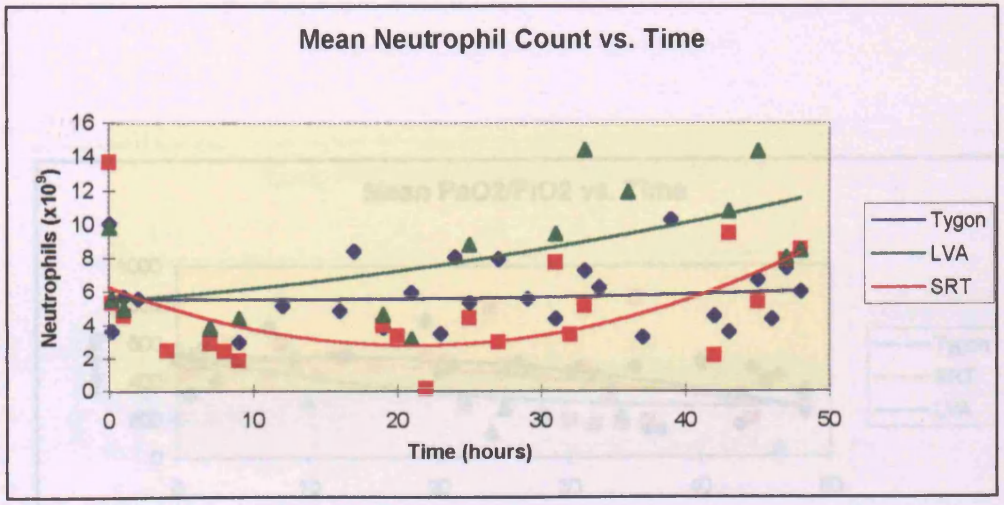
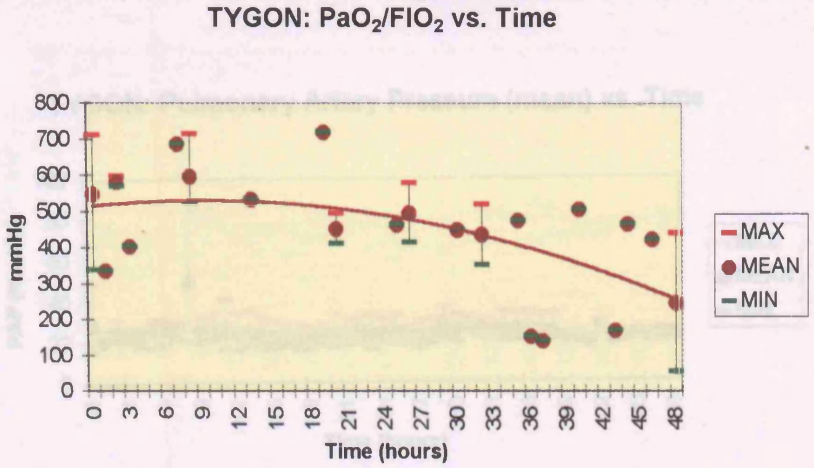
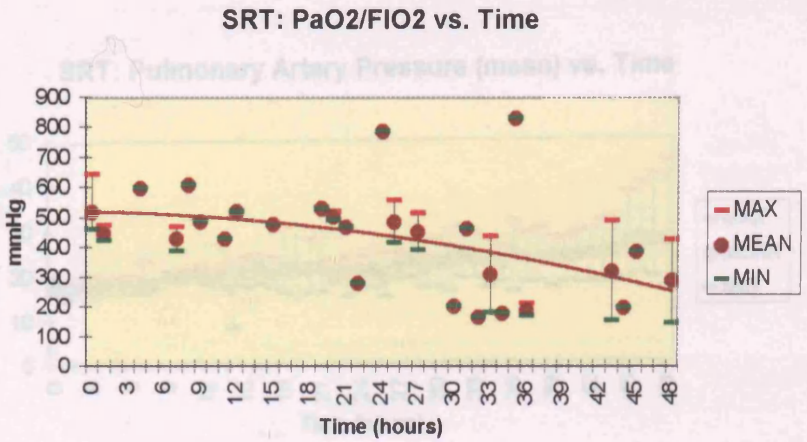
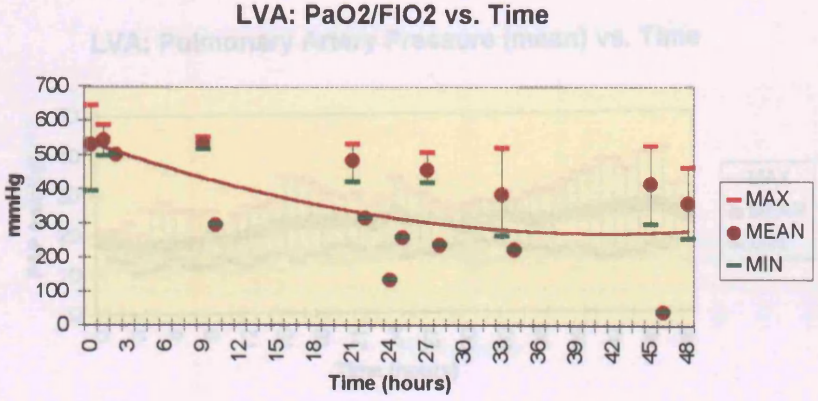


Figure 6.23: PaO_2 / FIO_2 RATIO vs. TIME







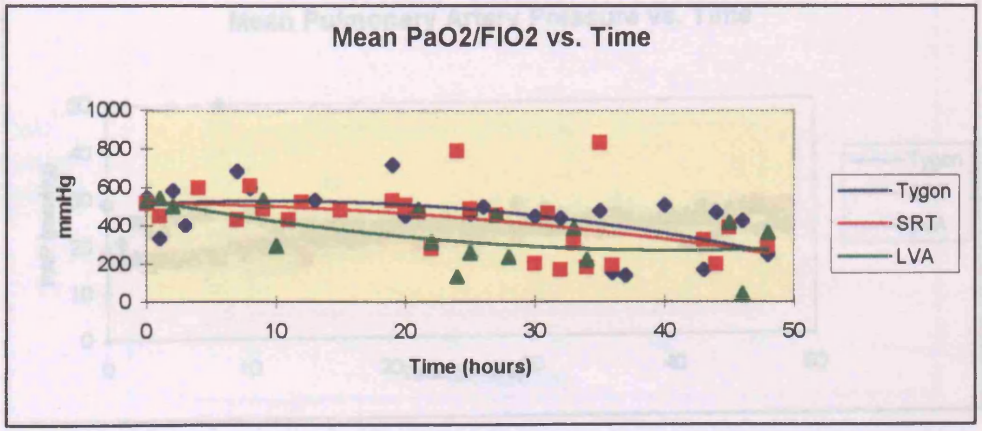
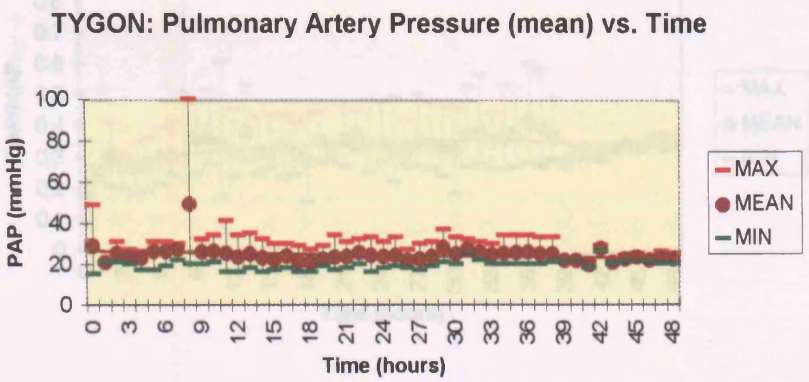
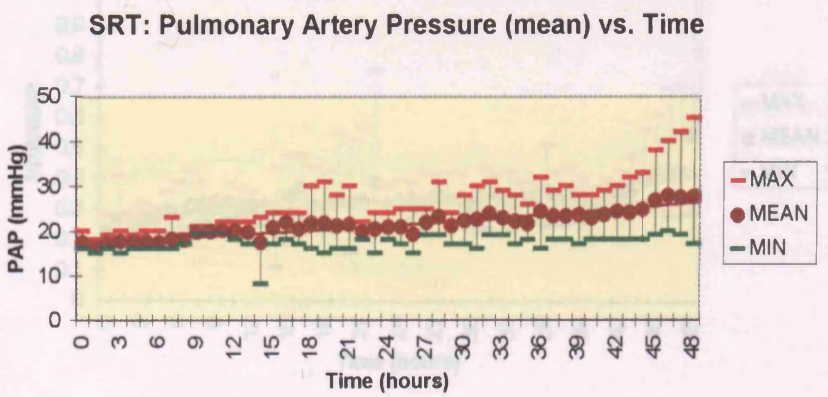
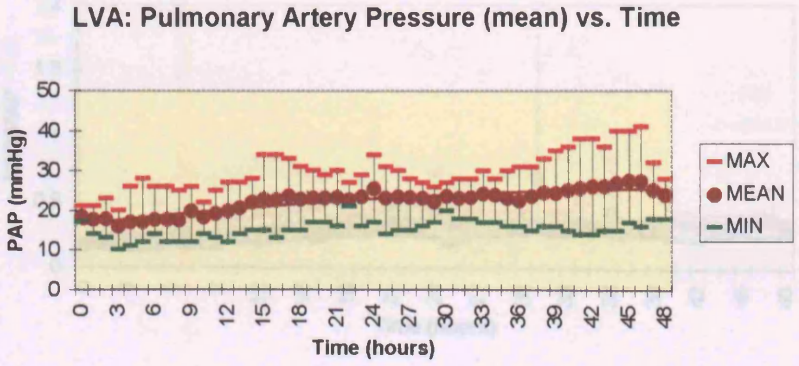


Figure 6.24: PULMONARY ARTERY PRESSURE (mean) vs. TIME







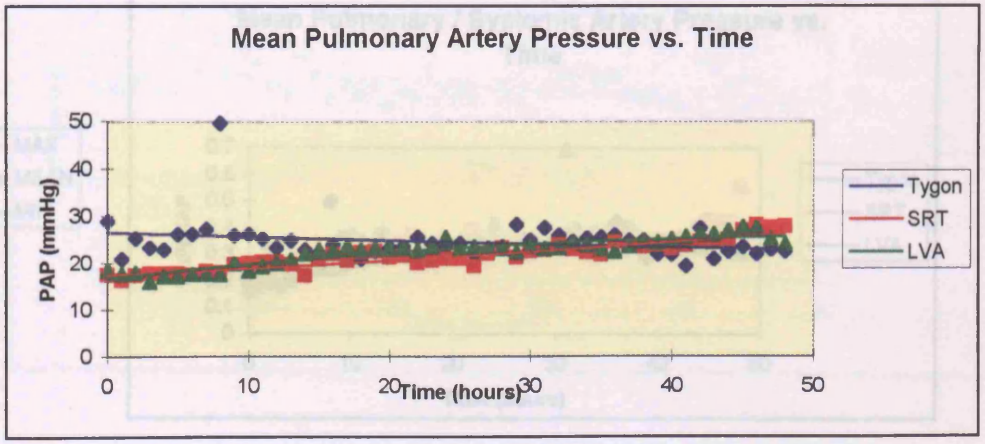
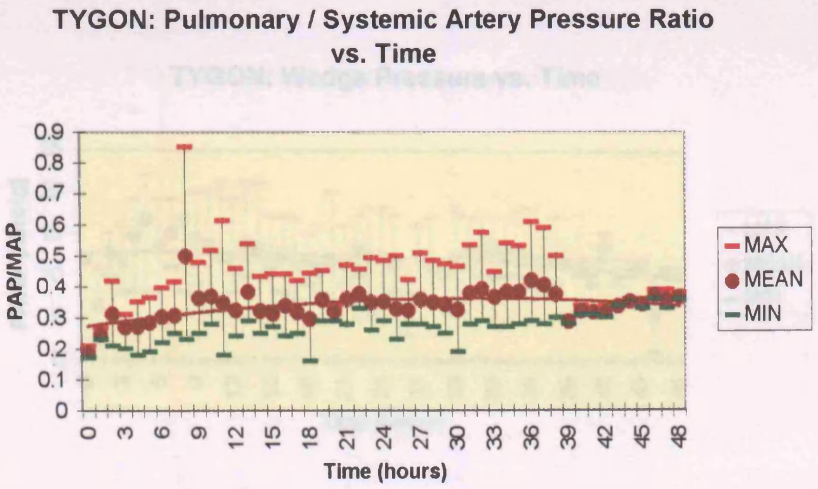
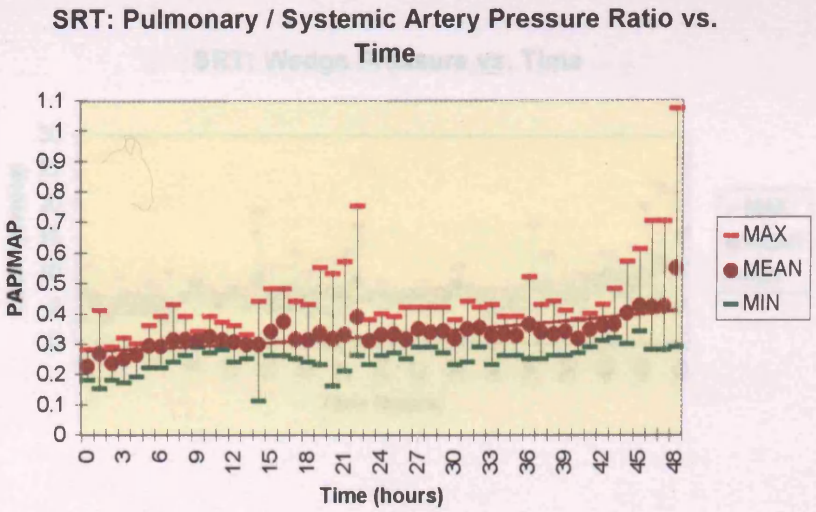
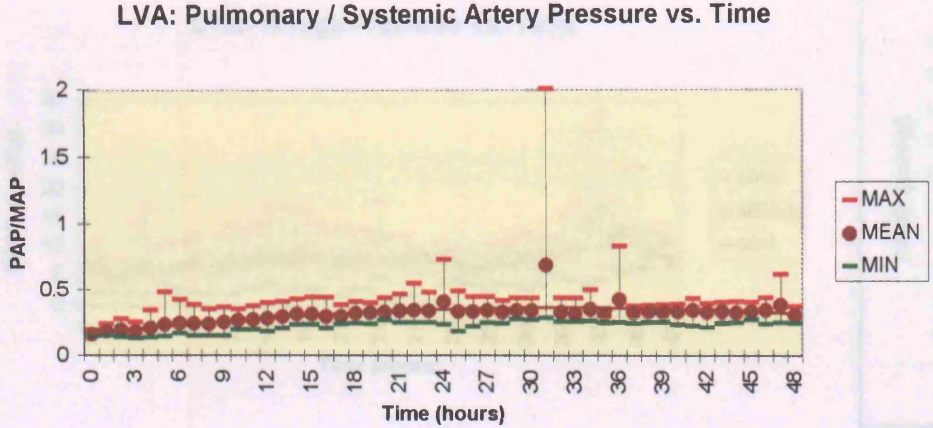


Figure 6.25: PULMONARY / SYSTEMIC MEAN ARTERIAL PRESSURE RATIOS vs. TIME







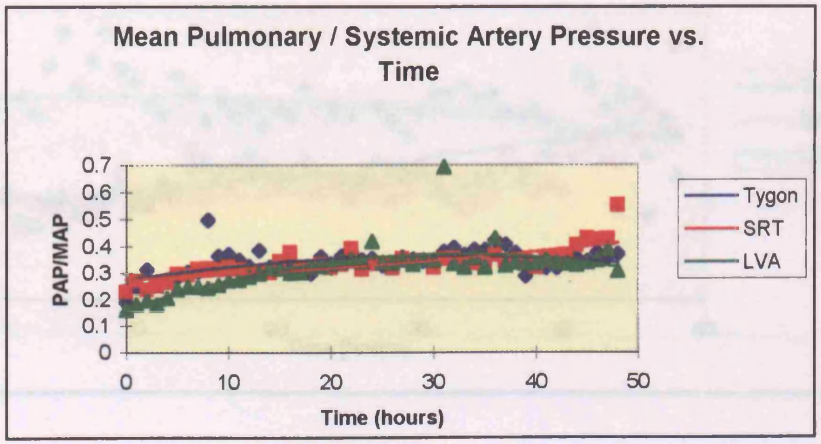
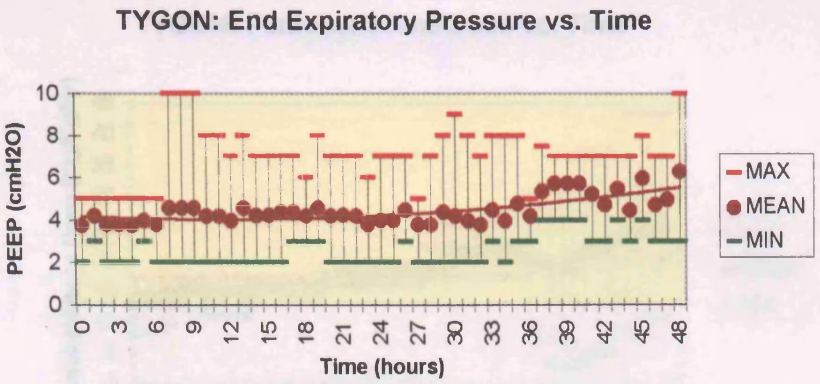
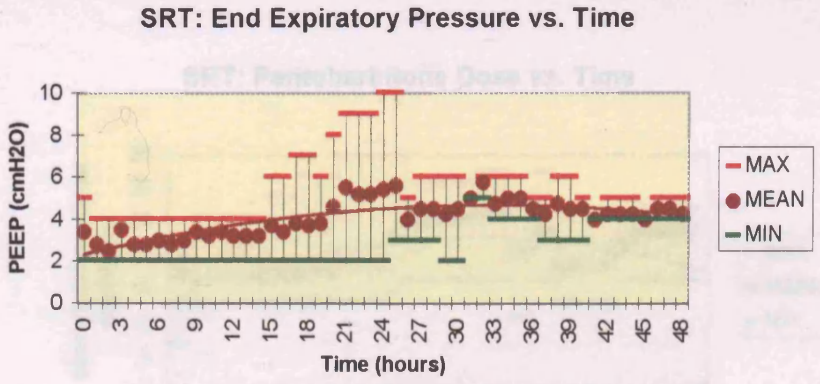
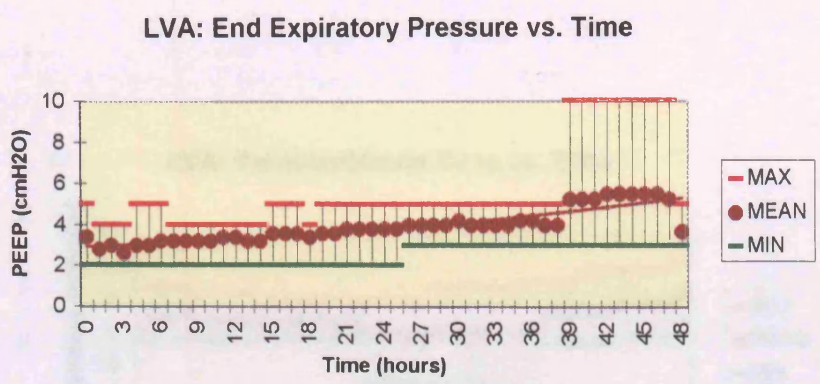


Figure 6.27: POSITIVE END EXPIRATORY PRESSURE vs. TIME







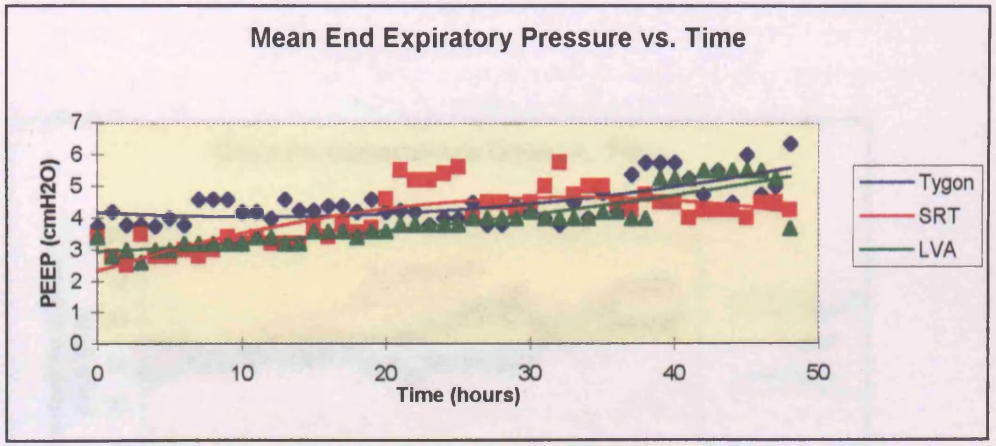
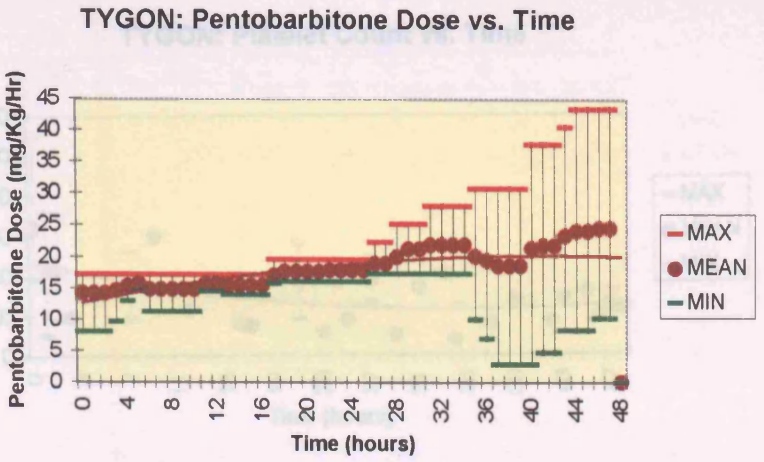
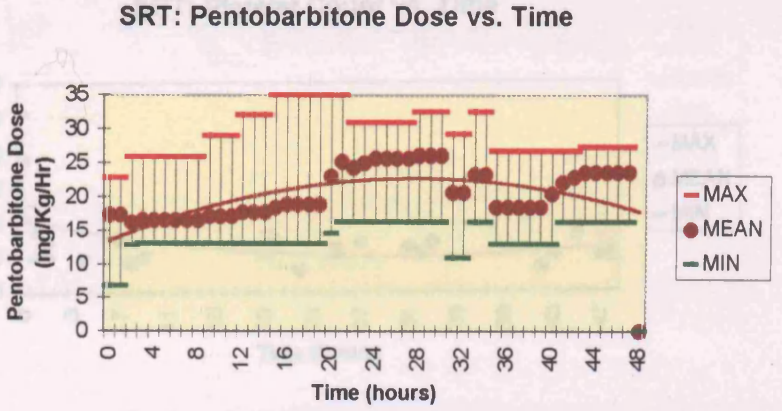
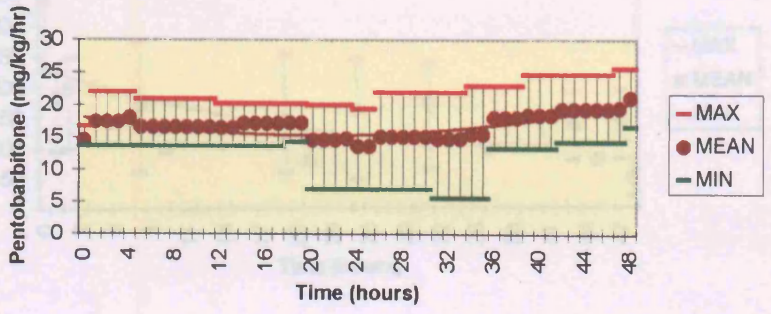


Figure 6.28: PENTOBARBITONE DOSE vs. TIME







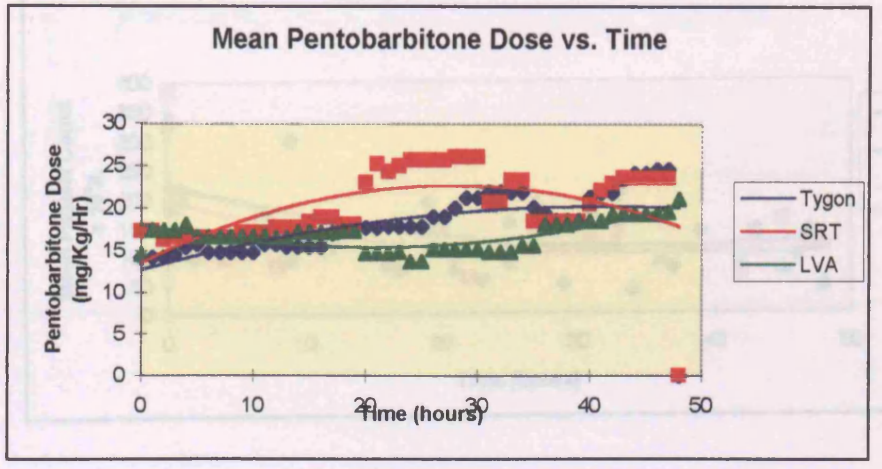
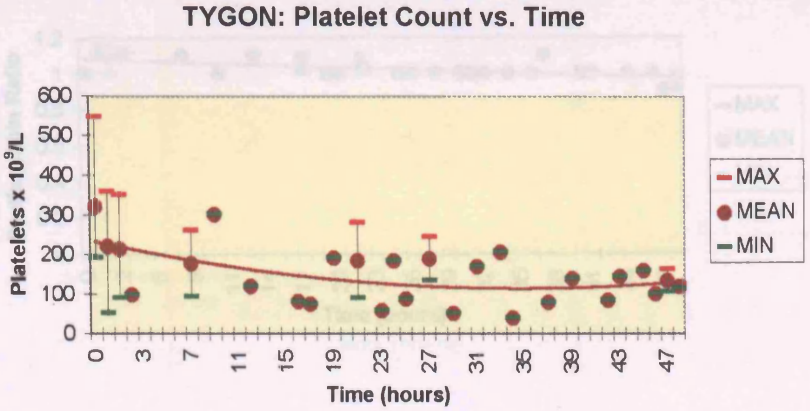
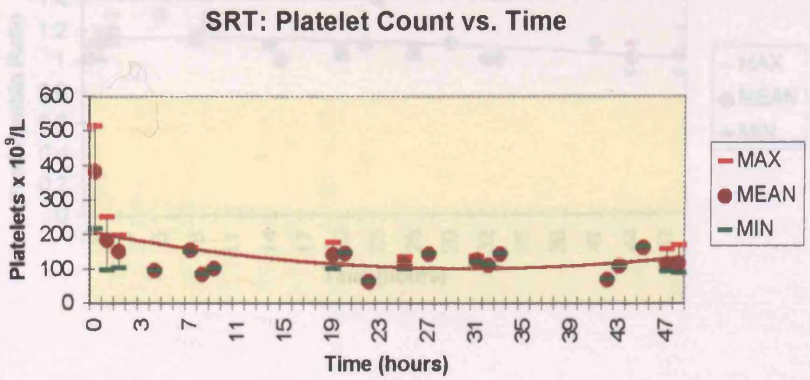
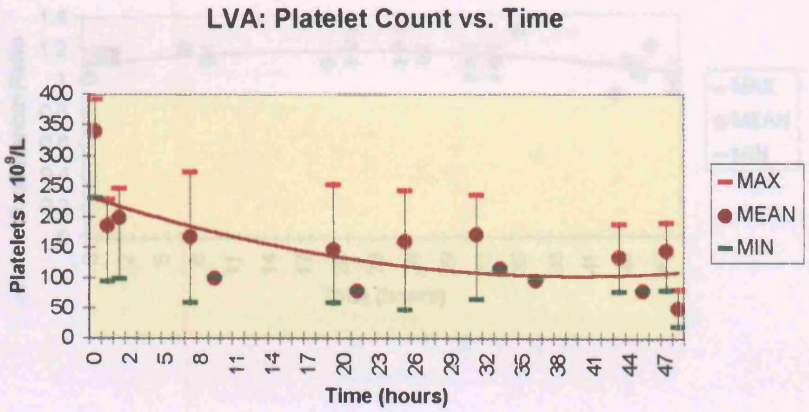


Figure 6.29: PLATELET COUNTS vs. TIME







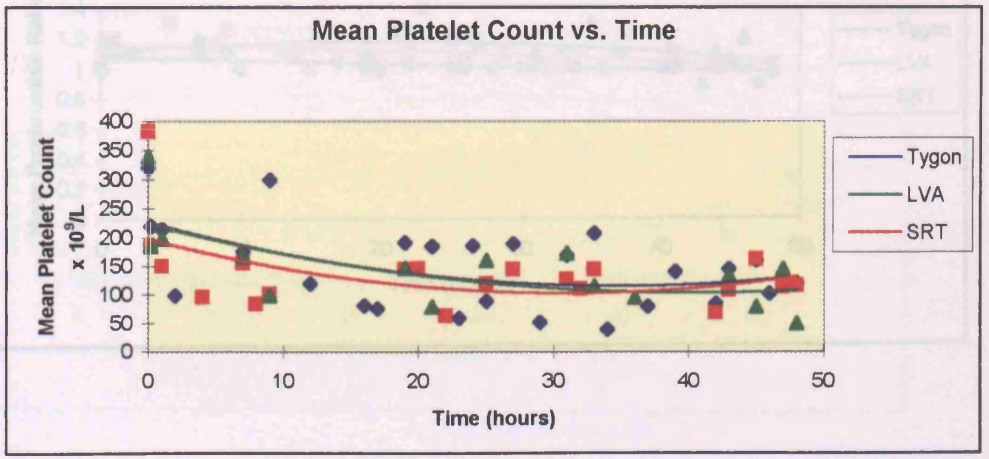
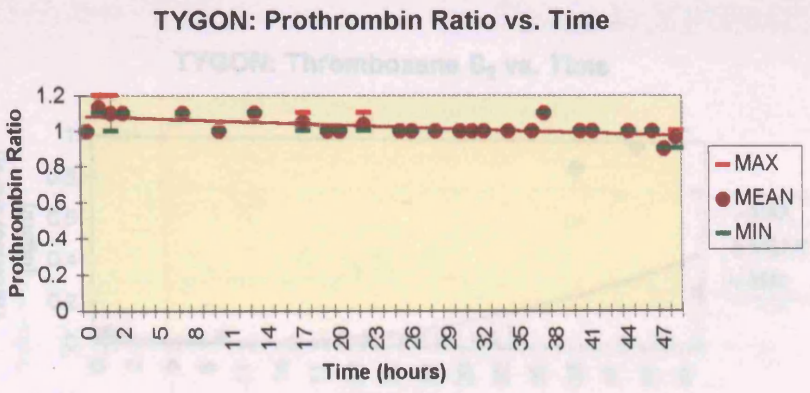
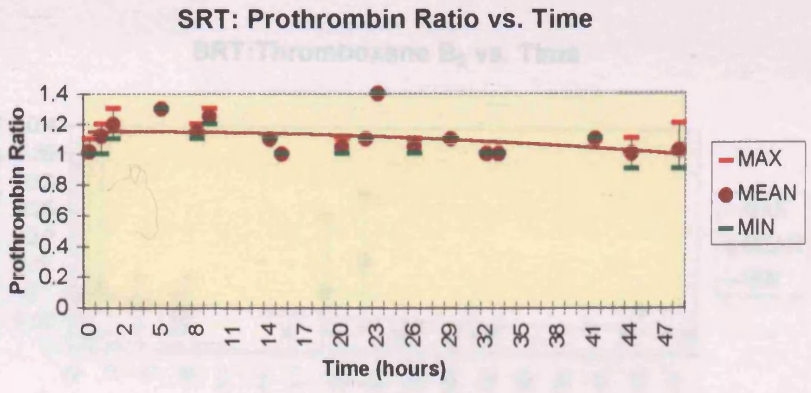
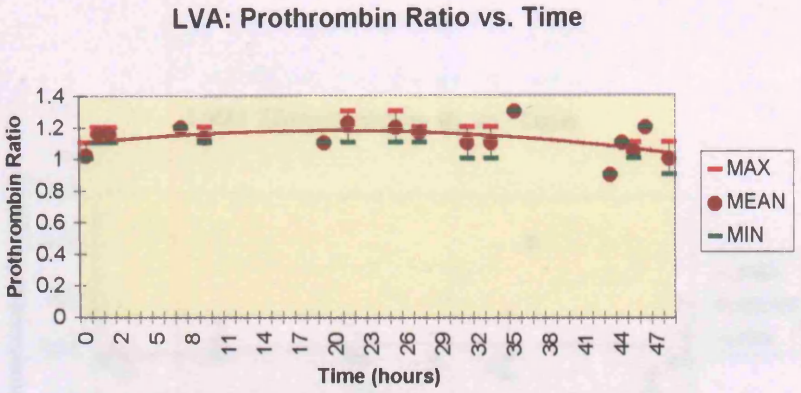


Figure 6.30 PROTHROMBIN RATIO vs. TIME







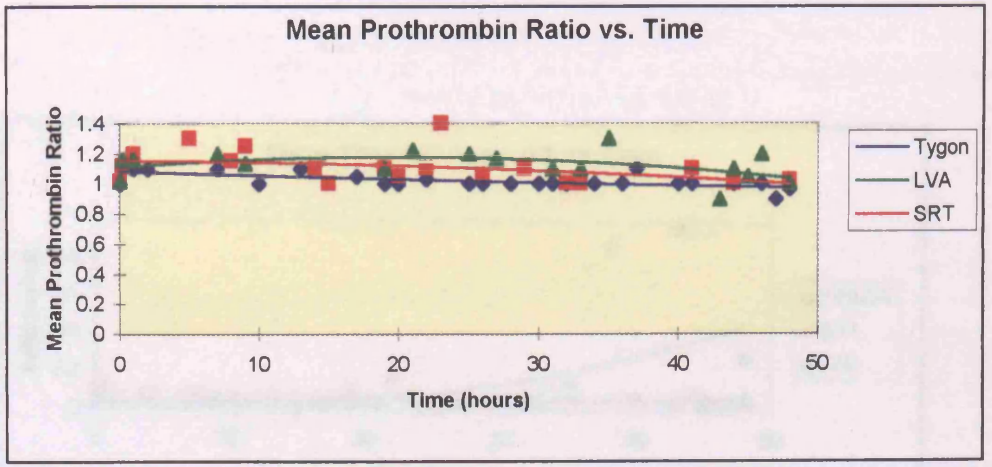
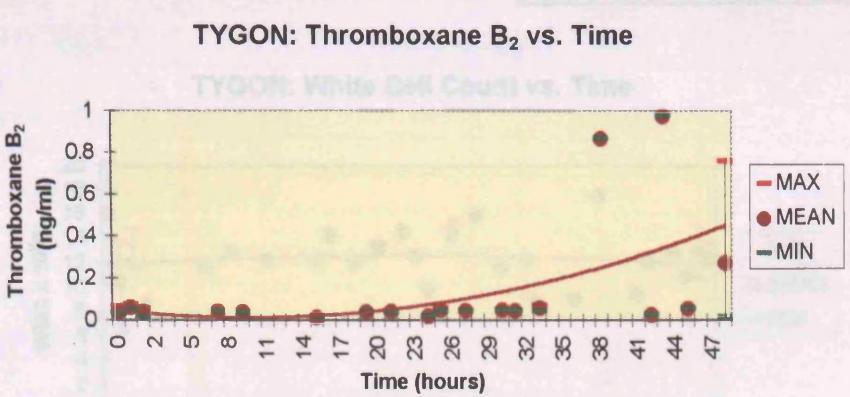
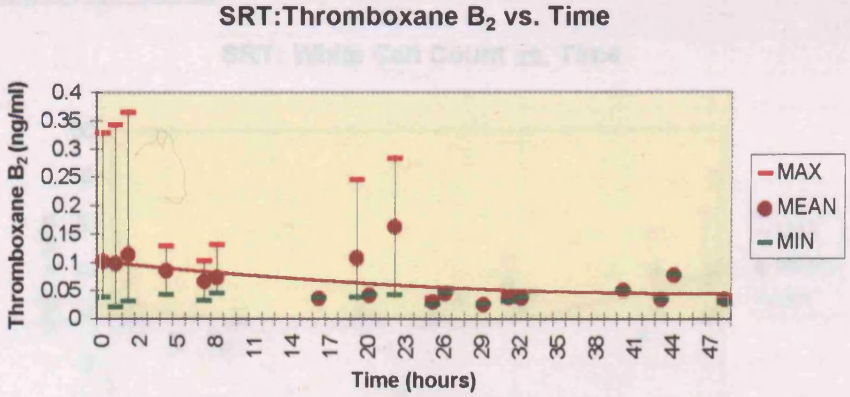
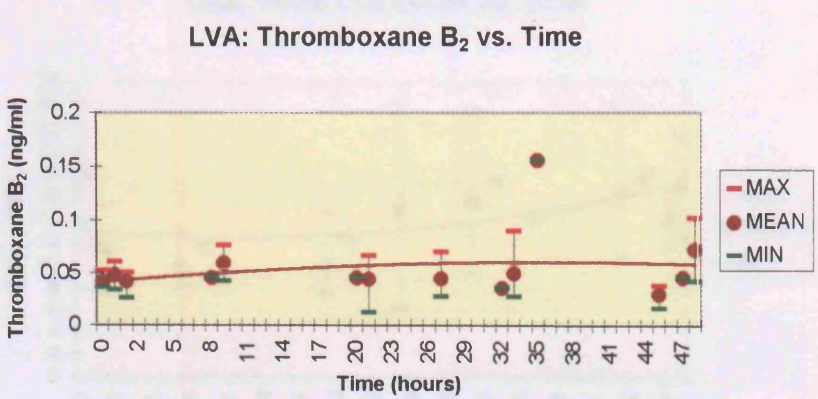


Figure 6.31: THROMBOXANE B2 vs TIME







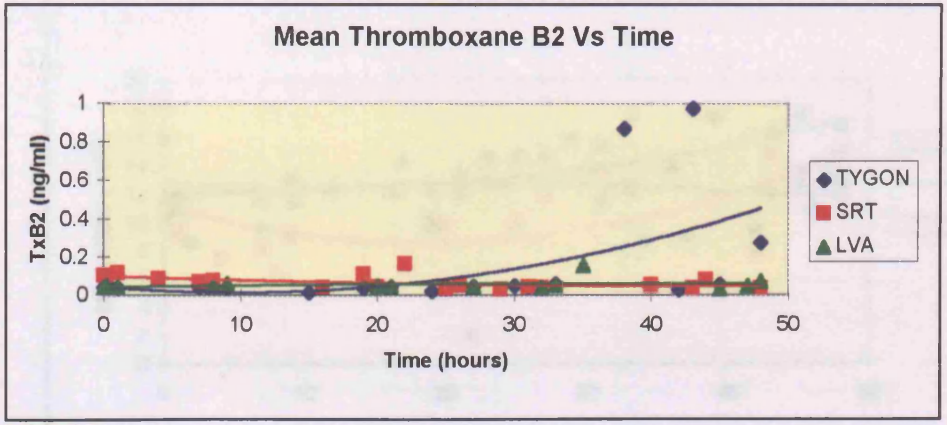
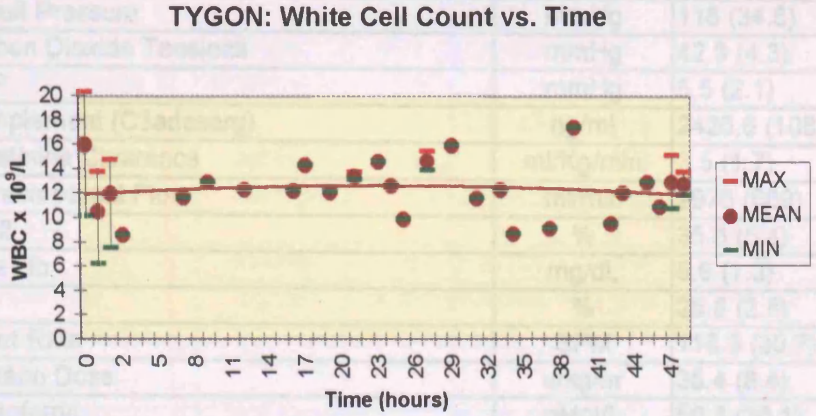
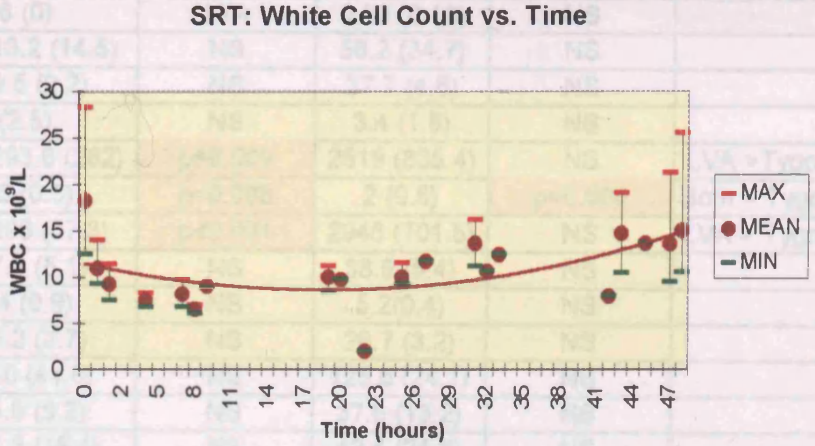
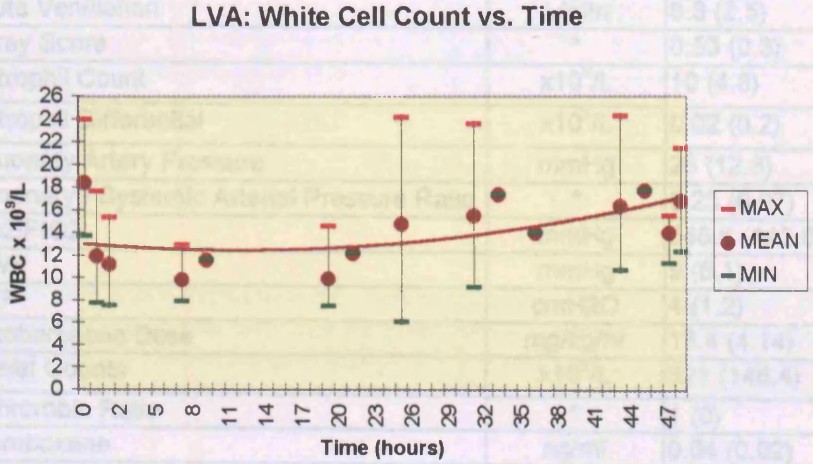


Figure 6.32: WHITE CELL COUNT vs. TIME







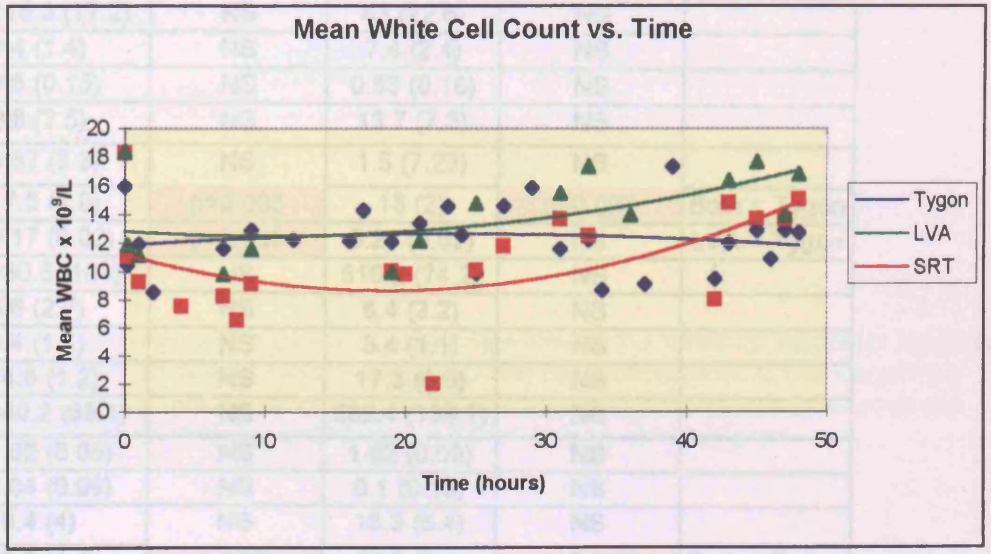


Figure 6.33: Baseline Variables

PARAMETER	units	Tygon Mean (SD)	LVA Mean (SD)	Tygon vs.LVA	SRT Mean (SD)	Tygon vs. SRT	COMMENTS
ACT	Secs	140 (32.3)	137.6 (10.3)	NS	106 (15.4)	p=0.008	SRT < Tygon
APTT Ratio	*	0.6 (0)	0.6 (0)	N/A	0.66 (0.13)	NS	
Circuit Pressure	mmHg	116 (34.6)	113.2 (14.5)	NS	56.2 (34.7)	NS	
Carbon Dioxide Tensions	mmHg	42.9 (4.3)	39.5 (2.2)	NS	37.7 (4.8)	NS	
CVP	mmHg	5.5 (2.1)	6 (2.5)	NS	3.4 (1.8)	NS	
Complement (C3adesarg)	ng/ml	2426.6 (1083.9)	3293.8 (282)	p=0.009	2619 (835.4)	NS	LVA >Tygon
Creatinine Clearance	ml/Kg/min	3.5 (1.7)	2.3 (0.5)	p=0.008	2 (0.5)	p=0.004	Both < Tygon
Extracorporeal Flow	ml/min	2976 (609)	3296 (67.3)	p<0.001	2946 (701.5)	NS	LVA > Tygon
FIO2	%	35.6 (5.4)	37.6 (5.1)	NS	38.9 (5.4)	NS	
Free Hb	mg/dL	5.6 (1.3)	5.4 (0.9)	NS	5.2(0.4)	NS	
Hct	%	25.8 (2.8)	26.2 (2.7)	NS	26.7 (3.2)	NS	
Heart Rate	BPM	118.3 (30.7)	110 (41.8)	NS	125.6 (24.1)	NS	
Heparin Dose	u/kg/hr	35.4 (8.4)	34.9 (5.2)	NS	37.6 (13.2)	NS	
Lactoferrin	pMol/L	56.8 (36.1)	41.3 (16.4)	NS	42.4 (24.8)	NS	
Lung Compliance	ml/cmH2O/Kg	0.62 (0.04)	0.64 (0.03)	NS	0.71 (0.03)	NS	
Mean Arterial Pressure	mmHg	114 (57)	115.3 (17.2)	NS	83 (22.6)	NS	
Minute Ventilation	L/min	8.3 (2.5)	6.4 (1.4)	NS	7.4 (2.4)	NS	
Murray Score	*	0.53 (0.3)	0.6 (0.15)	NS	0.53 (0.18)	NS	
Neutrophil Count	$x10^9/L$	10 (4.8)	9.8 (2.5)	NS	13.7 (7.3)	NS	
Neutrophil Differential	$x10^9/L$	0.02 (0.2)	0.67 (3.3)	NS	1.5 (7.23)	NS	
Pulmonary Artery Pressure	mmHg	26 (12.8)	17.5 (1.9)	p=0.003	18 (2)	p=0.001	Both < Tygon
Pulmonary / Systemic Arterial Pressure Ratio	*	0.23 (0.07)	0.17 (0.02)	p=0.005	0.23 (0.02)	NS	LVA < Tygon
PaO2/FIO2	mmHg	546.6 (143.8)	530.5 (103)	NS	516.2 (74.2)	NS	
PAWP	mmHg	9 (6.1)	5.6 (2.7)	NS	6.4 (3.2)	NS	
PEEP	cmH2O	4 (1.2)	3.4 (1.1)	NS	3.4 (1.1)	NS	
Pentobarbitone Dose	mg/kg/hr	13.4 (4.14)	14.6 (1.2)	NS	17.3 (6.6)	NS	
Platelet Counts	$x10^9/L$	321 (146.4)	340.2 (65.1)	NS	382.4 (138.1)	NS	
Prothrombin Ratio	*	1 (0)	1.02 (0.05)	NS	1.02 (0.05)	NS	
Thromboxane	ng/ml	0.04 (0.02)	0.04 (0.09)	NS	0.1 (0.13)	NS	
White Cell Count	$x10^9/L$	16 (4.1)	18.4 (4)	NS	18.3 (6.4)	NS	
Weight	Kg	51.2 (17)	39.6 (4.4)	p=0.004	38.3 (4.4)	p=0.003	Both < Tygon

Figure 6.34(a): Differences from Baseline and Control

PARAMETER	units	LVA Mean (SD) p	LVA vs Tygon p	SRT Mean (SD) p	SRT vs Tygon p	Tygon Mean (SD) p	Comment
ACT	Secs	46.4 (22.8) p=0.01	NS	162.4 (157.3) NS	NS	77 (68.2) NS	
Adrenaline Dose	mcg/kg/min	0.28 (0.6) NS	NS	0.53 (0.9) NS	NS	0.19 (0.26) NS	
APTT Ratio	*	0.72 (0.4) NS	NS	0.5 (0.2) p=0.009	NS	0.7 (0.47) NS	
Circuit Pressure	mmHg	21.9 (21.9) NS	NS	22.2 (66.5) NS	NS	33.2 (65.7) NS	
Carbon Dioxide Tensions	mmHg	1.1 (2.3) NS	NS	-4.5 (11.3) NS	NS	-2.3 (2.7) NS	
CVP	mmHg	6.4 (5.8) NS	NS	2.6 (4.7) NS	NS	1.8 (9.7) NS	
Complement (C3adesarg)	ng/ml	24.9 (134.7) NS	NS	315.5 (854) NS	NS	90 (318.6) NS	
Creatinine Clearance	ml/min/Kg	0.06 (1.6) NS	NS	0.04 (1.3) NS	NS	-1.7 (2.4) NS	
Dopamine Dose	mcg/kg/min	9 (5.1) NS	NS	5.6 (0.9) p<0.001	NS	4.6 (3.5) NS	
Extracorporeal Flow	ml/min	162 (519.2) NS	NS	178 (763.9) NS	NS	60 (513.3) NS	
FIO2	%	20.1 (28.7) NS	NS	15.4 (25.3) NS	NS	10.1 (37.3) NS	
Free Hb	mg/dL	-0.2 (0.44) NS	NS	0.8 (2.4) NS	p=0.002	1.2 (2.7) NS	SRT < Tygon
Hct	%	-10.9 (6.8) NS	NS	-10.7 (4.1) p=0.004	NS	-8.4 (5.7) NS	
Heart Rate	bpm	5.2 (56.8) NS	NS	-27.8 (20.4) NS	NS	0.8 (49.7) NS	
Heparin Dose	u/Kg/hr	-16.3 (10) NS	NS	1.4 (19.9) NS	NS	3.4 (17.2) NS	
Lactoferrin	pMol/L	61.8 (32.5) NS	NS	54.2 (48.7) NS	NS	3 (23.7) NS	

NB: This table shows variation of data from baseline levels (columns headed in green, yellow and mauve). Non-significant (NS) p values indicate that values did not change significantly from baseline. Significant variations from baseline are highlighted in red. A positive value indicates an increase from the beginning to the end of the experiment, and a negative a decrease. The changes in the study groups (LVA & SRT) are also compared to controls (Tygon) in the columns with the clear headings. Significant differences between study and control groups are highlighted in blue. A $p<0.001$ was taken to indicate significance. Table 6.34 (b), overleaf, is a continuation of these data in an identical format.

Figure 6.34 (b): Differences from Baseline and Control

PARAMETER	units	LVA Mean (SD) p	LVA vs Tygon p		SRT Mean (SD) p	SRT vs Tygon p		Tygon Mean (SD) p	Comment
Lung Compliance	ml/cmH2O/Kg	-0.24 (0.05) p=0.001	NS		-0.04 (0.06) NS	NS		-0.19 (0.11) NS	
Mean Arterial Pressure	mmHg	-44 (5.4) p<0.001	NS		-34.8 (29.9) NS	NS		-34.2 (45.9) NS	
Minute Ventilation	L	1.3 (2.4) NS	NS		1.3 (2) NS	NS		-0.3 (2.7) NS	
Murray Score	*	0.87 (0.9) NS	NS		0.34 (0.48) NS	NS		1 (0.9) NS	
Neutrophil Count	$x10^9/L$	-0.05 (2.8) NS	NS		-6.9 (2.9) p=0.006	NS		-3.2 (3.8) NS	
Neutrophil Differential	$x10^9/L$	0.67 (3.3) NS	NS		1.5 (7.2) NS	NS		0.02 (0.2) NS	
Pulmonary Artery Pressure	mmHg	6.6 (6.9) NS	NS		10.5 (13.2) NS	NS		-3 (23.9) NS	
Pulmonary / Systemic Arterial Pressure Ratio	*	0.3 (0.2) NS	NS		0.4 (0.3) NS	NS		0.2 (0.1) NS	
PaO2/FIO2	mmHg	-216.3 (200) NS	NS		-127.4 (260.2) NS	NS		-329.6 (152.3) NS	
PAWP	mmHg	4 (3.9) NS	NS		6.2 (10.4) NS	NS		3.7 (9.9) NS	
PEEP	cmH2O	1.8 (2.9) NS	NS		2 (2.3) NS	NS		1.6 (2.7) NS	
Pentobarbitone Dose	mg/Kg/Hr	2.2 (7.6) NS	NS		8.6 (8.1) NS	NS		10.1 (12.2) NS	
Platelet Counts	$x10^9$	-247.4 (85.7) p=0.003	NS		-277.2 (119.3) p=0.007	NS		-215.2 (134.6) NS	
Prothrombin Ratio	*	0.12 (0.15) NS	NS		0.08 (0.16) NS	NS		0 (0.07) NS	
Thromboxane	ng/ml	0.03 (0.07) NS	NS		-0.01 (0.2) NS	NS		0.18 (0.37) NS	
White Cell Count	$x10^9/L$	-2.3 (4.4) NS	NS		-5.8 (4.2) NS	NS		-3.5 (4.5) NS	

Figure 6.34 (c): Variables with only one time point

Parameter	Units	Tygon Mean	Tygon SD	SRT Mean	SRT SD	LVA Mean	LVA SD	Tygon vs LVA	Tygon vs SRT
Aminophyline Dose	mcg/Kg/Hr	86.7	125.65	63.26	141.45	64.88	145.08	NS	NS
Fluid Balance	ml/Kg/Hr	2.39	0.74	2.25	0.22	2.69	0.76	NS	NS
Frusemide Dose	mcg/Kg/Hr	51.4	92.16	72.2	125.53	23.8	23.8	NS	NS
Lung Neutrophil Infiltration	cells/Sq mm	479	666.3	569	730.5	942	699.1	NS	NS
Lung Water	%	82.80%	0.01	82.60%	0.06	84.00%	0.07	NS	NS
Survival	Number	3/5	*	4/5	*	3/5	*	NS	NS
Urine Output	ml/Kg/Hr	2.39	0.74	2.25	0.48	2.69	0.76	NS	NS

Fig. 6.35 Myocardium x200

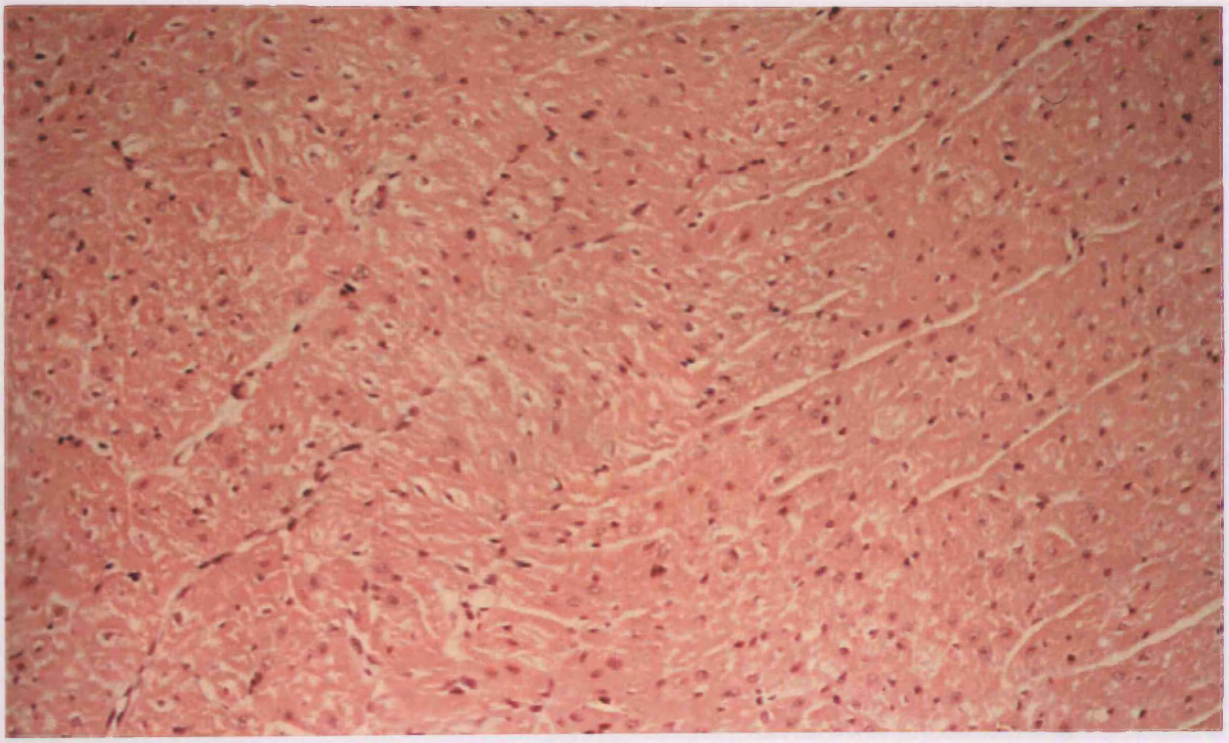


Fig. 6.36 Kidney x200

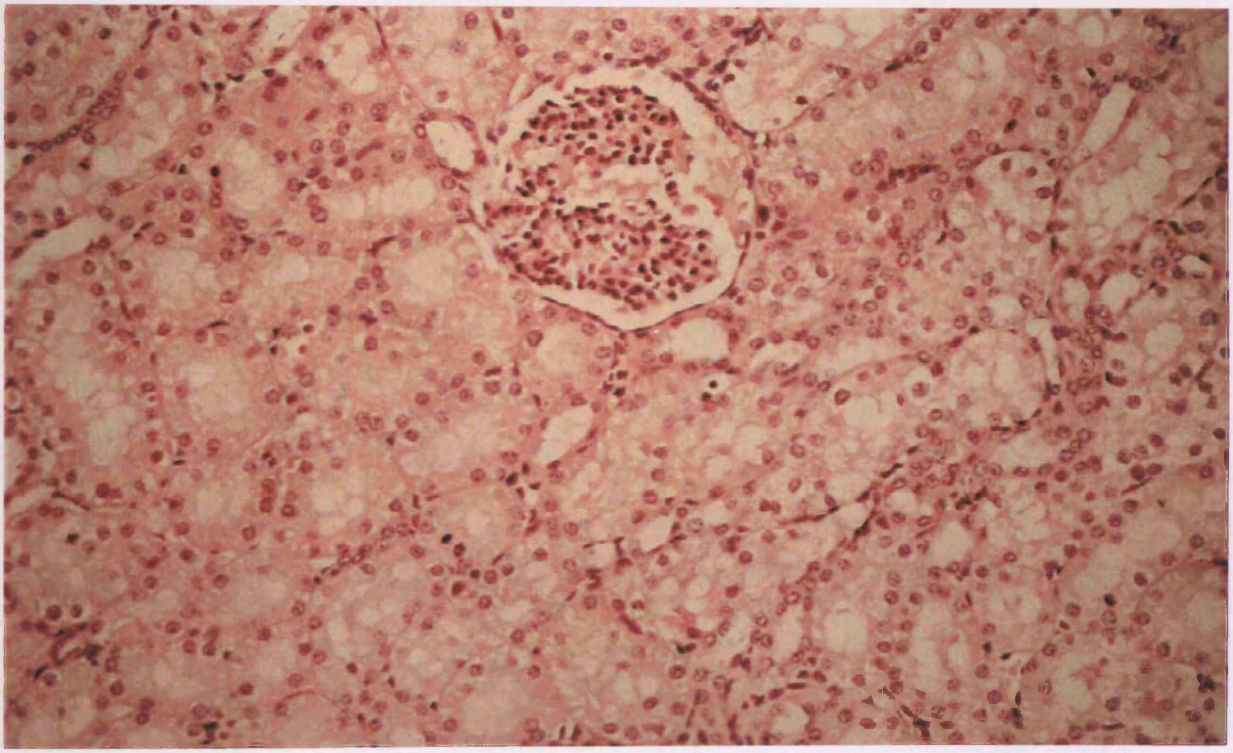


Fig. 6.37 Liver x200

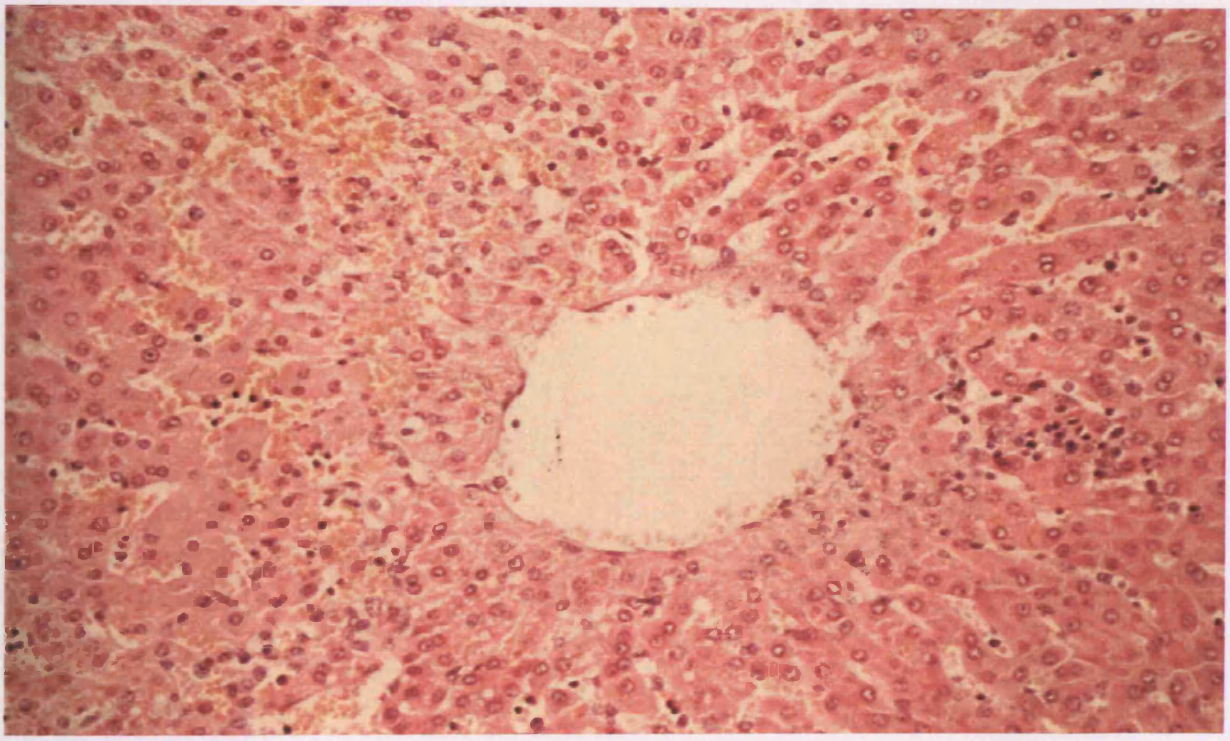


Fig. 6.38 Small Bowel x200

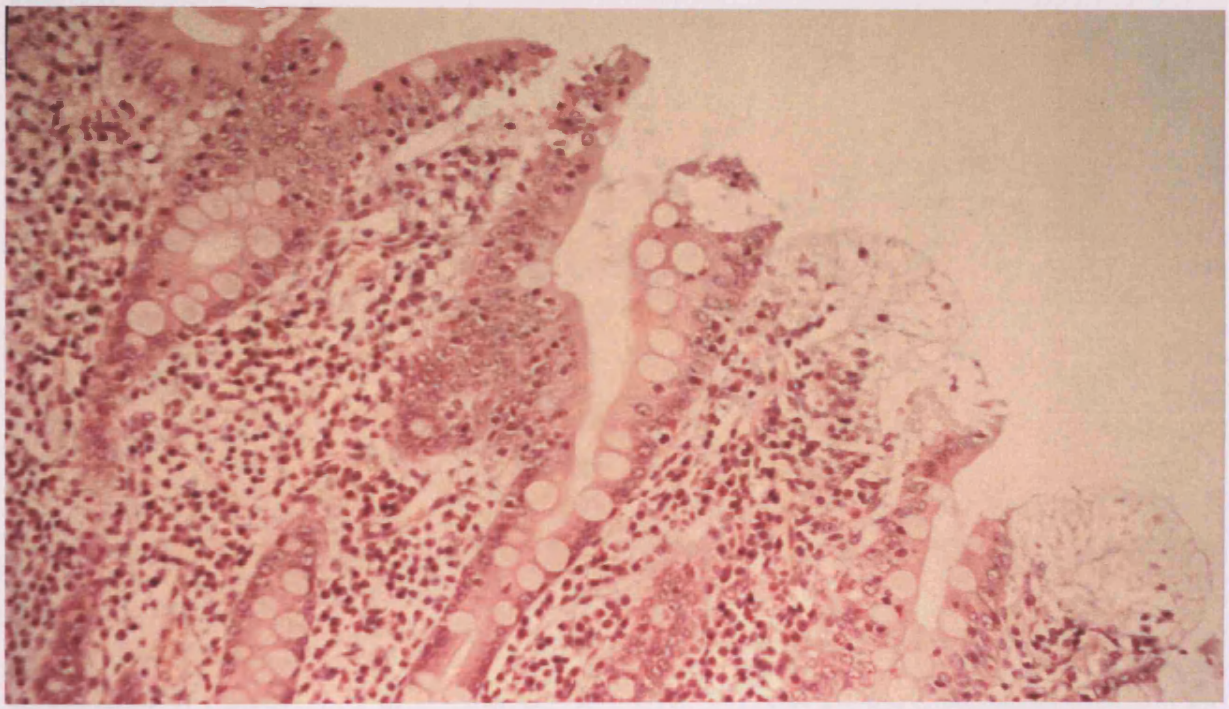


Fig. 6.39 Spleen x200

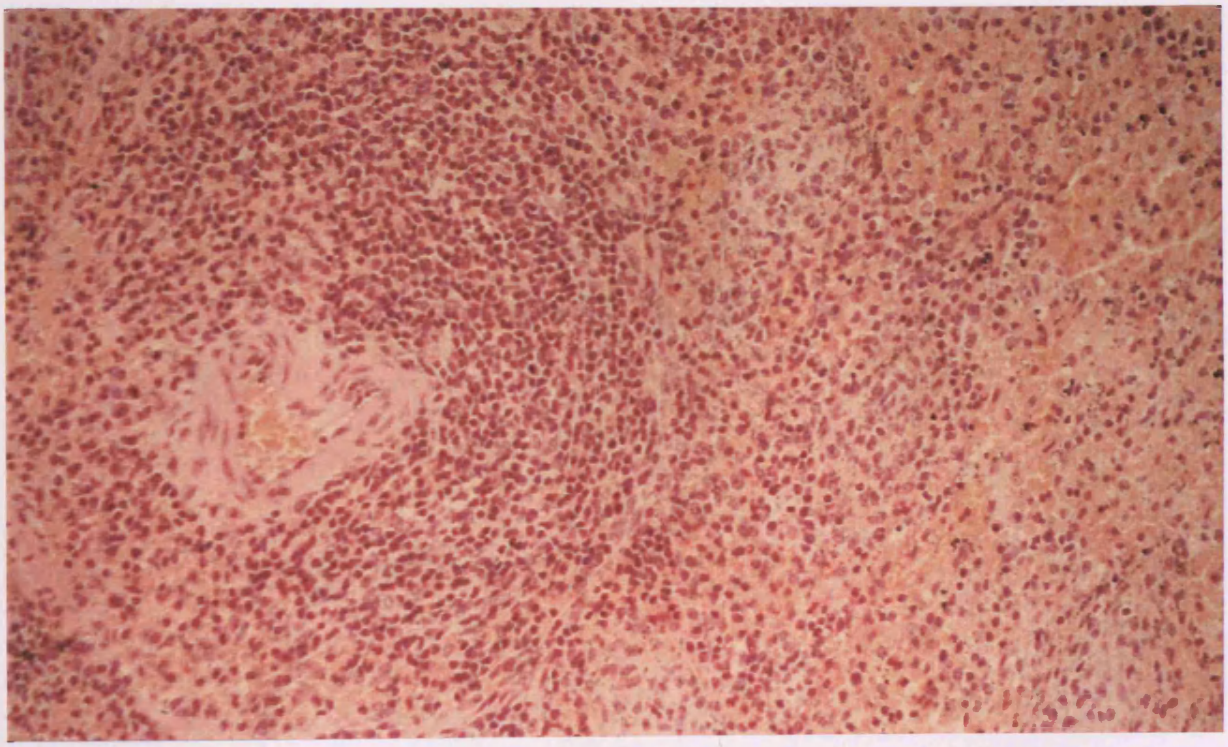


Fig. 6.40 Stomach x200

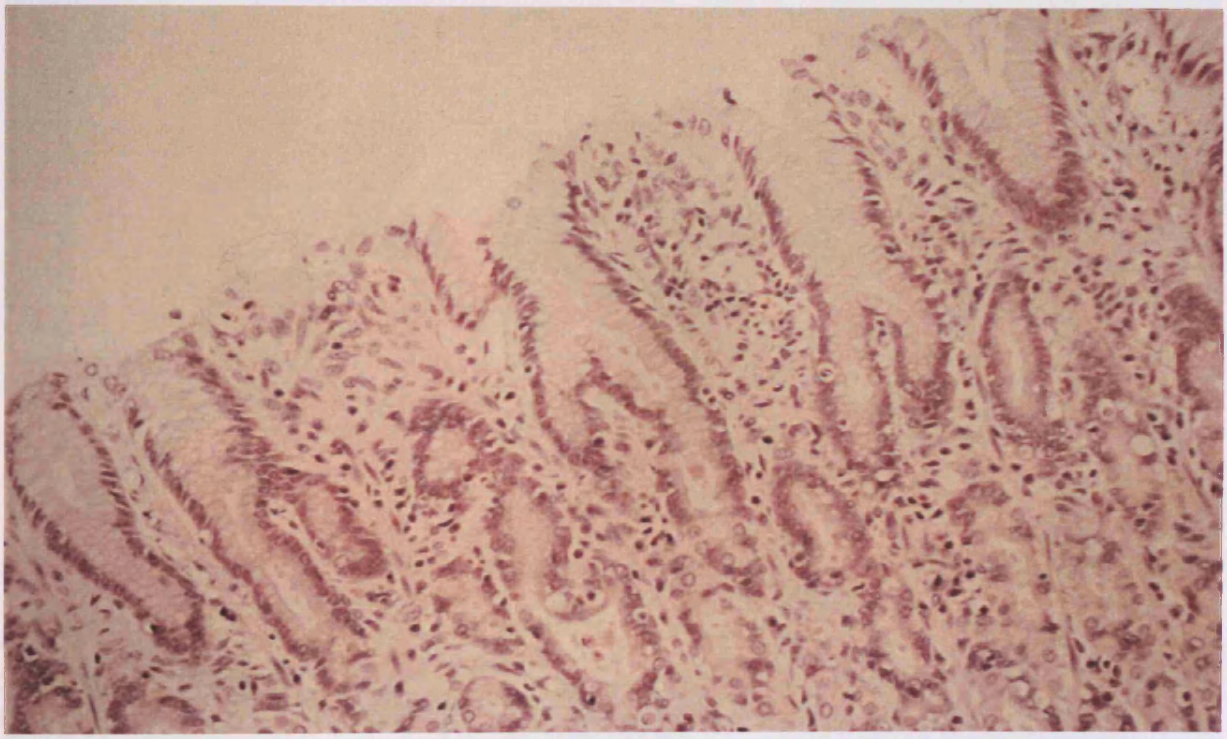


Fig. 6.41 Lung Macroscopic View showing areas of Consolidation/Pneumonia and Normal Lung

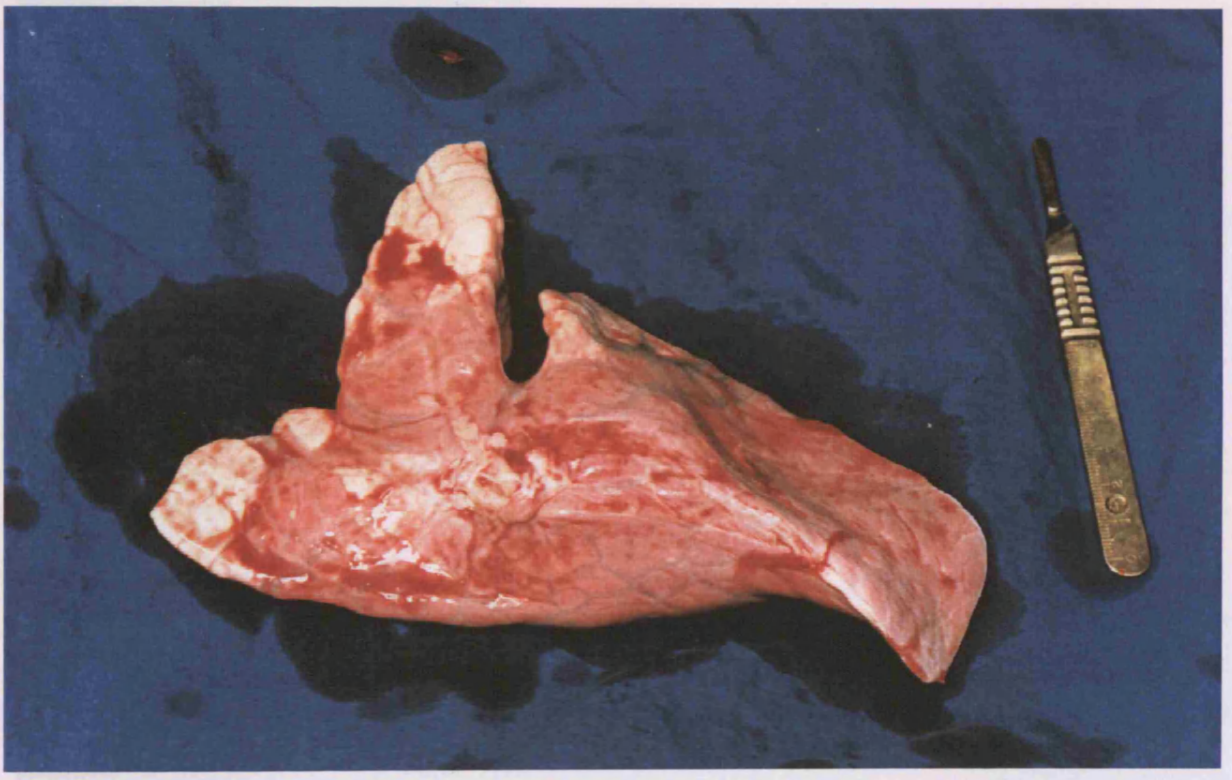


Fig. 6.42 Cut surface of lung showing Consolidation and Bronchitis

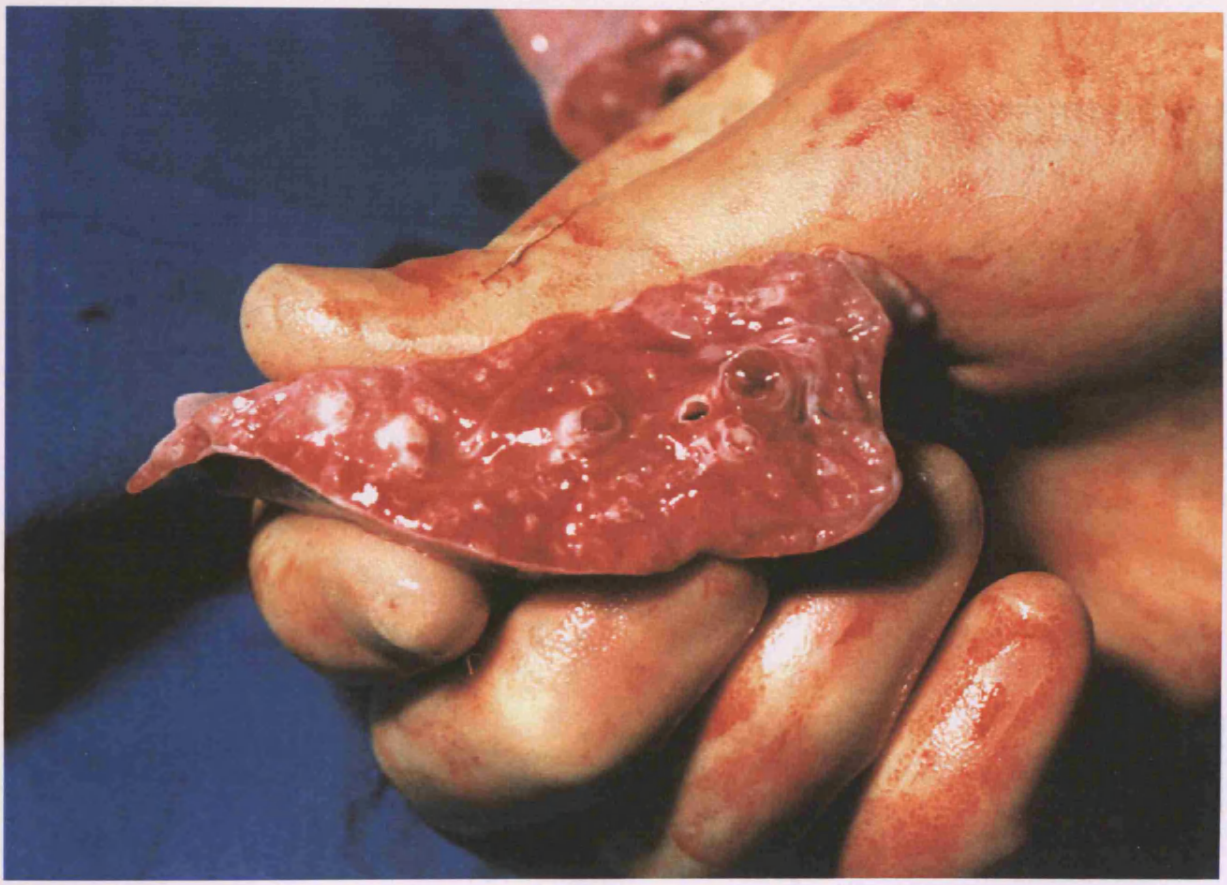


Fig. 6.43 Lung x40 showing severe Bronchitis and Bronchopneumonia

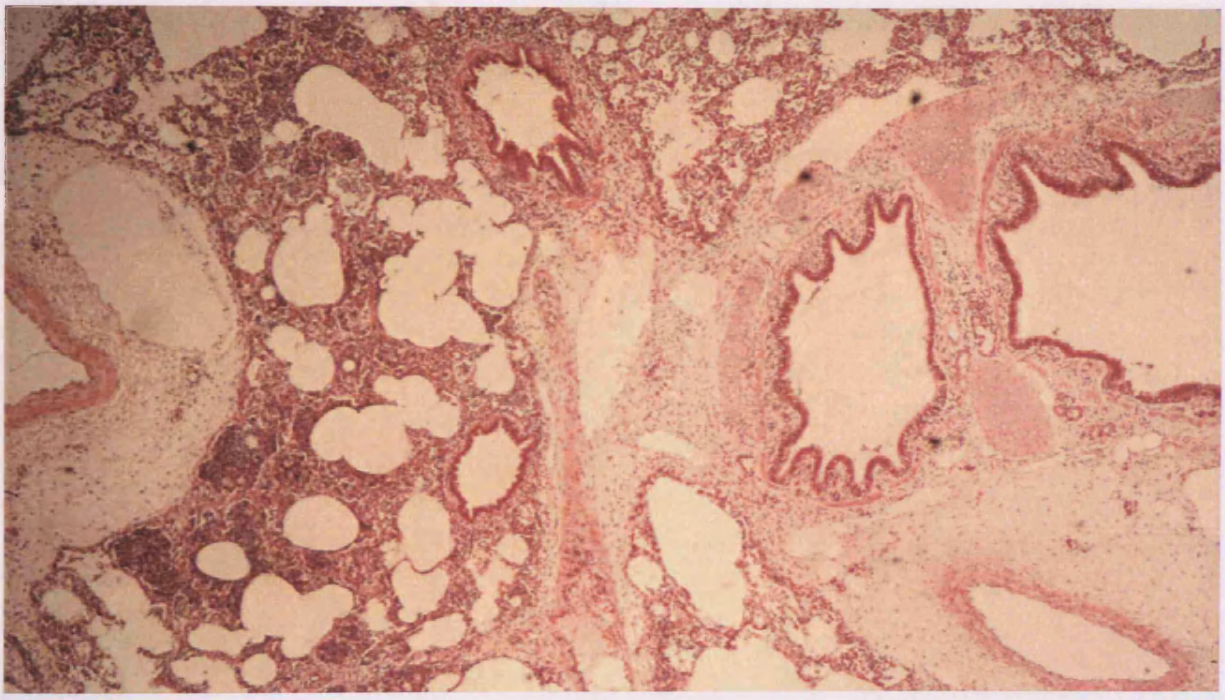


Fig. 6.44 Lung x200 showing severe Bronchitis

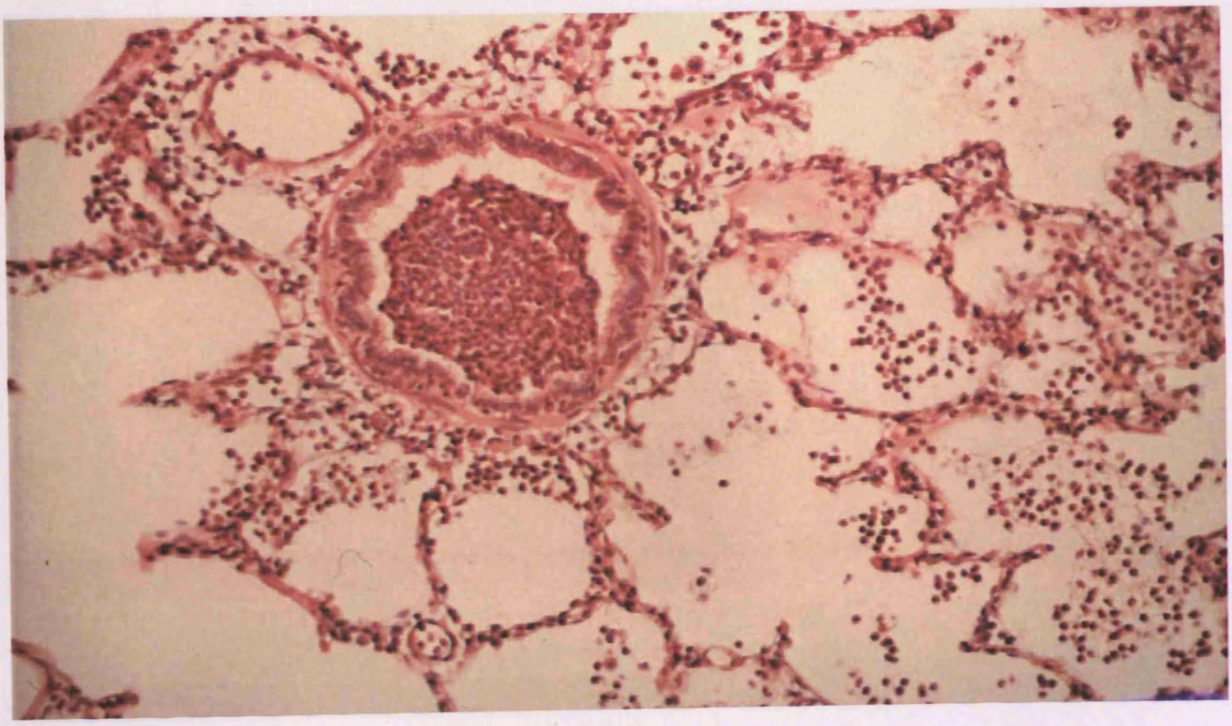


Fig. 6.45 Lung x40 showing foreign material in the Pulmonary Artery

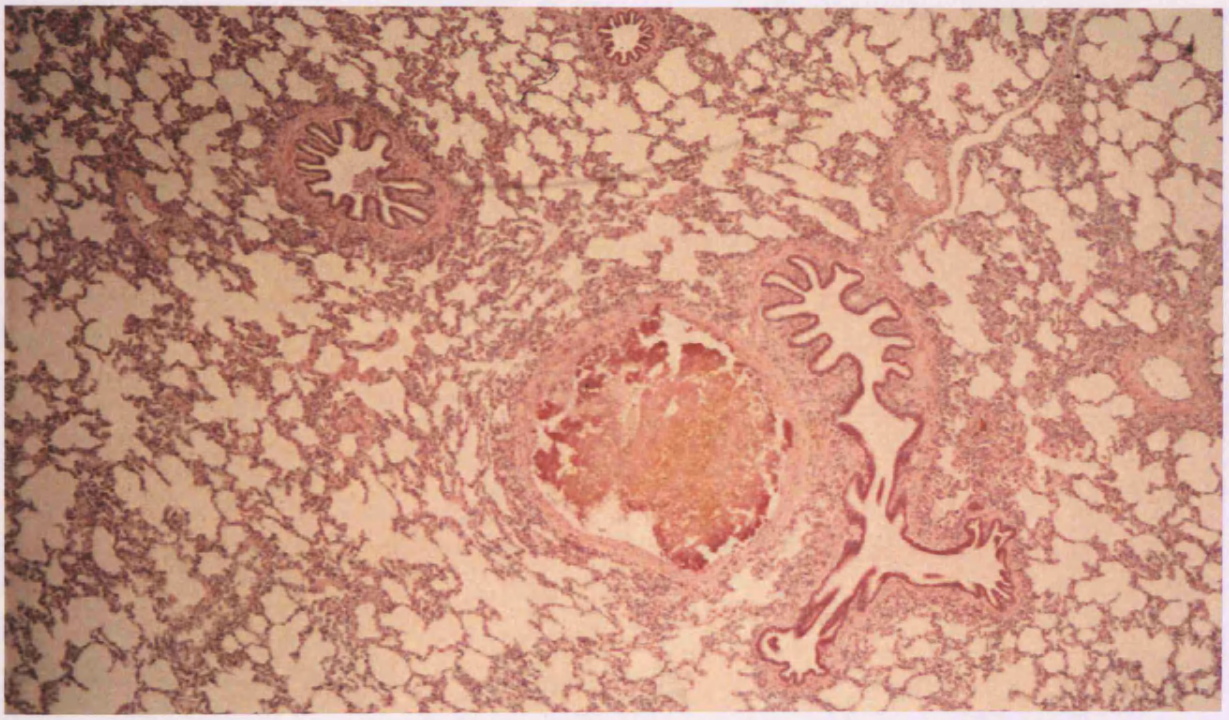


Fig. 6.46 Lung x200: Normal

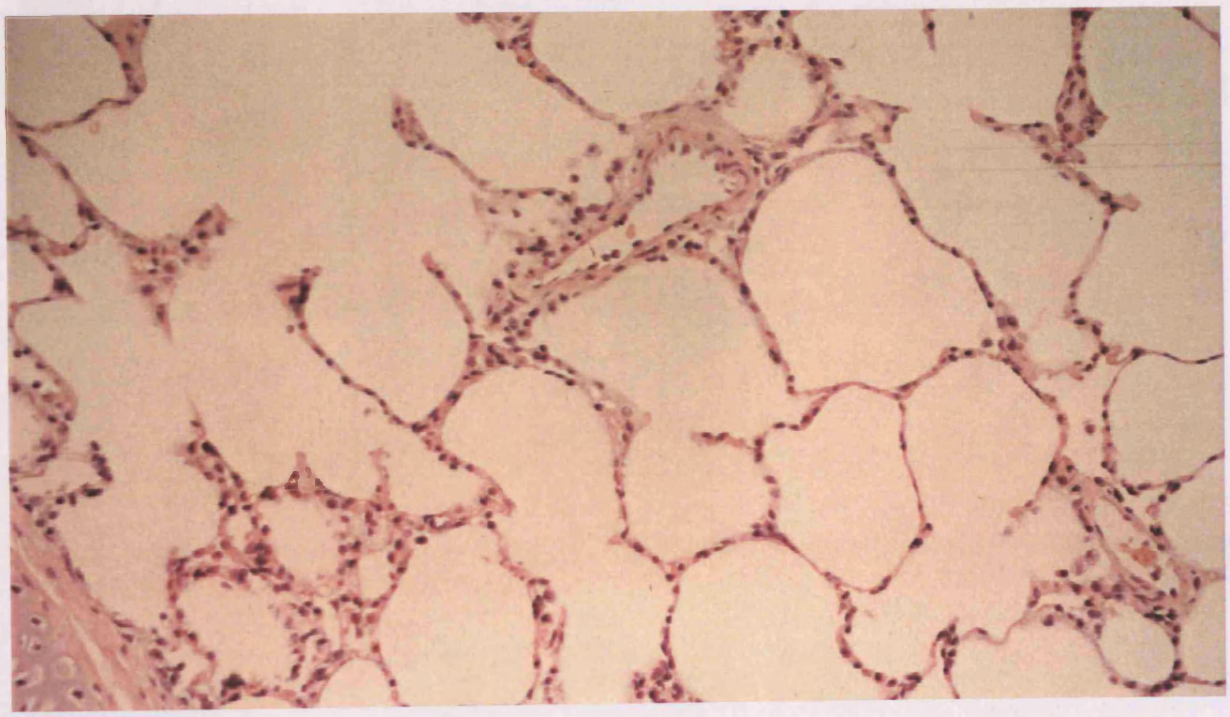


Fig. 6.47 Lung x40 showing Pulmonary Embolus

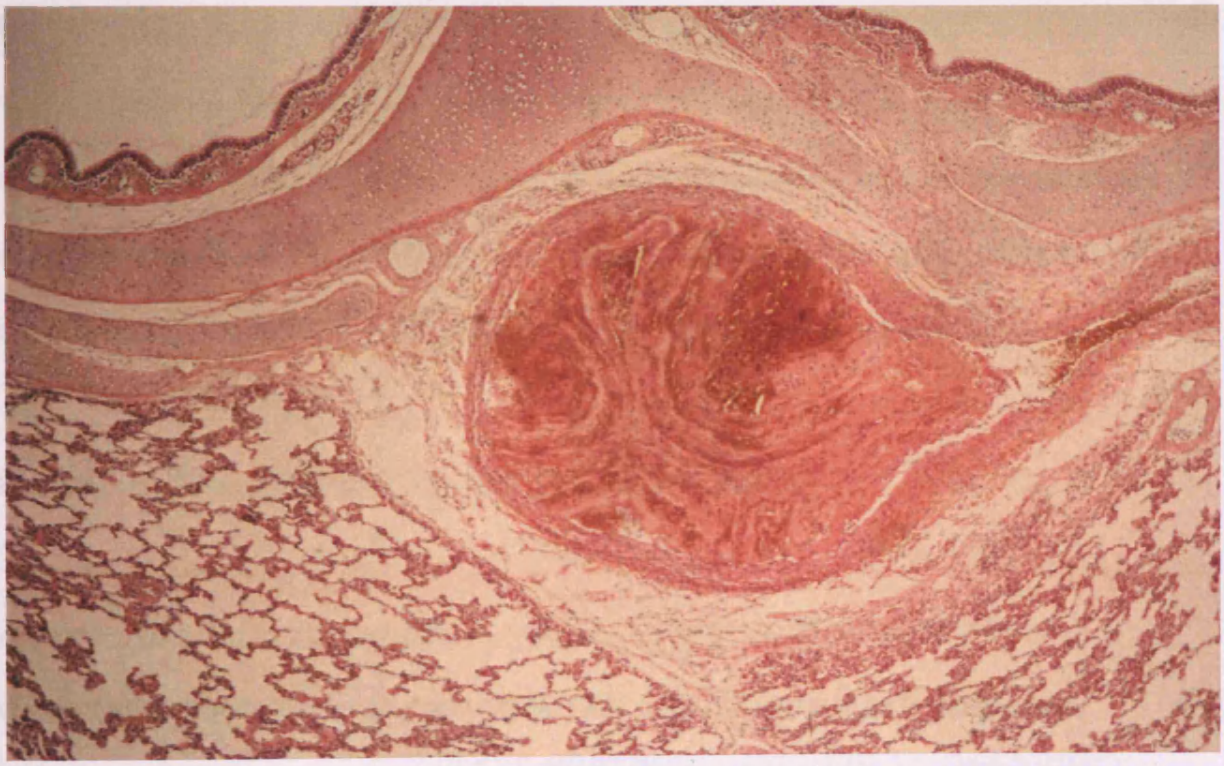


Figure 6.48: Histology Summary

FINDING	TYGON	SRT	LVA
Centrilobular hepatic congestion	2	2	0
Liver necrosis	3	2	1
Congestion	4	4	0
Bronchitis	4	2	5
Bronchopneumonia	3	3	3
Collapse	3	2	2
Other	Embolus of foreign material x 1	Pulmonary Embolism x 1	Focal haemorrhage x 1
	Duodenum, mild chronic inflammation x 1	*	Myocardial Infarction x 1

DISCUSSION

The main object of this experiment was to develop the porcine model to assess the effect of perfusion with different materials on the coagulation and inflammatory pathways of the intact organism. It also allows us to measure the general effects on each individual organ system. The discussion of results will therefore proceed along these lines.

COAGULATION, ANTICOAGULATION & HAEMOLYSIS

Activated Clotting Times, Heparin Dose & APTT Ratio: ACTs remained relatively constant and close to the target range of 160-200 seconds throughout the procedures. There were significant differences between the groups with both LVA and SRT having higher values than Tygon, although this difference was only significant for Tygon vs SRT at baseline. The ACTs increased gradually during the experiment in all groups but the increase was only significant in the LVA group. Neither SRT or LVA had increases significantly higher than control. The heparin dose varied between 20-60 u/kg/hour, similar to doses used during human ECMO. There were no significant between baseline heparin doses or change in dose for any material.

The APTT ratios also remained steady, with all groups increasing slightly throughout the experiment. This increase was significant for SRT. There were no significant differences in change of APTT in either group compared to control. Values were slightly lower, around 1.5, than the ratios of ~2 seen clinically.

Prothrombin Ratio, Circuit Pressure & Extracorporeal Flow: There were no differences in baseline Prothrombin ratios between groups. During the experiment the Prothrombin ratio in the Tygon group was unchanged, but ratios rose slightly in the SRT and LVA animals, none of these changes reached significance. There were no differences between SRT and LVA and controls. There were no differences between baseline circuit pressures. Pressures increase in all groups, but not significantly. There were no differences in increase between control and study groups. The baseline extracorporeal flow was significantly higher in the LVA group compared to control. Flows increased slightly in all groups during the procedure, but these increases were not significant. There was no significant difference in the size of the increases between LVA and SRT compared to Tygon. This difference at baseline, although statistically significant was not greatly different in clinical terms. The increase in flow over time and the large intra-group variability shown by the standard deviations, meant that flows were broadly similar for the majority of the experiment. It is possible that differences in host response to perfusion could result from the differences in flow rate (figure 6.10). However, on the whole there was little difference in response between groups.

Platelet Count & Thromboxane: The Platelet counts in all groups fell gradually during the procedure to reach a steady state at around 30 hours of perfusion. Platelet counts were significantly lower in both the LVA and SRT group at the end of the experiment compared

to baseline. But these falls were not significantly different from Tygon. There were no significant differences in Thromboxane B_2 between groups with levels remaining low throughout. There was an increase in Thromboxane levels in the Tygon group towards the end of the perfusion, but this did not reach significance. It is possible that the late rise in Thromboxane levels is a result of the preservation of platelet numbers in the Tygon group. These platelets could then be activated by thrombus build up in the circuit, releasing Thromboxane during the second day of perfusion.

Free Haemoglobin & Haematocrit: The haematocrit fell from around 26% to 16% on initiation of extracorporeal circulation, as a result of haemodilution. This fall in haematocrit was significant for the SRT animals. The haematocrit then stayed stable throughout the rest of the experiment. There were no significant differences between the two study groups and controls. Haemolysis, as measured by free haemoglobin, remained steady at around 6 mg/dl throughout the experiments. There was a trend towards higher levels at the initiation of extracorporeal circulation in the Tygon group. There was significantly less haemolysis in the SRT group compared to Tygon. This was the only significant difference between study and control groups (other than baseline variability) found in the entire study.

INFLAMMATION

Complement (C3adesarg): Complement levels remained high in all groups throughout the course of the experiments. There was a significant difference in complement activation at baseline with the LVA animals being significantly higher than controls. C3adesarg levels did not increase significantly during the experiments compared to baseline and control.

Leukocytes & Lactoferrin: Serum lactoferrin levels rose to a maximum in all groups at around 26 hours of perfusion, and then declined. The eventual difference between baseline and end of the experiments was therefore not significant. This follows the pattern of neutrophil activation described by Plotz et al (Plotz et al. 1993). There were no differences between the changes in lactoferrin levels (baseline-48 hours) between study and control groups. In case there was a difference that had been missed by the long sampling time (i.e. the peak occurred before 48 hours) the differences between groups were re-examined using the maximum deflection from baseline. Maximum increases (peak lactoferrin - baseline) were, mean (SD); Tygon 54.8 (98.4), LVA 141.8 (45.1) & SRT 131 (90). The values for SRT and LVA were not different from control. Tygon therefore only shows a trend for lower neutrophil activation than the other materials.

Differences in neutrophil activity were apparent in the total neutrophil counts. Neutrophil counts fell from baseline slightly in all groups. This fall was largest and reached significance in the SRT group. The size of the decreases, however, were not significantly different compared to the control. Overall, the SRT neutrophil counts were lower than the Tygon animals for the majority of the experiment. The fluctuations in neutrophil counts in the SRT group must be due to sequestration rather than leukopenia because lactoferrin levels remain elevated, indicating continued neutrophil granule release. It is possible that these neutrophils are sequestered in the lungs, although the evidence for this is tenuous. But the lung neutrophil infiltration on immunohistochemistry shows increased neutrophil occupation in the lung compared to Tygon (again, non-significant). Also the time course of the changes in trans-pulmonary neutrophil differential matches the total neutrophil count, however, neither of these differences reach significance.

The LVA perfused animals have neutrophil differentials (trans-pulmonary neutrophil gradients) that indicate that there is not preferential sequestration in the lungs

(gradient is always positive). The shape of the neutrophil differential curves for SRT and Tygon are consistent with pulmonary sequestration of neutrophils as a mechanism for these materials. Although these changes were not significant.

There were no statistical differences in lung water between the groups. This failure to find any difference in lung water is likely to be due to the fact that all the lungs were severely, possibly maximally, oedematous. Values for lung water being in excess of 80% in all groups. The small standard deviations (0.01-0.07%) and small differences between the groups (82.6-84%) lend support to the idea that lung water had reached a maximum.

The total white cell count followed an identical pattern to the neutrophil count with the Tygon animals staying constant, the LVA group steadily increasing, and the SRT animals decreasing below the level of the controls and then exceeding them by the end of the experiment. These differences were not significantly different from control.

LUNG MECHANICS & FUNCTION

Carbon Dioxide Tensions & Minute Ventilation: These remained constant at around 40 mmHg throughout the experiment. $PaCO_2$ was slightly higher in the LVA animals compared to controls. Neither $PaCO_2$ or minute ventilation changed significantly during the experiment. In addition the LVA and SRT groups showed no differences compared to Tygon.

FIO_2 and PaO_2/FIO_2 Ratio: The PaO_2/FIO_2 ratio fell steadily during the experiment, presumably as pulmonary oedema developed. Interestingly these falls from baseline were not statistically significant despite a mean decrease of over 300 mmHg in the Tygon group. Once again this is probably explained by the large intra-group variability and large standard deviations. There was no difference between the groups. The FIO_2 increased

during the experiments but not significantly, there was no difference between control and study groups.

Lung Compliance, PEEP and Murray Score: Lung compliance fell steadily in the LVA animals, reaching significance. Compliance remained almost constant in the SRT group, and fell slightly, but non-significantly, in the Tygon group. There were no differences between the study groups and controls. PEEP gradually increased in all groups during the experiment but was not significantly different either from baseline or control, as can be seen from the graph (fig 6.27). Combination of PaO_2/FIO_2 ratio, PEEP and Compliance to give a Murray lung injury score did not detect any significant differences between groups. Murray scores increased steadily in all groups throughout the experiment.

Pulmonary Artery Pressure & Pulmonary/Systemic Arterial Pressure Ratios: The PA pressures in the Tygon group were significantly higher than those of both the SRT and LVA groups at baseline. Also the pulmonary to systemic pressure ratios in the LVA group were lower than Tygon. This could be a result of the Tygon animals being significantly larger (older) than the other animals, as the pulmonary artery pressure (but not the ratio) increases with age. However, the mean arterial pressures were not different at baseline which is against this hypothesis. These differences were statistically important but values for each group were very similar (see fig. 6.24-6.25).

CARDIO-VASCULAR SYSTEM

Adrenaline & Dopamine Dose: Both increased during the experiment. Doses of adrenaline were higher than control for both LVA and SRT, but not significantly. Dopamine dose increased significantly from baseline in the SRT group. Dopamine increased in LVA and Tygon groups but did not reach significance. The increases in both study groups were not significantly different from control. The increasing inotrope requirement may have been

due to minor differences in cardiotoxicity of the materials, or could be related to the pentobarbitone. Pentobarbitone is a known myocardial suppressant, but there were no significant increases in dose or differences between controls and study groups.

CVP, Mean Arterial Pressure, Heart Rate & PAWP: Central Venous and Pulmonary Artery Wedge Pressures (CVP & PAWP) both followed similar patterns. In all groups both CVP and PAWP slowly increased throughout the perfusions. Increases were not significant compared to baseline or controls. Mean arterial pressure and heart rate fell slowly in all animals during the procedure. The fall in mean arterial pressure was significant compared to baseline for LVA, but there were no differences from control.

RENAL FUNCTION

Fluid balance, urine output and frusemide dose and aminophylline dose were not significantly different between groups.

SURVIVAL

There were no differences in number of animals surviving to the end of the protocol between the groups, compared to control.

HISTOLOGY

Representative examples of the histology of all of the organs retrieved at post mortem are shown in figures 6.35-6.47. Figure 6.48 is a summary of the abnormal findings. All other tissues examined were normal unless reported otherwise. A table of contents is given below to aid location of the photomicrographs.

Table of contents; Histology

Figure	Tissue	Description	Magnification
6.35	Myocardium	Normal	200
6.36	Kidney	Normal	200
6.37	Liver	Normal	200
6.38	Small Bowel	Normal	200
6.39	Spleen	Normal	200
6.40	Stomach	Normal	200
6.41	Whole Lung	Consolidation/Pneumonia	1
6.42	Lung	Cut surface of 6.41	1
6.43	Lung	Bronchitis & Bronchopneumonia	40
6.44	Lung	Bronchitis	200
6.45	Lung	Foreign material in PA	40
6.46	Lung	Normal	200
6.47	Lung	Pulmonary Embolism	40

Aside from the quantitative assessment of pulmonary neutrophil infiltration already presented above, no statistical comparison of histological findings was performed. It was felt that the small numbers and qualitative nature of the findings made this impossible. Instead the incidence of different histological findings in each group are presented in fig 6.48.

Aside from the lung and liver, all organs in all three groups were unremarkable. Exceptions were mild chronic (probably pre-existing) inflammation in the duodenum of one of the Tygon animals and a myocardial infarction in one of the LVA animals. The hepatic changes were centrilobular hepatic congestion and necrosis. Some hepatic changes were recorded in all three groups, these could be due to hepatic toxicity of substances such as plasticisers leached from the tubing, or be due to venous obstruction from the ECC cannula in the IVC. The later is unlikely as it was the drainage cannula which was situated in the IVC in most cases, and the IVC was much larger than the cannula.

The macroscopic appearances of the lungs was similar in all cases (6.41-6.42), showing marked collapse and consolidation with purulent sputum in the cut bronchi. There were also patchy areas of normal lung. Histologically all three groups showed a similar incidence of, bronchitis, bronchopneumonia and collapse. The Tygon and SRT

animals also showed congestion of the pulmonary vasculature, these findings were absent from the LVA group. Other notable findings were an embolus of foreign material (perhaps a tubing spall) in one of the Tygon animals, a pulmonary embolism in the SRT group and a focal pulmonary haemorrhage in one of the LVA cases. The absence of congestion in the lungs of the LVA animals correlates well with the lower pulmonary artery pressure and pulmonary/systemic arterial pressure ratio seen in these animals compared to the controls.

CONCLUSIONS

Each material has been compared in its performance to Tygon in a number of areas, namely: activation of the coagulation cascade, haemolysis, inflammation, cardiotoxicity, pneumotoxicity and nephrotoxicity. The responses of the animals to each material were similar with only one significant difference (in haemolysis) between groups. It is possible that this difference is due to chance. It is likely that the small numbers in each group contributed to the inability to distinguish between tubing materials. This was confounded by the impossibility of randomly assigning animals between groups. With larger numbers of animals in each group it may be possible to use statistical manipulations such as multiple logistic regression to correlate the results of the numerous investigations performed on these animals in order to generate a final "Biocompatability Score". We cannot distinguish between tubing materials on the basis of the animal model alone. We have been successful in establishing a novel animal model of prolonged closed chest veno-venous perfusion and have characterised the animals responses during perfusion. Haemodilution, progressive thrombocytopenia, neutrophil activation and development of severe, some-times fatal "ARDS" occurring in all animals.

Larger numbers of animals and strict randomisation should eliminate many of the problems seen during this experiment. Unfortunately time and cost constraints made this

impossible in the present study. This model could be developed to investigate any aspect of veno-venous ECMO, haemofiltration or extracorporeal liver support, including longer term experiments where animals are recovered.

CHAPTER 7

IN VITRO I: RADIO-LABELLED FIBRINOGEN UPTAKE

INTRODUCTION

The ideal biomaterial is completely inert, and does not activate any of the body's inflammatory, coagulation or immunological defence mechanisms. Unfortunately such a material does not exist, and we must compromise between biocompatability and acceptable mechanical properties. We have seen in chapter 2 that binding of fibrinogen to the circuit is one of the first steps in the activation of the coagulation system that occurs on initiation of extracorporeal circulation (Urlesberger et al. 1996) . This fibrinogen uptake in turn leads to platelet adhesion and activation (Colman et al. 1987) (Remuzzi and Boccardo, 1993). Fibrinogen uptake can be inhibited by surface modification (Yu et al. 1994) , or by pre-washing the circuit with albumin prior to the initiation of ECC (Kelly et al. 1989; Whittlesey et al. 1988). Thus a material which has a high affinity for albumin, will bind less fibrinogen, and be more inert or "biocompatible". In this chapter we will investigate the relative reduction in fibrinogen uptake after albumin immersion of the three materials Tygon (control) SRT and LVA by the use of a radio-isotope technique. I am greatly indebted to Ms Rachel Carter of the Department of Surgery, University of Leicester, who handled the radio-isotopes during this experiment.

AIM

To measure the fibrinogen uptake of SRT and LVA before and after albumin washing, and compare it with that of Tygon as a control.

MATERIALS & METHODS

The classical index of biocompatability is the fibrinogen / albumin ratio, determined by measuring the uptake of radio-labelled fibrinogen and albumin (Eberhart et al. 1987; Wesslen et al. 1994). Unfortunately an exhaustive world-wide search of suppliers of radio-labelled reagents revealed that it is no longer possible to obtain labelled albumin as a standard preparation. Since the object of this index is to quantify the reduction in fibrinogen binding that occurs following albumin adsorption we modified the method of Yu et al, (Yu et al. 1994) to measure exactly that.

Ten discs of approximately 9mm in diameter were cut from ½ inch Tygon S-65-HL (control) and the two test materials, LVA and SRT using a custom made punch (Garmed, Leicester). Discs were immersed in buffer (see below) overnight.

SOLUTIONS.

I) ^{125}I labelled human fibrinogen (Amersham) in isotonic solution (0.75% sodium citrate, 0.65% sodium chloride and 20mg/ml albumin) to a final fibrinogen concentration of 0.8mg/ml.

II) Buffer:5mM Tris, 0.9% saline, pH7.4.

III) 20% Human Albumin solution (Bio Products Laboratory, Elstree, Herts) used as received.

Five discs of each material were removed from buffer and stirred in 20% albumin solution at 37 Celsius for 1 hour. All discs were then immersed in aliquots of the fibrinogen uptake solution at room temperature. This solution was made by adding 40 microlitres of the fibrinogen stock solution (I) above, to 20 ml of buffer. A separate 20 ml aliquot of solution was used for each group of 5 discs. Adsorption was allowed to occur for 20 minutes, and then discs were removed from solution, washed once with buffer and

then dried with tissue. Discs were then placed in sample tubes for gamma counting. Positive controls were 100 microlitre samples of each of the six aliquots of fibrinogen uptake solution. A negative control (empty tube) was also included. Automated Gamma counting for 1 minute per sample was performed using the Cobra Auto-Gamma (Canberra-Packard). Counts were automatically corrected for background by the machine, and were expressed as counts per minute (CPM).

Fibrinogen uptake was calculated using the positive control for each five discs as a standard:

40 microlitres of fibrinogen stock solution contains 32 micrograms of fibrinogen.

This is diluted to 20 ml, 100 microlitres of this solution contains 160 picograms of fibrinogen.

STATISTICAL METHODS

Data for LVA and SRT were compared with control (Tygon) using an un-paired t-test. A P value of less than 0.05 was taken to indicate significance.

RESULTS

All results are tabulated below.

TABLE 1: Gamma Counts (counts per minute).

MATERIAL	CONTROL/PLAIN	PLAIN	CONTROL/ALBUMIN	ALBUMIN
TYGON	26880	650	27243	61
		375		33
		345		78
		234		41
		488		37
LVA	30480	829	26997	31
		717		47
		1159		29
		931		42
		915		30
SRT	26283	604	27644	97
		697		188
		725		71
		669		72
		601		84

TABLE 2: Fibrinogen Uptake (picograms) and Albumin / Plain Ratios

MATERIAL	CONTROL/PLAIN	PLAIN	CONTROL/ALBUMIN	ALBUMIN	RATIO
TYGON	168	3.87	170.27	0.358	0.093
		2.23		0.194	0.087
		2.05		0.458	0.223
		1.39		0.239	0.172
		2.95		0.214	0.073
LVA	190.5	4.35	168.7	0.184	0.043
		3.28		0.278	0.074
		6.1		0.169	0.035
		4.89		0.248	0.051
		4.81		0.179	0.037
SRT	164.27	3.68	172.78	0.561	0.151
		4.22		1.09	0.257
		4.39		0.411	0.093
		4.05		0.42	0.102
		3.64		0.49	0.133

TABLE 3: Fibrinogen Uptake (picograms), Means and Standard Deviations

	Tygon		LVA		SRT	
	Mean	SD	Mean	SD	Mean	SD
Albumin/Plain Ratio	0.13	0.065	0.048	0.016	0.147	0.066
Plain	2.5	0.95	4.7	1.02	4	0.33
Albumin	0.29	0.11	0.21	0.05	0.59	0.28

TABLE 4: Statistics

	Tygon vs LVA	Tygon vs SRT	Comments
Albumin/Plain Ratio	P<0.001	NS	Tygon > LVA
Plain	P=0.004	P=0.001	Tygon < Both
Albumin	P=0.021	NS	(SRT > Tygon > LVA)

DISCUSSION

Plain Tygon binds significantly less fibrinogen than LVA and SRT, but fibrinogen uptake is similar for SRT and Tygon when coated with albumin. Fibrinogen uptake of coated LVA is significantly lower than coated Tygon Since the fibrinogen binding of plain Tygon is lower, the ratio between fibrinogen uptake by the albumin treated tubing and the plain material is higher than SRT and LVA, although the difference is only significant for LVA. The ratio between fibrinogen uptake of the plain tubing and the albumin treated material is confounded in its application as a measure of bio-compatibility by the absolute levels of fibrinogen adsorption. Since it is the amount of fibrinogen adsorption that occurs after albumin washing that we are interested in, it would seem logical to take this figure as our main index of biocompatability. These values showed no difference between materials.

The much lower fibrinogen uptake of plain Tygon, almost half that of the other materials, may indicate that in the longer term Tygon would be more bio-compatible that SRT and LVA as there could be reduced potential for exchange of albumin for fibrinogen as ECC proceeds (Colman et al. 1987) . It obviously indicates that fibrinogen uptake

would be lower if the albumin wash were omitted from the prime. Since this is occasionally necessary when ECC must be established emergently, Tygon would have a bio-compatibility advantage.

The marked differences between the apparent bio-compatibility between the three materials in both the animal model (chapters 5 & 6) and the whole blood in vitro model (Chapter 8) indicate that fibrinogen uptake is a much cruder test. It is possible that the previously described fibrinogen/albumin ratio measurements (Yu et al. 1994), would detect these differences, although the above assay actually measures the mechanism thought to be responsible for tubing passivation i.e. reduction in fibrinogen uptake as a result of albumin pre-treatment. It is therefore difficult to understand how direct measurement of albumin binding could give a different qualitative answer, unless fibrinogen is not the main effector in the response to ECC. Albumin uptake onto the tubing would then be important in absolute terms as surface bound albumin could prevent blood/surface interaction of other mediators such as white cell adhesion molecules (Finn et al. 1993), platelets (Musial et al. 1990) or complement (Gillinov et al. 1993).

Measurement of fibrinogen uptake would therefore be irrelevant. Since radio-labelled albumin is only available at great expense as a custom made reagent, it would seem more relevant, simpler, quicker and cheaper to perform whole blood bio-compatibility testing in a similar manner to the experiment described in chapter 8.

CONCLUSIONS

Fibrinogen/albumin binding ratio has long been accepted as an easily performed index of bio-compatibility for extracorporeal circuit components. Radio-labelled albumin is no longer freely available and therefore fibrinogen binding was measured before and after washing tubing materials with albumin. There was no difference in fibrinogen uptake

following albumin immersion between the three materials, but fibrinogen levels for the untreated materials were lower for Tygon. We conclude that measurement of fibrinogen uptake alone is not a good index of biocompatability , probably because albumin binding does much more that inhibit fibrinogen uptake. Unless radio-labelled albumin is available it would seem more appropriate to use whole blood or in vivo responses to ECC to determine the bio-compatibility of extracorporeal circuit components.

CHAPTER 8

IN-VITRO II: RE-CIRCULATION OF FRESH HUMAN BLOOD

INTRODUCTION

In the evaluation of possible new extracorporeal circuit materials animal experiments are essential to measure the effects of perfusion on the intact organism. However, there may be important species differences between experimental animals and man which have resulted in disastrous consequences when animal data is extrapolated directly to man (Wollam, 1962). In order to minimise the risk of such effects we have also assessed the effect of the two new materials (LVA & SRT) on fresh human blood during recirculation, and compared it to Tygon S-65-HL as a control.

AIM

To measure the inflammatory and coagulative response of freshly donated human blood during recirculation through circuits made of Tygon, LVA and SRT.

METHODS

DONOR CRITERIA

Volunteer donors were recruited from the University of Leicester and Glenfield Hospital. All donors gave informed consent, and the donation protocol was approved by the Leicestershire Health Ethical Committee. Donors fulfilled the following criteria:

- ADULT (18-60 years).
- weight >60 kg.
- No history of abnormality of the haematological system or other serious illness.
- No regular medication including OCP/POP, inhaled bronchodilatators are acceptable provided none are taken for 24 hours before blood donation, inhaled steroids are not acceptable.

- No NSAIDS such as aspirin, brufen, indomethacin etc. for 1 week prior to the study.
- No alcohol for 24 hours prior to study.
- Fasted from midnight, water and fruit juice are allowed ad lib, but fat must be avoided (no milk).
- No history of allergy to local anaesthetics or heparin.

Donor criteria were selected in order to avoid factors which would affect the function of the blood such as oral contraceptive use which can lead to a hypercoagulable state(Laurence and Bennett, 1987) . Non Steroidal Anti-Inflammatory Drugs (NSAIDS) such as Aspirin are well known to inhibit platelet function (Laurence and Bennett, 1987). Whilst serum lipids can affect red cell fragility (Bernstein and Gleason, 1967) (Bernstein et al. 1967). A minimum donor weight of 60 kg was selected since a minimum of 500 ml of blood was required. Since 15% of the blood volume can be lost without major haemodynamic consequences (Anonymous1993) 630mls could be taken from a 60Kg patient whilst still leaving a sufficient safety margin.

DONATION OF BLOOD

Donors were weighed, consented and compliance with the donor criteria above was checked. The procedure was performed in a side room on the Paediatric Intensive Care Unit at Glenfield Hospital, ensuring full access to resuscitation equipment. All procedures were performed by the author. High flow (15l/min) oxygen was administered by Hudson mask ~60% O2, to optimise the oxygenation of the venous blood. Donors were monitored throughout by pulse oximetry. Under local anaesthesia a 6FG sheath (Cordis) was inserted into an ante-cubital vein using the Seldinger technique (Seldinger, 1953). Heparin 50u/kg was administered intra-venously and allowed to circulate for two minutes prior to baseline

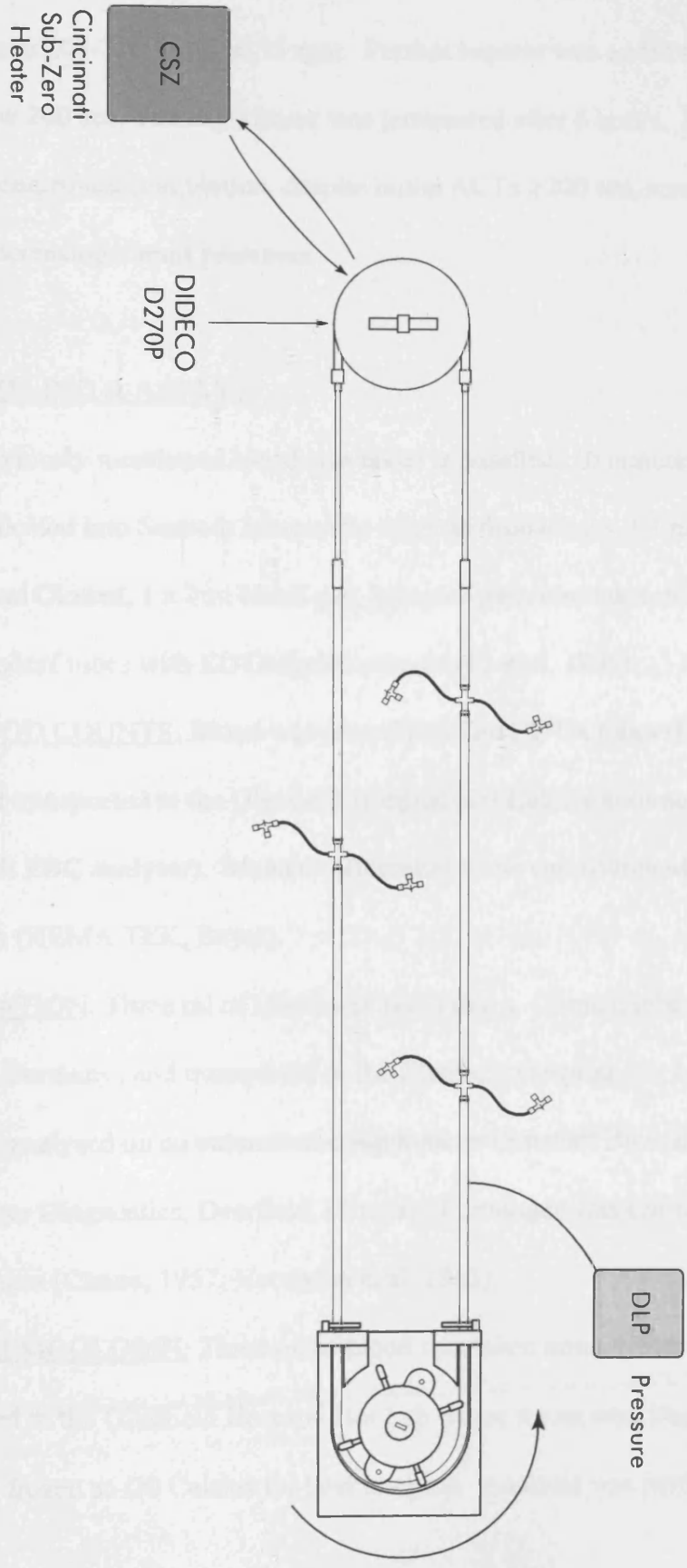
blood sampling. If the activated clotting time (ACT) was less than 200 seconds, further heparin was administered. Once a satisfactory (>200 sec) ACT was achieved, 500-600 ml of blood was drained via a standard Baxter blood giving set into a preservative free transfer bag (Baxter). This blood was then immediately added to the test circuit (see below). One litre of 0.9% saline solution was then administered to the donors via the intra-venous sheath to restore normo-volaemia. The sheath was then removed and haemostasis achieved with pressure. Donors were then observed for 30 minutes prior to discharge.

PERFUSION PROTOCOL

Fifteen identical circuits were constructed (fig 8.1), 5 each, from ½ inch Tygon S-65-HL (control) and the two test materials, LVA and SRT. Circuits comprised a closed loop of tubing with polycarbonate leur connectors to allow priming and pressure monitoring. Each circuit also had a standard length of ¼ inch PVC (Cobe) to allow later connection to a heat exchanger (Dideco D-720P). Circuits were then sterilised by Ethylene Oxide in the Central Sterile Supplies Department using their standard clinical procedure. The total circuit volume with the heat exchanger was 500 ml. Circuits were assembled and occlusion was set in the same manner as employed when priming standard clinical ECMO circuits, i.e. slightly under occlusive to just allow passage of air past the roller. Stockert roller pumps were used throughout. Circuits were primed with Plasmalyte-A (Baxter), and 100u Heparin, they were then re-circulated at 37 Celsius. Normothermia was maintained by a Cincinnati Sub Zero ECMO heater, connected to the Dideco D-720P. Mean post pump pressure was measured using standard DLP transducers and pressure boxes.

Donor blood was added to the circuit, completely displacing the clear prime. The pump speed was then set to 75 rpm, equivalent to a flow of 3.45 L/min. Plasmalyte-A was then added to the circuit until a post pump pressure of between 200-300mmhg was

Fig. 8.1 Re-circulation Circuit



obtained. Samples were taken at 10 minutes, 1 hour, 2,4 and 6 hours. During sampling the pump was switched off, and following withdrawal of blood an equivalent volume of Plasmalyte-A was replaced, further fluid was added or withdrawn to keep the post pump pressure between 200-300mmhg at 75 rpm. Further heparin was added to the circuit if the ACT fell below 200 sec. The experiment was terminated after 6 hours. Runs were also curtailed if uncontrolled coagulation, despite initial ACTs >200 sec, resulted in dangerously increasing circuit pressures.

BLOOD SAMPLING & ASSAYS

As previously mentioned blood was taken at baseline, 10 minutes, 1,2,4 & 6 hours. Blood was collected into Sarstedt Monovette tubes as follows: 2 x 2.7 ml EDTA, 2 x 3ml Citrate, 1 x 9 ml Clotted, 1 x 2ml blood gas. Samples were also taken for ACT (Hemocron) and into eppendorf tubes with EDTA/Indomethacin (Li et al. 1995).

i) FULL BLOOD COUNTS: Blood was placed in 2.7ml EDTA tubes (EDTA KE, Sarstedt, Germany) and transported to the Glenfield Hospital Hot Lab for automated analysis (Coulter STKR FBC analyser). Manual differential white cell counts were also performed in the Hot Lab (HEMA TEK, Bayer).

ii) COAGULATION: Three ml of blood was taken into a Citrated tube (Coagulation 9 NC, Sarstedt, Germany) and transported to the Glenfield Hospital Hot Lab for analysis. Samples were analysed on an automated coagulometer (Sarstedt Biomatic B10) using Dade reagents (Baxter Diagnostics, Deerfield, Illinois). Fibrinogen was estimated after the method of Clauss (Clauss, 1957; Vermylen et al. 1963).

iii) FREE HAEMOGLOBIN: Three ml of blood was taken into a citrated tube as for (ii) and transported to the Glenfield Hospital Hot Lab where it was centrifuged and serum separated and frozen at -20 Celsius for later analysis. Analysis was performed at LRI

Department of Haematology by colorimetric determination (Sigma Diagnostics, Catalog No. 527). This assay is based on the catalytic activity of haemoglobin on the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) by H_2O_2. The rate of colour formation at 600nm is proportional to the haemoglobin concentration (Lijana and Williams, 1979; Standefeder and Vanderjagt, 1977) .

iv) PLATELET ACTIVATION: Plasma Thromboxane B2 (TXB2) was taken as an index of platelet activation (Li et al. 1995). 1.5ml of blood was taken and collected into cold eppendorf tubes containing 15mcg indomethacin (Sigma) and 1.5mg EDTA (Sigma). These were centrifuged at 1000rpm at 4 Celsius for 10 minutes and then two 0.5ml aliquots of Plasma were removed. Plasma samples were acidified with two drops of 1N Hydrochloric acid, and then TXB2 was extracted with Ethyl-Acetate. Samples were then evaporated under nitrogen to dryness and stored at -70 Celsius for later Enzyme Immunoassay (Li et al. 1995) . All assays were carried out by Mr Paul Whitaker of the Department of Biochemistry, Leicester Royal Infirmary to whom I am most indebted. ELISA was performed using commercially available assay kit (BIOTRAK Thromboxane B_2, Amersham, UK). Samples were analysed in duplicate and the mean activity calculated.

v) COMPLEMENT ACTIVATION: One 2.7ml EDTA tube (EDTA KE,) was filled with blood, plasma was separated and then frozen at -20 Celsius for later analysis. Plasma was analysed for activity of the Terminal Complement Complex, C5b9 (Roitt, 1994). This was measured using a commercially available radioimmunoassay which uses a monoclonal antibody to C5b9 / Protein S complexes (SC5b9) to give an index of C5b9 activity (SC5b-9 (TCC) Enzyme Immunoassay from Quidel, San Diego, CA, USA). All complement assays were performed by Dr Jonathan North, Dept. Immunology, Leicester Royal Infirmary.

vi) LACTOFERRIN: Serum was collected in plain tubes (Z9 Sarstedt, Germany), separated and frozen at -20 Celsius for later analysis. All assays were carried out by Mr Paul Whitaker of the Department of Biochemistry, Leicester Royal Infirmary to whom I am most indebted. Samples were analysed by ELISA after the method of Hegnoj et al (Hegnhoj and Schaffalitzky de Muckadell, 1985). The lactoferrin for the standard was obtained from Sigma (Poole, Dorset, UK), and the antibodies from Dako (Copenhagen). All analyses were duplicated and the mean value taken.

STATISTICAL METHODS

Data was collected throughout the experiment and is displayed on the graphs as variation with time. The line of best fit is drawn through the mean values, using the polynomial trendline function in Microsoft Excel. Maxima and minima are also displayed. Variables that had a significant difference from the control group (see below) are highlighted with a blue background.

The difference between values at the beginning of the and the end of the experiment was found by subtracting the baseline value from the final value. A negative sign indicates a decrease from baseline and vice versa. These differences were compared with the mean difference found in the controls (Tygon) using an unpaired t-test. The differences from baseline were also analysed to determine whether any change was significant, an un-paired t-test was used to acomplish this using zero as the test value. $P < 0.05$ was taken to indicate significance. The 95% significance level was taken rather than the 99% level used in the porcine experiments because it was felt that this in-vitro model was much more reproducible.

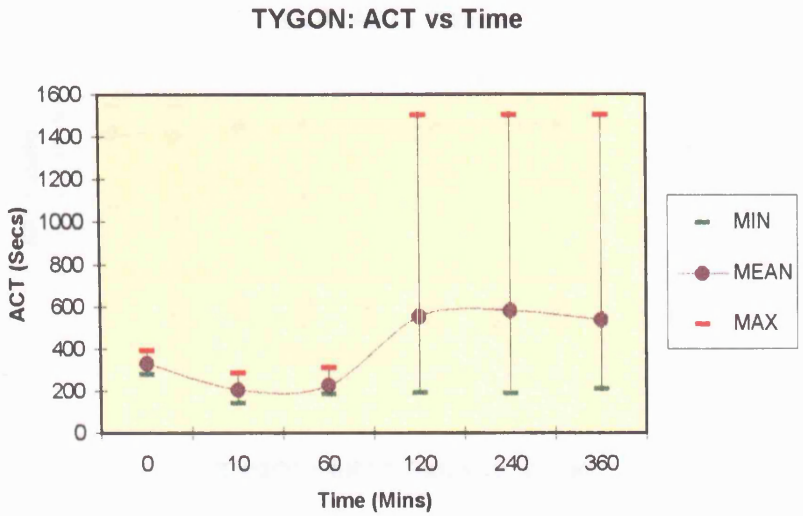
RESULTS

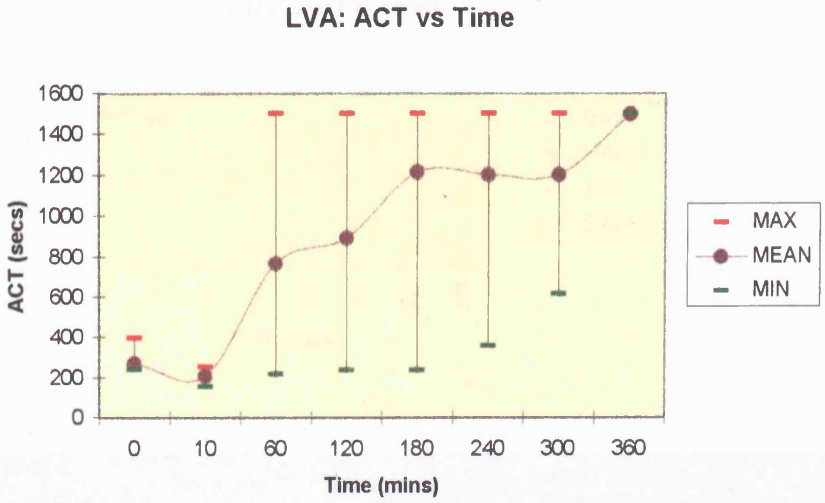
Baseline results are displayed in figure 8.17. There were no differences from control values in any of the variables with the exception of fibrinogen. The baseline fibrinogen levels, mean (SD), were significantly lower in the LVA group, 2.5 (0.1) compared to control 2.8 (0.6), p=0.011. Both groups were still within the normal range. It is reasonable to expect one significant difference out of a total of 26 t-tests by chance. Since all other parameters were not only not significantly different from control, but also all within the normal range it is likely that this significant difference is not relevant.

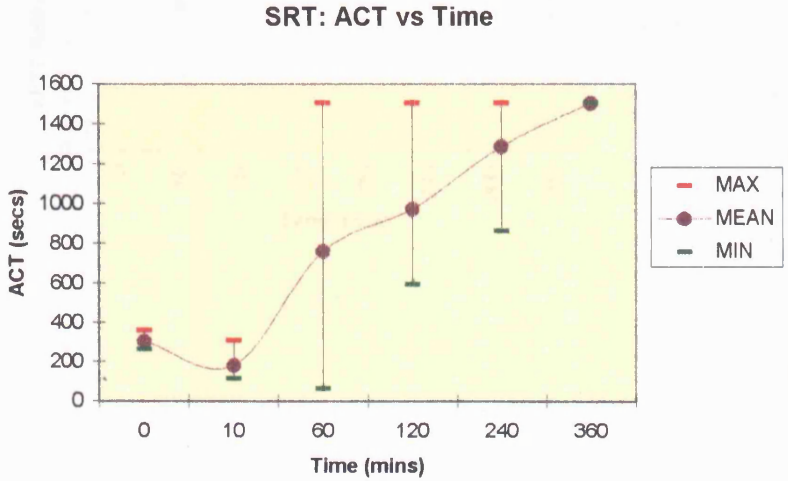
Thirteen out of the 15 experiments ran the full 6 hours. Two experiments were curtailed prematurely after 45 and 60 minutes respectively, both were in the SRT group. These runs were terminated because of pressurisation of the circuits resulting in spraying of blood past the tie straps on the post pump connectors. Both circuits had apparently adequate baseline ACTs (358 and 299 sec's), and pressurisation was thought to be due to uncontrolled coagulation despite this.

Loading doses of heparin, mean and (SD), were: SRT 50u/kg (0), LVA 50u/Kg (0) and Tygon 55.4 u/kg (12.2), there was no significant difference between groups (unpaired t-test). Circuit heparin doses, mean and (SD), were: SRT 260u (138.7), LVA 180u (97.5) and Tygon 120u (27.4), these values were not significantly different (unpaired t-test). All other results are given graphically below (fig 8.2-8.15), parameters whose change over time differ significantly from the control are highlighted in blue. The results are also tabulated at the end of the section (fig.8.16), in this table variables which have a significant change from baseline are highlighten in red. Variables whose change from baseline is significantly diferent from the control group (Tygon) are highlighted in blue. Baseline values are given in 8.17.

Fig 8.2: ACTs DURING RECIRCULATION







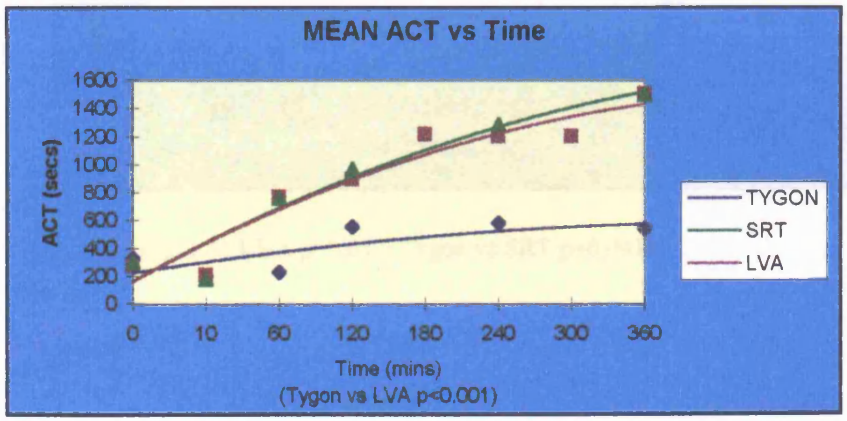
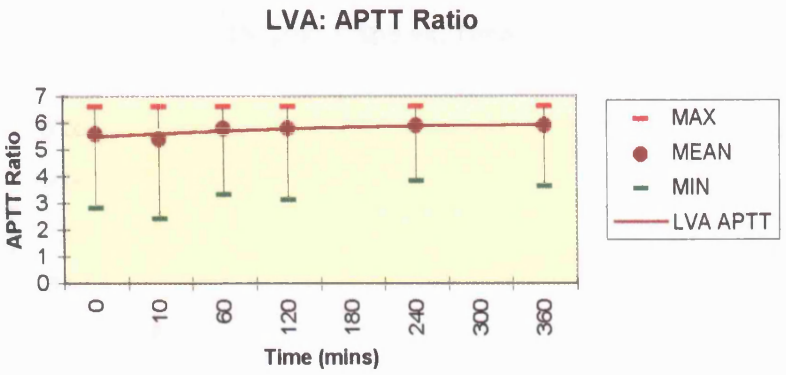
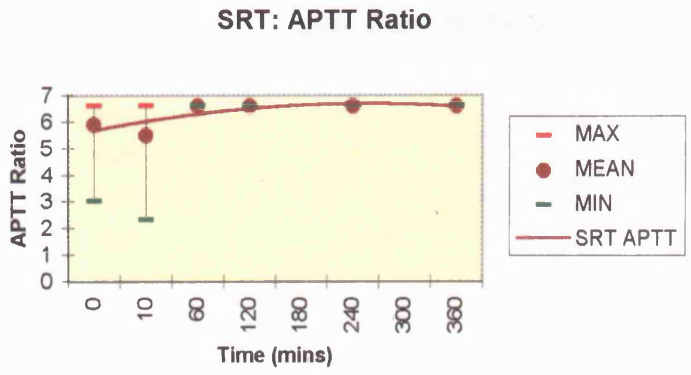
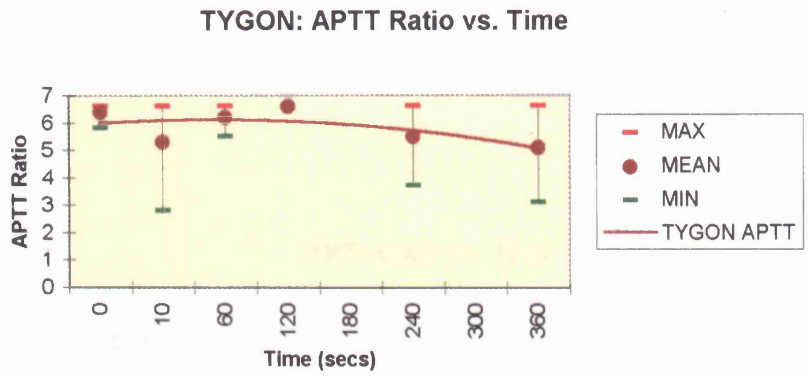
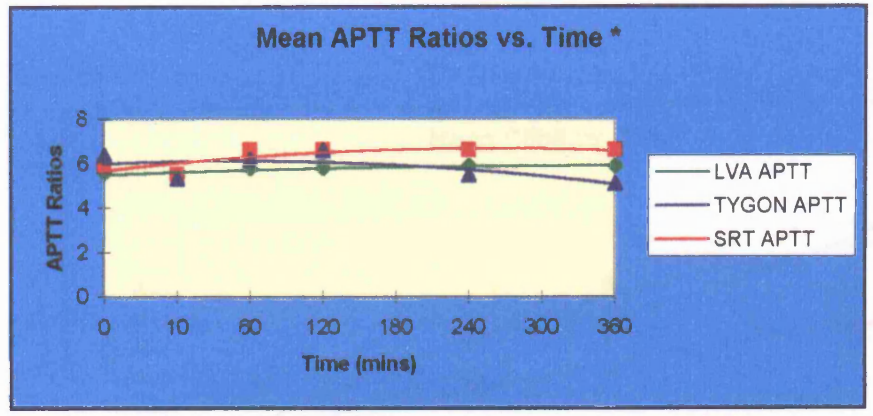


Fig 8.3: APTT RATIOS DURING RECIRCULATION



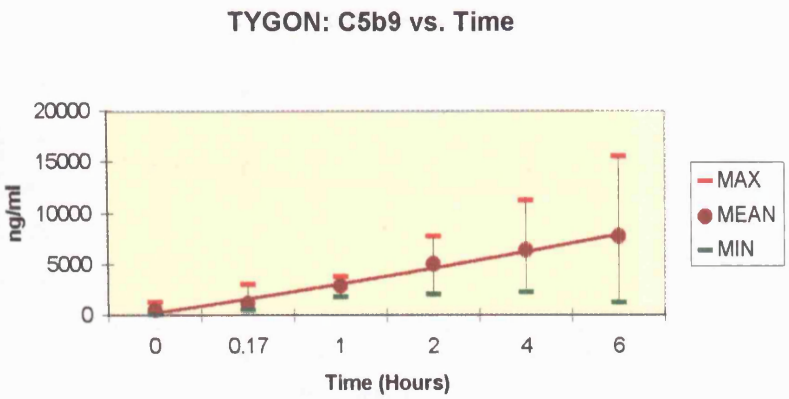


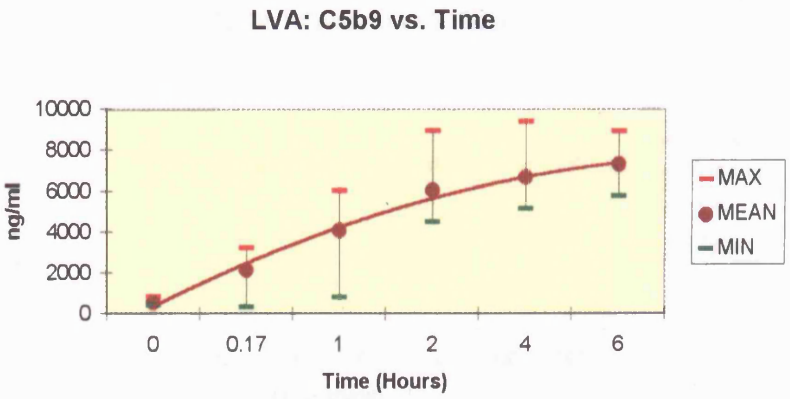


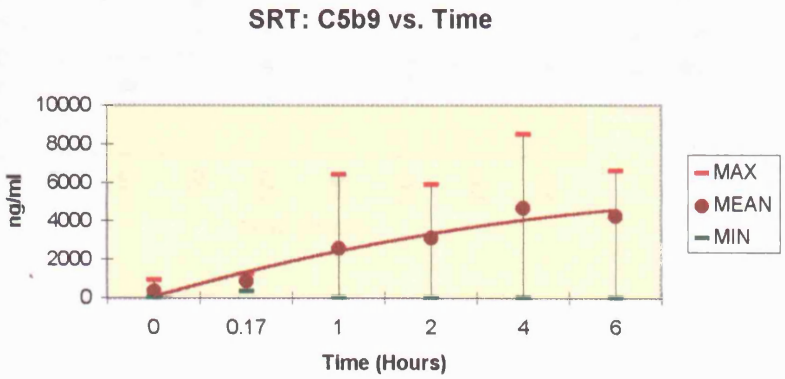


* (Tygon vs LVA p=0.017, Tygon vs SRT p=0.041)

Fig 8.4: C5b9 DURING RE-CIRCULATION







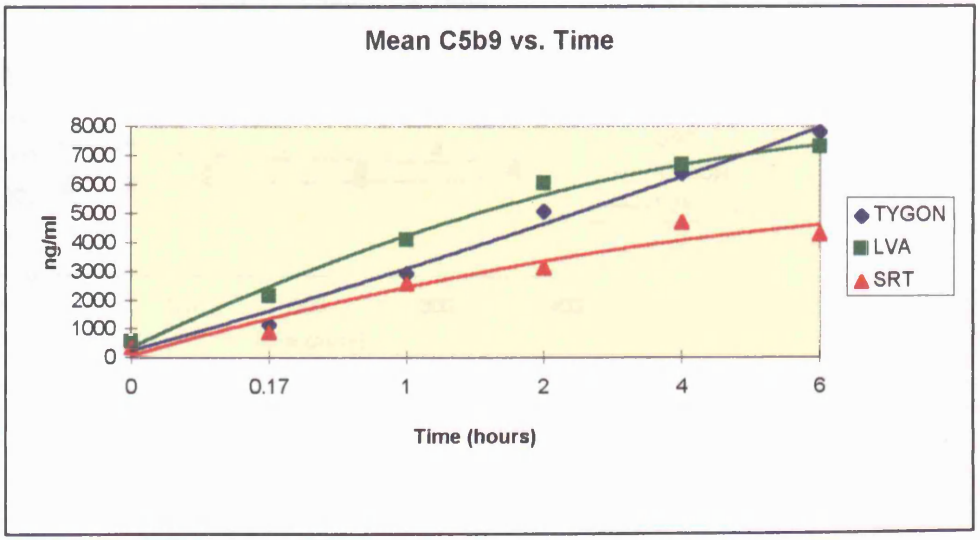
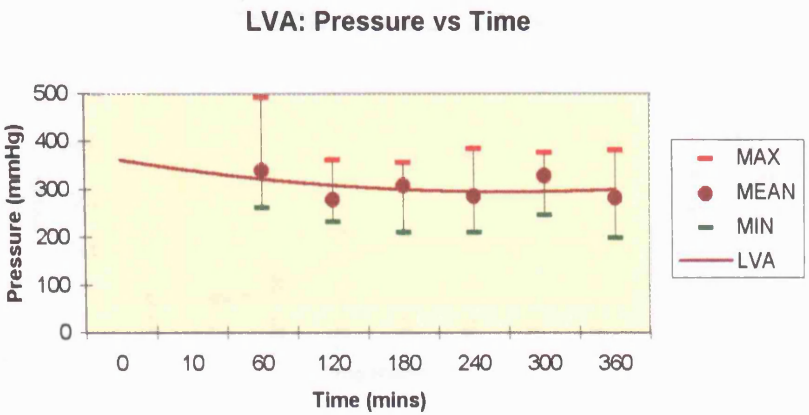
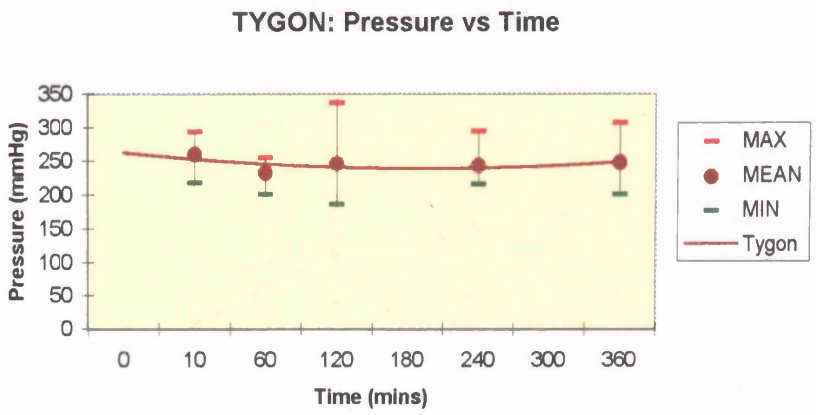
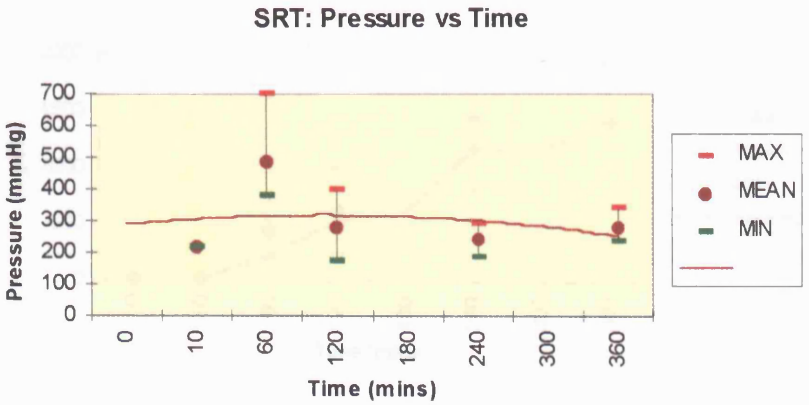


Fig 8.5: CIRCUIT PRESSURES DURING RECIRCULATION







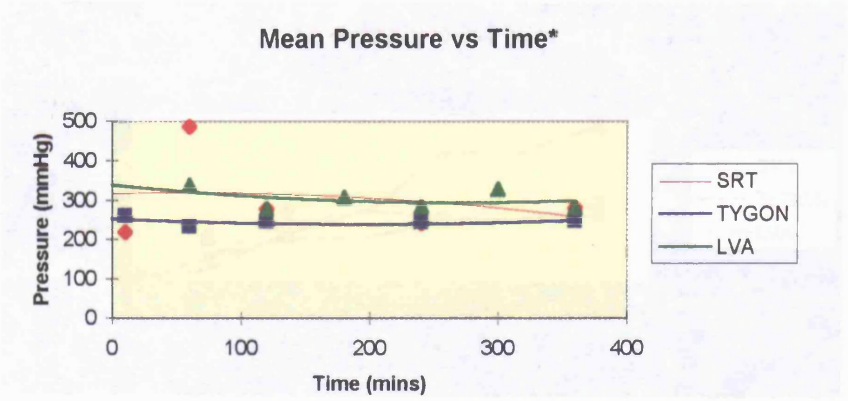
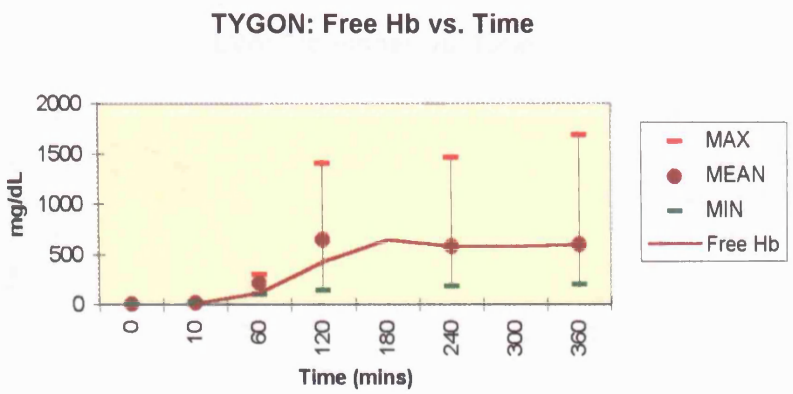
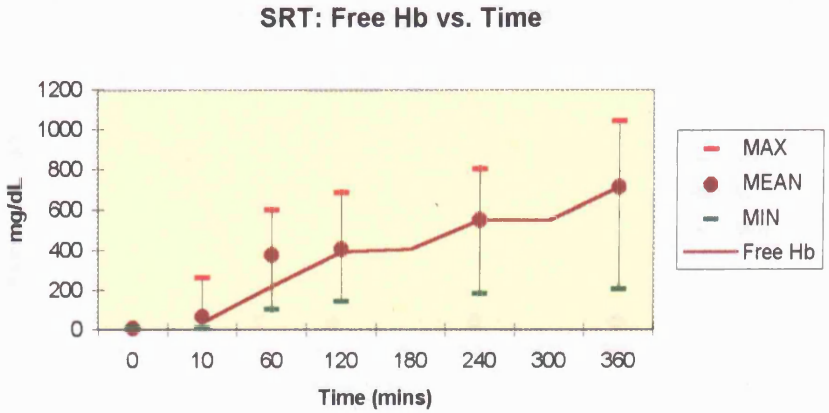
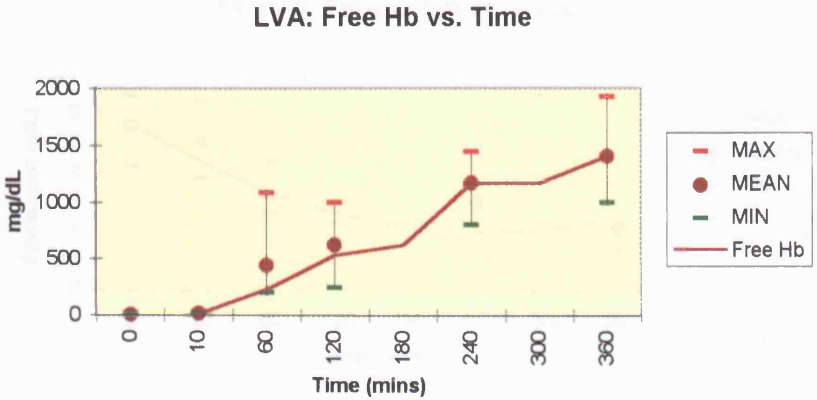
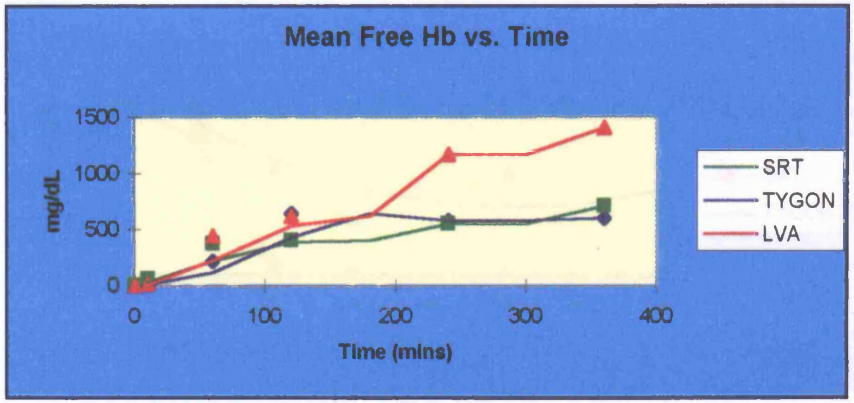


Fig 8.6: FREE HAEMOGLOBIN DURING RECIRCULATION



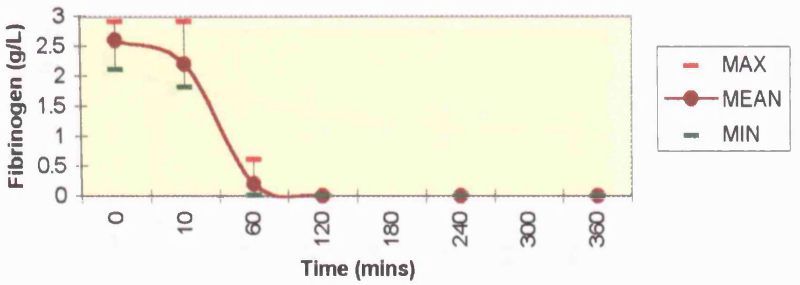


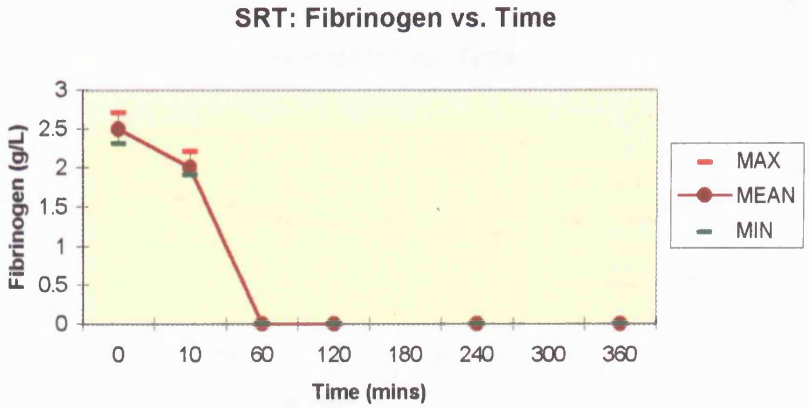


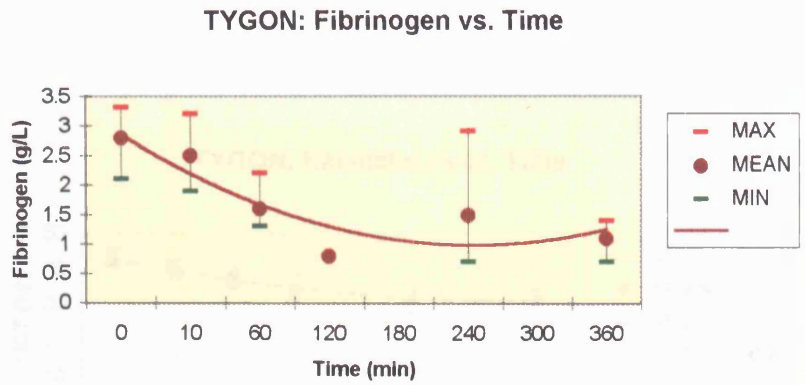


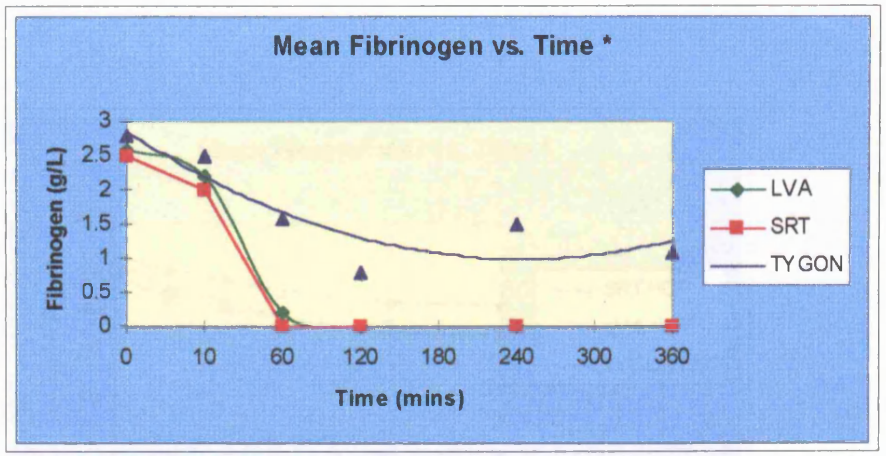
* (LVA vs Tygon p=0.005)

Fig 8.7: FIBRINOGEN CONCENTRATIONS DURING RECIRCULATION



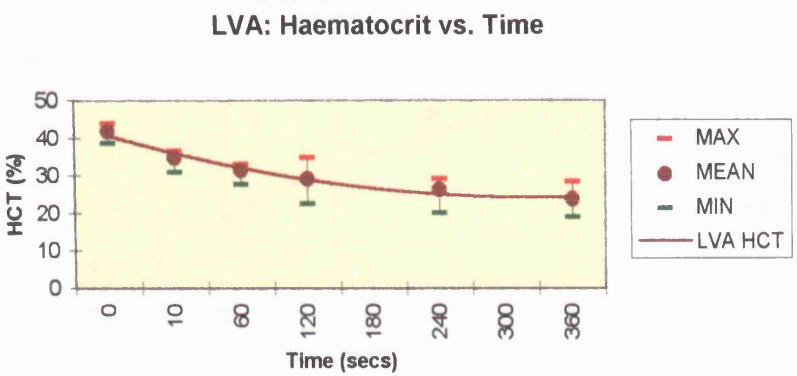


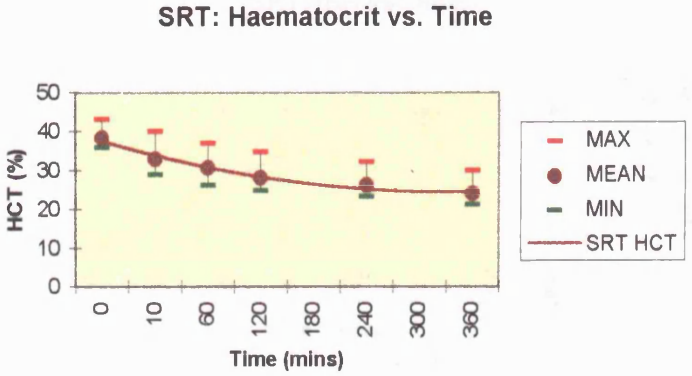


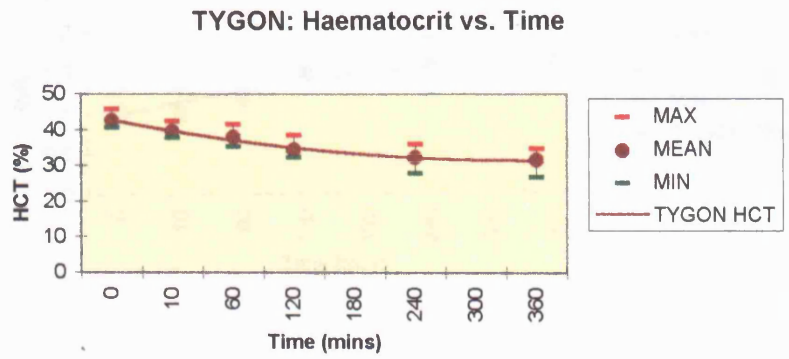


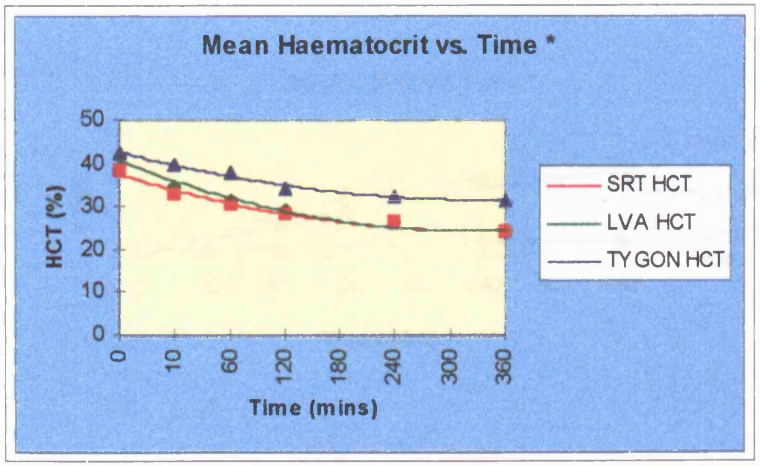
* (Tygon vs. LVA p=0.01)

Fig 8.8: HAEMATOCRIT DURING RECIRCULATION



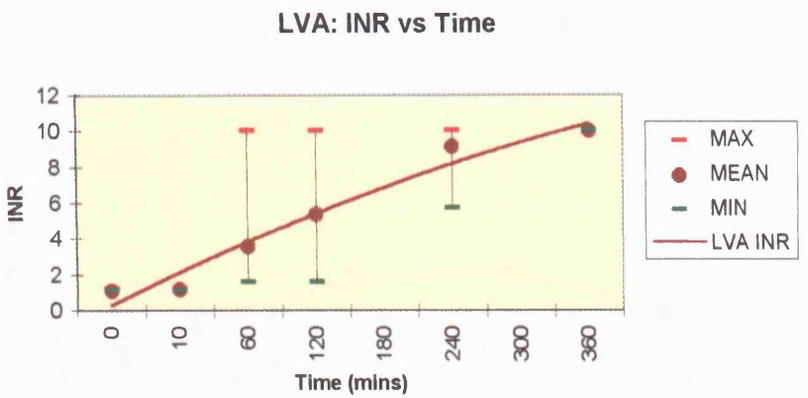


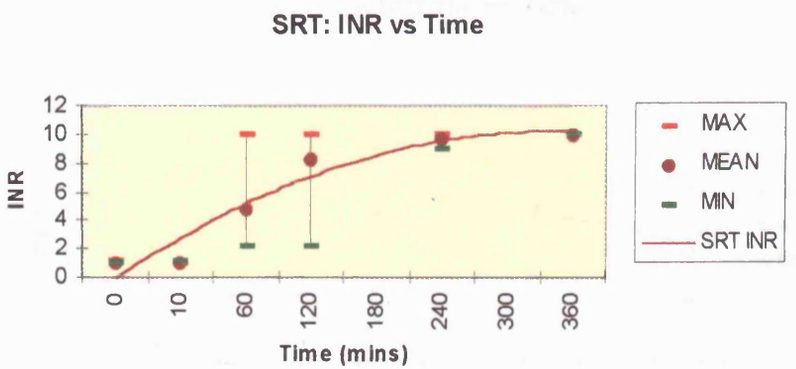


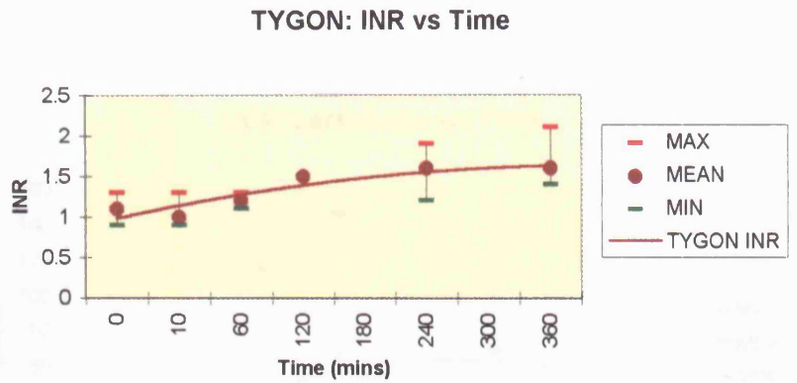


* (Tygon vs. LVA p=0.02)

Fig 8.9: INR DURING RECIRCULATION







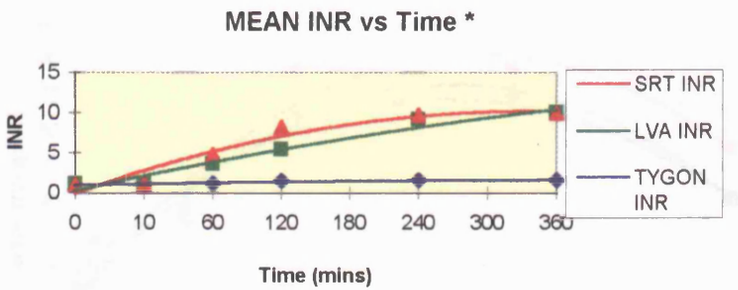
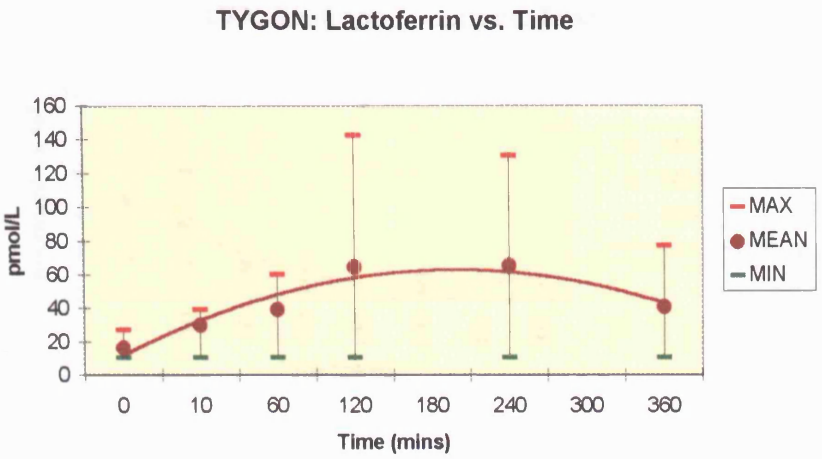
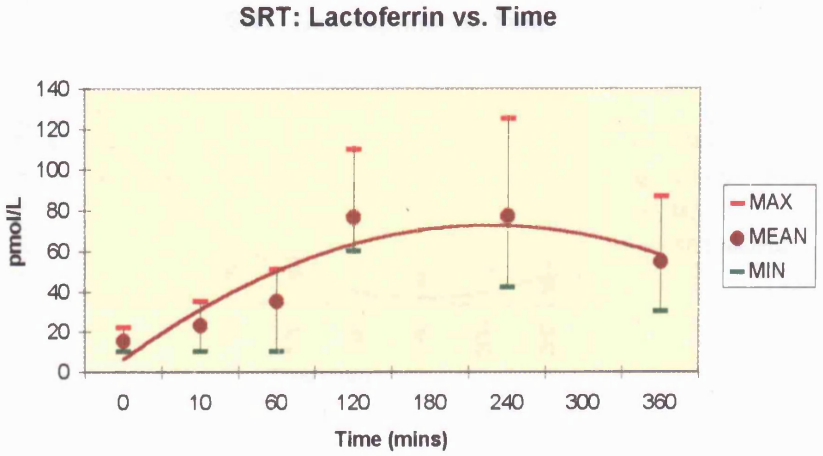
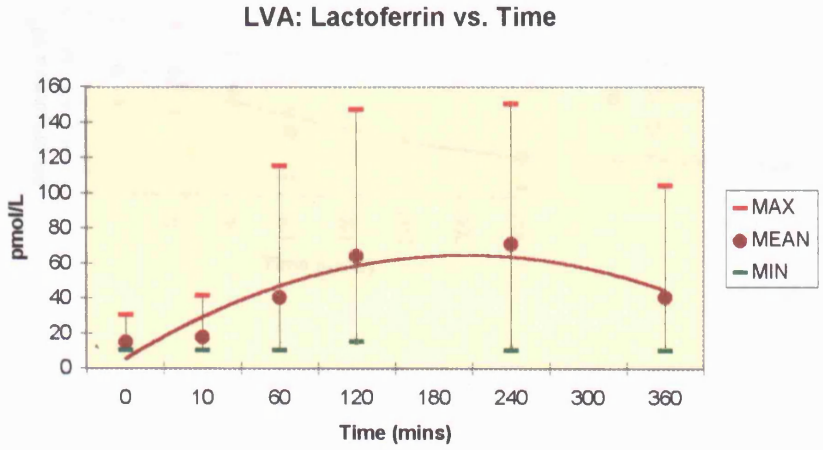


Fig 8.10: LACTOFERRIN DURING RECIRCULATION







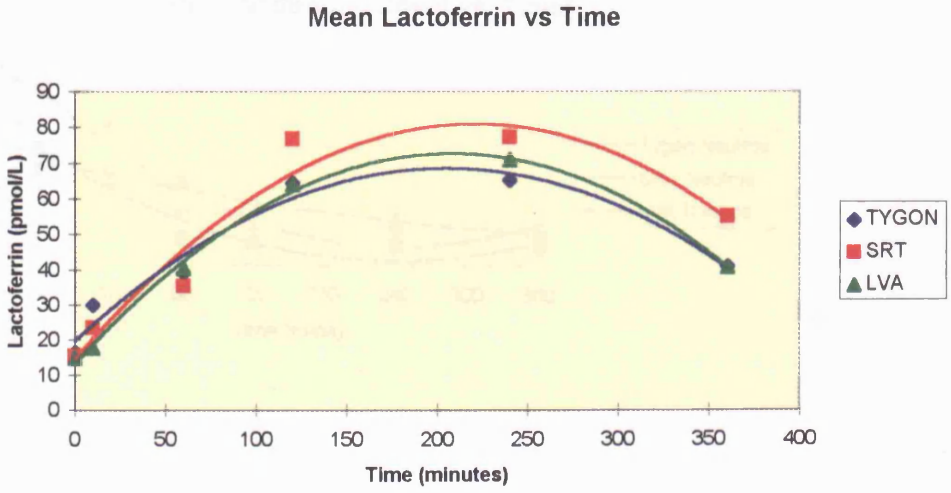
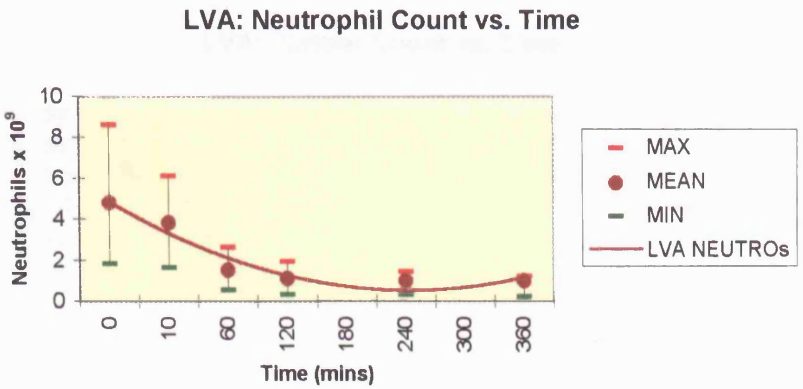
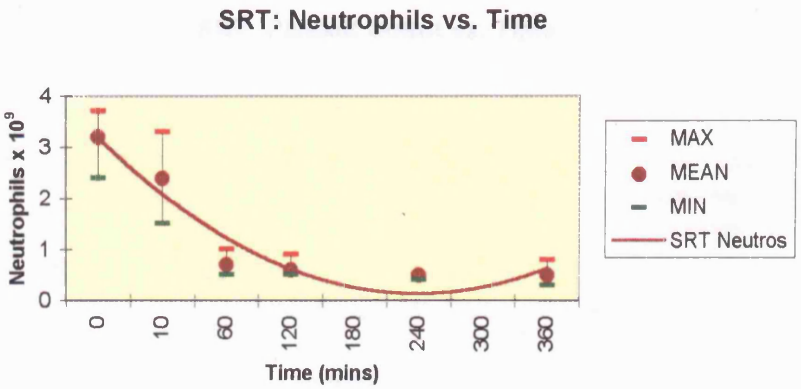
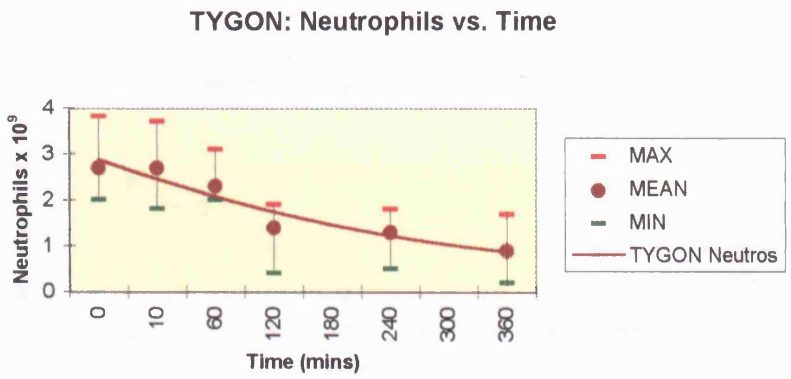


Fig 8.11: NEUTROPHIL COUNTS DURING RE-CIRCULATION







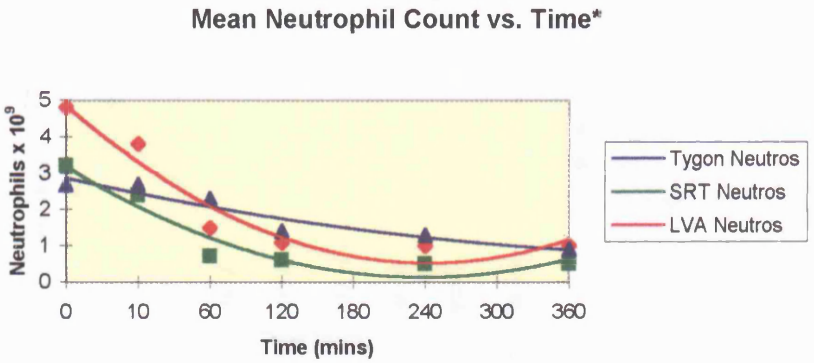
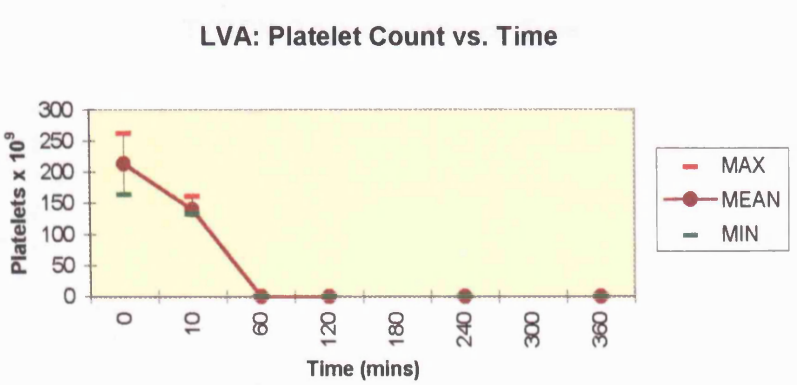
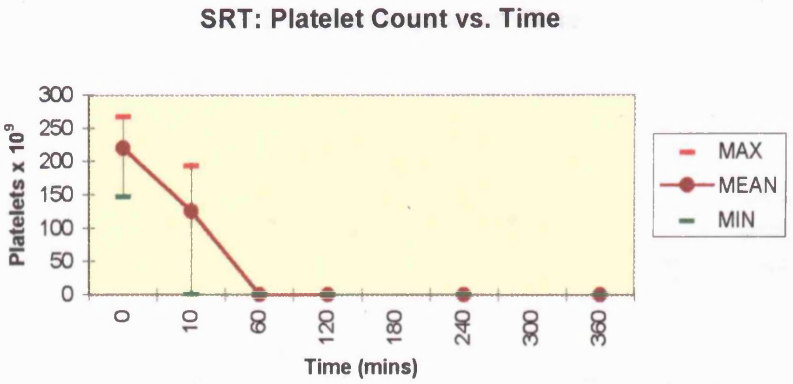
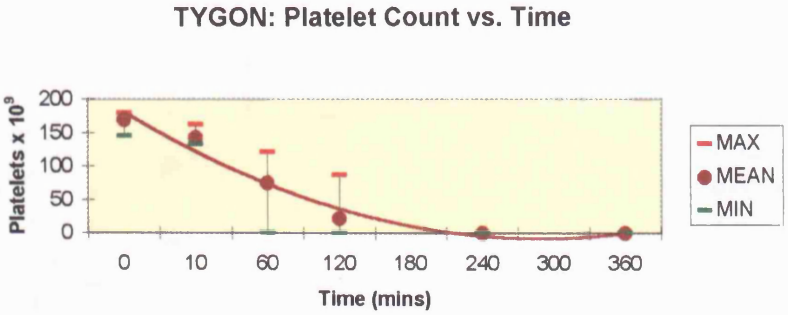


Fig 8.12: PLATELET COUNTS DURING RE-CIRCULATION







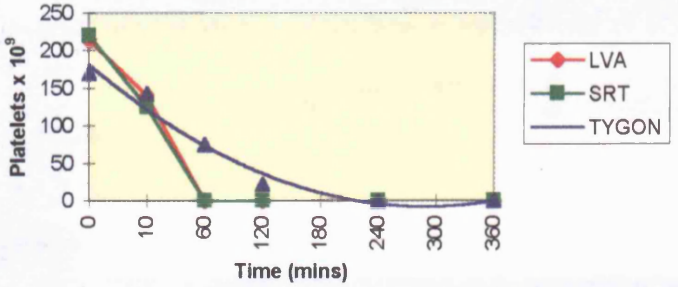
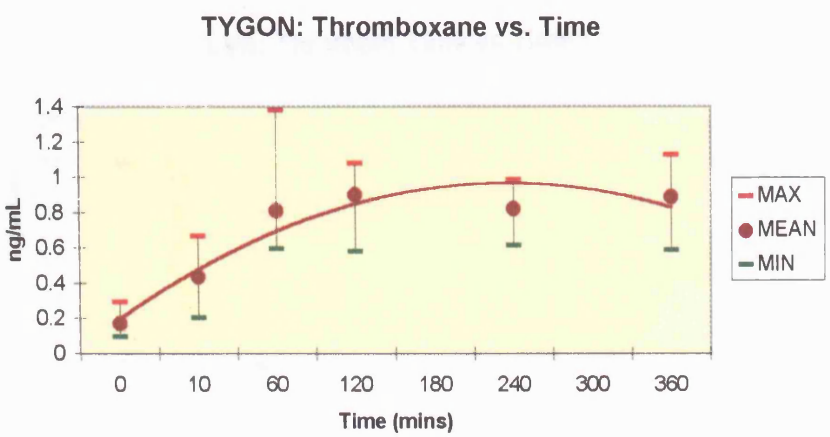
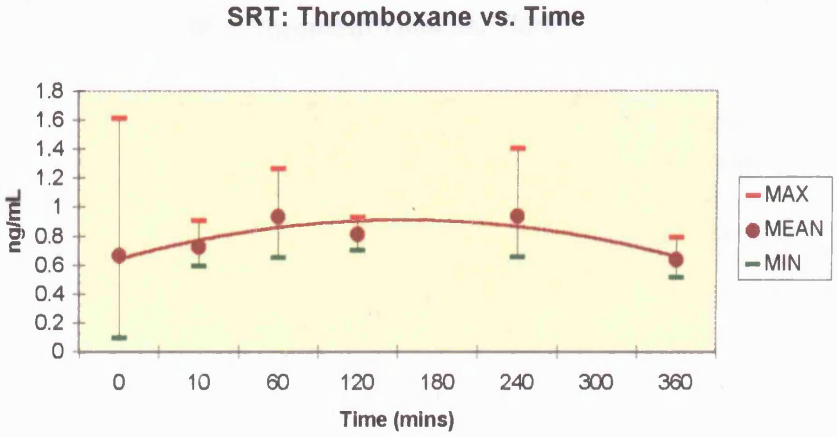
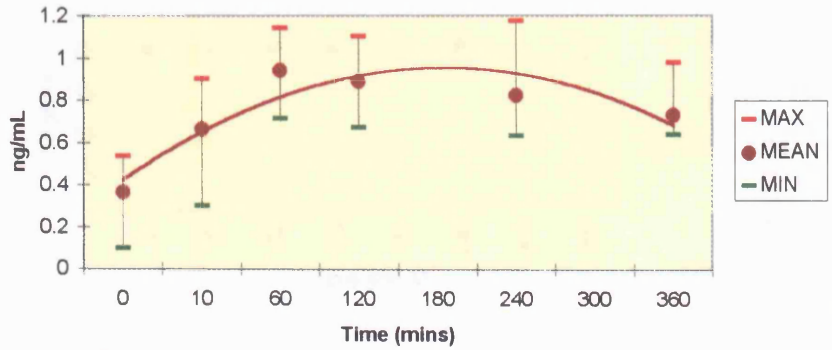
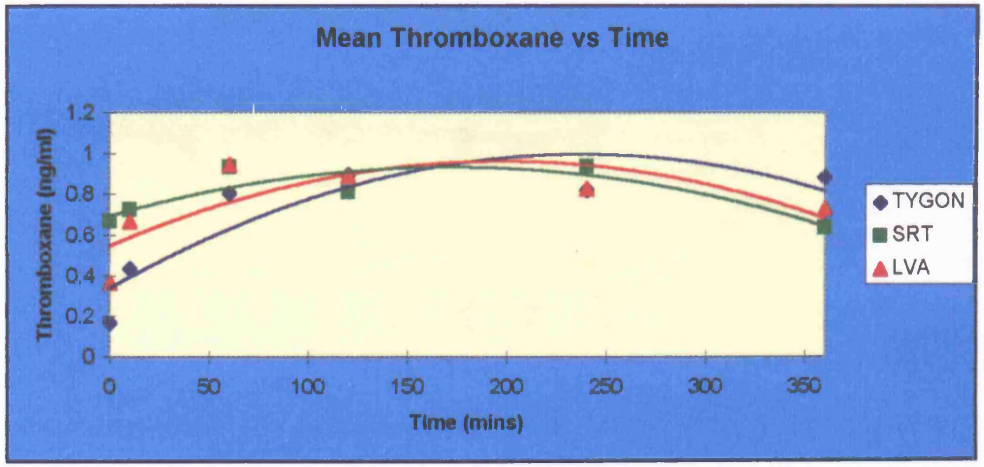


Fig 8.13: THROMBOXANE B_2 DURING RECIRCULATION



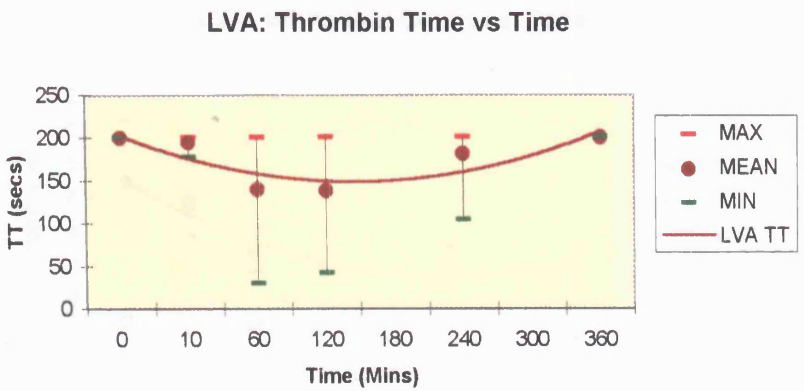


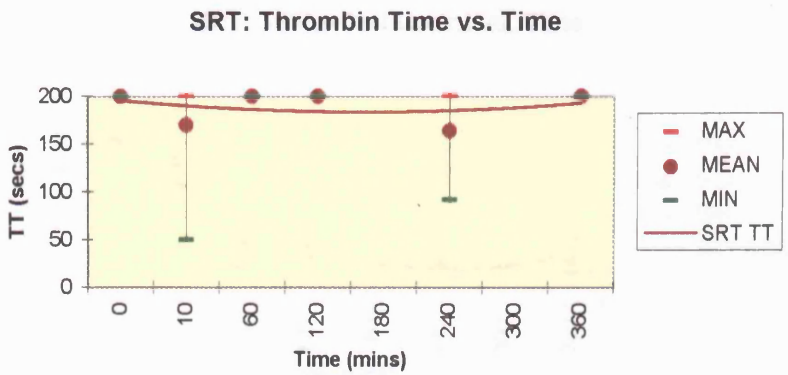


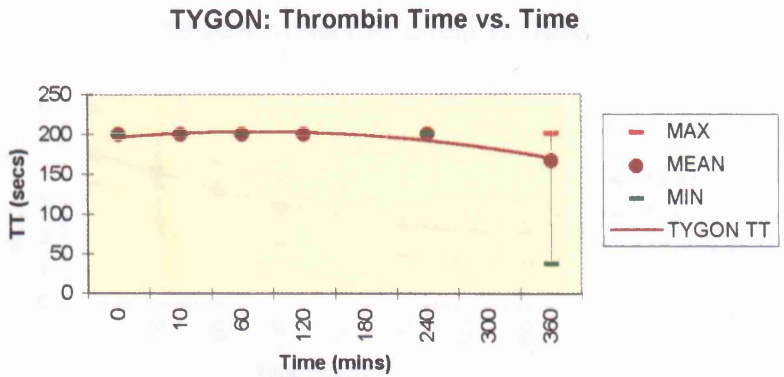


* (Tygon vs LVA p=0.019)

Fig 8.14: THROMBIN TIME DURING RECIRCULATION







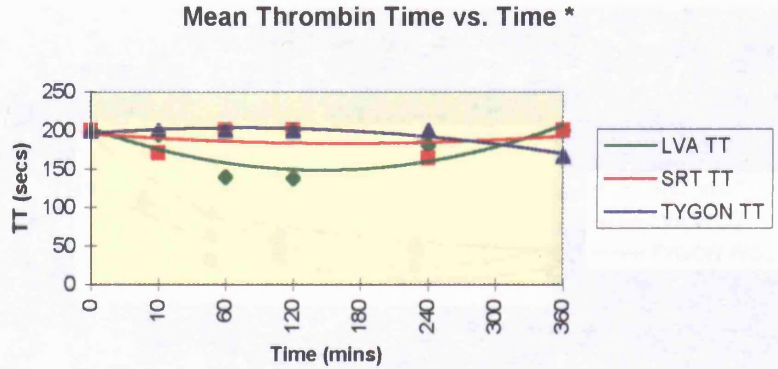
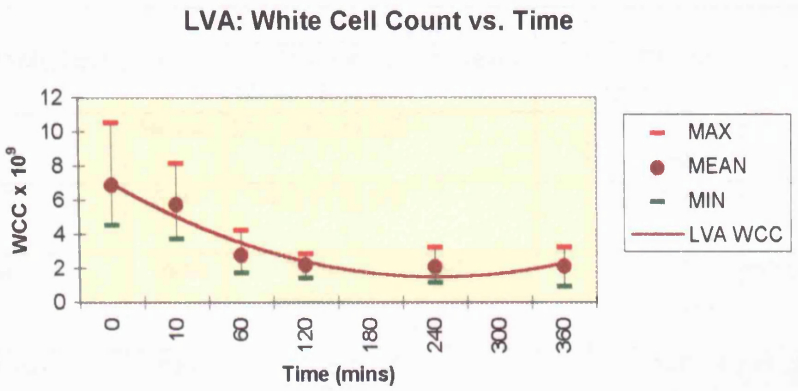
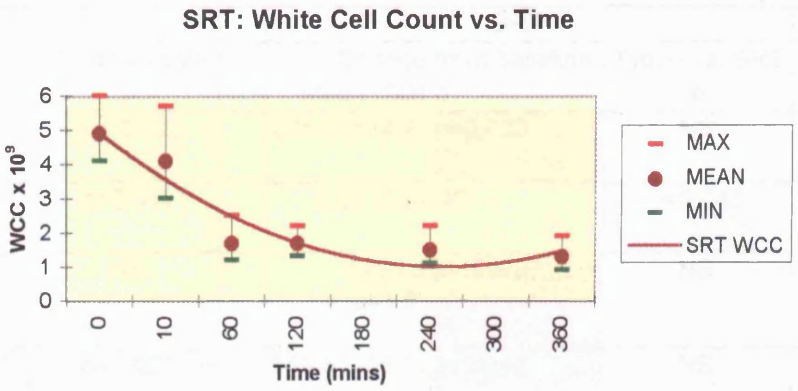
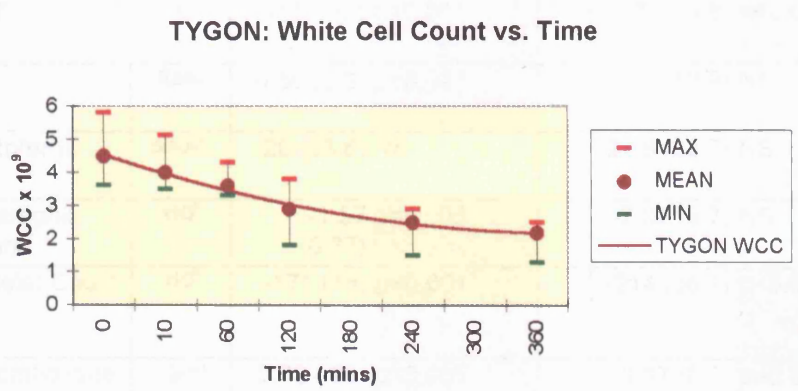
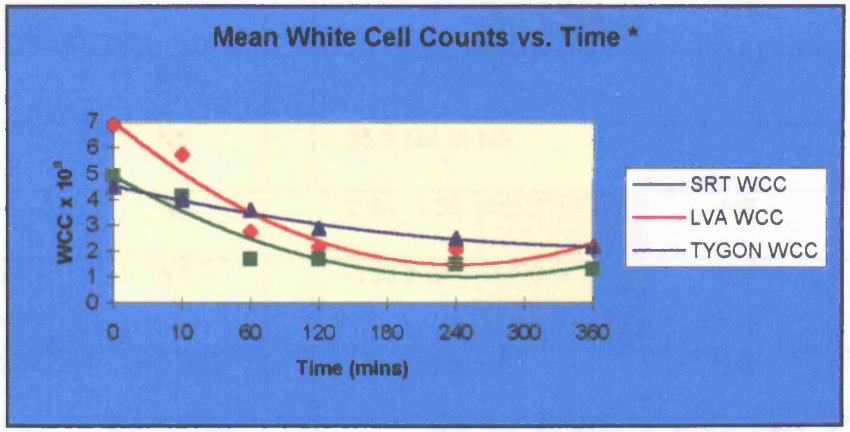


Fig 8.15: WHITE CELL COUNTS DURING RE-CIRCULATION









* (Tygon vs LVA p=0.027)

Figure 8.16: Summary of Results

PARAMETER	units	Tygon	LVA		SRT		
		Change from baseline Mean (SD) p	Change from baseline Mean (SD) p	Tygon vs.LVA p	Change from baseline Mean (SD) p	Tygon vs. SRT p	COMMENTS
ACT	Seconds	211 (580.4) NS	1227.8 (68.6) p<0.001	p < 0.001	878.8 (614.4) p=0.033	NS	Tygon Lower
APTT	Ratio	-1.3 (1.9) NS	0.47 (0.4) NS	p=0.017	0.66 (1.5) NS	p=0.041	Tygon Lower
C5b9	ng/ml	7244.4 (5660.5) p=0.046	5751.2 (1829) p=0.002	NS	5220.3 (1353.9) p=0.022	NS	Trend for SRT to be Lower
Free Hb	mg/dl	596.8 (617.6) NS	1400.4 (360.5) p=0.001	p=0.005	548.8 (435.2) p=0.048	NS	LVA Higher
Fibrinogen	g/L	-1.92 (0.49) p=0.001	-2.6 (0.3) p<0.001	p=0.01	-2.04 (0.9) p=0.006	NS	Tygon Higher
HCT	%	-11.6 (2) p=0.001	-17.8 (3.8) p<0.001	p=0.02	-11.4 (3.6) p=0.002	NS	Tygon Higher
INR	Ratio	0.56 (0.3) p=0.018	5.3 (12.9) NS	NS	5.6 (4.5) NS	NS	
Lactoferrin	pmol/L	20 (33.6) NS	27.8 (33.7) NS	NS	35.3 (34.9) NS	NS	
Neutrophil Count	$x10^9$	-1.57 (0.37) p=0.003	-2.87 (3.7) NS	NS	-2.35 (1.36) p=0.018	NS	
Platelet Count	$x10^9$	-171 (18) p<0.001	-214 (36.1) p<0.001	NS	-198.8 (91) p=0.008	NS	Trend for Tygon to be higher
Thromboxane B2	ng/ml	0.72 (.28) p=0.005	0.37 (0.2) p=0.017	p=0.019	0.31 (0.6) NS	NS	Tygon lower initially
Thrombin Time	Seconds	-32.6 (72.9) NS	0(0) N/A (SD=0)	N/A (SD=0)	0(0) N/A (SD=0)	N/A (SD=0)	
WCC	$x10^9$	-1.95 (0.7) p=0.01	-4.76 (0.8) p=0.004	p=0.027	-3.22 (1.3) p=0.005	NS	Tygon Higher

Figure 8.17 Baseline Values

Figure	PARAMETER	units	Tygon	LVA	Tygon vs.LVA	SRT	Tygon vs. SRT	COMMENTS
			Mean (SD)	Mean (SD)	p	Mean (SD)	p	
8.2	ACT	Seconds	329.2 (48.8)	272.2 (68.6)	NS	301.8 (35.5)	NS	
8.3	APTT	Ratio	6.4 (0.4)	5.3 (1.7)	NS	5.8 (1.6)	NS	
8.4	C5b9	ng/ml	534.6 (620.8)	557.4 (166.3)	NS	345.2 (160.7)	NS	
8.6	Free Hb	mg/dl	6.0 (1)	7.6 (5.81)	NS	5.8 (0.84)	NS	
8.7	Fibrinogen	g/L	2.8 (0.6)	2.6 (0.3)	NS	2.5 (0.1)	p=0.011	SRT Lower than Tygon
8.8	HCT	%	42.5 (2.2)	41.8 (2.1)	NS	38.4 (3.4)	NS	
8.9	INR	Ratio	1.08 (0.16)	1.12 (0.04)	NS	1.06 (0.09)	NS	
8.10	Lactoferrin	pmol/L	16.5 (8.2)	15.0 (8.7)	NS	15.4 (6.1)	NS	
8.11	Neutrophil Count	$x10^9$	2.7 (0.9)	4.8 (2.7)	NS	3.2 (0.6)	NS	
8.12	Platelet Count	$x10^9$	170.4 (15.7)	214.0 (36.1)	NS	220.0 (46.0)	NS	
8.13	Thromboxane B2	ng/ml	0.17 (0.08)	0.27 (0.17)	NS	0.67 (0.66)	NS	
8.14	Thrombin Time	Seconds	200 (0)	200 (0)	N/A	200 (0)	N/A	
8.15	WCC	$x10^9$	4.46 (0.9)	6.88 (2.5)	NS	4.88 (0.8)	NS	

Figure 8.18: Graph Contents

Figure	PARAMETER
8.2	ACT
8.3	APTT
8.4	C5b9
8.5	Circuit Pressure
8.6	Free Hb
8.7	Fibrinogen
8.8	Haematocrit
8.9	HCT
8.10	INR
8.11	Lactoferrin
8.12	Neutrophil Count
8.13	Platelet Count
8.14	Thromboxane B2
8.15	Thrombin Time
8.16	WCC

DISCUSSION

The discussion of the results will follow a similar format to Chapter 6, with subdivision of results into those concerned mainly with inflammation, coagulation or haemolysis.

COAGULATION

Activated Clotting Time & Fibrinogen: The baseline ACTs of around 300 indicate adequate heparinisation prior to withdrawal of blood from the donors. ACTs then fell in all groups as the blood was added to the circuits. The subsequent rise in ACTs in all groups can be related to the activation of fibrinogen by the circuits, resulting in defibrinated blood which does not clot. Another important factor acting to prolong the ACT is the thrombocytopenia which developed after 60 minutes in the SRT and LVA circuits and 240 minutes in the Tygon. The changes in ACT from baseline were significant for both LVA and SRT but not for Tygon. The change for LVA was also significant when compared to the control (Tygon). The fibrinogen concentration mirrors the ACT, falling to zero in both the LVA and SRT circuits by about 60 minutes of recirculation. Fibrinogen levels do fall in the Tygon circuits, but never below 1g/L, a level which is compatible with haemostasis in the intact patient. The differences in fibrinogen levels between both the SRT and LVA circuits are significantly lower than the controls (Tygon).

Thrombin Time, INR & APTT: Both the APTT and the Thrombin time remain almost constant throughout the recirculation. Interestingly even though there is no significant difference between change of APTT from baseline when compared to zero both LVA and SRT show a significant difference when compared to Tygon. This is because the APTT for Tygon decreases slightly whilst the others rise. There were no significant differences in Thrombin time. APTTs and Thrombin times remain steady despite gross failure of the

haemostatic cascade as a whole, due to de-fibrinogenation. This indicates the necessity of measuring pathways at different points. The International Normalised Ratio (INR) is equivalent for practical purposes to the Prothrombin time. The INR in both the SRT and LVA circuits increase progressively from baseline, in the same manner as the ACT. This increase is marked but does not reach significance. The Tygon INRs undergo a slight rise. There is little intra-group variability for Tygon and this increase reaches significance despite being an order of magnitude smaller than the other materials. SRT and LVA do not differ significantly from controls. The fall in fibrinogen levels is the probable explanation for the increase in INR.

Circuit Pressure: The circuit pressures remain constant during recirculation the Tygon circuits have a slightly lower pressure at around 250 mmHg. The circuit pressure changes were not analysed statistically as baseline pressures were not available. It is important to remember, however, the uncontrolled coagulation and over-pressurisation of the circuit that occurred after 45 and 60 minutes in 2 circuits in the SRT group. These runs were terminated , both circuits had apparently adequate baseline ACTs (358 and 299 sec's), and pressurisation was thought to be due to uncontrolled coagulation despite this. These results would not have influenced the mean circuit pressures which are recorded and displayed in fig 8.5. It is safe to conclude, however, that SRT does cause a clinically significant increase in circuit pressure, due to coagulation, compared to Tygon.

Platelet Count & Thromboxane B_2: The platelet count fell to zero in both the SRT and LVA groups after approximately 60 minutes. Platelet counts fall to zero in the Tygon circuits but take longer to fall, around 240 minutes. These falls are significant compared to baseline, but not when compared to control. Thrombocytopenia is presumably a result of platelet adherence to the circuit. The thrombocytopenia is an important contributor to the prolongation of the ACT. The Thromboxane B_2 levels reflect levels of platelet activation.

Levels rise significantly from baseline in both Tygon and LVA circuits. The difference in the LVA circuits is also significantly different from the control. Although actually a smaller rise in Thromboxane occurs in the LVA circuits compared to Tygon. This difference could be due to increased platelet activation or a result of the slightly higher platelet numbers later in the experiment in the Tygon circuits. Levels rise to a maximum at around 200 minutes and then decline as all the platelets have been activated.

HAEMATOCRIT & HAEMOLYSIS

The haematocrit gradually falls during the experiment as blood samples are taken and haemolysis occurs. Fall of haematocrit from baseline is significant in all three groups. The fall is significantly larger in the LVA circuits compared to control. It is likely that red cells disappear from the fluid phase of the recirculating blood as they become involved in thrombosis of the circuit. This could explain the difference in haematocrit. This mechanism also correlates with the demonstrated differences in activation of the coagulation cascade; especially platelet numbers, fibrinogen levels and INR.

The levels of free haemoglobin rose significantly from baseline in both the study groups, but not in the control group. The size of these differences was significantly different from control for LVA but not SRT. These findings indicate higher haemolysis with LVA and a trend towards increased haemolysis with SRT.

INFLAMMATION

Complement (C5b9): The levels of the Membrane Attack Complex (MAC) or C5b9 increase in all groups throughout the experiment. Increases from baseline are significant for Tygon and LVA. There are no significant differences in size of increase between the study groups and control.

Lactoferrin & Neutrophils: Lactoferrin levels rise in all groups to a maximum at around 200 minutes and then fall slightly, there are no significant differences between the control and study groups. The neutrophil counts fall steadily throughout the study, the LVA and SRT groups reach a nadir at around 200 minutes, mirroring the activation profile. Falls in neutrophil count from baseline are significant for Tygon and SRT. The size of the falls for SRT and LVA is not significantly different from control. Presumably neutrophils disappear from the circulation as they adhere to the tubing or heat exchanger and become activated, releasing lactoferrin. Relative preservation of neutrophil numbers in the Tygon group, but without a reduction in neutrophil activation (lactoferrin) may indicate neutrophils activated in the fluid phase of the blood, or surface activated neutrophils moving back into the circulation.

White Cell Count: The total white cell counts fall in an identical pattern to the neutrophil count. The falls are significant compared to baseline in all groups. In the LVA group the fall is significant compared to control.

CONCLUSIONS

The relative suitability of materials for ECMO use is easier to judge in this in vitro model than in the intact organism (Ch6). The rapid and complete disappearance of platelets and fibrinogen from the blood in both the SRT and LVA circuits excludes them both from extracorporeal use. A suitable tubing would have to match Tygon in this respect, with relative preservation of fibrinogen levels and a slower fall in platelet numbers (although the latter was not statistically significant). The circuit failures due to thrombosis in 2/5 SRT circuits would also make this material dangerous in conditions of low range heparinisation. Paradoxically SRT caused the least complement activation of the three materials, which may indicate a limitation in the use of complement assays as a measure of biocompatability.

CHAPTER 9

SUMMARY AND CONCLUSIONS

INTRODUCTION

In this chapter the clinical and laboratory data presented in chapters 3-8 will be summarised and discussed. In addition the conclusions will be collated in order to provide an overview of the findings as a whole. Particular attention will be focused on integration of the comparisons of the two potential ECMO tubing materials, LVA and SRT with Tygon, the currently used material, in order to reach a final conclusion on their relative suitability's for ECMO use. In this chapter the work will be subdivided into clinical, mechanical and biocompatability sections. The direction of possible future research will also be discussed.

CLINICAL ECMO

NEONATAL ECMO

The efficacy of ECMO as a treatment for neonates with severe respiratory failure is now proven following the publication of the UK Collaborative ECMO Trial, a fully randomised controlled trial (Anonymous. 1996). The longer term follow up results of this study will answer outstanding questions concerning the prognosis for babies with congenital diaphragmatic hernia. The long term outcome in the other patients will also be recorded, and is expected to be better than the CDH babies, with the majority of patients being normal. The situation in the neonatal age group is therefore clear and further research should be directed to refining clinical indications and improving the technology.

PAEDIATRIC & ADULT RESPIRATORY ECMO

The efficacy of ECMO in these patient groups is not proven by a randomised controlled trial (RCT). Despite the seeming incongruity of discussing paediatric and adult patients together there do seem to be marked similarities between the two groups. Both groups present with an approximately equal number of cases of ARDS and Pneumonia, and seem to require longer periods of ECMO support than the neonatal patients. The clinical course of paediatric and adult patients with respiratory failure on ECMO is often more stormy than their neonatal counterparts with a higher incidence of septic complications and a need for surgery on ECMO. The difference from the neonatal population is likely to be due to the different mechanisms of right to left shunting which cause hypoxia in the two groups. In the neonate persistent fetal circulation is the usual cause, which resolves relatively quickly in most cases. In the older patient shunting is usually intra-pulmonary through areas of collapsed and consolidated lung, which takes much longer to recover, allowing more potential for nosocomial infections, iatrogenesis and other complications. Despite these clinical challenges the overall outcome in these patients seems acceptable with survival of 77% and 66% for paediatric and adult patients who have mean PaO_2/FIO_2 ratios of 61 and 65 mmHg respectively.

There are no RCTs of ECMO versus conventional treatment in paediatric respiratory failure. The two RCTs of adult ECLS (Zapol et al. 1979; Morris et al. 1994) have methodological and technical flaws which make it difficult to apply their findings to ECMO as practised in the modern era (Anonymous1995; Peek et al. 1997). This deficit of applicable RCTs should be put into perspective. Positive pressure ventilation is the accepted standard treatment for respiratory failure, but there is no RCT of this treatment. The justification for not performing such a trial is that a large cohort study (Lassen, 1953) showed such marked improvement in mortality compared to the pre-existing treatment.

There are no randomised trials of the more advanced forms of "conventional" respiratory intensive care such as prone ventilation and inhaled nitric oxide, and yet intensivists have no conceptual problem with using these therapies, which are undoubtedly effective in many cases. The expected survival in patients with an equivalent degree of respiratory failure to our population of patients receiving ECMO is between 0 and 42% (Tamburro et al. 1991; Morris et al. 1994; Vasilyev et al. 1995). The recorded survivals of over 60% for adult and paediatric respiratory patients treated with ECMO at our institution would, at face value, seem to indicate a marked improvement over the expected outcomes. Only a properly designed RCT can really confirm that this is the case, and until such a trial is performed we are forced to use the best available evidence to make management decisions for our patients.

I do not believe it is reasonable to dismiss the potential benefits of ECMO treatment on the basis of the paucity of RCT data when the alternative treatments are equally unproved. A more rational approach is to see respiratory intensive care as a continuum starting with mechanical positive pressure ventilation, moving through prone ventilation and perhaps inhaled nitric oxide with ECMO for patients who fail to respond to treatment. In this way ECMO will continue to be available to patients with the highest risk of mortality who have failed other therapies. We are currently involved in the planning of an international RCT of ECMO versus conventional treatment in adult respiratory failure in conjunction with ELSO. Hopefully the conclusion of such a study will clarify patient management decisions.

CARDIAC ECMO

The situation of ECMO for cardiac support is even less clear. In some respects, however, the management decisions are easier. This is because it is widely accepted that

the population to whom cardiac support is offered have an extremely high mortality. This is especially true of patients who fail to wean from CPB. The survival of 61% of 28 children supported with ECMO at Glenfield would therefore seem acceptable. The poorer survival in adults (only 3/8, 38%), however, is less encouraging. The numbers in both of these series are extremely small, especially if one tries to examine individual diagnoses. Early impressions from our experience, borne out by the ELSO registry data, are that it is reasonable to offer ECMO as cardiac support to children if the University of Michigan Criteria outlined in Chapter 1 are adhered to. It is much more difficult to justify using ECMO for adult cardiac support. The 2 patients with massive pulmonary embolism did well, but other diagnoses did almost universally badly, all dying except one. Decisions to use ECMO in an adult cardiac patient must therefore be individualised, primarily with regard to the potential reversibility of the patients condition. We believe that this difference in outcome is due to the pattern of ventricular failure, right ventricular failure being effectively supported and off-loaded by VA ECMO. Unfortunately VA ECMO results in increased left ventricular afterload which probably reduces the survival in patients with left ventricular failure It is unlikely that an RCT could be constructed to define patient selection in the cardiac patients, as the patient numbers are so small and the diagnoses so diverse. However, continued collection of registry data by ELSO at least gives some indication of expected outcomes.

MECHANICAL

The mechanical assessment of materials in Chapter 4 compared the durability and spallation performance of the silicone rubber; LVA, poly-olefin; SRT and poly-vinyl chloride; Tygon (S-65-HL). In addition a more detailed investigation of the forces acting on the tubing during roller pump use was conducted for Tygon. During destruction testing

the time to tubing rupture during supra-normal roller pump use was measured for each material. Both LVA and SRT were significantly worse than Tygon, with SRT having a mean failure time of 6.6 hours, which would barely be sufficient for safe cardio-pulmonary bypass use, let alone ECMO. There was great variability in the failure times for Tygon, from 99 hours up to nearly 500 hours. Also there was a variability in the circuit pressures with Tygon, which seemed to correlate with tubing durability (Fig 4.7), tubing with higher circuit pressures taking less time to rupture. It is likely that this relationship is a function of a variation in the mechanical properties of the Tygon itself rather than the different rupture times being a result of differing imposed conditions. The reason for this is that it was almost impossible to control the pressure in the circuit (which was identical for all experiments). Each circuit found its own pressure, manipulation of circuit filling, or application of a gate clamp to increase the resistance merely resulted in vigorous shaking of the circuit and severe vibration of the apparatus. It is thought that the eventual pressure is a function of the tubing compliance, stiff tubing requiring more circuit tone in order to fill and empty without vibration, and therefore running at a higher pressure. The reduced durability seen in the tubing at higher pressure is therefore due to a combination of the higher distending force of the increased pressure, as well as the higher energy required to compress a stiffer material.

The spallation performance of the three materials followed a similar pattern with Tygon showing less spallation than the others, except in one instance. This unpredictable performance was not consistent with the pattern of spallation seen in the rest of the experiments and was thought to confirm the variability in the tubing material seen in the durability experiments.

The investigation of the mechanisms of tubing failure showed that there were two distinct forces acting on the material. Firstly compression, as the material is squashed flat

by the rollers, and secondly sheer stress, caused by the rotation of the rollers sheering the tubing longitudinally against the stationary pump boot. The LVA failed mostly through compression, whilst the Tygon showed both mechanisms. Since SRT is not even close to having acceptable mechanical properties its failure mechanism will not be further discussed. When sheer was eliminated by examination of durability in a specially constructed test rig which produced pure compression, the Tygon tubing was unmarked after 3.67 million compression cycles ! However when the tubing was over occluded and the experiment repeated stress cracks appeared after 24 hours. It would seem therefore that careful occlusion setting to produce under occlusion would greatly increase tubing durability. Redesign of the roller pump head using low friction materials, or a semi-elliptical rather than semi-circular pump boot could reduce sheer stress and further increase durability.

The great variability in mechanical properties of Tygon is somewhat alarming since tubing safety is a function of the worst case scenario rather than the average properties. The minimum failure time of 99 hours at 200 rpm would imply that our current protocol is safe. That is raceways are walked after 24 hours if the rpm is over 100, giving a safety factor of 4 times. It was noticed during the experiments that the pieces of Tygon which lasted longest had a slightly green tinge: discussion with Norton Plastics in Ohio revealed that there were several different suppliers of the base polymer, but it was not possible to determine which had been used in these pieces of tubing. It may be that chemical analysis of the more durable tubing samples or more careful tracking during manufacturing and use could determine which polymer was the most durable.

BIOCOMPATABILITY

Biocompatability was examined in vitro with reference to fibrinogen uptake, with and without albumin pre-washing (Chapter 7). More complex experiments were also performed where a wide range of haematological, inflammatory and functional indicators were examined in vivo using a porcine model (Chapters 5 and 6) and in vitro using fresh human blood (Chapter 8).

In the fibrinogen uptake experiments Tygon bound significantly less fibrinogen than either SRT or LVA. This correlated well with the situation during recirculation of human blood where both SRT and LVA caused complete de-fibrinogenation of the blood, whereas Tygon did not. Pre-washing the tubing with 20% albumin solution reduced the fibrinogen uptake, such that there were no significant differences in fibrinogen binding between materials. The marked differences in biocompatability seen in the other in vitro and in vivo experiments show that this type of in-vitro test should be used for screening materials only.

The porcine and human experiments showed wide agreement in their results in the case of the majority of assays. Although none of the trends seen in the porcine model reached statistical significance. On the whole LVA and SRT caused more activation of the coagulation cascade and more thrombocytopenia than Tygon. Based on histology and trends the other data Tygon and LVA probably have comparable pneumotoxicity, but the silicone rubber LVA showed reduced pneumotoxicity compared to Tygon. This was evidenced by lower pulmonary artery pressures, an absence of pulmonary vascular congestion and reduced neutrophil infiltration.

The lack of any differences in complement activation between groups despite marked differences in biocompatability (especially with respect to the coagulation system) indicates a poor correlation with a materials overall biocompatability. Based on these

findings complement activation should not be used as an isolated screening test for biocompatability.

There was increased haemolysis seen with LVA compared to Tygon in the in-vitro model. This contrasts with the situation in-vivo where Tygon was more haemolytic than SRT but no different from LVA.

CONCLUSIONS

Unfortunately neither of the materials studied had acceptable durability, spallation performance or biocompatability to recommend their routine use during ECMO. The porcine model was demonstrated to be reliable and reproducible although the ECC cannulation did require special care. Unfortunately, however, the porcine model did not have sufficient power when used in small numbers to differentiate between the three tubing materials. The recirculation model was easier to manage and much cheaper that the porcine experiments, but obviously does not yield information about organ function and toxicity. This human in vitro model would make a good screening test for materials which could then be tested further in vivo if they proved biocompatable.

FUTURE DIRECTIONS

Other pumping systems such as the non-occlusive roller pump (M-pump, Avecor, or Mondiere / Rhone-Poullenc AREC pump) (Durandy et al. 1990) may increase tubing pump life. Certainly early work in our own laboratory with the Mondiere pump gave run times for standard silicone tubing of around 100 hours. The poly-urethane raceway of the M-pump is expected to be extremely durable, and early tests have confirmed this (R Bartlett, Personal communication). Non-occlusive pumping systems may also be gentler on the blood. Tubing durability during occlusive de-Bakey type pump (DeBakey, 1934)

use could be improved by under-occlusive roller settings. This would act to give the minimum amount of compression to allow forward flow, and to reduce sheer stress. It would also be possible to re-design pump heads and boots to reduce sheer stress, and probably also to automatically set, monitor and adjust occlusion.

The durability of Tygon S-65-HL could be improved by identification of the chemical and manufacturing differences between the more and less durable tubing samples. It is possible that apart from the different sources of base polymer already discussed the more durable tubing samples are extruded at a lower or higher temperature, or at a slightly different speed. Subtle variations in the manufacturing process may result in the large changes in durability demonstrated. A programme of tracking material through the manufacturing plant and then measuring durability could identify the optimum manufacturing conditions.

Further study of LVA and SRT is indicated to determine the reason for the reductions in nephrotoxicity and in the case of SRT alone, pneumotoxicity. Perhaps a cell culture bio-assay or an isolated organ perfusion system could be developed into which single chemical constituents of different materials were injected. Such a system could identify the most toxic compounds, which could perhaps then be removed or substituted. It is quite likely that it will not be possible to find an inert material that is passive with respect to the bodies defence systems of inflammation and coagulation. Indeed the vascular endothelium is not a passive tissue, but actively secretes substances such as prostacyclin (Beverelli et al. 1997) and nitric oxide (Palmer et al. 1988; Billiar, 1995) to prevent activation of the coagulation cascade. The utopian extracorporeal circuit would be lined by a monolayer of HLA matched human vascular endothelial cells, but even if this were possible, the area of tubing in the pump, even a non-occlusive pump, would become denuded of cells. Coating circuits with substances which release nitric oxide (R Bartlett,

Personal Communication) greatly reduces platelet activation. Perhaps a combination of nitric oxide, prostacyclin and heparin coating could create a prosthetic endothelium which would be "actively bio-compatible".

Such a circuit could run without heparin, and would cause little or no platelet damage. Although it is likely that a certain amount of platelet and inflammatory activation will occur as a result of turbulence in the oxygenator and cannulae. Overall, the risk of ECMO related bleeding would therefore be greatly reduced. If this were the case the indications for ECMO could be extended to patients with recent trauma or surgery, and to neonates of less than 34 weeks gestation. It is more physiological for a premature neonate to have extracorporeal gas exchange than to have his immature lungs ventilated. Such an approach could perhaps reduce the severity of Broncho-Pulmonary Dysplasia (BPD) in these babies. Another approach to development of an artificial placenta/womb is to use actual placentas (Akagi et al. 1991), perfusion of the maternal side of the placenta with oxygenated perfluorocarbon has successfully provided sufficient gas exchange to maintain life in the laboratory.

In addition to development of more biocompatable circuitry the ECMO circuit could be used to actually modulate the ARDS and SIRS process. Plasmapheresis (K Hultqvist, Personal Communication) and selective leucocyte depletion (Bando et al. 1990) could be used to speed resolution of septic and inflammatory disease processes.

It is essential that indications for ECMO in each age group are tested by RCTs, where possible. Only a properly constructed RCT will answer both the critics and enthusiasts. However, the technical aspects of ECMO support during such a trial must be of a high standard. The NIH randomised trial showed no difference between conventional treatment and ECMO (Zapol et al. 1979) because patients had irreversible lung disease as a result of prolonged high pressure ventilation. These patients then had VA perfusion,

reducing pulmonary blood flow and resulting in micro-thrombosis and lung fibrosis, whilst continued high pressure ventilation with high FIO_2 caused ongoing lung injury. All these problems were compounded by high range heparinisation and haemorrhage. Although this study did not have a positive outcome it was very important because it lead investigators to develop ECMO protocols which worked. It also demonstrated that new treatments should not be subjected to randomised trials before cohort studies demonstrate that the technique has been fully developed. The randomised trial of PCIRV and $ECCO_2R$ (Morris et al. 1994) demonstrated the importance of choosing the appropriate technique for the patients being treated, and being sufficiently experienced with that technology to do it safely and efficiently. It showed that low flow $ECCO_2R$ was no better than computer controlled PCIRV in patients with severe ARDS. High flow ECMO with lung rest has been shown to be more effective than conventional treatment for neonates with severe respiratory failure (Anonymous. 1996), we should conduct an RCT along similar lines for, initially adults, and then eventually children to confirm that this is the case in all age groups.

Any such RCT should have both the ECMO and control arms conducted by clinicians experienced in either ECMO or "conventional" intensive care. This will eliminate the bias seen in Morris's study (Anonymous. 1996) that resulted from inexperience with the procedure.

One important point about the conventional arm of an RCT is what is conventional treatment ? It is not possible to control multi-factorial treatment in intensive care in the same way that a single drug can be investigated. This is especially true when multiple centres are involved. Another problem is how quickly treatment is evolving , new modes of ventilation, antibiotics, monoclonal antibodies and other therapies are being introduced and then abandoned all the time. Despite these concerns we must adopt a pragmatic attitude. ECMO is fairly standardised in the majority of ELSO registered centres and

therefore is more easily comparable. In the UK collaborative neonatal ECMO trial the conventional centres had free rein to treat patients as they felt best. Such a trial actually gives a very accurate comparison of treatments because they are being compared in the real world rather that within the artificial confines of a treatment protocol. At the end of the day the question which needs answering is "should this patient with severe respiratory failure be sent for ECMO or continue on conventional treatment?". If there are further evolution's in ECMO or conventional treatment then these can be trialled against the best treatment determined by the trial, and so on.

On the 18th September 1997 a meeting was held at the University of Michigan, Ann Arbor, to discuss an adult ECMO trial. A draft of these proceedings is available from ELSO, on request, or via their web page at http://www.med.umich.edu/elso/ . A synopsis of the discussion as it applies to the UK is as follows: The UK RCT for adult respiratory ECMO will select patients who are failing conventional intensive care. These patients would be identified by a scoring system which would predict patients with a high risk of dying. The NIH ARDS study group is currently developing this scoring system based on physiological variables, by retrospective review of their database. A score equivalent to a 75% mortality risk will be identified, and then applied prospectively in the UK to ensure that it accurately identifies patients in this risk stratum. During the trial all hospitals in the UK will identify patients who fall into this 75% mortality category. Provided they do not have a contra-indication to ECMO they will then be entered into the study and randomised to either ECMO or Conventional arms. If they are allocated to receive ECMO they will be transported to Glenfield hospital and treated according to our standard protocols (Chapter 1). The hospitals providing the conventional care will be assessed by an independent committee, possibly from the Intensive Care Society, to ensure that a high intensity of treatment is available. This will exclude small district hospital ITUs who do not have a

dedicated intensivist from the trial. It will ensure that the intensity of treatment is similar between the two arms of the trial. Designated conventional treatment centres will then treat the patients according to institutional protocols, which will not be controlled, but will be recorded. If a patient originates in a smaller hospital and is randomised to receive conventional treatment then they will be transferred to the nearest designated centre. The primary outcome measure will be whether patients are alive at one year. The randomisation will take account of age and diagnosis which will be the main minimisation criteria. For an α probability of 0.2 (improvement of survival from ~30% to ~50%) and 95% confidence intervals the sample size will be 180 patients in total. It was thought that enough patients would be recruited within 2 years.

Improvements in survival could perhaps be achieved by the support of other organ systems that have failed. We already routinely support the kidney as well as the lungs and heart, by the simple expedient of adding a haemofilter to the circuit. However, the majority of patients who die develop Multi Organ Dysfunction Syndrome (MODS), resulting in severe impairment in liver function as well as respiratory and renal failure. An extra-corporeal liver support system or bioreactor (Mitzner et al. 1996) attached to the ECMO circuit could possibly reduce the mortality in patients with severe MODS.

The eventual aim is to evolve present day ECMO into a minimally invasive modular multiple organ support system which has no adverse effects on the patients systems. Such a system could support patients of any age without anticoagulation and with only limited transfusion. This goal is still many years away, but extracorporeal technology has come a long way since Dr Gibbon sat helplessly beside a young woman dying of pulmonary embolism.

APPENDIX 1

SUMMARY OF STATISTICAL METHODS

INTRODUCTION

In this section an overview of the statistical approach used in the thesis will be presented. I am most indebted to Mr Nick Taub, of the Department of Epidemiology & Public Health at the University of Leicester, for his statistical advice and expertise.

COMPUTER HARDWARE & SOFTWARE

A Gateway G6-300 Pentium I personal computer was used throughout. The statistical package used was SPSS 7.5 for windows. All spreadsheets and graphs were prepared using Microsoft Excel 6.0. The lines of best fit were drawn using the "trendline" function in Excel in the polynomial mode.

STATISTICAL TESTS

Baseline values for each material (SRT & LVA) were compared with the control values (Tygon) using an un-paired t-test. Levenes test is applied automatically by SPSS to ensure that data is normally distributed. This approach was also used to compare data that had only one time point such as lung water and lung neutrophil infiltration (see Chapters 5 & 6).

Data was collected throughout the porcine and in-vitro perfusion experiments (Chapters 5,6 & 8) and is displayed as variation with time. The line of best fit is drawn through the mean values, using the polynomial trendline function in Microsoft Excel. Maxima and minima are also displayed. Variables that had a significant difference from the control group (see below) are highlighted with a blue background.

The difference between values at the beginning of the porcine and in vitro experiments and the end was found by subtracting the baseline value from the final value. A negative sign indicates a decrease from baseline and vice versa. These differences were compared with the mean difference found in the controls (Tygon) using an unpaired t-test. An identical approach was used for similar data varying with time in other experiments i.e. spallation in Chapter 4.

To detertmine if variables had altered significantly from baseline an unpaired t-test was performed using zero as the test value.

The survival of animals in the porcine perfusion experiments (Chapters 5 & 6) was measured by tabulating the number of animals in each group alive and dead at the end of

the 48 hour experiment. These were then cross tabulated and compared with controls using Fishers exact test.

In the mechanical experiments failure times were compared using an unpaired t-test. However in the spallation experiments single observations are made at each increment of occlusion, pump speed or time. It is therefore appropriate to use the Wilcoxon signed ranks test for paired samples. Pairings being LVA-control and SRT-control, since all other conditions were unaltered.

In the detailed analysis of 50 adult respiratory ECMO patients presented in Chapter 3 the survival with ECMO was compared with two published series of conventionally ventilated patients. Study data was compared with multi-institutional survival data for conventional treatment taken from the paper of Vasilyev et al (Vasilyev et al. 1995) using logistic regression, and analysing for the effects of hypoxia ($PaO_2/FIO_2 < 60$ mmHg). Logistic regression was also used to estimate the odds ratios of survival with ECMO compared to the survival with conventional treatment in Vasilyevs patients (Vasilyev et al. 1995), both with and without adjustment for hypoxia. Survival was also compared to the control group receiving Pressure Controlled Inverse Ratio Ventilation (PCIRV) in the paper of Morris et al (Morris et al. 1994) . As these patients were not sub-divided on the basis of hypoxia a Chi-squared test was used to compare this group with our patients. A 2 tailed unpaired t-test was used to compare continuous variables which were normally distributed, the Mann-Whitney U test was used for ordinal variables, and also for continuous variables which were not normally distributed as detected by Levenes' test. Non-normally distributed variables were: Blood use, Cryoprecipitate use, Pulmonary artery wedge pressure, PaO_2/FIO_2 pre ECMO, Platelet use and Duration of perfusion. This study, including the comparison with historical controls, has been published in "Chest" (Peek et al. 1997).

LEVEL OF SIGNIFICANCE

A p value of < 0.05 was taken to indicate significance in all experiments except the porcine studies. A p value of < 0.01 was used for these studies. The higher level of significance was used in order to ensure that any conclusions were very robust. This was felt to be necesarry as the asignment of animals to groups was not randomised (due to problems in supply of tubing from the manufacturers). In addition n was only 5 per group. Whilst this was also true for the in-vitro experiments it was felt that the in-vitro system was much more reproducible and reliable than the animal model.

BIBLIOGRAPHY

Anonymous (1985) *Muir's Textbook of Pathology*, 12th edn. London: Edward Arnold.

Anonymous (1989a) Angiology. In: Williams, P.L., Warwick, R., Dyson, M. and Bannister, L.H. (Eds.) *Gray's Anatomy.* 37th edn. pp. 661-858. London: Churchill Livingstone]

Anonymous (1989b) *Mediators of the Inflammatory Response*, Amsterdam: Elsevier.

Anonymous (1991) *Glenn's Thoracic and Cardiovascular Surgery*, 5th edn. Englewood Cliffs, New Jersey: Prentice-Hall.

Anonymous (1993) *Advanced Trauma Life Support course for physicians*, 5th edn. Chicago: American College of Surgeons.

Anonymous (1995) *ECMO, Extracorporeal cardiopulmonary support in critical care.* Ann Arbor: ELSO.

Anonymous. (1996) UK collaborative randomised trial of neonatal extracorporeal membrane oxygenation. UK Collaborative ECMO Trail Group. *Lancet* **348**, 75-82.

Addonizio, V.P., Fisher, C.A., Bowen, J.C., Palatianos, G.C., Colman, R.W., Edmunds and LH Jr. (1981) Prostacyclin in lieu of anticoagulation with heparin for extracorporeal circulation. *Transactions - American Society for Artificial Internal Organs* **27**, 304-307.

Agostini, C., Zambello, R., Trentin, L., Cerutti, A., Enthammer, C., Facco, M., Milani, A., Sancetta, R., Garbisa, S. and Semenzato, G. (1995) Expression of TNF receptors by T cells and membrane TNF-alpha by alveolar macrophages suggests a role for TNF-alpha in the regulation of the local immune responses in the lung of HIV-1-infected patients. *Journal of Immunology* **154**, 2928-2938.

Akagi, H., Takano, H., Taenaka, Y., Nakatani, T., Tatsumi, E., Masuzawa, T., Goto, M., Sasaki, E., Inoue, K., Sakaki, M. and et al. (1991) Hybrid respiratory support system with extracorporeal placental oxygenation. *ASAIO Transactions* **37**, M409-10.

Allen, D.H. and Tracy, P.B. (1995) Human coagulation factor V is activated to the functional cofactor by elastase and cathepsin G expressed at the monocyte surface. *Journal of Biological Chemistry* **270**, 1408-1415.

Anderson, B.O., Moore, E.E., Moore, F.A., Leff, J.A., Terada, L.S., Harken, A.H. and Repine, J.E. (1991) Hypovolemic shock promotes neutrophil sequestration in lungs by a xanthine oxidase-related mechanism. *Journal of Applied Physiology* **71**, 1862-1865.

Anonymous. (1996) UK collaborative randomised trial of neonatal extracorporeal membrane oxygenation. UK Collaborative ECMO Trail Group. *Lancet* **348**, 75-82.

Aren, C., Feddersen, K. and Radegran, K. (1983) Effects of prostacyclin infusion on platelet activation and postoperative blood loss in coronary bypass. *Annals of Thoracic Surgery* **36**, 49-54.

Arnold, J.H., Truog, R.D., Orav, E.J., Scavone, J.M. and Hershenson, M.B. (1990) Tolerance and dependence in neonates sedated with fentanyl during extracorporeal membrane oxygenation. *Anesthesiology* **73**, 1136-1140.

Arnold, J.H., Truog, R.D., Scavone, J.M. and Fenton, T. (1991) Changes in the pharmacodynamic response to fentanyl in neonates during continuous infusion [see comments]. *Journal of Pediatrics* **119**, 639-643.

Ashbaugh, D.G., Bigelow, D.B., Petty, T.L. and Levine, B.E. (1967) Acute respiratory distress in adults. *Lancet* **2**, 319-323.

Atkinson, J.B., Hamid, R. and Steward, D.J. (1994) General anesthesia with isoflurane for diaphragmatic hernia repair during ECMO. *ASAIO Journal* **40**, 986-989.

Baier, R.E. (1977) The organization of blood components near interfaces. *Annals of the New York Academy of Sciences* **283**, 17-36.

Baier, R.E. and Dutton, R.C. (1969) Initial events and interactions of blood with a foreign surface. *Journal of biomedical materials research* **3**, 91

Bando, K., Tago, M. and Teramoto, S. (1988a) Prevention of free radical-induced myocardial injury by allopurinol. Experimental study in cardiac preservation and transplantation. *Journal of Thoracic & Cardiovascular Surgery* **95**, 465-473.

Bando, K., Teramoto, S., Tago, M., Seno, S., Murakami, T., Nawa, S. and Senoo, Y. (1988b) Oxygenated perfluorocarbon, recombinant human superoxide dismutase, and catalase ameliorate free radical induced myocardial injury during heart preservation and transplantation. *Journal of Thoracic & Cardiovascular Surgery* **96**, 930-938.

Bando, K., Teramoto, S., Tago, M., Teraoka, H., Seno, S. and Senoo, Y. (1989) Successful extended hypothermic cardiopulmonary preservation for heart-lung transplantation. *Journal of Thoracic & Cardiovascular Surgery* **98**, 137-45; discussion 145-6.

Bando, K., Pillai, R., Cameron, D.E., Brawn, J.D., Winkelstein, J.A., Hutchins, G.M., Reitz, B.A. and Baumgartner, W.A. (1990) Leukocyte depletion ameliorates free radical-mediated lung injury after cardiopulmonary bypass. *Journal of Thoracic & Cardiovascular Surgery* **99**, 873-877.

Barie, P.S. (1995) Organ-specific support in multiple organ failure: pulmonary support. [Review] [70 refs]. *World Journal of Surgery* **19**, 581-591.

Bartlett, R.H., Gazzaniga, A.B., Wilson, A.F., Medley, T. and Wetmore, N. (1975) Mortality prediction in adult respiratory insufficiency. *Chest* **67**, 680-684.

Bartlett, R.H., Andrews, A.F., Toomasian, J.M., Haiduc, N.J. and Gazzaniga, A.B. (1982) Extracorporeal membrane oxygenation for newborn respiratory failure: forty-five cases. *Surgery* **92**, 425-433.

Bartlett, R.H., Roloff, D.W., Cornell, R.G., Andrews, A.F., Dillon, P.W. and Zwischenberger, J.B. (1985) Extracorporeal circulation in neonatal respiratory failure: a prospective randomized study. *Pediatrics* **76**, 479-487.

Bartlett, R.H. (1995) Management of ECLS in adult respiratory failure. In: Zwischenberger, J.B. and Bartlett, R.H. (Eds.) *ECMO. Extracorporeal cardiopulmonary support in critical care.* pp. 401-414. Ann Arbor, Michigan: Extracorporeal life support organization]

Bartlett, R.H. and Andersen, J.C. (1982) Blood-Surface interactions: An overview. In: Stanley, J.C. (Ed.) *Biologic and Synthetic vascular prostheses*, pp. 63-81. Grune & Stratton]

Bartlett, R.H. and Cilley, R.E. (1993) Physiology of Extracorporeal Life Support. In: Arensman, R.M. and Cornish, J.D. (Eds.) *Extracorporeal life support*, pp. 89-104. Boston: Blackwell Scientific Publications]

Bernbaum, J., Schwartz, I.P., Gerdes, M., D'Agostino, J.A., Coburn, C.E., Polin and RA. (1995) Survivors of extracorporeal membrane oxygenation at 1 year of age: the relationship of primary diagnosis with health and neurodevelopmental sequelae. *Pediatrics* **96**, 907-913.

Bernstein, E.F., Blackshear, P.L., Jr. and Keller, K.H. (1967) Factors influencing erythrocyte destruction in artificial organs. *American Journal of Surgery* **114**, 126-138.

Bernstein, E.F. and Gleason, L.R. (1967) Factors influencing hemolysis with roller pumps. *Surgery* **61**, 432-442.

Beutler, B. and Cerami, A. (1987) Cachectin: more than a tumor necrosis factor. *New England Journal of Medicine* **316**, 379-385.

Beverelli, F., Bea, M.L., Puybasset, L., Giudicelli, J.F. and Berdeaux, A. (1997) Chronic inhibition of NO synthase enhances the production of prostacyclin in coronary arteries through upregulation of the cyclooxygenase type 1 isoform. *Fundamental & Clinical Pharmacology* **11**, 252-259.

Billiar, T.R. (1995) Nitric oxide. Novel biology with clinical relevance. [Review] [109 refs]. *Annals of Surgery* **221**, 339-349.

Black, M.M. (1995) Cardiovascular applications of biomaterials and implants--an overview. *Journal of Medical Engineering & Technology* **19**, 151-157.

Blackshear, P.L.J., Dorman, F.D. and Steinbach, J.H. (1965) Some mechanical effects that influence hemolysis. *ASAIO Transactions* **11**, 112-117.

Blauhut, B., Gross, C., Necek, S., Doran, J.E., Spath, P. and Lundsgaard-Hansen, P. (1991) Effects of high-dose aprotinin on blood loss, platelet function, fibrinolysis, complement, and renal function after cardiopulmonary bypass [see comments]. *Journal of Thoracic & Cardiovascular Surgery* **101**, 958-967.

Bone, R.C. (1991) The pathogenesis of sepsis. [Review] [141 refs]. *Annals of Internal Medicine* **115**, 457-469.

Bone, R.C. (1992a) Phospholipids and their inhibitors: A critical evaluation of their role in the treatment of sepsis. *Critical Care Medicine* **20**, 884-890.

Bone, R.C. (1992b) Inhibitors of complement and neutrophils: a critical evaluation of their role in the treatment of sepsis. [Review] [104 refs]. *Critical Care Medicine* **20**, 891-898.

Boretos, J.W. and Wagner, F.R. (1971) Particle fragmentation generated in pump sets (letter). *Journal of biomedical materials research* **5**, 411-412.

Borg, U.R., Reynolds, H.N. and Habashi, N.M. (1996) ECLA, ECMO, ECCO2R: Is your system safe? Part 1 ECLA Circuit. *5th European Congress of Extracorporeal life support* 27a(Abstract)

Bramson, M.L., Osborn, J.J. and Main, F.B. (1965) A new disposable membrane oxygenator with integral heat exchanger. *Journal of Thoracic & Cardiovascular Surgery* **50**, 391

Brunet, F., Mira, J.P., Belghith, M., Lanore, J.J., Schlumberger, S., Toulon, P. and Dhainaut, J.F. (1992) Effects of aprotinin on hemorrhagic complications in ARDS patients during prolonged extracorporeal CO2 removal. *Intensive Care Medicine* **18**, 364-367.

Bui, K.C., Hammerman, C., Hirschl, R., Snedecor, S.M., Cheng, K.J., Chan, L., Short, BL and Bartlett, R.H. (1991) Plasma prostanoids in neonatal extracorporeal membrane oxygenation. Influence of meconium aspiration. *Journal of Thoracic & Cardiovascular Surgery* **101**, 612-617.

Bui, K.C., Humphries, B., Kitagawa, H., Kosi, M., Dorio, R., Lew, C., Atkinson, J. and Platzker, A. (1992) Extracorporeal membrane oxygenation in lambs through umbilical vessel perfusion: cardiac and hepatic complications. *Biology of the Neonate* **61**, 351-357.

Butch, S.H., Knafl, P., Oberman, H.A. and Bartlett, R.H. (1996) Blood utilization in adult patients undergoing extracorporeal membrane oxygenated therapy. *Transfusion* **36**, 61-63.

Caballero, R., Clark, R.H. and Wright, J.A. (1996) Outcome of respiratory failure: a case-control study. *Clinical Pediatrics* **35**, 199-204.

Caplan, M.S., Hsueh, W., Sun, X.M., Gidding, S.S. and Hageman, J.R. (1990) Circulating plasma platelet activating factor in persistent pulmonary hypertension of the newborn. *American Review of Respiratory Disease* **142**, 1258-1262.

Cappelletti, R.R., Domingo, R.T., Dennis, C., Amer, N.S. and Stuckey, J.H. (1961) Controlled studies in three pumping systems. *Trans ASAIO* **7**, 162-166.

Casey, W.F., Hauser, G.J., Hannallah, R.S., Midgley, F.M. and Khan, W.N. (1992) Circulating endotoxin and tumor necrosis factor during pediatric cardiac surgery. *Critical Care Medicine* **20**, 1090-1096.

Chenoweth, D.E., Cooper, S.W., Hugli, T.E., Stewart, R.W., Blackstone, E.H., Kirklin and JW. (1981) Complement activation during cardiopulmonary bypass: evidence for generation of C3a and C5a anaphylatoxins. *New England Journal of Medicine* **304**, 497-503.

Chenoweth, D.E. (1986) Complement activation produced by biomaterials. *Transactions - American Society for Artificial Internal Organs* **32**, 226-232.

Chenoweth, D.E. (1987) Complement activation in extracorporeal circuits. *Annals of the New York Academy of Sciences* **516**, 306-313.

Chenoweth, D.E. (1991) Complement production produced by biomaterials. *Artificial Organs* **12**, 502-504.

Cheung, A.K., LeWinter, M., Chenoweth, D.E. and Henderson, L.W. (1986) Cardiopulmonary effects of cuprophane activated plasma in the swine: role of complement products. *Kidney International* **29**, 799-806.

Chevalier, J.Y., Couprie, C., Larroquet, M., Renolleau, S., Durandy, Y., Costil and J. (1993) Venovenous single lumen cannula extracorporeal lung support in neonates. A five year experience. *ASAIO Journal* **39**, M654-8.

Cicco, N.A., Lindeman, A., Content, J., Vandenbussche, P., Lubbert, M., Gauss, J., Merteismann, R. and Herman, F. (1990) Inducible production of interleukin-6 by human polymorphonuclear neutrophils. Role of granulocyte-macrophage colony stimulating factor and tumor necrosis factor-alpha. *Blood* **75**, 2049-2052.

Clauss, A. (1957) Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens. *Acta Haematol* **17**, 237-246.

Colman, R.W., Scott, C.F., Schmaier, A.H., Wachtfogel, Y.T., Pixley, R.A., Edmunds and LH Jr. (1987) Initiation of blood coagulation at artificial surfaces. *Annals of the New York Academy of Sciences* **516**, 253-267.

Connell, R.S., Page, U.S., Bartley, T.D., Bigelow, J.C. and Webb, M.C. (1973) The effect on pulmonary ultrastructure of dacron-wool filtration during cardiopulmonary bypass. *Annals of Thoracic Surgery* **15**, 217-229.

Copeland, J.G., Pavie, A., Duveau, D., Keon, W.J., Masters, R., Pifarre, R., Smith, RG and Arabia, F.A. (1996) Bridge to transplantation with the CardioWest total artificial heart: the international experience 1993 to 1995. *Journal of Heart & Lung Transplantation* **15**, 94-99.

Cornish, J.D., Gerstmann, D.R., Null, D.M., Jr., Smith, M.D. and Kuehl, T.J. (1989) Oxygen delivery rate and sufficiency of oxygenation during ECMO in newborn baboons. *Journal of Applied Physiology* **66**, 210-216.

Cottrell, E.D., Kappa, J.R., Stenach, N., Fisher, C.A., Tuszynski, G.P., Switalska, HI and Addonizio, V.P. (1988) Temporary inhibition of platelet function with iloprost (ZK36374) preserves canine platelets during extracorporeal membrane oxygenation. *Journal of Thoracic & Cardiovascular Surgery* **96**, 535-541.

Craddock, P.R., Hammerschmidt, D.E., White, J.G., Dalmasso, A.P. and Jacob, H.S. (1977) Complement (C5a)-induced granulocyte aggregation in vitro: a possible mechanism of complement mediated leukostasis and leukopenia. *Journal of Clinical Investigation* **60**, 261-264.

Crombleholme, T.M., Adzick, N.S., Hardy, K., Longaker, M.T., Bradley, S.M., Duncan, BW, Verrier, E.D. and Harrison, M.R. (1990) Pulmonary lobar transplantation in neonatal swine: a model for treatment of congenital diaphragmatic hernia. *Journal of Pediatric Surgery* **25**, 11-18.

Dagan, O., Klein, J., Gruenwald, C., Bohn, D., Barker, G. and Koren, G. (1993) Preliminary studies of the effects of extracorporeal membrane oxygenator on the disposition of common pediatric drugs. *Therapeutic Drug Monitoring* **15**, 263-266.

Das, D.K., Engelman, R.M., Liu, X., Maity, S., Rousou, J.A., Flack, J., Laksmipati, J, Jones, R.M., Prasad, M.R. and Deaton, D.W. (1992) Oxygen-derived free radicals and hemolysis during open heart surgery. *Molecular & Cellular Biochemistry* **111**, 77-86.

Davenport, R.D. (1996) Cytokines as intercellular signals in hemolytic transfusion reactions. [Review] [20 refs]. *Biological Signals* **5**, 240-245.

Davies, G.C., Sobel, M. and Salzman, E.W. (1980) Elevated plasma fibrinopeptide A and thromboxane A2 levels during cardiopulmonary bypass. *Circulation* **61**, 808-814.

Davies, M.J., Arsiwala, S.S., Moore, H.M., Kerr, S., Sosnowski, A.W. and Firmin, R.K. (1995) Extracorporeal membrane oxygenation for the treatment of massive pulmonary embolism. *Annals of Thoracic Surgery* **60**, 1801-1803.

de Smet, A.A., Joen, M.C., van Oeveren, W., Roozendaal, K.J., Harder, M.P., Eijsman, L and Wildevuur, C.R. (1990) Increased anticoagulation during cardiopulmonary bypass by aprotinin [see comments]. *Journal of Thoracic & Cardiovascular Surgery* **100**, 520-527.

DeBakey, M.E. (1934) Simple continuous-flow blood transfusion instrument. *New Orleans Medical and Surgical Journal* **87**, 386

Debets, R., Hegmans, J.P., Buurman, W.A., Benner, R. and Prens, E.P. (1996) Expression of cytokines and their receptors by psoriatic fibroblasts. II. decreased TNF receptor expression. *Cytokine* **8**, 80-88.

Despotis, G.J., Joist, J.H., Joiner-Maier, D., Alsoufiev, A.L., Triantafillou, A.N., Goodnough, L.T., Santoro, S.A. and Lappas, D.G. (1995) Effect of aprotinin on activated clotting time, whole blood and plasma heparin measurements. *Annals of Thoracic Surgery* **59**, 106-111.

Dewald, B., Bretz, U. and Baggiolini, M. (1982) Release of glatinase from a novel secretory compartment of human neutrophils. *Journal of Clinical Investigation* **70**, 518-525.

Dinarello, C.A. (1992) Interleukin-1 and tumour necrosis factor and their naturally occurring antagonists during hemodialysis. *Kidney International* **42**, S-68-S-77.

Dobyns, E.L., Wescott, J.Y., Kennaugh, J.M., Ross, M.N. and Stenmark, K.R. (1994) Eicosanoids decrease with successful extracorporeal membrane oxygenation therapy in neonatal pulmonary hypertension. *American Journal of Respiratory & Critical Care Medicine* **149**, 873-880.

Donnelly, S.C., Strieter, R.M., Kunkel, S.L., Walz, A., Robertson, C.R., Carter, D.C., Grant, I.S., Pollok, A.J. and Haslett, C. (1993) Interleukin-8 and development of adult respiratory distress syndrome in at-risk patient groups [see comments]. *Lancet* **341**, 643-647.

Dorrington, K.L., McRae, K.M., Gardaz, J.P., Dunnill, M.S., Sykes, M.K., Wilkinson and AR. (1989) A randomized comparison of total extracorporeal CO2 removal with conventional mechanical ventilation in experimental hyaline membrane disease. *Intensive Care Medicine* **15**, 184-191.

Dorrington, K.L. and Radcliffe, F.M. (1991) Effect of a single inflation of the lungs on oxygenation during total extracorporeal carbon dioxide removal in experimental respiratory distress syndrome. *Intensive Care Medicine* **17**, 469-474.

Dubravek, D.B., Spriggs, D.R., Mannick, J.A. and Rodrick, M.L. (1990) Circulating human peripheral blood granulocytes synthesize and secrete tumor necrosis factor a. *Proceedings of the National Academy of Sciences of the United States of America* **87**, 6758-6761.

Durandy, Y., Chevalier, J.Y. and Lecompte, Y. (1990) Single-cannula venovenous bypass for respiratory membrane lung support. *Journal of Thoracic & Cardiovascular Surgery* **99**, 404-409.

Dutton, R.C. and Edmunds, L.H., Jr. (1973) Measurement of emboli in extracorporeal perfusion systems. *Journal of Thoracic & Cardiovascular Surgery* **65**, 523-530.

Eberhart, R.C., Munro, M.S., Frautschi, J.R., Lubin, M., Clubb, F.J., Jr., Miller, C.W. and Sevastianov, V.I. (1987) Influence of endogenous albumin binding on blood-material interactions. [Review] [28 refs]. *Annals of the New York Academy of Sciences* **516**, 78-95.

Edmunds, L.H., Jr., Ellison, N. and Colman, R.W. (1982) Platelet function during open heart surgery: Comparison of the membrane and bubble oxygenators. *Journal of Thoracic & Cardiovascular Surgery* **83**, 805-812.

Edmunds, L.H., Jr. and Addonizio, V.P. (1987) Extracorporeal circulation. In: Colman, R.W. (Ed.) *Hemostasis & Thrombosis*, pp. 901-912. J.B. Lipincott]

Fallma, M., Lew, D.P., Stendahl, O. and Andersson, T. (1989) Receptor-mediated phagocytosis in human neutrophils is associated with increased formation of inositol phosphates and diacylglycerol. *Journal of Clinical Investigation* **84**, 886-891.

Fausles, J.W. (1995) Extracorporeal life support for infants and children with cardiac disease. In: Zwischenberger, J.B. and Bartlett, R.H. (Eds.) *ECMO extracorporeal cardiopulmonary support in critical care*, pp. 445-471. Ann Arbor, Michigan: Extracorporeal life support organization]

Federowicz, I., Barrett, B.B., Andersen, J.W., Urashima, M., Popovsky, M.A. and Anderson, K.C. (1996) Characterization of reactions after transfusion of cellular blood components that are white cell reduced before storage. *Transfusion* **36**, 21-28.

Ferrante, A., Nandoskar, M., Walz, A., Goh, D.H. and Kowanko, I.C. (1988) Effects of tumor necrosis factor alpha and interleukin-1 alpha and beta on human neutrophil migration, respiratory burst and degrannulation. *International Archives of Allergy and Applied Immunology* **86**, 82-91.

Finn, A., Naik, S., Klein, N., Levinsky, R.J., Strobel, S. and Elliott, M. (1993) Interleukin-8 release and neutrophil degranulation after pediatric cardiopulmonary bypass [see comments]. *Journal of Thoracic & Cardiovascular Surgery* **105**, 234-241.

Firmin, R.K., Waggoner, J. and Pearson, G.A. (1995) Cannulation for ECMO. In: Lewis, T. and Graham, T.R. (Eds.) *Mechanical circulatory support*, pp. 319-321. London: Edward Arnold]

Fish, K.J., Sarnquist, F.H., van Steennis, C., Mitchell, R.S., Hilberman, M., Jamieson, S.W., Linet, O.I. and Miller, D.C. (1986) A prospective, randomized study of the effects of prostacyclin on platelets and blood loss during coronary bypass operations. *Journal of Thoracic & Cardiovascular Surgery* **91**, 436-442.

Fletcher, M.P., Stahl, G.L. and Longhurst, J.C. (1993) C5a-induced myocardial ischemia: role for CD18-dependent PMN localization and PMN-platelet interactions. *American Journal of Physiology* **265**, H1750-H1761.

Ford-Hutchinson, A.W., Bray, M.A., Doing, M.V., Shipley, M.E. and Smith, J.H. (1980) Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature* **286**, 264-265.

Forman, M.B., Perry, J.M., Wilson, B.H., Verani, M.S., Kaplan, P.R., Shawl, F.A. and Friesinger, G.C. (1991) Demonstration of myocardial reperfusion injury in humans: results of a pilot study utilizing acute coronary angioplasty with perfluorochemical in anterior myocardial infarction. *Journal of the American College of Cardiology* **18**, 911-918.

Forman, M.B., Pitarys, C.J., 2d, Vildibill, H.D., Lambert, T.L., Ingram, D.A., Virmani, R. and Murray, J.J. (1992) Pharmacologic perturbation of neutrophils by Fluosol results in a sustained reduction in infarct size in the canine model of reperfusion. *Journal of the American College of Cardiology* **19**, 205-216.

Fortenberry, J.D., Bhardwaj, V., Niemer, P., Cornish, J.D., Wright, J.A. and Bland, L. (1996) Neutrophil and cytokine activation with neonatal extracorporeal membrane oxygenation. *Journal of Pediatrics* **128**, 670-678.

Fratacci, M.D., Frostell, C.G., Chen, T.Y., Wain, J.C., Jr., Robinson, D.R. and Zapol, W.M. (1991) Inhaled nitric oxide. A selective pulmonary vasodilator of heparin-protamine vasoconstriction in sheep. *Anesthesiology* **75**, 990-999.

Fujishima, S., Hoffman, A.R., Vu, T., Kim, K.J., Xheng, H., Daniek, D., Kim, Y., Wallace, E.F., Larrick, J.W. and Raffin, T.A. (1993) The regulation of neutrophil interleukin 8 gene expression and protein secretion by LPS, TNF-a and IL-1b. *Journal of Cell Physiology* **154**, 478-485.

Fujishima, S. and Aikawa, N. (1995) Neutrophil-mediated tissue injury and its modulation. *Intensive Care Medicine* **21**, 227-285.

Fukahara, K., Murakami, A., Watanabe, G., Kotoh, K. and Misaki, T. (1997) Inhaled nitric oxide after lung ischemia reperfusion; effect on hemodynamics and oxygen free radical scavenger system. *European Journal of Cardio-Thoracic Surgery* **11**, 343-349.

Furlaneto, C. and Campa, A. (1997) The effect in vitro of high-density lipoprotein from healthy and infected humans on the oxidative metabolism of polymorphonuclear leukocytes. *Cell Biochemistry & Function* **15**, 39-45.

Gabay, J.E. and Almeida, R.P. (1993) Antibiotic peptides and serine protease homologs in human polymorphonuclear leukocytes: defensins and azurocidin. [Review] [46 refs]. *Current Opinion in Immunology* **5**, 97-102.

Gadek, J.E., Fells, G.A., Wright, D.G. and Crystal, R.G. (1980) Human neutrophil elastase functions as a type III collagen collagenase. *Biochemical and Biophysical Research Communications* **95**, 1815-1822.

Gadek, J.E. (1992) Adverse effects of neutrophils on the lung. *American Journal of Medicine* **92**, 27S-30S.

Gafter, U., Kalechman, Y. and Sredni, B. (1996) Blood transfusion enhances production of T-helper-2 cytokines and transforming growth factor beta in humans. *Clinical Science* **91**, 519-523.

Gall, H., Schultz, K.D., Boehncke, W.H. and Kaufmann, R. (1996) Clinical and pathophysiological aspects of hydroxyethyl starch-induced pruritus: evaluation of 96 cases. *Dermatology* **192**, 222-226.

Gamulka, B.D. (1994) Ethical uncertainty: an approach to decisions involving extracorporeal membrane oxygenation. *Canadian Medical Association Journal* **150**, 565-568.

Gasson, J.C., Weisbart, R.H., Kaufman, S.E., Clark, S.C., Hewick, R.M. and Wong, G.C. (1984) Purified human granulocyte-macrophage colony-stimulating factor: Direct action on neutrophils. *Science* **226**, 1339-1342.

Gattinoni, L., Pesenti, A., Mascheroni, D., Marcolin, R., Fumagalli, R., Rossi, F, Iapichino, G., Romagnoli, G., Uziel, L., Agostoni, A. and et al. (1986) Low-frequency positive-pressure ventilation with extracorporeal CO2 removal in severe acute respiratory failure. *JAMA* **256**, 881-886.

Gattinoni, L., Pesenti, A., Avalli, L., Rossi, F. and Bombino, M. (1987) Pressure-volume curve of total respiratory system in acute respiratory failure. Computed tomographic scan study. *American Review of Respiratory Disease* **136**, 730-736.

Gattinoni, L., D'Andrea, L., Pelosi, P., Vitale, G., Pesenti, A. and Fumagalli, R. (1993) Regional effects and mechanism of positive end-expiratory pressure in early adult respiratory distress syndrome [see comments] [published erratum appears in JAMA 1993 Oct 20;270(15): 1814]. *JAMA* **269**, 2122-2127.

Gay, J.C. and Stitt, E.S. (1988) Platelet-activating factor induces protein kinase activity in the particulate fraction of human neutrophils. *Blood* **71**, 159-165.

Gentilello, L.M., Anardi, D., Mock, C., Arreola-Risa, C. and Maier, R.V. (1995) Permissive hypercapnia in trauma patients. *Journal of Trauma* **39**, 846-52; discussion 852-3.

Gibbon, J.H. (1937) Artificial maintenance of the circulation during experimental occlusion of the pulmonary artery. *Archives of Surgery* **34**, 1105-1131.

Gibbon, J.H. (1939) The maintenance of life during experimental occlusion of the pulmonary artery followed by survival. *Surgery, Gynecology & Obstetrics.* **69**, 602-614.

Gibbon, J.H. (1954) Application of a mechanical heart and lung apparatus to cardiac surgery. *Minnesota medicine* 171-180.

Gillinov, A.M., DeValeria, P.A., Winkelstein, J.A., Wilson, I., Curtis, W.E., Shaw, D., Yeh, C.G., Rudolph, A.R., Baumgartner, W.A., Herskowitz, A. and Cameron, D.E. (1993) Complement inhibition with soluble complement receptor type 1 in cardiopulmonary bypass. *Annals of Thoracic Surgery* **55**, 619-624.

Gillinov, A.M., Redmond, J.M., Winkelstein, J.A., Zehr, K.J., Herskowitz, A., Baumgartner, W.A. and Cameron, D.E. (1994a) Complement and neutrophil activation during cardiopulmonary bypass: a study in the complement-deficient dog. *Annals of Thoracic Surgery* **57**, 345-352.

Gillinov, A.M., Redmond, J.M., Zehr, K.J., Wilson, I.C., Curtis, W.E., Bator, J.M., Burch, R.M., Reitz, B.A., Baumgartner, W.A., Herskowitz, A. and et al. (1994b) Inhibition of neutrophil adhesion during cardiopulmonary bypass. *Annals of Thoracic Surgery* **57**, 126-133.

Glauber, M., Szefner, J., Senni, M., Gamba, A., Mamprin, F., Fiocchi, R., Somaschini, M. and Ferrazzi, P. (1995) Reduction of haemorrhagic complications during mechanically assisted circulation with the use of a multi-system anticoagulation protocol. *International Journal of Artificial Organs* **18**, 649-655.

Gleason, C.A. (1993) ECMO and the brain. In: Arensman, R.M. and Cornish, J.D. (Eds.) *Extracorporeal life support*, pp. 138-155. Boston: Blackwell scientific publications]

Goldblum, S.E., Wu, K. and Jay, M. (1995) Lung myeloperoxidase as a measure of pulmonary leukostasis in rabbits. *Journal of Applied Physiology* **59**, 1978-1985.

Goldstein, G. and Luce, J.M. (1990) Pharmaocologic treatment of the adult respiratory distress syndrome. *Clinics in Chest Medicine* **11**, 773-787.

Goris, R.J., te Boekhorst, T.P., Nuytinck, J.K. and Gimbrere, J.S. (1985) Multiple-organ failure. Generalized autodestructive inflammation? *Archives of Surgery* **120**, 1109-1115.

Goris, R.J., Boekholtz, W.K., van Bebber, I.P., Nuytinck, J.K. and Schillings, P.H. (1986) Multiple-organ failure and sepsis without bacteria. An experimental model. *Archives of Surgery* **121**, 897-901.

Green, T.P., Timmons, O.D., Fackler, J.C., Moler, F.W., Thompson, A.E. and Sweeney, M.F. (1996) The impact of extracorporeal membrane oxygenation on survival in pediatric patients with acute respiratory failure. Pediatric Critical Care Study Group. *Critical Care Medicine* **24**, 323-329.

Greenough, A., Emery, E., Hird, M.F. and Gamsu, H.R. (1993) Randomised controlled trial of albumin infusion in ill preterm infants. *European Journal of Pediatrics* **152**, 157-159.

Greinacher, A., Eckhardt, T., Mussmann, J. and Mueller-Eckhardt, C. (1993a) Pregnancy complicated by heparin associated thrombocytopenia: management by a prospectively in vitro selected heparinoid (Org 10172). *Thrombosis Research* **71**, 123-126.

Greinacher, A., Philippen, K.H., Kemkes-Matthes, B., Mockl, M., Mueller-Eckhardt, C. and Schaefer, K. (1993b) Heparin-associated thrombocytopenia type II in a patient with end-stage renal disease: successful anticoagulation with the low-molecular-weight heparinoid Org 10172 during haemodialysis. *Nephrology, Dialysis, Transplantation* **8**, 1176-1177.

Grotendorst, G.R., Smale, G. and Pencev, D. (1989) Production of transforming growth factor beta by human peripheral blood monocytes and neutrophils. *Journal of Cell Physiology* **140**, 396-402.

Gu, Y.J., Wang, Y.S., Chiang, B.Y., Gao, X.D., Ye, C.X. and Wildevuur, C.R. (1991) Membrane oxygenator prevents lung reperfusion injury in canine cardiopulmonary bypass [see comments]. *Annals of Thoracic Surgery* **51**, 573-578.

Gulliver, G. (1846) *Works of William Hewson*, London: Sydenham Society.

Guzman, F., Hedley Brown, A., Been, M., Cook, S., Wren, C. and Richens, D. (1989) *Cardiorespiratory critical care.* London: Butterworths.

Hachenberg, T., Tenling, A., Nystrom, S.O., Tyden, H. and Hedenstierna, G. (1994) Ventilation-perfusion inequality in patients undergoing cardiac surgery. *Anesthesiology* **80**, 509-519.

Haeffner-Cavaillon, N., Cavaillon, J.M., Laude, M. and Kazatchkine, M.D. (1987) C3a (C3adesarg) induces production and release of interleukin-1 by cultured human monocytes. *Journal of Immunology* **139**, 794-799.

Haeffner-Cavaillon, N., Roussellier, N., Ponzio, O., Carreno, M.P., Laude, M., Carpentier, A. and Kazatchkine, M.D. (1989) Induction of interleukin-1 production in patients undergoing cardiopulmonary bypass. *Journal of Thoracic & Cardiovascular Surgery* **98**, 1100-1106.

Hakim, R.M., Briellatt, J., Lazarus, J.M. and Port, F.K. (1984) Complement activation and hypersensitivity reactions to dialysis membranes. *New England Journal of Medicine* **311**, 878-882.

Hakim, R.M. (1993) Complement activation by biomaterials. In: Anonymous *Cardiovascular Pathology*, No.3 (Suppl) edn. pp. 187s-197s. Elsevier Science]

Hallgren, R., Borg, T., Venge, P. and Modig, J. (1984) Signs of neutrophil and eosinophil activation in adult respiratory distress syndrome. *Critical Care Medicine* **12**, 14-18.

Hammerschmidt, D.E., Weaver, L.J., Hudson, L.D., Craddock, P.R. and Jacob, H.S. (1980) Association of complement activation and elevated plasma-C5a with adult respiratory distress syndrome. Pathophysiological relevance and possible prognostic value. *Lancet* **1**, 947-949.

Harder, M.P., Eijsman, L., Roozendaal, K.J., van Oeveren, W. and Wildevuur, C.R. (1991) Aprotinin reduces intraoperative and postoperative blood loss in membrane oxygenator cardiopulmonary bypass. *Annals of Thoracic Surgery* **51**, 936-941.

Harker, L.A., Marzec, U.M. and Ginsberg, M.H. (1983) Thrombospondin levels in plasma, platelets and urine in normal subjects, subjects receiving heparin, thoracotomy patients and patients undergoing cardiopulmonary bypass. *Thrombosis & Haemostasis* **50**, 40

Hegnhoj, J. and Schaffalitzky de Muckadell, O.B. (1985) An enzyme linked immunosorbent assay for measurements of lactoferrin in duodenal aspirates and other biological fluids. *Scandinavian Journal of Clinical & Laboratory Investigation* **45**, 489-495.

Heideman, M. and Hugli, T.E. (1984) Anaphylatoxin generation in multisystem organ failure. *Journal of Trauma* **24**, 1038-1043.

Hennessy, V.L., Jr., Hicks, R.E., Niewiarowski, S., Edmunds, L.H., Jr. and Colman, R.W. (1977) Function of human platelets during extracorporeal circulation. *American Journal of Physiology* **232**, H622-8.

Henson, P.M. (1981) Platelet activating factor (PAF) as a mediator of neutrophil-platelet interactions in inflammation. *Agents Action* **11**, 545-547.

Henwick, S., Koehler, M. and Patrick, C.C. (1993) Complications of bacteremia due to Stomatococcus mucilaginosus in neutropenic children. [Review] [25 refs]. *Clinical Infectious Diseases* **17**, 667-671.

Hernandez, L.A., Grisham, M.B., Twohig, B., Arfors, K.E., Harlan, J.M. and Granger, D.N. (1987) Role of neutrophils in ischemia-reperfusion-induced microvascular injury. *American Journal of Physiology* **253**, H699-703.

Hetland, G., Moen, O., Bergh, K., Hogasen, K., Hack, C.E., Mollnes, T.E. and Fosse, E. (1997) Both plasma and leucocyte associated C5a are essential for assesment of C5a generation in vivo. *Annals of Thoracic Surgery* **63**, 1076-80.

Heulitt, M.J. and Pickert, C.B. (1995) ECLS fluids, electrolytes and renal function. In: Zwischenberger, J.B. and Bartlett, R.H. (Eds.) *ECMO, extracorporeal cardiopulmonary support in critical care.* pp. 123-136. Michigan: Extracorporeal life support oranization]

Hill, J.D., O'Brien, T.G., Murray, J.J., Dontigny, L., Bramson, M.L., Osborn, J.J. and Gerbode, F. (1972) Prolonged extracorporeal oxygenation for acute post-traumatic respiratory failure (shock-lung syndrome). Use of the Bramson membrane lung. *New England Journal of Medicine* **286**, 629-634.

Hill, J.D., Rodvien, R., Snider, M.T. and Bartlett, R.H. (1978) Clinical extracorporeal membrane oxygenation for acute respiratory insufficiency. *Transactions - American Society for Artificial Internal Organs* **24**, 753-763.

Hirschl, R.B., Palmer, P., Heiss, K.F., Hultquist, K., Fazzalari, F., Bartlett and RH. (1993) Evaluation of the right atrial venous oxygen saturation as a physiologic monitor in a neonatal model. *Journal of Pediatric Surgery* **28**, 901-905.

Hirthler, M., Simoni, J. and Dickson, M. (1992) Elevated levels of endotoxin, oxygen-derived free radicals, and cytokines during extracorporeal membrane oxygenation. *Journal of Pediatric Surgery* **27**, 1199-1202.

Hirthler, M.A., Blackwell, E., Abbe, D., Doe-Chapman, R., LeClair Smith, C., Goldthorn, J. and Canizaro, P. (1992) Coagulation parameter instability as an early predictor of intracranial hemorrhage during extracorporeal membrane oxygenation. *Journal of Pediatric Surgery* **27**, 40-43.

Hochman, J.S., Boland, J., Sleeper, L.A., Porway, M., Brinker, J., Col, J., Jacobs, A, Slater, J., Miller, D., Wasserman, H. and et al. (1995) Current spectrum of cardiogenic shock and effect of early revascularization on mortality. Results of an International Registry. SHOCK Registry Investigators [see comments]. *Circulation* **91**, 873-881.

Hocker, J.R., Wellhausen, S.R., Ward, R.A., Simpson, P.M. and Cook, L.N. (1991) Effect of extracorporeal membrane oxygenation on leukocyte function in neonates. *Artificial Organs* **15**, 23-28.

Holley, D.G., Short, B.L., Karr, S.S. and Martin, G.R. (1994) Mechanisms of change in cardiac performance in infants undergoing extracorporeal membrane oxygenation. *Critical Care Medicine* **22**, 1865-1870.

Holmer, E., Lindahl, U. and Backstrom, G. (1980) Anticoagulant activities and effects on platelets of a heparin fraction with high affinity for antithrombin. *Thrombosis Research* **18**, 861

Howes, E.L., Jr. and McKay, D.G. (1975) Circulating immune complexes. Effects on ocular vascular permeability in the rabbit. *Archives of Ophthalmology* **93**, 365-370.

Huet, F., Semama, D., Grimaldi, M., Guignard, J.P. and Gouyon, J.B. (1995) Effects of theophylline on renal insufficiency in neonates with respiratory distress syndrome. *Intensive Care Medicine* **21**, 511-514.

Iacoviello, L., Kolpakov, V., Salvatore, L., Amore, C., Pintucci, G., de, Gaetano, G. and Donati, M.B. (1995) Human endothelial cell damage by neutrophil-derived cathepsin G. Role of cytoskeleton rearrangement and matrix-bound plasminogen activator inhibitor-1. *Arteriosclerosis, Thrombosis & Vascular Biology* **15**, 2037-2046.

Ichinose, Y., Hara, N., Ohta, M., Aso, H., Chikama, H., Kawasaki, M., Kubota, I., Shimizu, T. and Yagawa, K. (1990) Recombinant granulocyte colony-stimulating factor and lipopolysaccheride maintain the phenotype of and superoxide anion generation by neutrophils. *Infection and Immunity* **58**, 1647-1652.

Ignarro, L.J., Buga, G.M., Wood, K.S., Byrns, R.E. and Chaudhuri, G. (1987) Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proceedings of the National Academy of Sciences of the United States of America* **84**, 9265-9269.

Ing, T.S., Wong, F.K., Cheng, Y.L. and Potempa, L.D. (1995) The 'first-use syndrome' revisited: a dialysis centre's perspective. [Review] [66 refs]. *Nephrology, Dialysis, Transplantation* **10 Suppl 10**, 39-44.

Jaffe, M.Z. (1886) Ueber den Niederschlag, welchen pikrinsaure in normalen harn erzeugt eine reaction des kreatinins. *Zeitschrift Fuer Physiologische Chemie* **10**, 391-400.

Janatova, J., Cheung, A.K. and Parker, C.J. (1991) Biomedical polymers differ in their capacity to activate complement. *Complement & Inflammation* **8**, 61-69.

Janoff, A. and Scherer, J. (1968) Mediators of inflammation in leukocyte lysosomes. IX. Elastinolytic activity in granules of human polymorphonuclear leucocytes. *Journal of Experimental Medicine* **128**, 1137-1155.

Jansen, N.J., van Oeveren, W., van den Broek, L., Oudemans-van Straaten, H.M., Stoutenbeek, C.P., Joen, M.C., Roozendaal, K.J., Eysman, L. and Wildevuur, C.R. (1991) Inhibition by dexamethasone of the reperfusion phenomena in cardiopulmonary bypass [see comments]. *Journal of Thoracic & Cardiovascular Surgery* **102**, 515-525.

Joyce, L.D., Kiser, J.C. and Eales, F.C. (1990) Experience with the Sarns centrifugal pump as a ventricular assist device. *ASAIO Transactions* **36**, 619-623.

Kajita, T. and Hugli, T.E. (1990) C5a induced neutrophilia. A primary humoral mechanism for the recruitment of neuterophils. *American Journal of Pathology* **37**, 467-477.

Karges, H.E., Funk, K.A. and Ronneberger, H. (1994) Activity of coagulation and fibrinolysis parameters in animals. *Drug Research* **44(I)**, 793-797.

Kasama, T., Strieter, R.M., Standiford, T.J., Burdick, M.D. and Kunkel, S.L. (1993) Expression and regulation of human neutrophil-derived macrophage inflammatory protein 1a. *Journal of Experimental Medicine* **178**, 63-72.

Kawahito, K., Ino, T., Adachi, H., Ide, H., Mizuhara, A. and Yamaguci, A. (1994) Heparin coated percutaneous cardiopulmonary support for the treatment of circulatory collapse after cardiac surgery. *ASAIO Journal* **40**, 972-976.

Keeling, D.M., Richards, E.M. and Baglin, T.P. (1994) Platelet aggregation in response to four low molecular weight heparins and the heparinoid ORG 10172 in patients with heparin-induced thrombocytopenia. *British Journal of Haematology* **86**, 425-426.

Keh, D., Rossaint, R., Streich, R., Gerlach, H., Pappert, D., Kramer, H., Falke and KJ. (1995) Extracorporeal membrane oxygenation with heparin-coated systems in a 13-month-old infant with acute hypoxic respiratory failure after correction of tetralogy of Fallot. *European Journal of Cardio-Thoracic Surgery* **9**, 226-229.

Kelly, A.T., Short, B.L., Rains, T.C., May, J.C. and Progar, J.J. (1989) Aluminum toxicity and albumin. *ASAIO Transactions* **35**, 674-676.

Kes, P. (1994) Reprocessing and multiple use of hemodialyzers: a critical appraisal. *Acta Medica Croatica* **48**, 129-139.

Keszler, M., Subramanian, K.N., Smith, Y.A., Dhanireddy, R., Mehta, N., Molina, B., Cox, C.B. and Moront, M.G. (1989) Pulmonary management during extracorporeal membrane oxygenation. *Critical Care Medicine* **17**, 495-500.

Kikta, M.J., Keller, M.P., Humphrey, P.W. and Silver, D. (1993) Can low molecular weight heparins and heparinoids be safely given to patients with heparin-induced thrombocytopenia syndrome? *Surgery* **114**, 705-710.

Kinsella, J.P., Gerstmann, D.R. and Rosenberg, A.A. (1992) The effect of extracorporeal membrane oxygenation on coronary perfusion and regional blood flow distribution. *Pediatric Research* **31**, 80-84.

Kirklin, J.K., Westaby, S., Blackstone, E.H., Kirklin, J.W., Chenoweth, D.E. and Pacifico, A.D. (1983) Complement and the damaging effects of cardiopulmonary bypass. *Journal of Thoracic & Cardiovascular Surgery* **86**, 845-857.

Kirklin, J.W., Donald, D.E., Harshbarger, H.G., Hetzel, P.S., Patrick, R.T., Swan, H.J.C. and Wood, E.H. (1956) Studies in extracorporeal circulation. 1. Applicability of Gibbon-type pump-oxygenator to human intracardiac surgery: 40 cases. *Annals of Surgery* **144**, 2-8.

Kita, H., Ohnishi, T., Okubu, Y., Weiler, D., Abrahms, J.S. and Gleich, G.J. (1991) Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. *Journal of Experimental Medicine* **174**, 745-748.

Klein, M.D., Andrews, A.F., Wesley, J.R., Toomasian, J., Nixon, C., Roloff, D. and Bartlett, R.H. (1985) Venovenous perfusion in ECMO for newborn respiratory insufficiency. A clinical comparison with venoarterial perfusion. *Annals of Surgery* **201**, 520-526.

Klein, M.D., Shaheen, K.W., Whittlesey, G.C., Pinsky, W.W. and Arciniegas, E. (1990) Extracorporeal membrane oxygenation for the circulatory support of children after repair of congenital heart disease. *Journal of Thoracic & Cardiovascular Surgery* **100**, 498-505.

Klein, M.D. and Whittlesey, G.C. (1994) Extracorporeal membrane oxygenation. [Review] [118 refs]. *Pediatric Clinics of North America* **41**, 365-384.

Koksch, M., Rothe, G., Kiefel, V. and Schmitz, G. (1995) Fluorescence resonance energy transfer as a new method for the epitope-specific characterization of anti-platelet antibodies. *Journal of Immunological Methods* **187**, 53-67.

Kolff, W.J. and Effler, D.B. (1956) Disposable membrane oxygenator (heart lung machine) and its use in experimental and clinical surgery whilst the heart is arrested with potassium citrate according to the melrose technique. *ASAIO Transactions* **2**, 13-21.

Koller, M., Hilger, R.A. and Konig, W. (1996) Effect of the PAF-receptor antagonist SM-12502 on human platelets. *Inflammation* **20**, 71-85.

Kolls, J.K., Xie, J., Lei, D., Greenberg, S., Summer, W.R. and Nelson, S. (1995) Differential effects of in vivo ethanol on LPS-induced TNF and nitric oxide production in the lung. *American Journal of Physiology* **268**, L991-8.

Kolobow, T., Rossi, F., Borelli, M. and Foti, G. (1988) Long-term closed chest partial and total cardiopulmonary bypass by peripheral cannulation for severe right and/or left ventricular failure, including ventricular fibrillation. The use of a percutaneous spring in the pulmonary artery position to decompress the left heart. *ASAIO Transactions* **34**, 485-489.

Kolobow, T. and Bowman, R.L. (1963) Construction and evaluation of an alveolar membrane artificial heart lung. *ASAIO Transactions* **9**, 238

Komai, H., Yamamoto, F., Tanaka, K., Yagihara, T. and Kawashima, Y. (1994) Prevention of lung injury during open heart operations for congenital heart defects. *Annals of Thoracic Surgery* **57**, 134-140.

Koul, B., Vesterqvist, O., Egberg, N. and Steen, S. (1992) Twenty-four-hour heparin-free veno-right ventricular ECMO: an experimental study. *Annals of Thoracic Surgery* **53**, 1046-1051.

Kundu, S.K., Salley, S.O., Whittlesey, G.C. and Klein, M.D. (1989) Extracorporeal membrane oxygenation without anticoagulation: a study using quantitative scanning electron microscopy. *Journal of Laboratory & Clinical Medicine* **114**, 58-62.

Kurz, M., Greinix, H., Hocker, P., Kalhs, P., Knobl, P., Mayr, W.R., Pober, M. and Panzer, S. (1996) Specificities of anti-platelet antibodies in multitransfused patients with haemato-oncological disorders. *British Journal of Haematology* **95**, 564-569.

Langlois, P.F., Gawryl, M.S., Zeller, J. and Lint, T. (1989) Accentuated complement activation in patient plasma during adult respiratory distress syndrome: A potential mechanism for pulmonary inflammation. *Heart & Lung* **18**, 71-84.

LaRosa, C.A., Rohrer, M.J., Benoit, S.E., Rodino, L.J., Barnard, M.R. and Michelson, A.D. (1994) Human neutrophil cathepsin G is a potent platelet activator. *Journal of Vascular Surgery* **19**, 306-18; discussion 318-9.

Lassen, H.C.A. (1953) A preliminary report on the 1952 epidemic of poliomyelitis in Copenhagen with special reference to the treatment of acute respiratory insufficiency. *Lancet* **i**, 37-41.

Laufer, N., Merin, G. and Grover, N.B. (1975) The influence of cardiopulmonary bypass on the size of human platelets. *Journal of Thoracic & Cardiovascular Surgery* **70**, 727

Laurence, D.R. and Bennett, P.D. (1987) *Clinical Pharmacology*, London: Churchill Livingstone.

Lazar, E.L., Abramson, S.J., Weinstein, S. and Stolar, C.J. (1994) Neuroimaging of brain injury in neonates treated with extracorporeal membrane oxygenation: lessons learned from serial examinations. *Journal of Pediatric Surgery* **29**, 186-90; discussion 190-1.

Lazzara, R.R., Magovern, J.A., Benckart, D.H., Maher, T.D., Jr., Sakert, T., Magovern and GJ Jr. (1993) Extracorporeal membrane oxygenation for adult post cardiotomy cardiogenic shock using a heparin bonded system. *ASAIO Journal* **39**, M444-7.

Lee, W.H., Krumhaar, D., Fonkalsrud, E.W., Schjeide, O.A. and Maloney, J.V. (1961) Denaturation of plasma proteins as a cause of morbidity and death after cardiac operations. *Surgery* **50**, 29-39.

Leuschen, M.P., Ehrenfried, J.A., Willett, L.D., Schroder, K.A., Bussey, M.E., Bolam, DL, Goodrich, P.D. and Nelson, R.M., Jr. (1991) Prostaglandin F1 alpha levels during and after neonatal extracorporeal membrane oxygenation. *Journal of Thoracic & Cardiovascular Surgery* **101**, 148-152.

Leuschen, M.P., Willett, L.D., Hoie, E.B., Bolam, D.L., Bussey, M.E., Goodrich, P.D., Zach, T.L. and Nelson, R.M., Jr. (1993) Plasma fentanyl levels in infants undergoing extracorporeal membrane oxygenation. *Journal of Thoracic & Cardiovascular Surgery* **105**, 885-891.

Levy, M.S., Share, J.C., Fauza, D.O. and Wilson, J.M. (1995) Fate of the reconstructed carotid artery after extracorporeal membrane oxygenation. *Journal of Pediatric Surgery* **30**, 1046-1049.

Li, J., Oliver, J.R., Chong-Yuan, L. and Phillips, J.B., 3rd (1995) Delayed thromboxane or tumour necrosis factor alpha, but not leukotriene inhibition, attenuates prolonged pulmonary hypertension in endotoxemia. *American journal of the medical sciences* **310**, 103-110.

Li, J. and Guillory, R.J. (1997) Purified leukocyte cytochrome b558 incorporated into liposomes catalyzes a cytosolic factor dependent diaphorase activity. *Biochemistry* **36**, 5529-5537.

Li, Y.P., Mold, C. and Du Clos, T.W. (1994) Sublytic complement attack exposes C-reactive protein binding sites on cell membranes. *Journal of Immunology* **152**, 2995-3005.

Liem, K.D., Kollee, L.A., Hopman, J.C., De Haan, A.F. and Oeseburg, B. (1995) The influence of arterial carbon dioxide on cerebral oxygenation and haemodynamics during ECMO in normoxaemic and hypoxaemic piglets. *Acta Anaesthesiologica Scandinavica* **Supplementum. 107**, 157-164.

Liem, K.D., Kollee, L.A., Klaessens, J.H., De Haan, A.F. and Oeseburg, B. (1996) The influence of extracorporeal membrane oxygenation on cerebral oxygenation and hemodynamics in normoxemic and hypoxemic piglets. *Pediatric Research* **39**, 209-215.

Lijana, R.C. and Williams, M.C. (1979) Tetramethylbenzidine. A substitute for benzidine in hemoglobin analysis. *Journal of Laboratory and Clinical Medicine* **94**, 266-,

Lindeman, A., Riedel, D., Oster, W. and Ziegler-Heitbrock, H.W.L. (1989) Granulocyte-macrophage colony-stimulating factor induces cytokine secretion by human polymorphonuclear leucocytes. *Journal of Clinical Investigation* **83**, 1308-1312.

Lochan, S., Baumgart, S., Assadi, F., Frey, B., Marcus, S. and Adeniyi-Jones, S. (1997) Enhanced diuretic effect of lasix after priming with theophylliline in edematous babies on ECMO-randomized, blinded study. *13th annual CNMC symposium ECMO & advanced therapy for respiratory failure* 50(Abstract)

Lohrer, R.M., Bejar, R.F., Simko, A.J., Moulton, S.L. and Cornish, J.D. (1992) Internal carotid artery blood flow velocities before, during, and after extracorporeal membrane oxygenation. *American Journal of Diseases of Children* **146**, 201-207.

Longmore, D.B., Bennett, J.G., Hoyle, P.M., Smith, M.A., Gregory, A., Osivand, T. and Jones, W.A. (1981) Prostacyclin administration during cardiopulmonary bypass in man. *Lancet* **1**, 800-804.

Loop, F.D., Szabo, J., Rowlinson, R.D. and Urbanek, K. (1976) Events related to microembolism during extracorporeal perfusion in man: effectiveness of in-line filtration recorded by ultrasound. *Annals of Thoracic Surgery* **21**, 412-420.

Lord, P.C.W., Wilmoth, L.M.G., Mizel, S.B. and McCall, C.E. (1991) Expression of interleukin-1a and b genes by human blood polymorphonuclear leukocytes. *Journal of Clinical Investigation* **87**, 1312-1321.

Lyman, D.J., Metcalf, L.C., Albo, D.J., Richards, K.F. and Lamb, J. (1974) The effect of chemical structure and surface properties of synthetic polymers on the coagulation of blood. III. In vivo adsorption of proteins on polymer surfaces. *Transactions - American Society for Artificial Internal Organs* **20**, 474-479.

Lyons, A.S. and Petrucelli, R.J.I. (1978) *Medicine, an illustrated history*, New York: Abradale Press.

Macartney, H.W. and Tschesche, H. (1983) Latent and activate human polymorphonuclear collagenases. Isolation purification and characterisation. *European Journal of Biochemistry* **130**, 71-78.

Magnani, H.N. (1993) Heparin-induced thrombocytopenia (HIT): an overview of 230 patients treated with organon (Org 10172) [published erratum appears in Thromb Haemost 1993 Dec 20;70(6):1072]. *Thrombosis & Haemostasis* **70**, 554-561.

Maillet, F., Petitou, M., Choay, J. and Kazatchkine, M.D. (1988) Structure-function relationships in the inhibitory effect of heparin on complement activation: independency of the anti-coagulant and anti-complementary sites on the heparin molecule. *Molecular Immunology* **25**, 917-923.

Mancebo, J., Vallverdu, I., Bak, E., Dominguez, G., Subirana, M., Benito, S. and Net, A. (1994) Volume-controlled ventilation and pressure-controlled inverse ratio ventilation: a comparison of their effects in ARDS patients. *Monaldi Archives for Chest Disease* **49**, 201-207.

Marshall, W.J. (1988) *Clinical Chemistry*, London: Gower Medical Publishing.

Martin, G.R., Chauvin, L. and Short, B.L. (1991a) Effects of hydralazine on cardiac performance in infants receiving extracorporeal membrane oxygenation. *Journal of Pediatrics* **118**, 944-948.

Martin, G.R., Short, B.L., Abbott, C. and O'Brien, A.M. (1991b) Cardiac stun in infants undergoing extracorporeal membrane oxygenation [see comments]. *Journal of Thoracic & Cardiovascular Surgery* **101**, 607-611.

Martin, G.R. and Short, B.L. (1988) Doppler echocardiographic evaluation of cardiac performance in infants on prolonged extracorporeal membrane oxygenation. *American Journal of Cardiology* **62**, 929-934.

Marty, C., Misset, B., Tamion, F., Fitting, C., Carlet, J. and Cavaillon, J.M. (1994) Circulating interleukin-8 concentrations in patients with multiple organ failure of septic and nonseptic origin [see comments]. *Critical Care Medicine* **22**, 673-679.

Masters, R.G., Hendry, P.J., Davies, R.A., Smith, S., Struthers, C., Walley, V.M., Veinot, J.P., Mussivand, T.V. and Keon, W.J. (1996) Cardiac transplantation after mechanical circulatory support: a Canadian perspective. *Annals of Thoracic Surgery* **61**, 1734-1739.

Matsushima, K., Morishita, K., Yoshimura, T., Lavu, S., Kobayashi, Y., Lee, W., Appella, E., Kung, H.F., Leonard, E.J. and Openheim, J.J. (1988) Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin-1 and tumor necrosis factor. *Journal of Experimental Medicine* **167**, 1883-1893.

Matthiasson, S.E., Bergqvist, D., Lundell, A. and Lindblad, B. (1995) Effect of dextran and enoxaparin on early ePTFE graft thrombogenicity in sheep. *European Journal of Vascular & Endovascular Surgery* **9**, 284-292.

Matzner, Y., Barner, M., Yahalom, J., Ishai-Michaeli, R., Fuks, Z. and Vlodavsky, I. (1985) Degredation of heparan sulfate in the subendothelial extracellular matrix by a readily released heparinase from human neutrophils. Possible role for invasion through basement membranes. *Journal of Clinical Investigation* **76**, 1306-1313.

McDonald, J.A. and Kelley, D.G. (1980) Degradation of fibronectin by leukocytes elastase. Release of biologically active fragments. *Journal of Biology and Chemistry* **255**, 8848-8858.

McLean, J. (1959) The discovery of Heparin. *Circulation* **19**, 75

Mellgren, K., Friberg, L.G., Mellgren, G., Hedner, T., Wennmalm, A. and Wadenvik, H. (1996) Nitric oxide in the oxygenator sweep gas reduces platelet activation during experimental perfusion. *Annals of Thoracic Surgery* **61**, 1194-1198.

Meri, S. and Pangburn, M.K. (1990) Discrimination between activators and non-activators of the alternative pathway of complement: regulation via a sialic acid/poly-anion binding site on factor H. *Proceedings of the National Academy of Sciences of the United States of America* **87**, 3982-3986.

Meyer, D.M. and Jessen, M.E. (1995) Results of extracorporeal membrane oxygenation in neonates with sepsis. The Extracorporeal Life Support Organization experience. *Journal of Thoracic & Cardiovascular Surgery* **109**, 419-425; discussion 425-.

Mickelson, H.E., Qayumi, A.K., Jamieson, W.R., Smith, C.L., Gillespie, K.D., van den and Broek, J.E. (1990) Swine as a model for cardiovascular research: improved cardiopulmonary bypass techniques. *Journal of Investigative Surgery* **3**, 253-260.

Mitzner, S., Stange, J., Freytag, J., Lindemann, S. and Schmidt, R. (1996) Role of transport proteins in bioartificial liver assist systems. *International Journal of Artificial Organs* **19**, 49-52.

Moat, N.E., Shore, D.F. and Evans, T.W. (1993) Organ dysfunction and cardiopulmonary bypass: the role of complement and complement regulatory proteins [editorial]. [Review] [131 refs]. *European Journal of Cardio-Thoracic Surgery* **7**, 563-573.

Modig, J., Samuelsson, T. and Hallgren, R. (1986) The predictive and discriminative value of biologically active products of eosinophils, neutrophils and complement in bronchoalveolar lavage and blood in patients with adult respiratory distress syndrome. *Resuscitation* **14**, 121-134.

Moler, F.W., Palmisano, J.M., Custer, J.R., Meliones, J.N. and Bartlett, R.H. (1994) Alveolar-arterial oxygen gradients before extracorporeal life support for severe pediatric respiratory failure: improved outcome for extracorporeal life support-managed patients? *Critical Care Medicine* **22**, 620-625.

Moller, J., Gilman, J.T., Sussmane, J., Raszynski, A. and Wolfsdorf, J. (1993) Changes in plasma levels of oxygen radical scavenging enzymes during extracorporeal membrane oxygenation in a lamb model. *Biology of the Neonate* **64**, 134-139.

Montoya, J.P., Merz, S.I. and Bartlett, R.H. (1991) A standardized system for describing flow/pressure relationships in vascular access devices. *ASAIO Transcripts* **37**, 4-8.

Moon, Y.S., Ohtsubo, S., Gomez, M.R., Moon, J.K. and Nose, Y. (1996) Comparison of centrifugal and roller pump hemolysis rates at low flow. *Artificial Organs* **20**, 579-581.

Moreno-Cabral, R.J., Dembitsky, W.P., Adamson, R.M. and Daily, P.O. (1994) Percutaneous extracorporeal membrane oxygenation. [Review] [15 refs]. *Advances in Cardiac Surgery* **5**, 163-179.

Morgan, B.P. (1989) Complement membrane attack on nucleated cells: resistance, recovery and non-lethal effects. *Biochemical Journal* **264**, 1-14.

Morishita, K., Tsuchiya, M., Asano, S., Kaziro, Y. and Nagata, S. (1987) Chromosomal gene structure of human myeloperoxidase and regulation of its expression by granulocyte colony-stimulating factor. *Journal of Biology and Chemistry* **262**, 15208-15213.

Morris, A.H., Wallace, C.J., Menlove, R.L., Clemmer, T.P., Orme, J.F., Jr., Weaver, L.K., Dean, N.C., Thomas, F., East, T.D., Pace, N.L. and et al. (1994) Randomized clinical trial of pressure-controlled inverse ratio ventilation and extracorporeal CO2 removal for adult respiratory distress syndrome [see comments] [published erratum appears in Am J Respir Crit Care Med 1994 Mar;149(3 Pt 1):838]. *American Journal of Respiratory & Critical Care Medicine* **149**, 295-305.

Mortensen, J., Bagley, B., Cosgrove, W., Yates, W. and Daniels, A. (1979) Durability of tubing subjected to roller pumps in extracorporeal blood perfusion systems. *Artificial Organs* 442-7.

Morton, A. (1975) Some observations on infective shock. *Anaesthesia & Intensive Care* **3**, 95-100.

Moulton, S.L., Delius, R.E. and Arensman, R.M. (1993) Vascular access for extracorporeal life support. In: Arensman, R.M. and Cornish, J.D. (Eds.) *Extracorporeal life support*, pp. 175-194. Boston: Blackwell scientific publications.]

Muller-Eberhard, H.J. (1984) The membrane attack complex. *Springer Seminars in Immunopathology* **7**, 93-141.

Murakami, T., Kino, K., Irie, H., Kioka, Y., Indo, S., Kawakami, S., Yamada, M. and Shimizu, N. (1994) Results of circulatory support for postoperative cardiogenic shock. *Artificial Organs* **18**, 691-697.

Murata, Y., Fujimori, K., Quilligan, E.J., Nagata, N., Ibara, S., Hirano, T. and Kamimura, T. (1996) Cardiac oxygenation by extracorporeal membrane oxygenation in exteriorized fetal lambs. *American Journal of Obstetrics & Gynecology* **174**, 864-870.

Murray, J.F., Matthay, M.A., Luce, J.M. and Flick, M.R. (1988) An expanded definition of the adult respiratory distress syndrome [published erratum appears in Am Rev Respir Dis 1989 Apr; 139(4):1065]. *American Review of Respiratory Disease* **138**, 720-723.

Musial, J., Niewiarowski, S. and Hershock, D. (1985) Loss of fibrinogen receptors from the platelet surface during simulated extracorproeal circulation. *Journal of Laboratory & Clinical Medicine* **105**, 514-522.

Musial, J., Niewiarowski, S., Rucinski, B., Stewart, G.J., Cook, J.J., Williams, JA and Edmunds, L.H., Jr. (1990) Inhibition of platelet adhesion to surfaces of extracorporeal circuits by disintegrins. RGD-containing peptides from viper venoms. *Circulation* **82**, 261-273.

Nathan, C., Srimal, S., Farber, C., Sanchez, E., Kabbash, L., Asch, A., Gailit, J. and Wright, S.D. (1989) Cytokine-induced respiratory burst and CD11/CD18 integrins. *Journal of Cell Biology* **109**, 1341-1349.

Nilsson, L., Brunnkvist, S., Nilsson, U., Nystrom, S.O., Tyden, H., Venge, P. and Aberg, T. (1988) Activation of inflammatory systems during cardiopulmonary bypass. *Scandinavian Journal of Thoracic & Cardiovascular Surgery* **22**, 51-53.

Nilsson, L., Bagge, L. and Nystrom, S.O. (1990a) Blood cell trauma and postoperative bleeding: comparison of bubble and membrane oxygenators and observations on coronary suction. *Scandinavian Journal of Thoracic & Cardiovascular Surgery* **24**, 65-69.

Nilsson, L., Nilsson, U., Venge, P., Johansson, O., Tyden, H., Aberg, T. and Nystrom, S.O. (1990b) Inflammatory system activation during cardiopulmonary bypass as an indicator of biocompatibility: a randomized comparison of bubble and membrane oxygenators. *Scandinavian Journal of Thoracic & Cardiovascular Surgery* **24**, 53-58.

Nilsson, L., Tyden, H., Johansson, O., Nilsson, U., Ronquist, G., Venge, P., Aberg, T. and Nystrom, S.O. (1990c) Bubble and membrane oxygenators--comparison of postoperative organ dysfunction with special reference to inflammatory activity. *Scandinavian Journal of Thoracic & Cardiovascular Surgery* **24**, 59-64.

Nishina, T., Okabayashi, H., Shimada, I., Ohno, N., Minatoya, K., Kameyama, N., Soga, Y. and Matsubayashi, K. (1995) [A successful case of emergency CABG using PCPS due to acute myocardial infarction]. [Japanese]. *Kyobu Geka - Japanese Journal of Thoracic Surgery* **48**, 773-775.

Nojiri, C., Hagiwara, K., Yokoyama, K., Kurbayashi, E., Hidaka, K., Ishida, N., Horiuchi, K., Oshiyama, H., Nogawa, A., Kido, T. and et al. (1995) Evaluation of a new heparin bonding process in prolonged extracorporeal membrane oxygenation. *ASAIO Journal* **41**, M561-7.

Nowlen, T.T., Salley, S.O., Whittlesey, G.C., Kundu, S.K., Maniaci, N.A., Henry, R.L. and Klein, M.D. (1989) Regional blood flow distribution during extracorporeal membrane oxygenation in rabbits. *Journal of Thoracic & Cardiovascular Surgery* **98**, 1138-1143.

O'Rourke, P.P., Crone, R.K., Vacanti, J.P., Ware, J.H., Lillehei, C.W., Parad, R.B. and Epstein, M.F. (1989) Extracorporeal membrane oxygenation and conventional medical therapy in neonates with persistent pulmonary hypertension of the newborn: a prospective randomized study [see comments]. *Pediatrics* **84**, 957-963.

Orenstein, J.M., Sato, N., Aaron, B., Buchholz, B. and Bloom, S. (1982) Microemboli observed in deaths following cardiopulmonary bypass surgery: silicone antifoam agents and polyvinyl chloride tubing as sources of emboli. *Human Pathology* **13**, 1082-1090.

Ostgaard, G. and Onarheim, H. (1996) Retention and distribution of polygeline (Haemaccel) in the rat. *Acta Anaesthesiologica Scandinavica* **40**, 96-101.

Page, U.S., Bigelow, J.C., Carter, C.R. and Swank, R.L. (1974) Emboli (debris) produced by bubble oxygenators. Removal by filtration. *Annals of Thoracic Surgery* **18**, 164-171.

Palmer, K., Ehren, H., Benz, R. and Frenckner, B. (1995) Carmeda surface heparinization in neonatal ECMO systems: long-term experiments in a sheep model. *Perfusion* **10**, 307-313.

Palmer, R.M., Ashton, D.S. and Moncada, S. (1988) Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* **333**, 664-666.

Pangburn, M.K., Schreiber, R.D. and Muller-Eberhard, H.J. (1981) Formation of the initial C3 convertase of the alternative complement pathway: Aquisition of C3b-like activities by spontaneous hydrolysis of the putative thiolester in native C3. *Journal of Experimental Medicine* **154**, 856-867.

Papadakos, P.J. and Apostolakos, M.J. (1996) High-inflation pressure and positive end-expiratory pressure. Injurious to the lung? Yes. [Review] [29 refs]. *Critical Care Clinics* **12**, 627-634.

Pardi, R., Inveradi, L. and Bender, J.R. (1992) Regulatory mechanisms in leukocyte adhesion: flexible receptors for sophisticated travelers. *Immunology Today* **13**, 224-229.

Pavie, A., Leger, P., Regan, M., Nataf, P., Bors, V., Szefner, J., Cabrol, C. and Gandjbakhch, I. (1995) Clinical experience with a total artificial heart as a bridge for transplantation: the La Pitie experience. *Journal of Cardiac Surgery* **10**, 552-558.

Pedersen, T.H., Videm, V., Svennevig, J.L., Karlsen, H., Ostbakk, R.W., Oyestein, J. and Mollnes, T.E. (1997) Extracorporeal Membrane Oxygenation using a centrifugal pump and a servo regulator to prevent negative inlet pressure. *Annals of Thoracic Surgery* **63**, 1333-1339.

Peek, G.J., Firmin, R.K., Moore, H.M. and Sosnowski, A.W. (1996a) Cannulation of neonates for venovenous extracorporeal life support. *Annals of Thoracic Surgery* **61**, 1851-1852.

Peek, G.J., Firmin, R.K. and Sosnowski, A.W. (1996b) Limb perfusion during cardiopulmonary support [letter; comment]. *Annals of Thoracic Surgery* **61**, 1291-1292.

Peek, G.J., Moore, H.M., Moore, N., Sosnowski, A.W. and Firmin, R.K. (1997) Extracorporeal Membrane Oxygenation for Adult Respiratory Failure. *Chest* **112**, 759-764.

Pennington, D.G., McBride, L.R. and Swartz, M.T. (1989) Use of the Pierce-Donachy ventricular assist device in patients with cardiogenic shock after cardiac operations. *Annals of Thoracic Surgery* **47**, 130-135.

Pennington, D.G. and Swartz, M.T. (1995) Mechanical circulatory support: patient and device selection. In: Lewis, T. and Graham, T.R. (Eds.) *Mechanical circulatory support.* pp. 159-168. London: Edward Arnold]

Perchinsky, M.J., Long, W.B., Hill, J.G., Parsons, J.A. and Bennett, J.B. (1995) Extracorporeal cardiopulmonary life support with heparin-bonded circuitry in the resuscitation of massively injured trauma patients. *American Journal of Surgery* **169**, 488-491.

Pevri, P., Walz, A., Dewald, B. and Baggiolini, M. (1988) A novel neutrophil-activating factor produced by human mononuclear phagocytes. *Journal of Experimental Medicine* **167**, 1547-1559.

Pierce, W.S., Sapirstein, J.S. and Pae, W.E., Jr. (1996) Total artificial heart: from bridge to transplantation to permanent use. *Annals of Thoracic Surgery* **61**, 342-346.

Pingleton, W.W., Coalson, J.J. and Guenter, C.A. (1975) Significance of leukocytes in endotoxic shock. *Experimental & Molecular Pathology* **22**, 183-194.

Plotz, F.B., van Oeveren, W., Aloe, L.S., Riley, M.K., Hultquist, K.A., Bartlett, RH and Wildevuur, C.R. (1991) Prophylactic administration of tranexamic acid preserves platelet numbers during extracorporeal circulation in rabbits. *ASAIO Transactions* **37**, M416-7.

Plotz, F.B., van Oeveren, W., Bartlett, R.H. and Wildevuur, C.R. (1993) Blood activation during neonatal extracorporeal life support. *Journal of Thoracic & Cardiovascular Surgery* **105**, 823-832.

Pollack, M.M., Ruttimann, U.E., Getson, P.R. and et al (1988) The pediatric risk of mortality (PRISM) score. *Critical Care Medicine* **16**, 1110-1116.

Prescott, S.M., Zimmerman, G.A. and Seeger, A.R. (1984) Leukotriene B4 is an incomplete agonist for the activation of human neutrophils. *Biochemical and Biophysical Research Communications* **122**, 535-541.

Purohit, D.M., Swindle, M.M., Smith, C.D., Othersen, H.B., Jr. and Kazanovicz, J.M. (1993) Hanford miniature swine model for extracorporeal membrane oxygenation. *Journal of Investigative Surgery* **6**, 503-508.

Rabhi-Sabile, S., Pidard, D., Lawler, J., Renesto, P., Chignard, M. and Legrand, C. (1996) Proteolysis of thrombospondin during cathepsin-G-induced platelet aggregation: functional role of the 165-kDa carboxy-terminal fragment. *FEBS Letters* **386**, 82-86.

Rady, M.Y., Kirkman, E., Cranley, J. and Little, R.A. (1996) Nociceptive somatic nerve stimulation and skeletal muscle injury modify systemic hemodynamics and oxygen transport and utilization after resuscitation from hemorrhage. *Critical Care Medicine* **24**, 623-630.

Rais-Bahrami, K., Rivera, O., Mikesell, G.T., Seale, W.R., Sell, J.E. and Short, B.L. (1995) Improved oxygenation with reduced recirculation during venovenous extracorporeal membrane oxygenation: evaluation of a test catheter. *Critical Care Medicine* **23**, 1722-1725.

Reed, C.C., Romagnoli, A., Taylor, D.E. and Clark, D.K. (1974) Particulate matter in bubble oxygenators. *Journal of Thoracic & Cardiovascular Surgery* **68**, 971-974.

Remuzzi, A. and Boccardo, P. (1993) Albumin treatment reduces in vitro platelet deposition to PMMA dialysis membrane. *International Journal of Artificial Organs* **16**, 128-31.

Revenis, M.E., Glass, P. and Short, B.L. (1992) Mortality and morbidity rates among lower birth weight infants (2000 to 2500 grams) treated with extracorporeal membrane oxygenation. *Journal of Pediatrics* **121**, 452-458.

Richardson, B.W. (1865) An enquiry into the possibility of restoring the life of warm blooded animals in certain cases where respiration, the circulation and the ordinary manifestations of organic motion are exhausted or have ceased. *Proceedings of the Royal Society of London*

Rivera, R.A., Butt, W. and Shann, F. (1990) Predictors of mortality in children with respiratory failure: possible indications for ECMO. *Anaesthesia & Intensive Care* **18**, 385-389.

Roberts, C.S., D'Armini, A.M. and Egan, T.M. (1996) Canine double-lung transplantation with cadaveric donors. *Journal of Thoracic & Cardiovascular Surgery* **112**, 577-583.

Robinson, T.M., Kickler, T.S., Walker, L.K., Ness, P. and Bell, W. (1993) Effect of extracorporeal membrane oxygenation on platelets in newborns. *Critical Care Medicine* **21**, 1029-1034.

Rock, C.S. and Lowry, S.F. (1991) Current research review. Tumor necrosis factor-alpha. *Journal of Surgical Research* **51**, 434-445.

Roitt, I. (1994) *Essential Immunology*, 8th edn. London: Blackwell.

Roohk, H.V., Pick, J., Hill, R., Hung, E. and Bartlett, R.H. (1976) Kinetics of fibrinogen and platelet adherence to biomaterials. *Transactions - American Society for Artificial Internal Organs* **22**, 1-8.

Rossaint, R., Slama, K., Lewandowski, K., Streich, R., Henin, P., Hopfe, T., Barth, H., Nienhaus, M., Weidemann, H., Lemmens, P. and et al. (1992) Extracorporeal lung assist with heparin-coated systems. *International Journal of Artificial Organs* **15**, 29-34.

Roupie, E., Dambrosio, M., Servillo, G., Mentec, H., el Atrous, S., Beydon, L., Brun-Buisson, C., Lemaire, F. and Brochard, L. (1995) Titration of tidal volume and induced hypercapnia in acute respiratory distress syndrome. *American Journal of Respiratory & Critical Care Medicine* **152**, 121-128.

Royston, B.D., Royston, D. and Pearson, J.D. (1992) Aprotinin inhibits platelet adhesion to endothelial cells. *Blood Coagulation & Fibrinolysis* **3**, 737-742.

Royston, D. (1990) The serine antiprotease aprotinin (Trasylol): a novel approach to reducing postoperative bleeding. [Review] [74 refs]. *Blood Coagulation & Fibrinolysis* **1**, 55-69.

Sakamaki, F., Ishizaka, A., Handa, M., Fujishima, S., Urano, T., Sayama, K., Nakamura, H., Kanazawa, M., Kawashiro, T., Katayama, M. and et al. (1995) Soluble form of P-selectin in plasma is elevated in acute lung injury. *American Journal of Respiratory & Critical Care Medicine* **151**, 1821-1826.

Salama, A., Hugo, F., Heinrich, D., Hoge, R., Muller, R., Kiefel, V., Mueller-Eckhardt, C. and Bhakdi, S. (1988) Deposition of terminal C5b-9 complement complexes on erythrocytes and leukocytes during cardiopulmonary bypass. *New England Journal of Medicine* **318**, 408-414.

Salzman, E.W., Rosenberg, R.D. and Smith, M.H. (1980) Effect of heparin and heparin fractions on platelet aggregation. *Journal of Clinical Investigation* **65**, 64

Salzman, E.W. and Merrill, E.W. (1987) Interaction of blood with artificial surfaces. In: Colman, R.W. (Ed.) *Hemostasis & Thrombosis*, pp. 1335-1347. J.B.Lippincott]

Samama, M.M., Bara, L. and Gouin-Thibault, I. (1996) New data on the pharmacology of heparin and low molecular weight heparins. [Review] [24 refs]. *Drugs* **52 Suppl 7**, 8-14; discussion 14-5.

Scala, J.L., Jew, R.K., Lyons, S., Kearney, M., Polin, R. and Poon, C.Y. (1996) In vitro analysis of furosemide disposition during neonatal extracorporeal membrane oxygenation. *12th annual CNMC symposium on ECMO & advanced therapies for respiratory failure.* 51(Abstract)

Schierwagen, C., Bylund-Fellenius, A. and Lundberg, C. (1990) Improved Method for Quantification of Tissue PMN Accumulation Measured by Myeloperoxidase Activity. *Journal of Pharmacological Methods* **23**, 179-186.

Schiffman, E., Corcoran, B.A. and Wahl, S.A. (1975) N-formylmethionyl peptides as chemoattractants for leukocytes. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 1059-1062.

Schilero, G.J., Oropello, J. and Benjamin, E. (1995) Impairment in gas exchange after granulocyte colony stimulating factor (G-CSF) in a patient with the adult respiratory distress syndrome [see comments]. *Chest* **107**, 276-278.

Schreiber, R.D. and Muller-Eberhard, H.J. (1979) Complement and renal disease. In: Wilson, C.B., Brenner, B.M. and Stein, J.H. (Eds.) *Immunologic mechanisms of renal disease*, pp. 67-105. New York: Churchill Livingstone]

Schueler, S., De Valeria, P.A., Hatanaka, M., Cameron, D.E., Bando, K., Zeebley, M, Hutchins, G.M., Reitz, B.A. and Baumgartner, W.A. (1992) Successful twenty-four-hour lung preservation with donor core cooling and leukocyte depletion in an orthotopic double lung transplantation model. *Journal of Thoracic & Cardiovascular Surgery* **104**, 73-82.

Schumacher, R.E., Barks, J.D., Johnston, M.V., Donn, S.M., Scher, M.S., Roloff, D.W. and Bartlett, R.H. (1988) Right-sided brain lesions in infants following extracorporeal membrane oxygenation. *Pediatrics* **82**, 155-161.

Seldinger, S.E. (1953) Catheter replacement of the needle in percutaneous arteriography. *Acta Radiologica* **39**, 368-376.

Sessler, C.N., Windsor, A.C., Schwartz, M., Watson, L., Fisher, B.J., Sugerman, H.J. and Fowler, A.A.I. (1995) Circulating ICAM-1 is increased in septic shock. *American Journal of Respiratory & Critical Care Medicine* **151**, 1420-1427.

Shalaby, M.R., Aggarwal, B.B., Rinderknecht, E., Svedersky, L.P., Finkle, B.S. and Palladino, M.A.J. (1985) Activation of human polymorphonuclear neutrophil functions by interferon-gamma and tumor necrosis factors. *Journal of Immunology* **135**, 2069-2073.

Shann, F., Duncan, A., Butt, W., Henning, R., South, M. and Tibballs, J. (1994) *Drug doses.* 8th edn. Melbourne: Collective Pty Ltd.

Shapiro, A. (1995) Antithrombin deficiency in special clinical syndromes--Part I: neonatal and pediatric/physiologic deficiency: extracorporeal membrane oxygenation. [Review] [10 refs]. *Seminars in Hematology* **32**, 33-36.

Shatney, C.H., Dietzman, R.H. and Lillehei, R.C. (1976) The effects of corticosteroids on systemic oxygenation and pulmonary shunting in septic shock. pp. 92-101. [Review] [29 refs]. *In: Shoemaker WC, Tavares BM, ed* **Current topics in Critical Care Medicine**,

Shigeta, O., Gluszko, P., Downing, S.W., Lu, W., Niewiarowski, S., Edmunds, L.H. and Jr. (1992) Protection of platelets during long-term extracorporeal membrane oxygenation in sheep with a single dose of a disintegrin. *Circulation* **86**, II398-404.

Short, B.L. (1993a) Clinical management of the neonatal ECMO patient. In: Arensman, R.M. and Cornish, J.D. (Eds.) *Extracorporeal life support.* pp. 195-206. Boston. Blackwell scientific publications.]

Short, B.L. (1993b) Pre-ECMO considerations for neonatal patients. In: Arensman, R.M. and Cornish, J.D. (Eds.) *Extracorporeal life support.* pp. 156-174. Boston: Blackwell scientific publications]

Short, B.L., Bender, K., Walker, L.K. and Traystman, R.J. (1994a) The cerebrovascular response to prolonged hypoxia with carotid artery and jugular vein ligation in the newborn lamb. *Journal of Pediatric Surgery* **29**, 887-891.

Short, B.L., Walker, L.K. and Traystman, R.J. (1994b) Impaired cerebral autoregulation in the newborn lamb during recovery from severe, prolonged hypoxia, combined with carotid artery and jugular vein ligation [see comments]. *Critical Care Medicine* **22**, 1262-1268.

Shumway, N. (1993) The development of heart and heart-lung transplantation at Stanford. Honored guest's lecture [editorial]. *European Journal of Cardio-Thoracic Surgery* **7**, 5-7.

Sinard, J.M., Merz, S.I., Hatcher, M.D., Montoya, J.P. and Bartlett, R.H. (1991) Evaluation of extracorporeal perfusion catheters using a standardized measurement technique--the M-number. *ASAIO Transactions* **37**, 60-64.

Skinner, R., Abrahams, J.P., Whisstock, J.C., Lesk, A.M., Carrell, R.W., Wardell and MR. (1997) The 2.6 A structure of antithrombin indicates a conformational change at the heparin binding site. *Journal of Molecular Biology* **266**, 601-609.

Smith, H.G., Whittlesey, G.C., Kundu, S.K., Salley, S.O., Kuhns, L.R., Chang, C.H. and Klein, M.D. (1989) Regional blood flow during extracorporeal membrane oxygenation in lambs. *ASAIO Transactions* **35**, 657-660.

Snyder, E.L., Mechanic, S., Baril, L. and Davenport, R. (1996) Removal of soluble biologic response modifiers (complement and chemokines) by a bedside white cell-reduction filter. *Transfusion* **36**, 707-713.

Solis, R.T., Kennedy, P.S., Beall, A.C., Jr., Noon, G.P. and DeBakey, M.E. (1975) Cardiopulmonary bypass. Microembolization and platelet aggregation. *Circulation* **52**, 103-108.

Solomon, H.M., Randall, J.R. and Simmons, V.L. (1995) Heparin-induced increase in the international normalized ratio. Responses of 10 commercial thromboplastin reagents [see comments]. *American Journal of Clinical Pathology* **103**, 735-739.

Souhami, R.L. and Moxham, J. (1994) *Textbook of Medicine*, 2nd edn. Edinburgh: Churchill Livingstone.

Stallion, A., Cofer, B.R., Rafferty, J.A., Ziegler, M.M. and Ryckman, F.C. (1994) The significant relationship between platelet count and haemorrhagic complications on ECMO. *Perfusion* **9**, 265-269.

Standefeder, J.C. and Vanderjagt, D. (1977) Use of tetramethylbenzidine in plasma hemoglobin assay. *Clinical Chemistry* **23**, 749-,

Starnes, V.A., Barr, M.L., Cohen, R.G., Hagen, J.A., Wells, W.J., Horn, M.V., Schenkel and FA. (1996) Living-donor lobar lung transplantation experience: intermediate results. *Journal of Thoracic & Cardiovascular Surgery* **112**, 1284-90; discussion 1290-.

Steinhorn, R.H., Isham-Schopf, B., Smith, C. and Green, T.P. (1989) Hemolysis during long-term extracorporeal membrane oxygenation. *Journal of Pediatrics* **115**, 625-630.

Steinmeyer, J. and Kalbhen, D.A. (1996) The inhibitory effects of antirheumatic drugs on the activity of human leukocyte elastase and cathepsin G. *Inflammation Research* **45**, 324-329.

Streletz, L.J., Bej, M.D., Graziani, L.J., Desai, H.J., Beacham, S.G., Cullen, J. and Spitzer, A.R. (1992) Utility of serial EEGs in neonates during extracorporeal membrane oxygenation. *Pediatric Neurology* **8**, 190-196.

Sugiura, M., McCulloch, P.R., Wren, S., Dawson, R.H. and Froese, A.B. (1994) Ventilator pattern influences neutrophil influx and activation in atelectasis-prone rabbit lung. *Journal of Applied Physiology* **77**, 1355-1365.

Sukura, A., Konttinen, Y.T., Sepper, R., Kaartinen, L., Sorsa, T. and Lindberg, L.A. (1995) Collagenases and the serine proteinases elastase and cathepsin G in steroid-induced Pneumocystis carinii pneumonia. *Journal of Clinical Microbiology* **33**, 829-834.

Szefner, J. (1995) Control and treatment of hemostasis in cardiovascular surgery. The experience of La Pitie Hospital with patients on total artificial heart. *International Journal of Artificial Organs* **18**, 633-648.

Takeda, A., Okumura, S., Miyamoto, T., Hagio, M. and Fujinaga, T. (1995) Comparison of extravascular lung water volume with radiographic findings in dogs with experimentally increased permeability pulmonary edema. *Journal of Veterinary Medical Science* **57**, 481-485.

Tamburro, R.F., Bugnitz, M.C. and Stidham, G.L. (1991) Alveolar-arterial oxygen gradient as a predictor of outcome in patients with nonneonatal pediatric respiratory failure [see comments]. *Journal of Pediatrics* **119**, 935-938.

Tatsumi, E., Takano, H., Taenaka, Y., Akagi, H., Nakatani, T., Sasaki, E., Goto, M, Masuzawa, T., Sakaki, M., Matsuo, Y. and et al. (1991) An integrated artificial heart-lung device. *ASAIO Transactions* **37**, M301-3.

Taylor, G.A., Lotze, A., Kapur, S. and Short, B.L. (1986) Diffuse pulmonary opacification in infants undergoing extracorporeal membrane oxygenation: clinical and pathological correlations. *Radiology* **161**, 347-50.

Taylor, G.A., Short, B.L. and Fitz, C.R. (1989) Imaging of cerebrovascular injury in infants treated with extracorporeal membrane oxygenation [see comments]. *Journal of Pediatrics* **114**, 635-639.

Taylor, K.M. (1995) Pulsatile and non-pulsatile perfusion in cardiac surgery: the continuing controversy. In: Lewis, T. and Graham, T.R. (Eds.) *Mechanical circulatory support*, pp. 105-117. London: Edward Arnold]

Terashima, T., Kanazawa, M., Sayama, K., Urano, T., Sakamaki, F., Nakamura, H., Waki, Y., Soejima, K., Tasaka, S. and Ishizaka, A. (1995) Neutrophil-induced lung protection and injury are dependent on the amount of Pseudomonas aeruginosa administered via airways in guinea pigs. *American Journal of Respiratory & Critical Care Medicine* **152**, 2150-2156.

Tervahartiala, T., Konttinen, Y.T., INgman, T., Hayrinen-Immonen, R., Ding, Y. and Sorsa, T. (1996) Cathepsin G in gingival tissue and crevicular fluid in adult periodontitis. *Journal of Clinical Periodontology* **23**, 68-75.

Till, G.O., Johnson, K.J., Kunkel, R. and Ward, P.A. (1982) Intravascular activation of complement and acute lung injury. Dependency on neutrophils and toxic oxygen metabolites. *Journal of Clinical Investigation* **69**, 1126-1135.

Timmons, O.D., Havens, P.L. and Fackler, J.C. (1995) Predicting death in pediatric patients with acute respiratory failure. Pediatric Critical Care Study Group. Extracorporeal Life Support Organization. *Chest* **108**, 789-797.

Tonnessen, T., Tollofsrud, S., Kongsgaard, U.E. and Noddeland, H. (1993) Colloid osmotic pressure of plasma replacement fluids. *Acta Anaesthesiologica Scandinavica* **37**, 424-426.

Tracey, K.J., Vlassara, H. and Cerami, A. (1989) Cachectin / tumour necrosis factor. *Lancet* **1**, 1122-1126.

Treasure, T. (1994) A surgeon's view of adult respiratory distress syndrome. [Review] [24 refs]. *British Journal of Hospital Medicine* **52**, 108-114.

Tsai, C.C., Frautschi, J.R. and Eberhart, R.C. (1988) Enhanced albumin affinity of silicone rubber. *ASAIO Transactions* **34**, 559-563.

Tsai, C.C., Huo, H.H., Kulkarni, P. and Eberhart, R.C. (1990) Biocompatible coatings with high albumin affinity. *ASAIO Transactions* **36**, M307-10.

Tsuno, K., Miura, K., Takeya, M., Kolobow, T. and Morioka, T. (1991) Histopathologic pulmonary changes from mechanical ventilation at high peak airway pressures. *American Review of Respiratory Disease* **143**, 1115-1120.

Tulunay, M., Demiralp, S., Tastan, S., Akalin, H., Ozyurda, U., Corapcioglu, T. and Akarsu, E.S. (1993) Complement (C3, C4) and C-reactive protein responses to cardiopulmonary bypass and protamine administration. *Anaesthesia & Intensive Care* **21**, 50-55.

Underwood, M.J., Pearson, J.A., Waggoner, J., Lunec, J., Firmin, R.K. and Elliot, M.J. (1995) Changes in "inflammatory" mediators and total body water during extra-corporeal membrane oxygenation (ECMO). A preliminary study. *International Journal of Artificial Organs* **18**, 627-632.

Uretzky, G., Landsburg, G., Cohn, D., Wax, Y. and Borman, J.B. (1987) Analysis of microembolic particles originating in extra-corporeal circuits. *Perfusion* **2**, 9-17.

Urlesberger, B., Zobel, G., Zenz, W., Kuttnig-Haim, M., Maurer, U., Reiterer, F., Riccabona, M., Dacar, D., Gallisti, S., Leschnik, B. and Muntean, W. (1996) Activation of the clotting system during extracorporeal membrane oxygenation in term newborn infants. *Journal of Pediatrics* **129**, 264-268.

Van Meurs, K.P., Rhine, W.D., Benitz, W.E., Shochat, S.J., Hartman, G.E., Sheehan, AM and Starnes, V.A. (1994) Lobar lung transplantation as a treatment for congenital diaphragmatic hernia. *Journal of Pediatric Surgery* **29**, 1557-1560.

van Oeveren, W., Dankert, J. and Wildevuur, C.R. (1987a) Bubble oxygenation and cardiotomy suction impair the host defense during cardiopulmonary bypass: a study in dogs. *Annals of Thoracic Surgery* **44**, 523-528.

van Oeveren, W., Jansen, N.J., Bidstrup, B.P., Royston, D., Westaby, S., Neuhof, H and Wildevuur, C.R. (1987b) Effects of aprotinin on hemostatic mechanisms during cardiopulmonary bypass. *Annals of Thoracic Surgery* **44**, 640-645.

van Oeveren, W., Harder, M.P., Roozendaal, K.J., Eijsman, L. and Wildevuur, C.R. (1990a) Aprotinin protects platelets against the initial effect of cardiopulmonary bypass. *Journal of Thoracic & Cardiovascular Surgery* **99**, 788-96; discussion 796-7.

van Oeveren, W., Wildevuur, C.R. and Kazatchkine, M.D. (1990b) Biocompatability of extracorporeal circuits in heart surgery. *Transfusion Science* **11**, 5-33.

van Oeveren, W., van Oeveren, B. and Wildevuur, C.R. (1992) Anticoagulation policy during use of aprotinin in cardiopulmonary bypass [letter; comment]. *Journal of Thoracic & Cardiovascular Surgery* **104**, 210-211.

Vardi, A., Jessen, M.E., Chao, R.Y., Brink, L.W., Levin, D.L. and Johnson, R.L. (1995) Effect of extracorporeal membrane oxygenation flow on pulmonary capillary blood flow. *Critical Care Medicine* **23**, 726-732.

Vasilyev, S., Schaap, R.N. and Mortensen, J.D. (1995) Hospital survival rates of patients with acute respiratory failure in modern respiratory intensive care units. An international, multicenter, prospective survey. *Chest* **107**, 1083-1088.

Vermylen, C., de Vreker, R.A. and Verstraete, M. (1963) A rapid enzymatic method for assay of fibrinogen fibrin polymerisation time (FPT test). *Clin Chem Acta* **8**, 418-424.

Videm, V., Fosse, E., Mollnes, T.E., Ellingsen, O., Pedersen, T. and Karlsen, H. (1989) Different oxygenators for cardiopulmonary bypass lead to varying degrees of human complement activation in vitro [see comments]. *Journal of Thoracic & Cardiovascular Surgery* **97**, 764-770.

Videm, V., Fosse, E., Mollnes, T.E., Garred, P. and Svennevig, J.L. (1990) Complement activation with bubble and membrane oxygenators in aortocoronary bypass grafting. *Annals of Thoracic Surgery* **50**, 387-391.

Videm, V. and Mollnes, T.E. (1994) Human complement activation by polygeline and dextran 70. *Scandinavian Journal of Immunology* **39**, 314-320.

Wachtfogel, Y.T., Kucich, U., Greenplate, J., Gluszko, P., Abrams, W., Weinbaum, G, Wenger, R.K., Rucinski, B., Niewiarowski, S., Edmunds, L.H., Jr. and et al (1987) Human neutrophil degranulation during extracorporeal circulation. *Blood* **69**, 324-330.

Walker, I.D., Davidson, J.F., Faichney, A., Wheatley, D.J. and Davidson, K.G. (1981) A double blind study of prostacyclin in cardiopulmonary bypass surgery. *British Journal of Haematology* **49**, 415-423.

Walker, L.K., Short, B.L., Gleason, C.A., Jones, M.D., Jr. and Traystman, R.J. (1994) Cerebrovascular response to carbon dioxide in lambs receiving extracorporeal membrane oxygenation. *Critical Care Medicine* **22**, 291-298.

Walker, L.K., Short, B.L. and Traystman, R.J. (1996) Impairment of cerebral autoregulation during venovenous extracorporeal membrane oxygenation in the newborn lamb [see comments]. *Critical Care Medicine* **24**, 2001-2006.

Ward, P.A., Till, G.O., Kunkel, R. and Beauchamp, C. (1983) Evidence for role of hydroxyl radical in complement and neutrophil-dependent tissue injury. *Journal of Clinical Investigation* **72**, 789-801.

Ward, P.A., Till, G.O., Hatherill, J.R., Annesley, T.M. and Kunkel, R.G. (1985) Systemic complement activation, lung injury, and products of lipid peroxidation. *Journal of Clinical Investigation* **76**, 517-527.

Weber, T.R. and Kountzman, B. (1996) The effects of venous occlusion on cerebral blood flow characteristics during ECMO. *Journal of Pediatric Surgery* **31**, 1124-1127.

Webster, R.O., Hong, S.R., Johmston, R.B.J. and Henson, P.M. (1980) The biological effects of the human complement fragments C5a and C5a desarg on neutrophil function. *Immunopharmacology* **2**, 201-219.

Weerwind, P.W., Maessen, J.G., van Tits, L.J., Stad, R.K., Fransen, E.J., de Jon, DS and Penn, O.C. (1995) Influence of Duraflo II heparin-treated extracorporeal circuits on the systemic inflammatory response in patients having coronary bypass. *Journal of Thoracic & Cardiovascular Surgery* **110**, 1633-1641.

Wesslen, B., Kober, M., Freij-Larsson, C., Ljungh, A. and Paulsson, M. (1994) Protein adsorption of poly(etherurethane) surfaces modified by amphiphilic and hydrophilic polymers. *Biomaterials* **15**, 278-284.

Westaby, S. (1996) The need for artificial hearts. *Heart* **76**, 200-206.

Westfall, S.H., Stephens, C., Kesler, K., Connors, R.H., Tracy, T.F., Jr. and Weber, T.R. (1991) Complement activation during prolonged extracorporeal membrane oxygenation. *Surgery* **110**, 887-891.

Whittlesey, G.C., Kundu, S.K., Salley, S.O., Nowlen, T.T. and Klein, M.D. (1988) Is heparin necessary for extracorporeal circulation? *ASAIO Transactions* **34**, 823-826.

Wildevuur, C.R., Eijsman, L., Roozendaal, K.J., Harder, M.P., Chang, M., Van and Oeveren, W. (1989) Platelet preservation during cardiopulmonary bypass with aprotinin. *European Journal of Cardio-Thoracic Surgery* **3**, 533-7; discussion 537-8.

Wildevuur, C.R. (1991) Blood damage and activation in cardiopulmonary bypass. In: Friedel, N., Hetzer, R. and Royston, D. (Eds.) *Blood use in cardiac surgery*, pp. 269-275. Darmstadt: Steinkopff]

Williford, S.K., Salisbury, P.L., 3d, Peacock, J.E., Jr., Cruz, J.M., Powell, B.L., Lyerly, E.S. and Capizzi, R.L. (1989) The safety of dental extractions in patients with hematologic malignancies. *Journal of Clinical Oncology* **7**, 798-802.

Wittner, M., Di Stefano, A., Wangemann, P. and Greger, R. (1991) How do loop diuretics act?. [Review] [34 refs]. *Drugs* **41 Suppl 3**, 1-13.

Wollam, D.H.M. (1962) Thalidomide disaster considered as an experiment in mammalian tetralogy. *BMJ* **2**, 236

Wright, S.D., Ramos, R.A., Tobias, P.S., Ulevitch, R.J. and Mathiason, J.C. (1990) CD14, a receptor for complexes of lipopolysaccheride (LPS) and LPS binding protein. *Science* **249**, 1431-1433.

Yanagi, F., Terasaki, H., Matsukawa, A., Ohkawara, S., Morioka, T., Yoshinaga and M. (1996) Cytokine generation in rabbits during extracorporeal lung assist with a mini hollow fiber lung. *Artificial Organs* **20**, 209-217.

Young, J.A. (1982) Coagulation abnormalities with cardiopulmonary bypass. In: Utley, J.R. (Ed.) *Pathophysiology and techniques of cardiopulmonary bypass*, pp. 88-105. Baltimore: Williams & Wilkins]

Yu, J., Lamba, N.M.K., Courtney, J.M., Whateley, T.L., Gaylor, J.D.S. and Lowe, G.D.O. (1994) Polymeric biomaterials: Influence of phosphorylcholine polar groups on protein adsorption and complement activation. *International Journal of Artificial Organs* **17**, 499-504.

Yu, M., Burchell, S., Takiguchi, S.A. and McNamara, J.J. (1996) The relationship of oxygen consumption measured by indirect calorimetry to oxygen delivery in critically ill patients. *Journal of Trauma* **41**, 41-8; discussion 48-50.

Zach, T.L., Steinhorn, R.H., Georgieff, M.K., Mills, M.M. and Green, T.P. (1990) Leukopenia associated with extracorporeal membrane oxygenation in newborn infants. *Journal of Pediatrics* **116**, 440-444.

Zapol, W.M., Snider, M.T., Hill, J.D., Fallat, R.J., Bartlett, R.H., Edmunds, L.H., Morris, A.H., Peirce, E.C., 2d, Thomas, A.N., Proctor, H.J., Drinker, P.A., Pratt, P.C., Bagniewski, A. and Miller, R.G., Jr. (1979) Extracorporeal membrane oxygenation in severe acute respiratory failure. A randomized prospective study. *JAMA* **242**, 2193-2196.

Zehr, K.J., Poston, R.S., Lee, P.C., Uthoff, K., Kumar, P., Cho, P.W., Gillinov, A.M., Redmond, J.M., Winkelstein, J.A., Herskowitz, A. and et al. (1995) Platelet activating factor inhibition reduces lung injury after cardiopulmonary bypass [see comments]. *Annals of Thoracic Surgery* **59**, 328-335.

Zilla, P., Fasol, R., Groscurth, P., Klepetko, W., Reichenspurner, H. and Wolner, E. (1989) Blood platelets in cardiopulmonary bypass operations: recovery after initial stimulation, rather than continual stimulation. *Journal of Thoracic & Cardiovascular Surgery* **97**, 379-88.

Zimmerman, G.A. and Amory, D.W. (1982) Transpulmonary polymorphonuclear leukocyte number after cardiopulmonary bypass. *American Review of Respiratory Disease* **126**, 1097-1098.

Zobel, G., Pierer, G., Dacar, D., Berger, J. and Novak, J. (1990) Extracorporeal CO2 removal in a lung lavage induced respiratory distress syndrome. *International Journal of Artificial Organs* **13**, 430-435.

Zobel, G., Dacar, D., Kuttnig, M., Rodl, S. and Rigler, B. (1992) Mechanical support of the left ventricle in ischemia induced left ventricular failure: an experimental study. *International Journal of Artificial Organs* **15**, 114-119.

Zwischenberger, J.B., Cox, C.S., Jr., Minifee, P.K., Traber, D.L., Traber, L.D., Flynn, JT, Linares, H.A. (1993) Pathophysiology of ovine smoke inhalation injury treated with extracorporeal membrane oxygenation. *Chest* **103**, 1582-1586.