

Gastrointestinal Injury following Cardiopulmonary Bypass

A thesis submitted for the degree of

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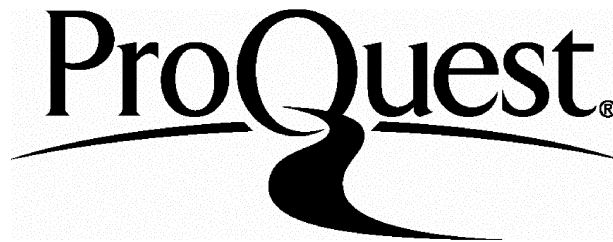
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Abstract

The gastrointestinal (GI) tract may be the source of a number of bacterial and non-bacterial mediators, which may contribute to the development of morbidity and mortality following episodes of gut hypoperfusion/ ischaemia. The aim of this thesis has been to identify the changes in gut blood flow, oxygenation and function following cardiopulmonary bypass (CPB) and their relationship to the development of post-CPB morbidity. The findings are summarised below:

- The retrospective study identified age (>65 yr) and CPB time as risk factors for the development of post-CPB intra-abdominal complications
- Tonometrically determined values for intramucosal pH (pHi) need temperature correction to avoid calculation of erroneously high values during hypothermic CPB
- Considerable hypoperfusion occurs during hypothermic CPB, with laser Doppler flowmetry (LDF) falling to approximately 45% of pre-CPB values
- The gastric and colonic pHi becomes acidotic (<7.35) during the re-warming and immediate post-CPB period
- Intramucosal acidosis occurs at a time when mucosal LDF blood flow is normal or supranormal
- CPB increases gut permeability and reduces the absorption of the monosaccharides, 3-O-m-D-glucose, D-xylose & L-rhamnose
- Post-CPB gut permeability has a temporal relationship with the CPB time
- Pulsatile flow attenuates the increase in post-CPB gut permeability
- Endotoxaemia occurs during CPB but is not associated with the production of TNF α ; pulsatile flow attenuates this endotoxaemia
- When examining perfusion and patient factors, the best predictor for a protracted ventilation & ICU stay for patients was a low gastric pHi (<7.35)
- A canine model of CPB supported the clinical findings, but also found that:
 - (a) changes in large vessel blood flow do not indicate more dynamic alterations in small vessel blood flow
 - (b) blood flow is prioritised to the mucosa at the expense of the serosal aspects of the bowel wall
 - (c) in the re-warming phase of hypothermic CPB & the immediate post-CPB period, when intramucosal acidosis occurs, there is a disparity between gut oxygen consumption & delivery
 - (b) increased expression of vasoactive intestinal peptide was found in the neural plexus of the submucosa post-CPB, which may indicate a role in preserving mucosal blood flow during periods of hypoperfusion

Dedication

*I dedicate this work to my parents Anita & Manohar Lal Ohri
& my wife Sonia who have been the engine of my academic work and constant
source of inspiration, encouragement and support*

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Statement of Originality

The work undertaken in this thesis is in large part original and the conception of the author. The retrospective study is one the larger studies reported in the literature. Only a few previous studies have examined the influence of pulsatile flow, but none has addressed the problem of both the nature of flow and the type of oxygenator employed during CPB and the development of post-CPB complications.

Previous studies using gastric tonometry have reported the development of intramucosal acidosis following CPB, but the influence of temperature has not been investigated. Previous studies have inferred the development of mucosal hypoperfusion during CPB, but this has not been previously reported. This thesis describes the application of laser Doppler flowmetry for determining mucosal blood flow during CPB, and reports the relationship between laser Doppler mucosal blood flow with mucosal oxygenation.

No previous study, to the author's knowledge, has previously reported the effect of CPB on gut permeability and absorption, and the influence of the CPB time, the use of pulsatile flow and the type of oxygenator employed.

Studies simultaneously examining the effect of CPB on endotoxaemia, $\text{TNF}\alpha$, gut permeability and gastric intramucosal pH and the influence of these parameters on post-CPB morbidity are to the author's knowledge original.

The animal model is unique in providing a greater understanding of the relationship between gut blood flow, gut oxygen utilisation and the development of intramucosal acidosis. One hormone (VIP) has been identified, which may be important in local vasoregulation of blood flow during CPB and ischaemia.

Publications & Abstracts arising from Thesis

1. Desai J B, Ohri S K 1990 Gastrointestinal damage following cardiopulmonary bypass. *Perfusion* 5: 161-168
2. Ohri S K, Desai J B, Gaer J A R, Roussak J B, Hashemi M, Smith P L C, Taylor K M 1991 Intraabdominal complications following cardiopulmonary bypass. *Ann Thorac Surg* 52: 826-831
3. Ohri S K, Bjarnason I, Pathi V, Somasundaram S, Bowles C T, Keogh B E, Khaghani A, Menzies I S, Yacoub M H, Taylor K M 1993 Cardiopulmonary bypass impairs small intestinal transport & increases gut permeability. *Ann Thorac Surg* 55: 1080-1086

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4. Ohri S K, Somasundaram S, Keogh BE, Menzies I S, Taylor K M, Bjarnason I
The effect of cardiopulmonary bypass on gut permeability. *Gastroenterology*
5. Ohri S K, Bowles C T, Siddiqui A A, Khaghani A, Keogh B E, Wright G, Yacoub M H, Taylor K M
The effect of cardiopulmonary bypass on gastric and colonic mucosal perfusion: a tonometric assessment. *Perfusion*
6. Ohri S K, Becket J, Brannan J, Keogh B E, Taylor K M
The effects of cardiopulmonary bypass upon gut mucosal blood flow, oxygen utilisation and intramucosal pH. *Ann Thorac Surg*

Submitted for Publication

7. Ohri S K, Bowles C T, Yacoub M H, Taylor K M The effect of temperature on tonometrically determined gastric intramural pH during cardiopulmonary bypass. *Crit Care Med*
8. Bowles C T, Ohri S K, Keogh B E, Yacoub M H Plasma endotoxin measurement in patients undergoing cardiopulmonary bypass: limitations and refinements of the Limulus chromogenic microassay. *Clin Science*
9. Ohri S K, Bjarnason I, Somasundaram S, Bowles C T, Keogh B E, Menzies I S, Taylor K M Pulsatile perfusion during cardiopulmonary bypass attenuates gut injury. *Ann Thorac Surg*
10. Ohri S K, Bishop A E, Abrams D C, Facer P, Arnold J A, Keogh B E, Polak J M, Taylor K M Increased gut vasoactive intestinal peptide expression following cardiopulmonary bypass and gut ischaemia. *Gastroenterology*
11. Ohri S K, Bjarnason I, Lawrence D, Bowles C T, Somasundaram S, Keogh B E, Menzies I S, Yacoub M H, Taylor K M The relationship between mucosal perfusion and gut permeability in patients undergoing cardiopulmonary bypass. *Crit Care Med*
12. Bowles C T, Ohri S K, Bjarnason I, Lawrence D, Somasundaram S, Keogh B E, Menzies I S, Yacoub M H, Taylor K M Endotoxemia, gut permeability and cytokine release following cardiopulmonary bypass: the influence of oxygenator type and pulsatile perfusion. *Ann Thorac Surg*

Abstracts

1. Ohri S K, Desai J B, Hashemi M, Smith P L C, Taylor K M Intra-abdominal complications following cardiopulmonary bypass. 5th Meeting, Mediterranean Association of Cardiology & Cardiac Surgery, 23-27 September 1991.
2. Ohri S K, Somasundaram S, Pathi V, Keogh B E, Menzies I S, Taylor K M, Bjarnason I The effect of cardiopulmonary bypass on gastric mucosal blood flow & small intestinal function. *Gut* 1992; 33: S42.
3. Ohri S K, Bowles C T, Pathi V, Wright G, Khaghani A, Keogh B E, Yacoub M H The effect of cardiopulmonary bypass on splanchnic blood flow & mucosal perfusion. *Eur J Cardiol* 1992; 13 (Suppl): 384.
4. Ohri S K, Bjarnason I, Siddiqui A, Somasundaram S, Keogh B E, Khaghani A, Menzies I, Yacoub M H Cardiopulmonary bypass modifies small gut function & permeation. Annual Meeting, The Association of Cardiothoracic Surgeons of Great Britain and Ireland, 31 March 1992.
5. Ohri S K, Bjarnason I, Somasundaram S, Keogh B E, Khaghani A, Yacoub M H, Taylor K M Cardiopulmonary bypass impairs intestinal saccharide transport and increases gut permeability. 77th Meeting, The Surgical Research Society, 7-8 January 1993
6. Ohri S K, Keogh B E, Becket J, Brannan J, Taylor K M The effects of cardiopulmonary bypass upon gut mucosal blood flow, oxygen utilisation and intramucosal pH in a canine model: implications for gut barrier function. *Br Heart J* 1993; 69; 85

Chapter 1

Introduction

The Development of Cardiopulmonary Bypass

The development of extracorporeal circulation, facilitated by modern anaesthesia, has accelerated advancements in cardiac surgical technique over the last 30 years. In this short time, surgery has become a routine treatment for coronary artery disease, and cardiac transplantation an accepted modality of therapy for end-stage cardiac failure. Without the early pioneering work in cardiopulmonary bypass (CPB), with considerable contributions from this country, these achievements could not have been realised.

Researchers in this field faced an uphill struggle, the burden of which was not eased by the myopic prediction of earlier medical practitioners.

'Surgery of the heart has reached the limits set by Nature toward all surgery, no new method and no new technique will overcome the natural obstacles surrounding a wound of the heart'

Sir Steven Paget

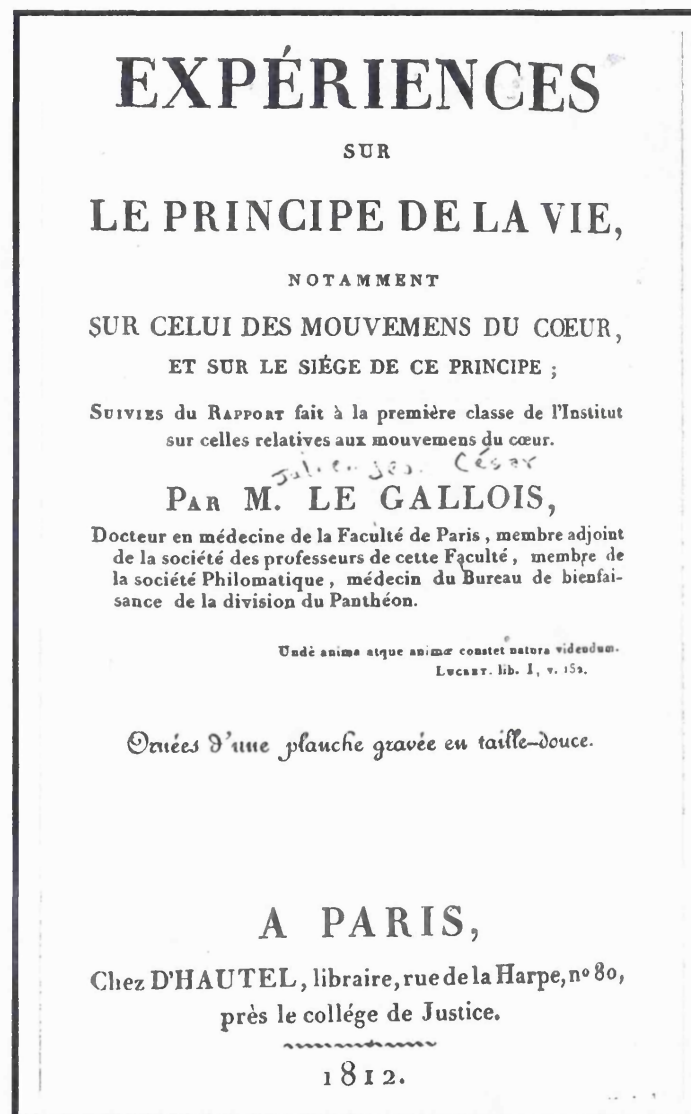
The more boisterous gastric surgeon, Bilroth, is purported to have said:

'Any man who should operate upon the heart should lose the respect of his colleagues'

Bilroth

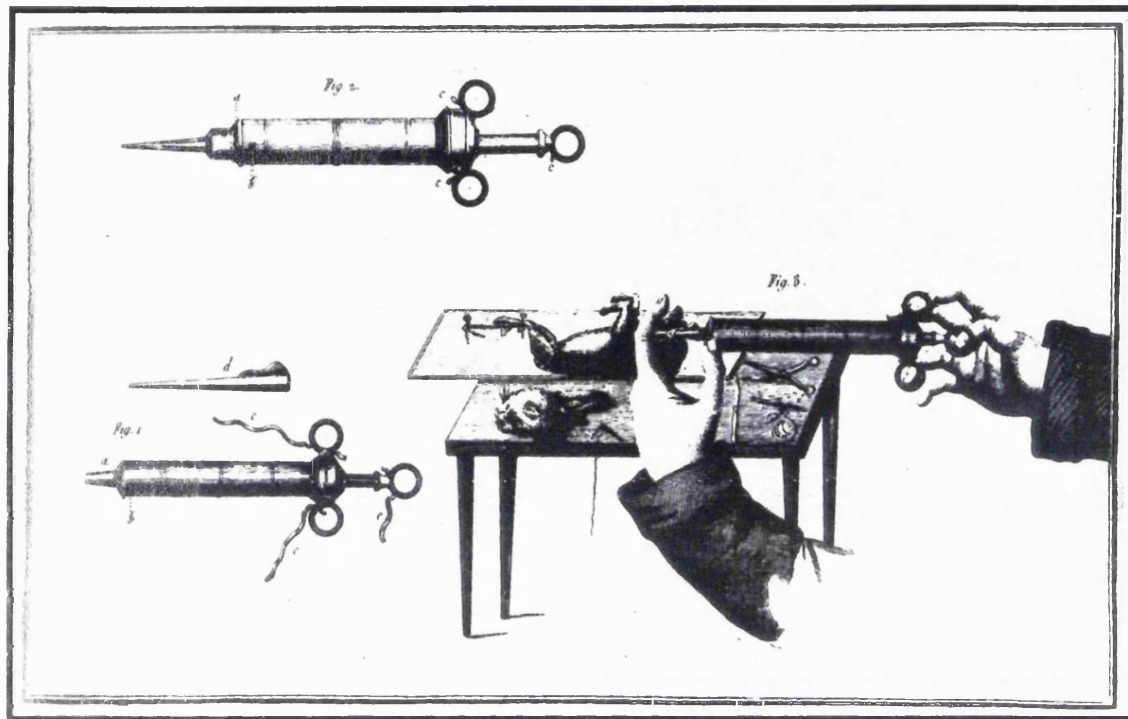
The origins of extracorporeal perfusion can be traced to Julien-Jear Cesar Le Gallois who in 1812 documented the concept of artificial perfusion (LeGallois 1812). He hypothesised that an isolated part of the body could be kept viable if arterial blood was provided by a means other than the pumping action of the heart (Fig 1).

Fig 1 : The frontpiece of LeGallois's thesis describing the principle of extracorporeal perfusion



Le Gallois described perfusion of decapitated rabbits by injection (Fig 2), but validation of his hypothesis seemed impossible until 1821, when Prévost and Dumas discovered the non-clotting properties of defibrinated blood (Prévost and Dumas 1821).

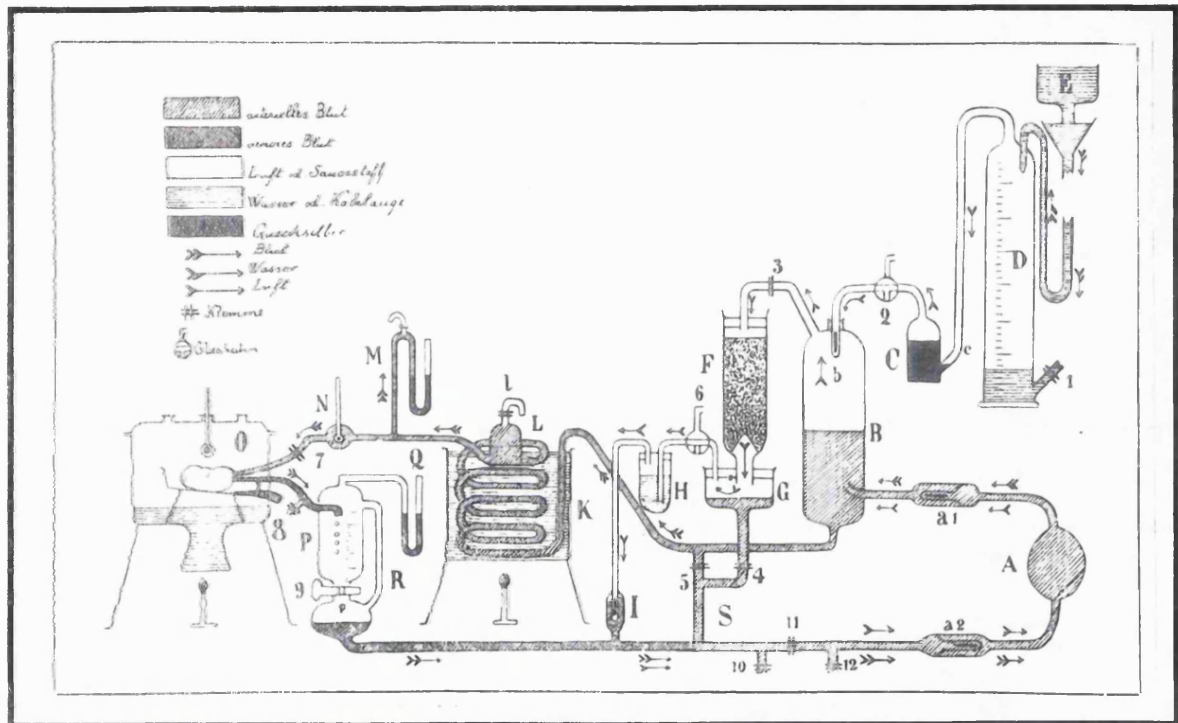
Fig 2: Perfusion of decapitated rabbits by Le Gallois



This enabled Loebell in 1849 to perform isolated perfusion of the kidney using naturally oxygenated blood (Loebell 1849), but it was not until 1869 that Ludwig and Schmidt described artificial blood oxygenation (Ludwig and Schmidt 1862). The method was simple: defibrinated blood was shaken in a balloon filled with gas during extracorporeal circulation. This basic method was refined by von Schröder in 1882, who may be credited for the development of the first bubble oxygenator (von Schröder 1882). He described oxygenation by bubbling air through blood in the venous reservoir of an extracorporeal circuit. The rise in pressure of the reservoir from insufflated air forced the blood into the arterial reservoir, from which the organ was perfused by hydrostatic pressure. Three years later, Frey and Gruber introduced the first film oxygenator, which permitted oxygenation of blood with a rotating cylinder without interruption of blood flow (von Frey and Gruber 1885). A syringe acted as the driving pump for the flow.

The physiological importance of pulsatility was perhaps recognised by Jacobj, who in 1890 designed the first pulsatile pump (Jacobj 1890). Air was introduced into a stream of blood propelled by a balloon, which created a pulsatile flow, and finally to a helical de-bubbling reservoir (Fig 3).

Fig 3: Diagram of Jacobj's pulsatile pump-oxygenator



Subsequently, Brodie who was director of the research laboratories at the Royal College of Physicians, London, at the turn of the century, devised a bubble oxygenator, which was driven by a piston pump, but did not require blood for priming (Brodie 1903). Over the next 25 years, further developments in oxygenation technology were made. Hooker introduced a film oxygenator which he employed for isolated perfusion of the head. Blood was oxygenated as it was filmed over a rotating hard disc in an inverted bell jar filled with oxygen. This concept was refined by Bayliss and associates, who used a column of cones that rotated on a vertical axis (Bayliss et al 1928). As blood flowed through the unit it was centrifugally filmed over stationary plates by the rotation of the cones.

Although considerable improvements were made during this period in the design of pumps, artificial support of the circulation was accelerated by the discovery of heparin and its anti-coagulation properties in 1916 by McLean (McLean 1916).

This landmark discovery was a catalyst in the advancement from isolated organ perfusion to total body perfusion.

Pumping technology was improving synchronously with advances in oxygenation. An ingenious pump was designed by Dale and Schuster in 1928 for the perfusion of the pulmonary and systemic circulation, although it was only employed for the perfusion of the hindquarters of cats and dogs (Dale and Schuster 1928). The mechanism consisted of a crankshaft driven by a pulley, which moved a vertical pumping rod connected to a diaphragm. The diaphragm moved water in and out of a vertical finger stall, which alternatively expanded to force blood from a glass dome cylinder and contracted to allow filling, thus imitating systole and diastole, in a fashion not dissimilar to the driving chamber of the modern intra-aortic balloon pump.

Perhaps the single most important contribution in the history of extracorporeal circulation was made by Gibbon. In 1937 he described the first total bypass of the heart and lungs in experimental animals (Gibbon 1937). Gibbon employed a vertical rotating cylinder as a film oxygenator and a modified Dale-Schuster pump for the maintenance of blood flow.

However, two years prior to Gibbon's report, Craaford in Stockholm, Sweden had found that the flow of blood to all organs could be suspended for up to 25 minutes without obvious signs of damage, provided blood flow to the brain was maintained. Björk, who worked in Craaford's laboratory subsequently developed a disc oxygenator, which he employed and described in his classic paper on brain perfusion in dogs (Björk 1948). Further advances in extracorporeal technology were frozen

with the advent of the Second World War. However, surgeons who had seen a number of soldiers survive injuries to the heart were encouraged to develop safer techniques for cardiac surgery. The post-war research began to bear fruit in the early fifties when groups in England and America reported improvements in the pump-oxygenator, which would finally culminate in the first human cardiopulmonary bypass (CPB).

Jongbloed in 1951 reported complete circulatory and respiratory support of dogs for 1-4 hours, and suggested that the pump-oxygenator may one day be employed for the repair of congenital heart anomalies and for supporting the circulation of patients with heart failure (Jongbloed 1951).

The development of the bubble oxygenator had remained largely static until Clark and associates advocated dispersion of oxygen through a sintered glass filter to transmit minute bubbles into the venous blood (Clark et al 1950). This development was further refined with the introduction of siloxine, which permitted dispersion of oxygen into the blood without foaming.

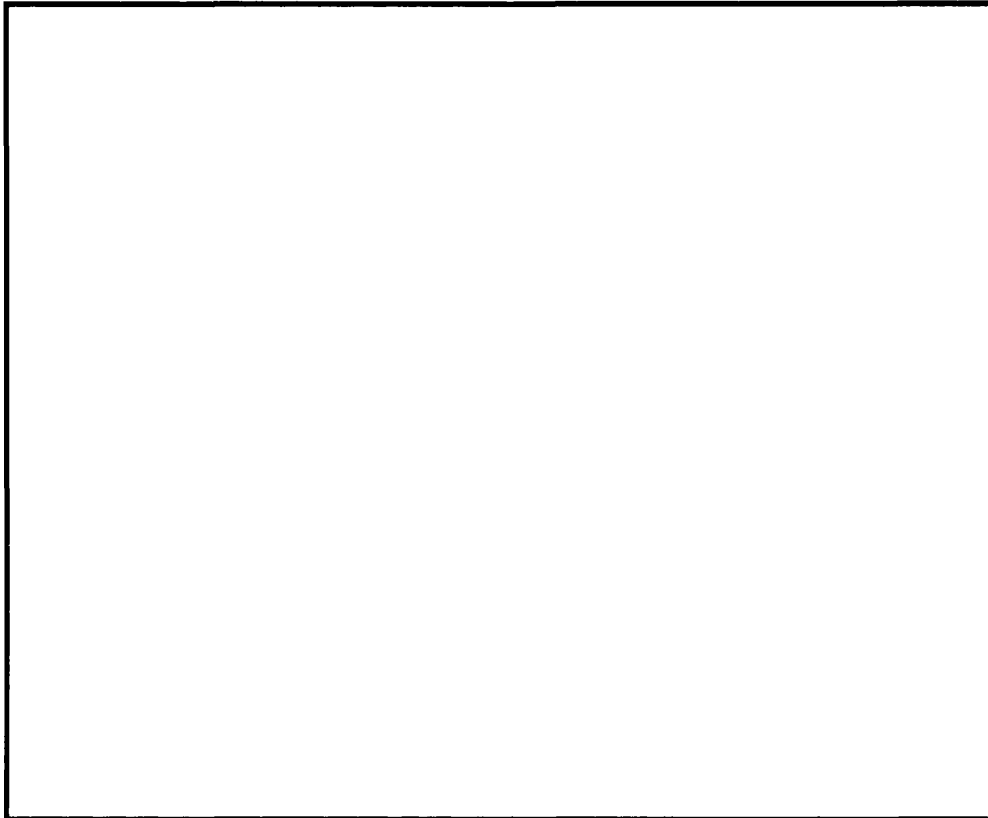
The first human application of the pump-oxygenator was reported in 1951 by Dennis and associates from America, for the repair of what was thought to be pre-operatively an atrial septal defect, in a six year old (Dennis et al 1951). However, the child died, and an autopsy showed that the lesion was in fact a partial atrio-ventricular canal defect, which may have contributed to the subsequent death. The first successful bypass was performed by Dogliotte and Costantini, for the removal of a mediastinal tumour. Only the right side of the heart was bypassed, with a bypass time of 20 minutes (Dogliotte and Costantini 1951).

However, Gibbon who had pioneered much of the early development of CPB, had his work interrupted by the Second World War. Nevertheless, he was rewarded by performing the first successful total bypass in the world on an 18 year old woman for repair of an atrial septal defect (Gibbon 1954). Unfortunately his next four cases all died from a variety of problems and he became disillusioned with the technique.

Around this period a pump-oxygenator was also being developed in London at the Royal Postgraduate Medical School between 1949 and 1952 (Melrose 1981 p.259). This development culminated in the sixth recorded bypass in the world and the fourth successful one (Aird et al 1954). This British pump-oxygenator was perhaps more important because it was the first in the world to become commercially available in 1954, and therefore contributed to the spread of cardiac surgery around the world.

In the previous year, Andreasen and Watson working at the Royal College of Surgeons of England's Research Farm at Downe determined the least cardiac output that would safely maintain the circulation in dogs for half an hour. This later became to be known as the 'azygous flow', since with both the cavae clamped, the blood returning via the azygous vein into the right atrium was sufficient to maintain the circulation. They decided this represented approximately 10% of the cardiac output (Andreasen and Watson 1953). This principle of 'low flow' was adopted by Lillehei and Varco at the University of Minnesota, USA, who in 1954 performed a series of almost 50 operations using 'cross-circulation'. The technique employed a simple pump and a donor adult, usually the mother or father as the oxygenator (Lillehei and Varco 1955). Although medico-legal considerations led to abandonment of the technique, it paved the way to modern cardiac surgery (Fig 4).

Fig 4: Cardiac surgery using cross-circulation from Lillehei's original description



In 1955 Kirklin and associates at the Mayo Clinic, Minnesota, USA, using a modified version of Gibbon's pump-oxygenator, started the first series of intra-cardiac operations (Kirklin et al 1955). At this time Melrose and colleagues at the Royal Postgraduate Medical School, London, observed in animal studies the benefit of priming the pump with crystalloid/ colloid mixture as opposed to heparinised blood. They substituted 6% dextran in saline for blood, and found that blood pressure, pulse and respiratory rates remained virtually unchanged, compared to a blood prime, which caused an immediate and persistent fall in blood pressure on commencement of CPB (Melrose et al 1953). Unfortunately, these findings were not utilised in the first clinical applications of CPB, which probably contributed to the high mortality at that time. It was over a decade later, that studies alluded to tissue flow with haemodilution during CPB, by reducing blood viscosity (Brenner et al 1969, Utley et al 1976).

Despite advances in oxygenation and circulation of blood, cardiac surgery was still limited by the techniques of myocardial preservation. Bigelow had shown in 1950, that animals could withstand circulatory arrest for much longer periods of time, if they were made hypothermic. In a study in dogs, surface cooling to 20°C was undertaken, with complete recovery after 15 minutes of total circulatory arrest (Bigelow et al 1950). Unfortunately, Bigelow was never allowed to use this technique clinically because of objections from the Hospital Ethics Committee, which left him very bitter. Delorme suggested direct cooling of blood rather than the slower surface cooling employed (Delorme 1952). To this end, Brock and Ross, devised a pumping circuit and heat exchanger (Ross 1954), which led to the development of 'profound hypothermia' by Drew in 1959. This technique employed two pumps and no oxygenator. The patient was cooled to 15°C and the circulation arrested (Drew et al 1959); artificial oxygenation was not undertaken but the lungs allowed to naturally oxygenate the blood. The main advantage of this technique was the prolongation of safe circulatory arrest, which was particularly important for repair of congenital heart and great vessel defects.

The ability electively to arrest and re-commence the effective heart beat was the result of pioneering work on dogs, rabbits and human fetuses published by Melrose and colleagues in 1955 and 1957 (Baker et al 1957, Melrose et al 1955). Based upon these observations, elective cardiac arrest in combination with hypothermia provided the foundations for myocardial protection during CPB. Melrose et al showed that when potassium citrate in a concentration of over 1 mg/ml was added to Locke's solution used to perfuse the heart, diastolic arrest occurred. This always happened within 20 seconds, and could be maintained as long as the coronary vessels contained the perfusate. Spontaneous beating was reinstated within 90 seconds of perfusing with plain Locke's solution, with recovery almost complete by 3 minutes. However, hearts arrested by stopping coronary flow recovered very slowly, even after short periods of

arrest. This latter technique was known as 'simple asphyxia', and was initially promoted by Cooley in the USA. He simply clamped the aorta and induced arrest within minutes, but the subsequent mortality from the so-called 'stone heart', saw an end to this technique. The cardioplegic technique described by Melrose et al was subsequently shown to be clinically effective and safe. Effler et al employed potassium citrate electively to arrest the first human heart, and later reported a series of 97 patients using the same technique (Effler et al 1957). Since then a number of cardioplegic solutions have been developed, and controversies continue as to the most effective composition, mode of delivery, temperature and dose requirements (Buckberg 1990).

In the late 1950's, the safe and effective oxygenation of blood still remained a problem. The bubble oxygenators had become available as disposable units, which made them very popular. However, concern persisted over the dangers of direct oxygenation of blood, in particular the risks of gas embolisation. The idea of oxygenating blood through a semi-permeable membrane was recognised by Kolf and Berk, who observed the arterialisation of blood as it passed through the cellophane chambers of the artificial kidney (Kolf and Berk 1944). This concept was developed further by Clowes et al, who in 1958 reported a membrane oxygenator with a surface area of 25m² (Clowes et al 1956). This massive gas exchange surface area was reduced by the development of silicone elastomers in the late 1950s, which were both highly permeable to oxygen and carbon dioxide and biologically inert. Membranes were developed for clinical use which had a surface area of 2.5m² (Lande et al 1969). The gas exchange may now take place over a surface area of only 0.8m², by using micro-porous teflon or polypropylene.

The Pathophysiology of Cardiopulmonary Bypass

In the 1990's we can perform CPB with a high degree of assurance and reliability. The number of bypass procedures performed each year world-wide increases with the continuing dissemination of extracorporeal perfusion knowledge and training. In the USA, 590, 000 open heart procedures were reported in 1987 (National Hospital Discharge Survey 1987), whilst the United Kingdom Cardiac Register recorded 21,907 open heart procedures in 1989. With this growth in cardiac surgery has come the recognition that a significant degree of organ and tissue damage occurs following CPB. A greater understanding of the mechanisms of this tissue injury is required, if refinements in bypass technique and technology are to be made with the goal of eliminating CPB-related morbidity and mortality.

CPB-induced dysfunction has been reported for all the systems of the body (Alfieri and Kotler 1990) but attention is now turning to the splanchnic bed with the recognition that it may be a source of a number of mediators responsible for post-CPB myocardial depression (Haglund and Lundgren 1973, Lundgren et al 1976).

The pathophysiology of CPB is becoming increasingly complex but it may be broadly divided into haemodynamic and inflammatory changes. The latter phenomenon has been referred to as the 'whole body inflammatory response to bypass' and is a result of blood flowing over bio-incompatible surfaces during extracorporeal perfusion.

An outline of this pathophysiology is provided as a background to different mechanisms which may play a role in the development of splanchnic injury.

Haemodynamic Alterations during CPB

Haemodilution

Haemodilution occurs as a result of priming the pump-oxygenator with crystalloid solutions; the haematocrit may fall on commencement of bypass to 20-25%. The decrease in oxygen content of the blood is thought to be offset by improvements in tissue flow and free water clearance by the kidney. Haemodilution improves tissue flow by reducing blood viscosity and ameliorates the vasoconstrictive effect of hypothermia during cardiopulmonary bypass (Utley et al 1976). By reducing the blood viscosity, haemodilution improves renal blood flow, urine flow, free water clearance, glomerular filtration and filtration fraction (Utley et al 1981). These beneficial effects may be reversed by the addition of albumin into the priming fluid.

Fluid Balance & Tissue Oedema

Total body water increases during cardiopulmonary bypass, and the increase is related to the length of CPB. The excess fluid is located in the extracellular compartment (Breckenridge et al 1970). This may be due to the more immediate increases in capillary permeability (Smith et al 1987) and reductions in tissue oncotic pressure, and later to reductions in renal clearance of free water and sodium. The increased water content has been demonstrated in the stomach, intestine, liver, medulla of the kidney and myocardium. This increased water content is also manifest as a rise in the interstitial fluid pressure. Following CPB, the rise in interstitial fluid pressure has been found to be greatest in the myocardium, intermediate in the stomach, and least in skeletal muscle (Utley 1990). The resultant tissue oedema is dependent upon the intrinsic compliance of the tissues and organs and the permeability of the capillaries. The association between tissue oedema and organ dysfunction is not commonly reported except for pulmonary and myocardial function. Recent advocates of blood cardioplegia have suggested that myocardial tissue oedema may be reduced because

of the higher oncotic pressure of blood, compared to crystalloid cardioplegic solutions (Buckberg 1990). However, the organs of greatest oedema formation, the liver, stomach and intestines, have not been extensively investigated for cellular dysfunction, because of the relatively low incidence of post-operative intra-abdominal complications (Leitman et al 1987).

Systemic Haemodynamic Consequences of Cardiopulmonary Bypass

Physiological blood flow rates at rest are 3.0-3.2 l/min/m² (Tarhan and Moffit 1971), but during normothermic CPB flow rates are generally maintained between 2.2-2.4 l/min/m². With hypothermic CPB, as tissue oxygen requirements fall with reduction in the metabolic rate, the flow rate is reduced using a nomogram so that at 28°C the flow rate may be reduced to 1.6 l/min/m² (Fox et al 1982). Further reductions in blood flow are limited by the requirements of vital organs such as the brain. With normal cerebral circulation, the flow may be reduced to 20% of normal, without subsequent brain damage (Astrup et al 1977). However, the effect of low flow during hypothermic CPB on other organ systems in the presence of diseased vessels is yet to be determined. The gastrointestinal tract may be expected to receive a smaller proportion of the total flow, which is prioritized to more vital organs such as the brain and kidney.

Upon this background of reduced flow, the neuro-hormonally mediated response of the body to CPB is progressive peripheral vasoconstriction (Dunn et al 1974). This vasoconstriction results in a rise in the peripheral vascular resistance, thus increasing the afterload on the heart. This may result in heart failure following CPB, especially if myocardial preservation has been inadequate. Under resting conditions, the splanchnic circulation contains approximately one-third of the total circulatory volume, mainly in the post-capillary venous vasculature. Vasoconstriction of this capacitance bed results in an autotransfusion and increases the preload to the heart

(Rothe 1983). This vasoconstriction is mediated via both neurohormonal and other vasoactive substances. Hormonal mediators that have been implicated include catecholamines, angiotensin II (Taylor et al 1980), vasopressin (Levine et al 1981), and local tissue vasoconstrictors such as thromboxane A₂ (Watkins et al 1982). The relationship between catecholamine levels and the haemodynamic changes of CPB have yet to be established. A lack of pulsatility in the circulation during conventional non-pulsatile flow increases the release of both vasopressin and renin (Many et al 1967). Renin results in increased plasma and tissue angiotensin II, a potent vasoconstrictor and mediator of subendocardial ischaemia. There is a significant correlation between rises in plasma angiotensin II and vasoconstriction (Taylor et al 1979). The neural arm of the vasoconstrictive response is believed to be mediated via sympathetic nerves; baroreceptors in the carotid sinus discharge with increased frequency when arterial flow is changed from pulsatile to non-pulsatile flow (Angell-James and de Burgh Daly 1971).

The Effect of Pulsatility on Blood Flow during Cardiopulmonary Bypass

Although physiological flow is pulsatile in character, the majority of cardiac operations requiring CPB are conducted at present using non-pulsatile flow. This is largely historical: creating pulsatile flow has been a more challenging engineering problem than producing a pump for non-pulsatile blood flow. However, the body of evidence for the benefits of pulsatile flow is mounting, and some may argue is already weighted in its favour.

Burton suggested in 1954, that blood flow through capillaries only occurred when the pressure exceeded the critical closing pressure of the pre-capillary arterioles (Burton 1954). For a given pressure, he hypothesised that pre-capillary arterioles would be kept open longer by the systolic peaks of pulsatile flow. Shepard theorised in 1966, the concept of 'energy equivalent pressure' (eep) which was defined as:

$$eep = \frac{\int p \cdot f \cdot dt}{\int f \cdot dt}$$

p=pressure (mmHg)
f=flow (ml/s)
dt=change in time at a specific point in time

For a given pressure, Shepard showed that 1.0 - 2.3x the energy was required to produce pulsatile flow than for non-pulsatile flow. Although never proven, the extra available energy to tissues is thought to help maintain capillary patency and thus improve tissue blood flow, tissue oxygenation and metabolism (Shepard et al 1966).

Reduction in capillary blood flow leads to tissue hypoperfusion, and subsequently tissue hypoxia, acidosis, and impaired oxygen and glucose utilisation. These effects on tissue metabolism have been documented for non-pulsatile flow and ameliorated by pulsatile flow (Chun-Hsiu et al 1981, Shepard and Kirklin 1969). Further, there is reduced release of vasopressin, angiotensin II and thromboxane A₂ during pulsatile bypass. This has beneficial implications for both tissue perfusion and for afterload reduction during and following CPB. Some groups have reported a reduced requirement for intra-aortic balloon counter-pulsation and inotropic drugs following pulsatile CPB (Maddoux et al 1976).

The Bioincompatibility of Cardiopulmonary Bypass

Microembolism

Microemboli are generated during cardiopulmonary bypass and may be classified into gaseous, biological and non-biological emboli (Uretzky et al 1987). These microemboli have been implicated in organ damage following CPB (Brennan et al 1971, Connell et al 1973). More recently, microemboli have been directly visualised in the retina of patients during CPB using fluorescein retinal angiography (Blauth et al 1986). The majority of these emboli are biological in nature and probably consist of cellular components of the blood (Page et al 1974). Although patients undergoing CPB are heparinised this does not prevent the activation of blood cells, in particular platelets and leukocytes (Harker et al 1980). These cells are activated as a result of exposure to the bioincompatible surface (Salzman et al 1978), which occurs during bypass as the blood flows through tubing, oxygenators and reservoirs. Small vessel damage may occur directly by a mechanical effect of the emboli, but for the biological emboli the release of vasoactive substances such as histamine, serotonin and other kinins, by degranulation of the component cells, may be more damaging to the endothelium.

The clinical manifestations of post CPB pulmonary damage include atelectasis, permeability pulmonary oedema and, rarely, adult respiratory distress syndrome (ARDS). The development of ARDS appears to be heralded by a rise in the alveolar capillary membrane permeability, which has been demonstrated in 1.7% of patients following CPB (Fowler et al 1983). Neutrophils, which form a component of biological microemboli, may cause direct and indirect damage by lysosomal enzyme release and oxygen free radical (OFR) production (Royston 1990). However, a recent study demonstrated no relationship between neutrophil activation, as assessed by neutrophil n-formyl methionyl-leucyl-phenylalanine (FMLP) receptor status, and lung permeability (Tennenberg et al 1990).

The other main component of biological microemboli is platelets. Degranulation of these cells releases thromboxane A₂, a potent vasoconstrictor and activator of platelets themselves. As previously mentioned, there are significant rises in thromboxane A₂ levels following CPB (Watkins et al 1982). It is currently unknown whether prostacyclin administered during CPB can significantly reduce the number of microemboli and their associated sequelae (Addonizio et al 1985).

The non-biological particles probably act by a mechanically occlusive action on the microcirculation, but unlike biological and gaseous emboli cannot be removed by phagocytosis following CPB. One of the sources of this particulate matter is the bypass tubing. The material from which the tube is constructed influences the time of particulate release, the size and number of particles generated. This has been shown to be a result of damage to the tubing by the recurrent compression action by the roller head of the CPB pump (Uretzky et al 1987).

There is no reason to believe that other organs such as the kidney, pancreas, liver and gut do not undergo microembolism which to date has only been documented for the brain during CPB.

Blood Cell Damage & Activation during Cardiopulmonary Bypass

Red Cell Damage

Activation and/or destruction of blood cells during cardiopulmonary bypass contributes to tissue and organ dysfunction in the post-CPB period. Red blood cells (RBC) may be damaged by the action of the roller head of the peristaltic pump, and by excessive cardiotomy suction (Wright and Sanderson 1979). The release of haemoglobin and the membrane ghosts both may have deleterious effects. Free haemoglobin contributes a plasma osmotic load which may alter the rheology of plasma flow and thereby decrease tissue perfusion. Haemoglobin may also be oxidised and produce toxic oxygen free radical (OFR) species. The membrane ghosts of RBC may mechanically block capillaries with resultant tissue ischaemia if collateral supply is also impaired. Adenosine diphosphate (ADP) is released on RBC destruction and has the ability to aggregate platelets which may embolise into the microcirculation. Other alterations of the RBC membrane are more subtle but nevertheless may still contribute towards the production of tissue ischaemia. It is now well recognised that RBC become less deformable following CPB. This may be due to mechanical damage from increased shear stresses upon erythrocytes (Haneda et al 1985) with resultant alterations in ionic pumping of the RBC membrane (Lumb 1987). Damage may also be non-mechanical by direct attack of the RBC membrane from the activated products of complement (Ridley et al 1990) or lipid peroxidation by OFR species (Fumero et al 1989). Some patients undergoing CPB may be at greater risk such as those with diabetic retinopathy (Lowe et al 1980) or peripheral vascular disease (Reid et al 1976), since reductions in red cell deformability have been demonstrated in these patients.

Neutrophil Activation

Neutrophils become activated during CPB by a number of different mechanisms, and may produce tissue ischaemia and damage by mechanical and/or functional alterations. On the institution of CPB the complement cascade produces C5a and C3b on contact activation with the extracorporeal circuit. Neutrophils possess adhesion receptors such as LFA-1 glycoprotein and the binding site for iC3b; iC3b is an opsonic fragment of the third component of complement. C5a enhances neutrophil expression of these adhesion receptors (Arnaout et al 1985) and promotes neutrophil margination in capillaries of tissues. The C3b component of complement can covalently bind to the bypass tubing and act upon C3 convertase to cleave more C5a. However other substances including the leukotrienes (Hoover et al 1984) and cytokines such as interleukin 1 (Schleimer and Rutledge 1986) and tumour necrosis factor may also promote neutrophil margination within the microvasculature. Activated neutrophils may produce tissue injury by mechanical occlusion of capillaries, by the release of oxidative and non-oxidative products, and through the production of oxygen free radical species at the time of reperfusion.

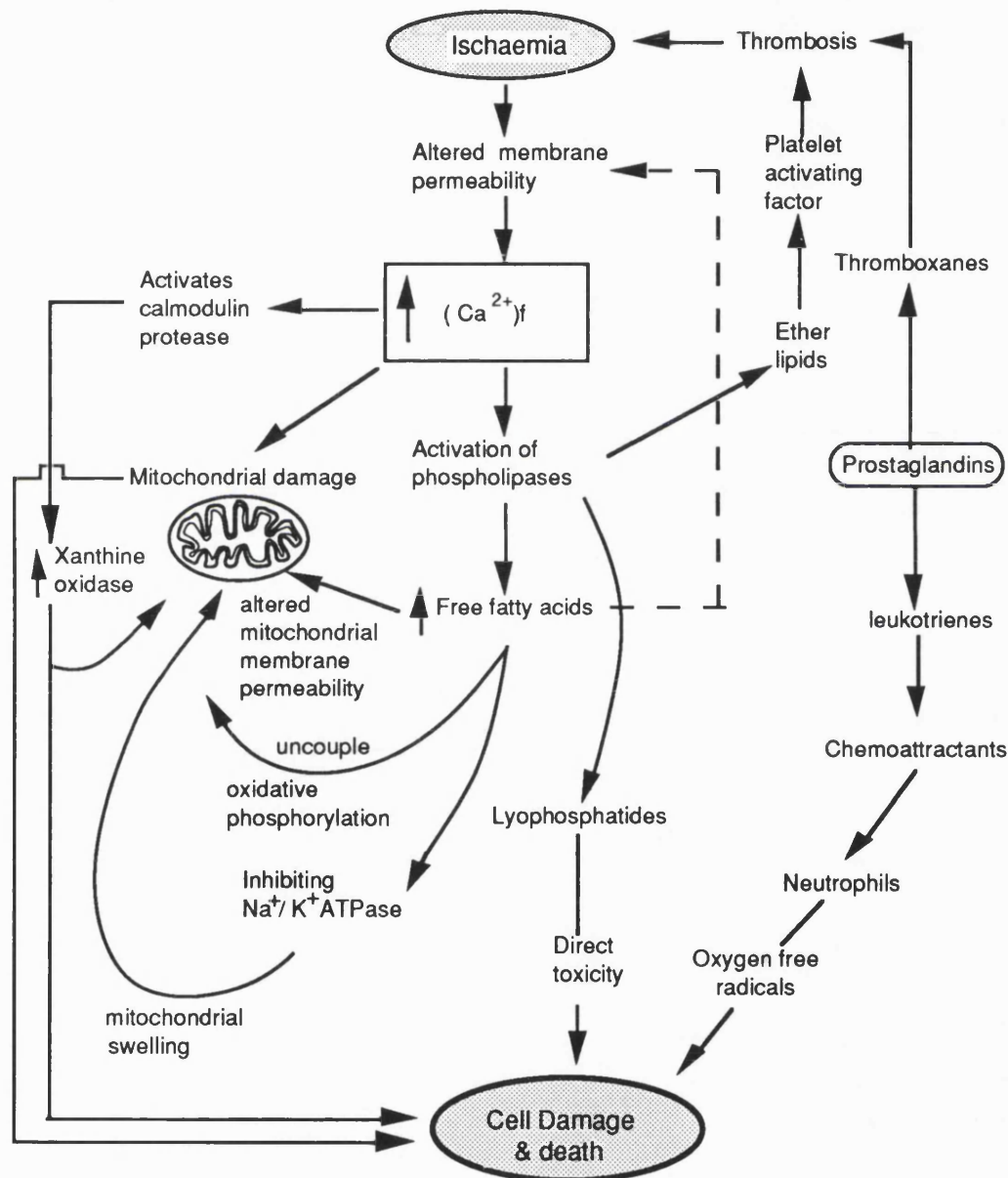
The degree of C3a activation and granulocyte trapping may be influenced by the type of oxygenator employed during CPB. Less neutrophil activation has been observed when a membrane oxygenator is used compared to a bubble oxygenator (Cavarocchi et al 1986), which may be due to greater activation of complement by bubble oxygenators (Tamiya et al 1988).

Activated neutrophils can cause mechanical occlusion of capillaries resulting in the 'no reflow' phenomenon (Engler et al 1983). It has been shown in locally ischaemic hearts that there can be perfusion of arterioles and venules with no flow in nearby capillaries (Engler et al 1983).

Mechanisms of Reperfusion Injury

Reperfusion injury is mediated via three main mechanisms; (a) intracellular calcium overload, (b) oxygen free radical species production, and (c) the products of neutrophils sequestered within the tissues.

Fig 5: The role of raised intracellular calcium in cellular damage.



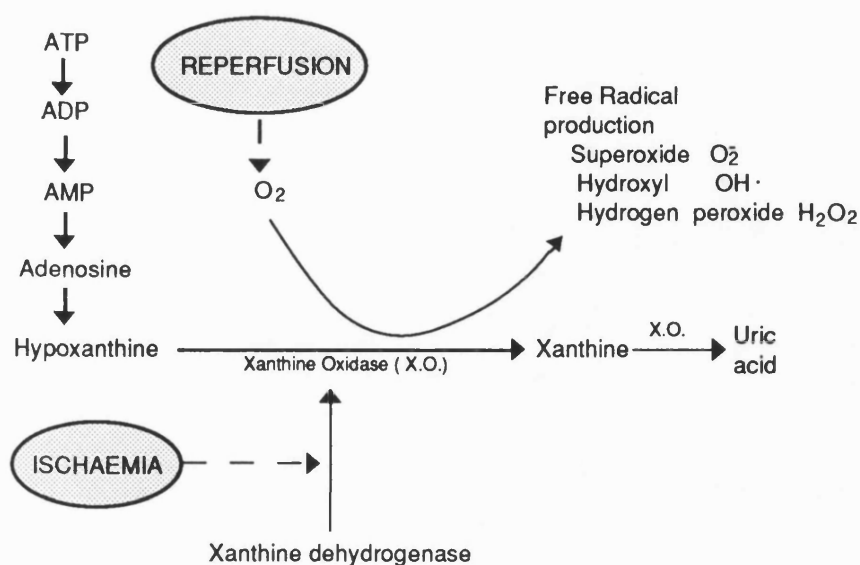
(a) Intracellular Calcium Overload

During ischaemia there is an increase in the intracellular calcium concentration ($[Ca^{2+}]_i$), which results in the release of free fatty acids and altered microtubular and mitochondrial function (Fig 5). The role of intracellular calcium in the production of tissue injury following CPB has been recently reviewed (Ohri and Abel 1991), but the inhibition of energy production by excess ionised calcium within the mitochondria is the postulated general mechanism for ischaemic damage (Wroegeman and Pena 1977).

(b) Oxygen Free radical Species

Oxygen free radicals (OFR) are generated predominantly during the reperfusion period when the supply of oxygen is again plentiful. During ischaemia there is the hydrolysis of adenosine triphosphate (ATP) to adenosine monophosphate (AMP) and eventually hypoxanthine (Fig 6). In ischaemic tissue the enzyme xanthine dehydrogenase is converted to xanthine oxidase (Engerson et al 1987). This enzyme utilizes molecular oxygen to generate the oxygen superoxide radical or anion and uric acid. The superoxide radical can react with hydrogen peroxide, which itself is produced by electron donation to superoxide, to produce the hydroxyl radical.

Fig 6: Oxygen free radical generation after ischaemia-reperfusion.



The hydrogen peroxide may react with hypochlorous acid, produced by the reaction of hydrogen peroxide with chlorine in the presence of myeloperoxidase in the neutrophil, to generate singlet oxygen. There are no naturally occurring scavengers of hydroxyl radical or singlet oxygen. Similar to tissue, neutrophils and macrophages are capable of producing oxygen free radical species by the activation of NADPH oxidase with the consumption of molecular oxygen and the production of the superoxide radical, otherwise referred to as the respiratory burst (Babior 1984).

The amount of superoxide generated may be increased by certain stimuli such as lipopolysaccharide or endotoxin (Guthrie et al 1984). Endotoxaemia has been documented to occur in most patients undergoing CPB and shows a temporal relationship between the cross-clamp and total bypass times (Nilsson et al 1990, Roche et al 1987).

Oxygen free radicals are capable of oxidizing the sulphhydryl group in methionine and cysteine amino acids, which are essential for membrane integrity and form the active site in a number of enzymes, such as Ca-ATPase, calmodulin and alpha 1 protease inhibitor.

Alpha 1 protease inhibitor is an antiprotease that prevents the actions of elastase and cathepsin G released from neutrophils. Similarly nucleic acids and the extracellular tissue matrix may be damaged. The disruption of the extracellular matrix will promote the escape of macromolecules from the intravascular compartment with subsequent tissue oedema.

The lipids of membranes are readily attacked by free radicals. The process is initiated by the removal of a hydrogen atom from a polyunsaturated lipid, thus producing a lipid radical. Following molecular rearrangement a lipid diene is produced from this radical. This diene may react with other lipids to produce a hydroperoxide, or undergo

transformation to an unstable endoperoxide. The production of these moieties results in alteration in membrane function and rises in intracellular calcium. Free radicals can also act upon arachidonic acid to produce the epoxide leukotriene A₄, which is subsequently converted to B₄, a potent chemoattractant for neutrophils and an oedemogenic agent. Leukotrienes C₄ and D₄ may also be generated which are powerful coronary (Michelassi et al 1983) and mesenteric vasoconstrictors (Bayorh et al 1985).

(c) The Role of the Neutrophil in Tissue Injury following CPB

There is a substantial body of evidence which implicates OFR species in microvascular and parenchymal ischaemia-reperfusion (IR) damage (Hearse et al 1986). The two pivotal enzymes involved in OFR production, as previously discussed, are tissue associated xanthine oxidase and neutrophil derived NADPH oxidase. Inhibition (allopurinol, pterin aldehyde) or inactivation (tungsten supplemented, molybdenum-deficient diet) of these enzymes has been found to protect against IR injury in the intestine (Granger et al 1981). These findings together with the high gut mucosal content of xanthine oxidase supports the hypothesis that the tissue xanthine oxidase is a major source of OFR species. The role of neutrophils in ischaemic injury was proposed by Romson et al on studies upon ischaemic myocardium (Romson et al 1983). They observed that neutrophil depletion reduced infarct size (by 45%) to a similar extent as pretreatment with oxygen free radical scavengers. Although the attenuation of IR injury may be due to improved haemodynamics by reduced neutrophil plugging, it may also be due to reduced neutrophil OFR release. Using anti-neutrophil serum to deplete neutrophil number or monoclonal antibodies against the neutrophil membrane glycoprotein CD18 (adherence protein), IR induced rise in capillary permeability could be prevented (Hernandez et al 1987). Neutrophil infiltration following IR could also be prevented by pretreatment with allopurinol or superoxide dismutase (Grisham et al 1986). The contribution of other non oxidising

compounds released by neutrophils on de-granulation remains largely undetermined in IR injury.

Neutrophils show increased oxygen free radical activity following CPB (van Oeveren et al 1985), and this may be correlated with the degree of complement activation (Cavarocchi et al 1986). The pulmonary bed has been found to undergo increases in pulmonary capillary permeability, neutrophil sequestration and evidence of oxygen free radical mediated injury (Braude et al 1986, Ratliff et al 1973).

As regards the myocardium, when reperfused after a period of ischaemia with agranulocytic blood, the no reflow phenomenon can be completely abolished (Engler et al 1986). Furthermore, there is a direct relationship between the degree of myocardial oedema following reperfusion and the number of granulocytes within the area of oedema (Engler et al 1986); the oedema can be prevented by reperfusion with agranulocytic blood (Engler et al 1986). In the clinical setting, neutrophil-depleted blood has been found to be of value in reducing reperfusion myocardial injury in neonatal hearts (Breda et al 1989). Other potentially therapeutic interventions such as the use of monoclonal antibodies against the neutrophil adherence receptor (Vedder et al 1988), the inhibition of neutrophil activation with prostacyclin (Simpson et al 1987) and the infusion of antioxidants and free radical scavengers with cardioplegia have been beneficial in animal and clinical studies (Menasche et al 1987).

Aprotinin, a non-specific antiprotease, which has the ability to reduce elastase release from neutrophils (Smedly et al 1986) and weakly inhibit the neutral lysosomal proteinases elastase and cathepsin G released from activated neutrophils (Fritz and Wunderer 1983), has been found to significantly improve post-CPB cardiac function in dogs when administered in high doses (Sunamori et al 1988).

Although it is unknown if the gut undergoes ischaemia-reperfusion injury following CPB, mechanisms which operate within the pulmonary bed may also be present in the

gut. Furthermore, using gastric tonometry, Fiddian-Green has shown that approximately 50% of patients who are undergoing elective cardiac surgery have episodes of gastric intramucosal acidosis, which indicates mucosal hypoxia. More importantly, the duration of mucosal acidosis is correlated with subsequent post-operative prognosis (Fiddian-Green and Baker 1987). Gut ischaemia-reperfusion injury has been implicated as a prime mechanism in the development of the multiple organ failure (Wilmore et al 1988). The post-CPB increase in pulmonary capillary leak may be secondary to ischaemia-reperfusion injury of the gut with the activated neutrophil acting as the effector cell. The ability of gut activated neutrophils to cause remote organ damage has been demonstrated in animal models of gut ischaemia. Schmeling et al showed that superior mesenteric occlusion in rodents for 120 minutes with subsequent reperfusion produced acute lung injury characterised by neutrophil sequestration, capillary endothelial cell injury and increased microvascular permeability (Schmeling et al 1989).

Other components of the Immune System & the development of Tissue Injury following CPB

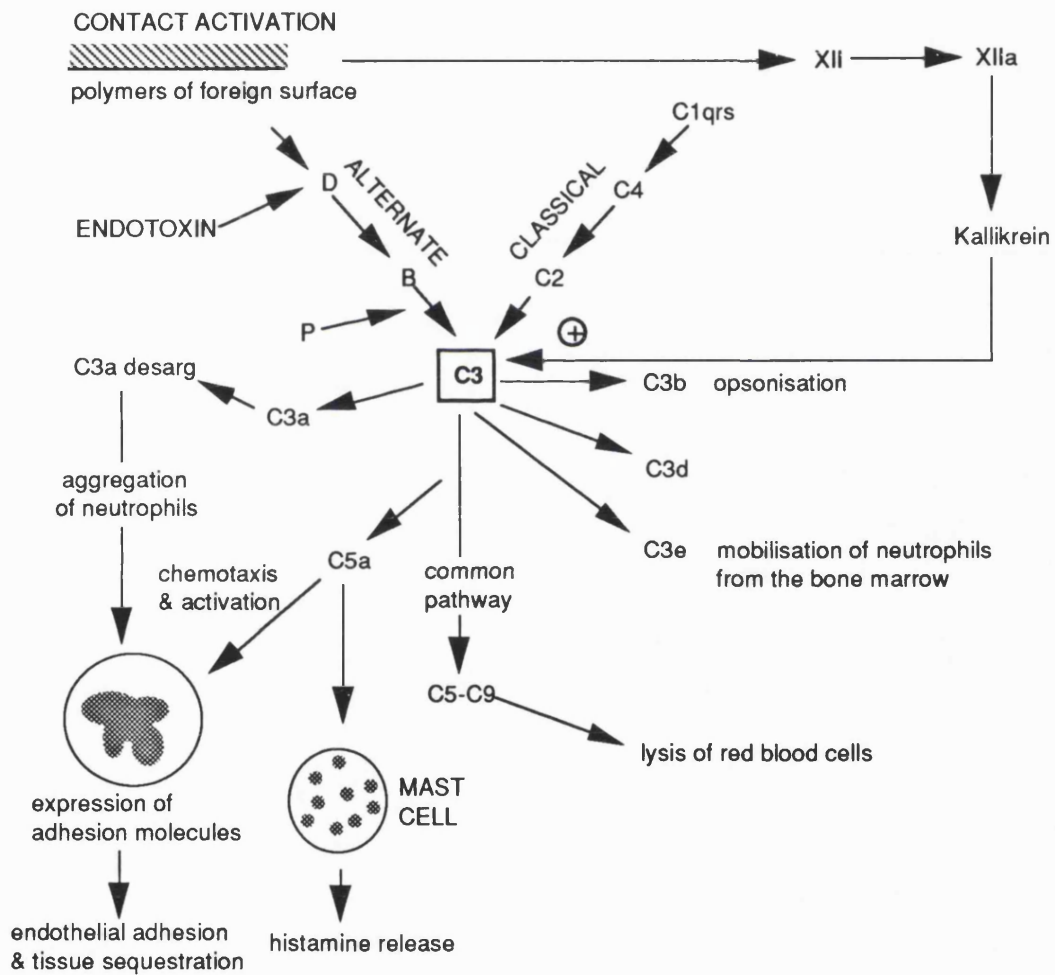
Historically, most research has centred upon the role of the neutrophil in CPB-induced tissue injury, largely because of the early recognition that neutrophilia occurred following bypass (Melrose et al 1953). However, with the relatively recent discovery of the role of cytokines and their role in tissue injury, a resurgence of research into the effects of CPB on other components of the immune system has become evident. Analogous to other immune disorders, the response of the body to the insults of CPB, surgery, blood transfusions and anaesthesia is so great that an unfortunate side effect is tissue injury.

Apart from tissue injury following CPB, immune dysregulation may have far-reaching consequences in terms of the immunocompetence of patients following CPB. This may be particularly relevant for those patients who sustain episodes of endotoxaemia during bypass (Nilsson et al 1990, Rocke et al 1987) and the minority who develop frank gut ischaemia (Leitman et al 1987). These patients may become predisposed to the development of post-operative infections which may be of gut origin (Ford et al 1991).

Soluble Proteins

One of the major problems of CPB is contact activation of blood components. This is a result of the extracorporeal circulation of blood through artificial tubes and chambers. The primary event is the activation of factor XII (Hageman factor), with the production of activated factor XII (XIIa), kallikrein and bradykinin. Kallikrein may then cleave the C5 component of complement, producing the anaphylatoxin C5a (Royston 1990).

Fig 7: The complement cascade & cellular activation during CPB



The complement system may be activated via the classical (requiring antibody) or alternate (independent of antibody) pathways. Contact activation leads to direct activation of the C3 component with production of C3a and C3b (Fig 7). The C3b then binds covalently to foreign surfaces where it may continue to activate the

complement cascade. The final effector sequence of C5-C9 has cytolytic, vasoactive and immunoregulatory properties (Royston 1990).

Complement, together with other acute phase proteins such as C-reactive protein are important for phagocytosis of foreign organisms, a process known as opsonisation. However, under normal circumstances only a small amount of complement and immunoglobulin is required for adequate opsonisation (Alexander 1980). Following CPB, the level of complement is reduced (Collett et al 1984, Parker et al 1972). This is due to a combination of consumption by excessive activation during CPB and haemodilution in the immediate post-operative period.

Complement activation may be determined by measuring the level of C3a desarg, an inactivation product of C3a (Moore et al 1988). The level of C3a desarg has been found to rise within the first ten minutes of CPB and remain constant thereafter, although further activation following the administration of protamine at the conclusion of CPB has been reported (Cavarocchi et al 1985, Kirklin et al 1986). The rise in C3a desarg was associated with the expression of neutrophil C3b receptors (Moore et al 1988). Although C3a desarg levels rise dramatically with CPB no similar rise in C5a desarg has been found. This is attributable to the rapid and avid uptake of C5a by high affinity C5a receptors on neutrophils (Chenoweth et al 1981, Hammerschmidt et al 1981). Massive complement activation may have a down-regulatory action upon neutrophil function. Binding of C5a results in membrane internalisation of the neutrophil C5a receptor, which may make neutrophils unresponsive to chemotactic C5a signalling. The expression of C3b receptors results in neutrophil aggregates which are ineffective for phagocytosis but maintain their capacity to damage tissue such as the lungs (Moore et al 1988). The expression of the C3i receptor which responds in parallel to the C3b receptor results in neutrophil degranulation, leaving neutrophils deficient in microbiocidal capacity but increasing plasma proteolytic activity (O'Shea et al 1985, Todd et al 1984).

Activation of complement by the classical pathway also occurs during CPB since the level of C4 falls. C-reactive protein, protamine complex and aggregated immunoglobulins have been suggested as activators of the classical pathway (White 1981), but C-reactive protein levels do not rise until the 5th post-operative day (Collett et al 1984).

Complement activation results in increased capillary permeability, mast cell degranulation with resultant tissue oedema and ultimately contributes to organ damage. Therefore strategies which attenuate complement activation may limit organ damage. Cavarocchi et al (Cavarocchi et al 1986) in a randomised prospective study found reduced C3a levels in patients who were (a) given corticosteroids or (b) had membrane oxygenation during CPB, compared to patients who had bubble oxygenation. This may have been due to corticosteroid-induced functional impairment of the first four components of the complement cascade (O'Flaherty et al 1977). More recently, Videm et al (Videm et al 1989) found no difference between bubble or membrane oxygenation for C5-C9 activation, although the sheet and capillary hollow fibre oxygenators were associated with greater C3 activation.

The alterations in the level of complement components, has been closely linked to neutrophil numbers and function during and following CPB. Pulmonary damage following CPB has been associated with neutrophil sequestration in pulmonary capillaries and elevated activated complement products (Cavarocchi et al 1986).

Macrophages

The effects of CPB on neutrophils has already been discussed and this section will focus upon the mononuclear phagocytes or macrophages, which play a central role in cell mediated immunity (CMI). Macrophages can present antigen with the major histocompatibility complex (MHC) class II molecules, thereby activating T-lymphocytes. The signal for this activation is interleukin-1 (IL-1), which is also a

pyrogenic molecule. Macrophages possess tumouricidal activity and can release a number of substances inducing tumour cell death, such as tumour cell necrosis factor (TNF), another pyrogenic molecule. Similar to neutrophils, after phagocytosis, bacterial killing is achieved via an oxygen-dependent process with the generation of OFR, or an oxygen-independent process using cationic proteins and enzymes. These cells are found both in the blood and tissues and together form the reticuloendothelial (RE) system (Roitt et al 1985 p.11.7).

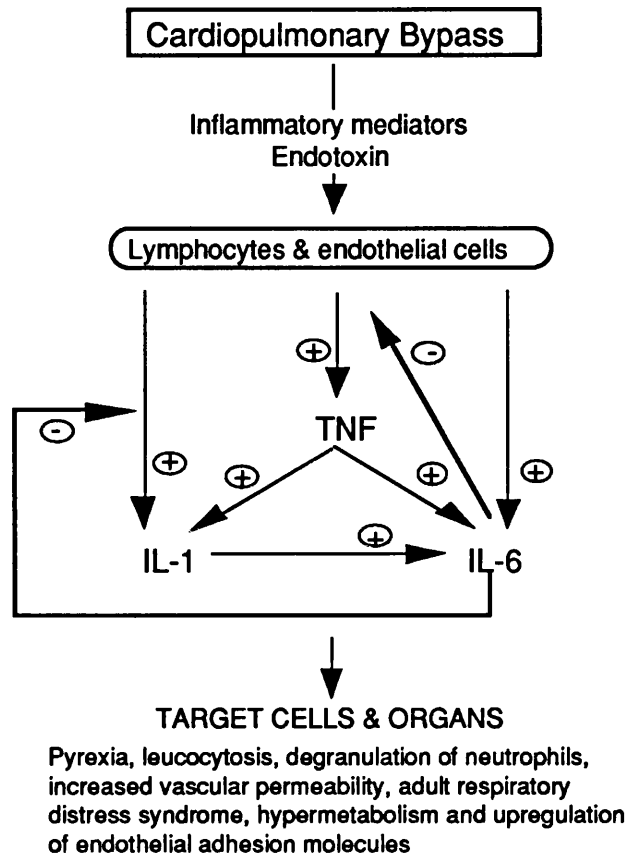
Unfortunately, little work has been undertaken examining the effects of CPB on the RE system, except for the work of Subramanian and colleagues in the late 1960's, in a rat model of CPB. They showed that the ability of the rat liver to clear colloidal gel (Au¹⁹⁸) following CPB was reduced compared to control and sham CPB rats (Subramanian et al 1968). The macrophages responsible for this clearance are the Kupffer cells. This is an important finding since any foreign material including bacteria entering the portal circulation from the gut is removed by these cells. The ability of Kupffer cells to clear bacteria (Benacerraf et al 1959) and endotoxin (Rutenberg et al 1967) is established and the contribution of impaired RE function to the development of endotoxaemia during CPB is implied but unestablished. In further experiments to elucidate the mechanism of Kupffer cell dysfunction, Subramanian et al examined the influence of blood cell destruction on Kupffer phagocytic ability. When the liver was perfused with fresh blood or plasma no impairment in Au¹⁹⁸ or Fe⁵⁹-labelled staphylococci clearance was observed. Liver perfused with circulated blood showed a degree of red blood cell fragmentation observed in patients following CPB and was associated with impaired Kupffer cell function (Subramanian et al 1968).

Cytokine Production and CPB

Cytokines are soluble mediators of inflammation which are produced in infection, trauma and neoplasia by a variety of cell types. Cytokines are essential for the mediation of the inflammatory response but when produced in excess they may have

deleterious actions. The cytokines which have been extensively studied are TNF, IL-1 and interleukin-6 (IL-6). These are produced by monocytes and endothelial cells (Atkins et al 1967, Miossec et al 1986, van Snick 1990). When generated in a controlled fashion, interleukin-8 (IL-8) and IL-6 upregulate the immune system (Dinarello 1988, Le and Vilcek 1989, Wong and Clark 1988), whilst TNF can destroy tumour cells as one of its many bioactivities (Tracey et al 1989). There is evidence that a 'cytokine network' exists (Fig 8). TNF and IL-1 can stimulate each other's production (Le and Vilcek 1987) and enhance IL-6 production (LeandVilcek 1989). IL-6 may be part of a negative feedback loop since it inhibits endotoxin induced TNF and IL-1 production by mononuclear cells (Schindler et al 1990). The production of TNF, IL-1 and IL-6 is stimulated by bacteraemia, endotoxin and complement products (Beutler et al 1985, Fong et al 1989, Haeffner-Cavaillon et al 1987, Hesse et al 1988)]. TNF is the first to appear after an endotoxin challenge followed by IL-1 at two hours and IL-6 at 6 hours (Fong et al 1989). The level of production of IL-6 has been correlated with the duration of operation and may be a marker of tissue injury (Shenkin et al 1989). It also mediates the response to injury such as fever, leucocytosis, increased vascular permeability and synthesis of acute phase proteins. These changes frequently occur following CPB (Freeman and Gould 1985, Kress et al 1987, Smith et al 1987, van Oeveren et al 1985) and may be further exacerbated in those patients who also sustain endotoxaemia during CPB.

Fig 8: The cytokine network



TNF may be one of the mediators of endotoxic induced tissue injury. Injection of high doses of recombinant TNF induce a syndrome with all the characteristics of septic shock (Tracey et al 1986, Tracey et al 1987). Intravenous TNF is also capable of provoking pulmonary injury with increased vascular permeability, resulting in a condition that resembles adult respiratory distress syndrome (ARDS) (Natanson et al 1989, Schirmer et al 1989) which occurs in approximately 1.7% of patients post-CPB (Fowler et al 1983).

To date, there have been only a few studies examining the consequence of CPB upon cytokine production. Haeffner-Cavallion et al, found no rise in TNF antigen in monocyte lysate or in the plasma of patients during or after CPB (Haeffner-Cavallion et al 1989), whilst in a smaller study three out of five patients had detectable levels of TNF in the plasma at the end of CPB (Laidler et al 1991). However, other clinical factors may also increase TNF levels such as myocardial infarction, cardiogenic

shock and severe heart failure (Levine et al 1990, Maury and Teppo 1989). More recently, Jansen et al (Jansen et al 1991) have found that TNF production peaks with endotoxaemia during CPB upon cross-clamp release. The release of the aortic cross clamp was also the time when a peak in complement receptor 3 (CR3), an adhesion receptor was found on circulating neutrophils during CPB together with a peak in leukotriene B4 (a chemoattractant for neutrophils) (Gu et al 1992). During CPB, neutrophils become activated to express adhesion molecules; however, TNF and endotoxin can upregulate the expression of adhesion molecules on endothelial cells (Pohlman et al 1986), thus promoting neutrophil margination and leukocyte mediated cell injury (Harlan 1985, Ward and Varani 1990).

Summary

In summary, CPB causes profound changes in haemodynamic parameters during and following extracorporeal perfusion. Perhaps more important are the consequences of the bioincompatibility of the extracorporeal circuit, which by activating cellular and non-cellular components of blood results in the generalised or so-called 'whole body inflammatory response of bypass'. These factors combine to act in synergy, promoting tissue and organ damage, which in large part is effected by neutrophils and orchestrated by components of the immune system. Before addressing some of the alterations in the haemodynamic parameters which may underlie splanchnic injury or factors promoting the inflammatory response of bypass, the extent of CPB associated intra-abdominal complications needs to be determined. The following chapter aims to provide an overview of the prevalence of splanchnic damage reported in the literature and that estimated from a retrospective study undertaken at the Hammersmith Hospital.

Chapter 2

Intra-abdominal Complications following Cardiopulmonary Bypass

Introduction & Aims

Over recent years, there has been a progressive reduction in post-operative morbidity and mortality after cardiac surgery (Miller et al 1983, Naunheim et al 1988). With this reduction in mortality due to cardiac related causes, the need to understand the pathogenesis of non-cardiac complications of CPB has assumed greater importance.

Intra-abdominal complications are serious sequelae of CPB and the mortality in this group of patients has been reported to be as high as 67% (Wallwork and Davidson 1980), which probably reflects generalised organ dysfunction in these patients.

To investigate the incidence of gastrointestinal (GI) complications following CPB at the Hammersmith Hospital, a retrospective study was undertaken to determine:

- (1) the incidence of GI complications following CPB,
- (2) to identify patient and operative characteristics, which may predispose to the development of intra-abdominal complications, and
- (3) the influence of pulsatile perfusion, oxygenator type and arterial filtration.

Materials & Methods

All adult patients who had undergone CPB at the Hammersmith Hospital during the period 1st January 1973 to 31st December 1989 were included in the study. A total of 4629 patients were identified, of whom 2568 patients underwent coronary artery bypass grafting (CABG) while valve or other procedures were performed in 2061 patients. The ratio of males to females was 2.6:1.

The medical notes and data available from a computerised data base from 1985 were reviewed. Patient, operation, perfusion and intensive care data were recorded on a proforma. Those patients who had sustained intra-abdominal complications were identified and their subsequent management and outcome noted. All patients received prophylactic antibiotics. The drugs used varied over the period studied. Nasogastric tubes were not inserted routinely. H₂-blocking drugs were not employed as a prophylaxis against peptic ulceration, but were continued in those patients already taking them, or those with a past history of gastrointestinal bleeding.

Cardiopulmonary Bypass Technique

Myocardial protection during CPB was effected by cold St Thomas' cardioplegia solution. Patients in addition were cooled to 28-32°C during bypass, and received either pulsatile or non-pulsatile flow at rates of 1.8-2.0 l/min/m², with a mean perfusion pressure of 55-65 mm Hg. Pulsatile perfusion was employed in 562 patients between 1984 & 1989. The selection of the type of perfusion was based upon the practice of the consultant responsible for the patient. One team had employed pulsatile flow during bypass routinely on all patients undergoing CPB since 1984.

Statistical Analysis

The non-paired Student's t, and the Chi-square test with Yates correction were employed to assess differences between means and groups for statistical significance. All data were calculated for two tailed values where $p < 0.05$ is a significant difference. All data are presented as mean \pm standard deviation.

Results

Excluding renal and hepatic dysfunction following CPB, 33 intra-abdominal complications occurred in 27 patients, an incidence of 0.58%. The characteristics of this group of patients are summarised (Tab 1). The ratio of males to females was 2.7:1. The incidence of intra-abdominal complications in those patients who had undergone CABG was 0.7% compared to 0.44% for non-CABG patients, which was not a significant difference (NS). There were 4 deaths (14.8%). The mean bypass and cross-clamp times were 96.7 ± 28.6 and 48.6 ± 15.7 minutes for intra-abdominal complication patients compared to 81.7 ± 48.4 minutes and 48.5 ± 26.1 minutes respectively for the group as a whole. There was no significant difference between the

two groups in cross-clamp time, but the intra-abdominal complication group had a total bypass time which was 15 minutes longer ($p<0.01$). The incidence of complications was 0.8% in those aged over 65 years compared to 0.53% in those less than 65 years (NS). The mortality was significantly higher in the intra-abdominal group (14.8%) than in the control group of patients (3.4%; $p<0.01$).

Tab 1: Patient characteristics of those with & without intra-abdominal complications following cardiopulmonary bypass

	Number	Sex		Operation		CPB (minutes \pm SD)		Age		Mortality %
		M	F	CABG	Valve/other	Pump time	Cross clamp	<65	>65	
Control	4602	3332	1270	2550	2052	81.7 \pm 48.4	48.5 \pm 26.1	3756	873	3.4
Complications	27	20	7	18	9	96.7 \pm 28.6	48.6 \pm 15.8	20	7	14.8
Oesophagitis	6	4	2	4	2	100.8 \pm 38.0	43.8 \pm 9.4	4	2	17
Gastritis	3	3	0	3	0	73 \pm 16.4	34.7 \pm 4.2	3	0	0
Gastric erosions	2	0	2	1	1	96 \pm 1.4	47.0 \pm 5.1	1	1	0
Duodenal ulcer	4	4	0	2	2	97 \pm 8.9	34.5 \pm 4.6	2	2	50
Perforated DU	2	1	1	1	2	99.5 \pm 27.6	47 \pm 17.9	1	1	0
GI Haemorrhage undetermined source	3	3	0	3	0	121.7 \pm 34	63.3 \pm 18	3	0	33
Cholecystitis	5	2	3	5	0	86.2 \pm 7.5	45.8 \pm 1.5	3	2	0
Pancreatitis	4	3	1	3	1	105.3 \pm 17.7	64.8 \pm 17	3	1	0
Diverticulitis	1	0	1	0	1	69	0	0	1	0
Colitis	1	1	0	1	0	66	0	1	0	0
Ogilvie's	1	1	0	1	0	85	0	0	1	100
Irreducible inguinal hernia	1	1	0	1	0	64	0	1	0	0

Analysis of the risk factors, following bypass of those with and without intra-abdominal complications was also undertaken, and the results are presented in Tab 1. There was a significantly higher incidence of arrhythmias ($p<0.01$), inotropic support ($p<0.001$) and the requirement of intra-aortic balloon pump ($p<0.01$) for those patients who subsequently developed intra-abdominal complications. Further assessment was undertaken for the incidence of renal and hepatic dysfunction in the two groups following CPB.

Tab 2: Postoperative profile of patients with & without intra-abdominal complications following cardiopulmonary bypass

	Patients with Intra-abdominal complications (n=27)	Patients without Intra-abdominal complications (n=4602)	p Value
Inotropic support	8 (29.6%)	326 (7.1%)	<0.001
Intra-aortic balloon pump	5 (18.5%)	119 (2.6%)	<0.01
Arrhythmias	8 (29.6%)	603 (13.1%)	<0.01
Renal dysfunction (urea >6.5mmol/l & creatinine >125 µmol/l)	14 (51.9%)*	492 (10.2%)*	<0.001
Renal failure**	11 (42.3%)	123 (2.7%)	<0.001
Hepatic dysfunction (bilirubin >14µmol/l)	11 (40.7%)	662 (14.4%)	<0.001
Ventilated for > 24 hrs	4 (14.8%)	199 (4.3%)	<0.05

* In complication group one patient had preoperative chronic renal failure (n=26 for patients without chronic renal failure), whilst in control group 27 patients had preoperative chronic renal failure (n=4575 for patients without chronic renal failure)

**Defined as those patients who developed a ceatinine >160µmol/l following CPB.

Renal dysfunction was defined as an elevation of the urea and creatinine above the upper limit of normal for our laboratory (urea >6.5 mmol/l and creatinine >125 µmol/l), in patients who had normal pre-operative values. Acute renal failure was defined as a rise in creatinine of >160 µmol/l following CPB, since records for the volume of urine production and urinary excretion of sodium were incomplete on retrospective analysis of the charts. For the intra-abdominal complication group of patients, one patient was excluded because he had chronic renal failure (IgA nephropathy), and required subsequent haemofiltration and haemodialysis. Two patients in the intra-abdominal complication group, who developed renal dysfunction, went on to develop oliguric renal failure and required haemofiltration in the post-

operative period. One of these patients had renal failure as part of multi-system organ failure and died, whilst the second patient recovered following haemofiltration.

Hepatic dysfunction was defined as an elevation of the bilirubin ($>14 \mu\text{mol/l}$), and an elevated alkaline phosphatase ($>120 \text{ IU/l}$), in patients that had normal pre-operative liver function tests. 14.4% of patients developed hepatic dysfunction following CPB without the development of other intra-abdominal complications (Tab 2), 18.4% ($n=122$) died following surgery, whilst 3 out of 4 patients (75%) died in the intra-abdominal complication group who had hepatic dysfunction.

Gastrointestinal haemorrhage or peptic ulceration occurred in 20 patients (5 women & 15 men); in three of these patients the site of haemorrhage was not found despite endoscopic investigation. Fifteen patients had CABG and five had valve repairs or combined valve/CABG operations. The mean CPB time was 98.4 ± 30.9 minutes ($n=20$), which was significantly longer than the control group of patients ($p<0.02$), and the mean aortic cross-clamp time was 45.3 ± 14.4 minutes ($n=15$), which was not significantly different from the control group ($p>0.09$). All the patients who died subsequent to the development of their intra-abdominal complication belonged to this group. An outline of the management of patients with GI blood loss is provided in Table 3, together with their requirements for blood transfusion.

Tab 3: Management & outcome of patients with gastrointestinal bleeding

	Mean Blood Transfusion Requirements for GI Blood Loss (Units)	Treatment	Outcome
Oesophagitis (n=6)	0.3	H ₂ antagonist	All alive, except one who also had colitis*
Gastritis (n=3)	1.7	H ₂ antagonist	All alive
Gastric erosions (n=2)	2.0	1 Bilroth I gastrectomy 1 Truncal vagotomy & pyloroplasty	Alive Alive
Duodenal ulcer (n=4)	2.8	2 H ₂ antagonist 2 Truncal vagotomy & pyloroplasty	Both alive Both died
Perforated DU (n=2)	4.0	2 Omentopexy	Both alive
GI Haemorrhage undetermined source (n=3)	2.0	Conservative	One patient died following development of multisystem failure
Colitis (n=1)	2.0	Antibiotics & parenteral nutrition	Died*

Diagnosis was established between 2 and 10 days post-operatively (mean 5 days). This varied with the nature of the underlying pathology. Perforated duodenal ulcers presented in the classical manner, with the development of sudden upper abdominal pain, tachycardia and pyrexia. Subsequent abdominal X-rays confirmed pneumoperitoneum in two cases, whose perforated ulcer was treated by simple omentopexy in one case and truncal vagotomy and pyloroplasty in the second. There was no mortality in this sub-group. The highest mortality in our series was for duodenal ulcer (DU) patients. Two out of 4 patients died subsequent to surgery. Both these patients underwent truncal vagotomy and pyloroplasty, whilst the two patients treated with H₂-antagonist and blood transfusion were subsequently discharged home without morbidity. The DU group of patients were diagnosed a mean of 7 days post-operatively (range 5 to 8 days) compared to a mean of 4.5 days (range 4 to 5 days) for the perforated DU group.

Acute cholecystitis developed in 5 patients (two men & three women). All five patients had undergone CABG ($p < 0.001$) with a mean pump time of 86.2 ± 7.5 minutes (NS). Two patients developed acalculous and three patients, calculous cholecystitis. Although pyrexia, nausea and upper abdominal pain were consistent findings, Murphy's sign was present in only one out of the five patients. Clinical diagnosis was confirmed by ultrasonography in each case. Patients were treated with intravenous fluids, broad spectrum antibiotics against gut coliforms and bowel rest. None of the five patients required cholecystectomy during their cardiac in-patient stay, and none died.

Four patients developed acute pancreatitis (three men & one woman), with a mean diagnosis time of 2.5 days post-operatively (2 to 3 days). The clinical presentation was notable for the absence of vomiting in any of the patients, although other symptoms such as fever, nausea and upper abdominal pain were present. The diagnosis was established on the finding of elevated serum pancreatic amylase levels and ultrasonographic findings. The mean bypass time for this sub-group of patients was 105.3 ± 17.7 minutes ($p < 0.01$). Patients were managed by intravenous hydration, nasogastric suction and complete bowel rest; prophylactic antibiotics were given only to the one patient who had undergone valve replacement. Subsequently patients were followed up by twice weekly abdominal ultrasound. Two patients developed pancreatic pseudocysts which, in one case was a cause of some post-prandial nausea, but these resolved 7 weeks post-operatively. There was no mortality in this group of patients.

Oesophagitis developed in one man, which was proven on endoscopy, and, 17 days post-operatively, rectal bleeding and diarrhoea. Colitis of undetermined aetiology was diagnosed, but fiberoptic colonoscopy was undiagnostic. He was treated with

antibiotics and parenteral nutrition, but at 36 days post-operation had a torrential haemorrhage and died. A post-mortem was not performed.

An irreducible inguinal hernia developed in one patient post-CPB who had a history of 2 previous herniorrhaphies. The hernia was reduced at operation and a further hernia repair was performed without complications.

Multi-system organ failure developed in 2 patients post-CPB. One patient had undergone ventricular aneurysectomy and CABG and subsequently developed renal failure, hepatic failure and melaena. Despite endoscopy, the source of the bleeding was not determined, and he died. The second patient, who underwent CABG, developed Ogilvie's syndrome (pseudocolonic obstruction) as well as pancreatitis, acalculous cholecystitis, and renal and hepatic failure. All these complications were managed non-operatively with parenteral nutrition and antibiotics. He survived to be discharged home 7 weeks post-operatively.

During 1984-1989 at the Hammersmith Hospital, 2145 patients underwent cardiopulmonary bypass surgery (Tab 1). Five hundred and sixty-two patients had pulsatile flow during their cardiac procedure, and only one abdominal complication was recorded (diverticulitis). This compares to 13 complications in 10 patients out of 1583 patients who had non-pulsatile flow during CPB. Although there were fewer complications in the pulsatile group, this was not statistically different ($p=0.14$). The total bypass time for the pulsatile flow patients was 83.7 ± 34.6 minutes compared to 80.9 ± 41.5 minutes for the non-pulsatile group (NS). The total bypass time for the pulsatile group with complications was 69 minutes ($n=1$), compared to a mean of 99.8 ± 24.9 minutes ($n=10$) for the non-pulsatile group with complications. There was a significant difference ($p<0.001$) between the total bypass time for non-pulsatile

control and the complication group of patients. For the pulsatile group only one patient sustained a complication and her total bypass time was actually shorter than the control group.

Further analysis was undertaken (Tab 4), in this group of patients, to see if there was any protective effect of using arterial line filters, and a membrane oxygenator. The pulsatile flow with arterial line group had fewer intra-abdominal complications, than a group with non-pulsatile flow and arterial line filters ($p < 0.05$).

Tab 4: Analysis of 2145 patients who underwent pulsatile & non-pulsatile flow for the use of arterial line filters, & the type of oxygenator employed.

			Arterial line filter		Without filter	
	Pulsatile	Non pulsatile	Pulsatile	non pulsatile	pulsatile	non pulsatile
Number	562	1583	562	540	0	1043
GI Complication	1	13	1	10	0	3
No. of patients	1	10	1	7	0	3
Incidence (%)	0.18	0.63	0.18	1.85	0	0.19
p Value	NS		$p < 0.05$			

	Pulsatile		Non pulsatile	
	membrane oxygenator	Bubble oxygenator	membrane oxygenator	Bubble oxygenator
Number	73	489	13	1570
GI Complication	1	0	0	13
No. of patients	1	0	0	10
Incidence (%)	1.36	0	0	0.64
p Value	NS		NS	

However, the beneficial effect was unlikely to be due to the filter, since in a similar group of patients without arterial line filters and non-pulsatile flow, had a lower

incidence of intra-abdominal complications (0.19%) than the non-pulsatile/arterial filter group (1.85%). No significant difference was found in the use of the membrane or bubble oxygenator, or for the type of flow employed during CPB.

Discussion

In the presented series and previous retrospective studies gastrointestinal haemorrhage has been the most common post-CPB intra-abdominal complication reported (Egleston et al 1993, Hanks et al 1982, Huddy et al 1991, Krasna et al 1988, Lawhorne et al 1978, Leitman et al 1987, Lucas and Max 1980, Moneta et al 1985, Pinson and Alberty 1983, Reath et al 1983, The Society of Thoracic Surgeons 1993, Wallwork and Davidson 1980, Welling et al 1986).

Tab 5: The Incidence of gastrointestinal complications following CPB

Author	Total Series	Incidence (%)	GI Bleeding	Intestinal	Cholecystitis	Pancreatitis	Other	Mortality (%)	
Lawhorne 1978	2,500	0.6	2	5	1	1	7	33	
Wallwork 1980	1,000	0.9	2	5	-	2	-	67	
Lucas 1980	3,000	0.3	-	4	3	-	1	25	
Hanks 1982	5,080	0.8	19	5	6	7	4	63	
Pinson 1983	5,682	0.6	9	17	8	-	2	27	
Reath 1983	3,200	0.8	10	5	2	1	5	24	
Moneta 1984	2,482	0.9	2	9	2	6	8	17	
Welling 1986	1,596	1.1	1	9	3	-	5	12	
Leitman 1987	6,452	0.9	20	18	11	5	6	59	
Krasna 1988	1,297	2.0	8	4	7	8	6	44	
Huddy 1991	4,473	0.8	19	15	-	6	-	63	
Egleston 1993	8,559	0.4	24	7	1	4	1	26	
Society of Thoracic Surgeons (USA) 1988-1992	81,033	1.68	NOT STATED						

This has been followed by intestinal ischaemia or infarction, acute cholecystitis, pancreatitis, perforated ulcer, small intestinal obstruction, hepatic necrosis and splenic injury respectively. Risk factors that have been identified for the development of gastrointestinal complications include prolonged bypass time, valve surgery, post-operative hypotension or bleeding, low cardiac output, the use of vasopressors, the need for an intra-aortic balloon pump, emergency operation and advanced age (>70 years)(Krasna et al 1988, Leitman et al 1987, Welling et al 1986). In the presented study, patients who subsequently developed intra-abdominal complications had a significantly longer total bypass time compared to the control population. We could demonstrate no significant differences for type of surgery or age. However, the Society of Thoracic Surgeons (USA) in an assessment of 81,033 patients reported a higher incidence of GI complications in those undergoing aortic (1.83%) and mitral (2.53%) valve replacements than those who had coronary bypass grafting (1.61%)(The Society of Thoracic Surgeons 1993). Patients who developed intra-abdominal complications, had a greater incidence of post-operative arrhythmias, and requirement of inotropic or intra-aortic balloon support, than those patients who did not develop similar complications. The incidence of post-operative renal dysfunction and hepatic dysfunction was also significantly higher in the former group.

Gastrointestinal bleeding due to peptic ulceration following CPB was first reported in 1957 in 6 patients(Berkowitz et al 1957). The cause was thought to be the Curling stress response, similar to that occurring in life-threatening illness. As a result, some centres employ routine H₂-blockade. This is not our current practice, and probably justifiable in the face of the low incidence (0.4%) of GI haemorrhage in our group of 4629 patients. The incidence of GI haemorrhage following CPB is variable depending upon the definition of gastrointestinal bleeding used in each series, but a mortality of up to 80% has been reported (Leitman et al 1987, Moneta et al 1985, Pinson and Alberty 1983, Reath et al 1983, Shocket et al 1977, Taylor et al 1973, Welling et al

1986). Recently, Rosen et al have examined the risk factors for GI haemorrhage post-CPB. In a study of 9,199 patients, the incidence of GI haemorrhage was 0.32%. The main risk factors were age, the need for re-operation and episodes of hypoperfusion. The mortality was 34.3% for patients with GI complications compared to 0% for a matched population of patients. Prophylactic regimens (H_2 -receptor antagonists and/or antacids) designed to reduce the risk of peptic ulceration did not influence the development of peptic ulceration post-CPB (Rosen et al 1992).

It is now recognised that acidity of the gastric lumen is not the only factor in the development of ulceration. Experimental models suggest that the maintenance of the mucosal barrier is dependent upon normal blood flow and prostaglandin production (Cheung and Ashley 1987, Levine 1987). Hypoperfusion may occur as a result of a combination of pre-existing mesenteric atherosclerosis in elderly patients and the vasoconstriction which occurs during CPB. The latter may be ameliorated by using pulsatile rather than non-pulsatile flow (Taylor et al 1979, Watkins et al 1982).

The mechanism of this ischaemic injury is as yet undetermined. Free-radical damage may play a pivotal role, as has been shown in ischaemic/reperfusion injury in the stomach (Perry et al 1986). The loss of blood from the stomach in rats, as determined by ^{51}Cr -labelled red blood cells, was reduced in those animals in which free-radical production was inhibited (Smith et al 1987). In our series, only 2 of the 20 patients with GI haemorrhage admitted to a previous history of peptic ulceration. If mucosal hypoperfusion is the underlying problem in these patients, then prophylactic agents which enhance gastric mucosal defence such as sucralfate or prostaglandin analogues may be more appropriate prophylactic drugs for patients undergoing CPB.

Post-CPB gastrointestinal haemorrhage is managed in the same way as that occurring in the non-cardiac patient. Endoscopic laser- and electro-coagulation are alternatives to surgery which were not available to surgeons at the beginning of the study period. These newer modes of non-invasive therapy may reduce the mortality, which for the duodenal ulcer group of patients was 50%.

The incidence of overt pancreatitis after CPB is low, 0.2–0.63% (Krasna et al 1988, Lawhorne et al 1978), but the sub-clinical incidence may be much higher. Recently, Castillo et al performed a prospective study of 300 patients and measuring serum amylase, pancreatic isoamylase and lipase, and found that 27% of their patients sustained pancreatic cellular injury following CPB (Castillo et al 1991). Unfortunately, in the study presented in this thesis, information as regards the incidence of post-CPB hyperamylasaemia was not available. However, a subsequent prospective study suggested a relationship between the development of hyperamylasaemia and the use of non-pulsatile CPB (Ohri et al 1992). Rattner, et al reported the incidence of post-CPB hyperamylasaemia to be as high as 32% (Rattner et al 1989). The mortality of patients with hyperamylasaemia was 9%, compared to 1% for those without hyperamylasaemia (Rattner et al 1989). The mechanisms of pancreatic damage are unresolved, but Castillo et al found that the administration of >800 mg calcium to be risk factor; the underlying mechanism is postulated to be hypoperfusion (Castillo et al 1991). Undoubtedly, a delay in diagnosis due to absence of the classical clinical picture contributes to the morbidity and mortality. Haas et al reported that all the patients had fever, pain and leucocytosis at the time of diagnosis (Haas et al 1985). This was also true for patients in this series, but vomiting which is described for classical acute pancreatitis was notably absent. Patients were managed by nasogastric aspiration and intravenous fluids with no mortality.

Leitman et al, in a review of 6542 CPB cases (Leitman et al 1987), reported 6 cases of acute calculous cholecystitis with a mortality of 73%. This compares with a mortality of 47% for cholecystitis following non-cardiac operations (Ottinger 1976). Fortunately, none of our patients with cholecystitis died; this may be due to the conservative policy adopted in our institution, although cholecystectomy has been recommended as the treatment of choice for acute acalculous cholecystitis (Krasna et al 1988). Fever and leucocytosis are usually present, but tenderness in the right upper quadrant was not a consistent finding (Krasna et al 1988, Leitman et al 1987), a finding mirrored in this series. Radio-isotopic scanning and ultrasonography may be adjuncts to clinical diagnosis, but both have a significant incidence of false positive results in the critically ill patient (Krasna et al 1988). Approximately 50% of patients with acalculous disease progress to gangrenous cholecystitis, which makes tube cholecystostomy a potentially hazardous procedure. As in post-CPB haemorrhage and pancreatitis, the incidence of cholecystitis correlates with age greater than 70 years, valve surgery and prolonged bypass time (Krasna et al 1988, Leitman et al 1987), although we could not demonstrate any significant differences for these factors.

Renal dysfunction and acute renal failure occurred with a greater incidence in patients with intra-abdominal complications. Renal failure following cardiac surgery is associated with a high mortality (Corwin et al 1989), and may represent generalised organ hypoperfusion during bypass and/ or the post-operative period. It has been shown that both low flow (42 ml/kg/min) and low blood pressure (50 mm Hg) during CPB can be tolerated with good preservation of renal function (Hilbermann et al 1977). However, at lower rates of flow, the glomerular filtration rate cannot be maintained by renal autoregulatory mechanisms, and renal failure ensues (Ohri and Abel 1991).

Liver dysfunction following CPB may present as mild hyperbilirubinaemia or much less commonly as liver failure as part of multi-system organ failure. In our patients a 14.4% incidence of post-CPB liver dysfunction was found in patients without other intra-abdominal complications whilst it was 40.7% in the group with other complications. This compares to 20% incidence of hyperbilirubinaemia, which has been documented in a prospective study of 248 patients undergoing CPB (Collins et al 1983). In the study by Collins et al, pre-operative alcohol intake, enzymes and bilirubin were not associated with subsequent liver dysfunction, but raised right atrial pressure and multiple valve replacement were significant prognostic factors. Mathie et al showed that hepatic blood flow is better preserved during CPB by pulsatile flow at normothermia (Mathie et al 1986), but this advantage is less apparent at hypothermic temperatures (28-30°C) (Desai et al 1993). Like other organs of the splanchnic bed, the liver may also sustain injury from oxidant stress following CPB. The liver is one of richest sources of xanthine oxidase (Al-Khalidi and Chaglassion 1965). This enzyme is required for the conversion of hypoxanthine to xanthine, and the subsequent production of oxygen free radicals.

In this retrospective study of 2145 patients, we were unable to demonstrate any significant advantage of pulsatile flow for the prevention of intra-abdominal complications, or for the employment of arterial line filters during CPB. However, Huddy et al in a larger study of 4438 patients found a significant lower number of patients developed GI complications who had received pulsatile perfusion during CPB (Huddy et al 1991). Although non-pulsatile flow during CPB was not found to be a significant risk factor for the development of post-CPB intra-abdominal complications, age of the patient (>65 years) and the duration of the CPB time were found to be the most important patient and perfusion risk factors.

Chapter 3

The Effect of CPB on Gut Mucosal Perfusion: *Tonometric assessment*

Introduction & Aims

The effect of CPB on gut mucosal perfusion needs to be addressed clinically, in order to understand the mechanisms underlying the development of gut injury. In experimental animals radioactive microspheres and flow probes have been employed to evaluate the adequacy of mucosal perfusion; approaches which are not easily amenable to use in patients. However, even if these methods could be performed on patients they would only have limited benefit, since the parameter of interest is not really the absolute value (in ml/min) of mesenteric blood flow but the adequacy of mucosal delivery relative to the metabolic demands of the epithelium of the gut.

To this end, relatively non-invasive clinical techniques have been employed to examine for alterations in mucosal blood flow and overall mucosal perfusion. Blood flow has been determined using laser Doppler flowmetry (LDF) and intramucosal pH (pHi) evaluated using gastric tonometry.

Gastric Tonometry

Background

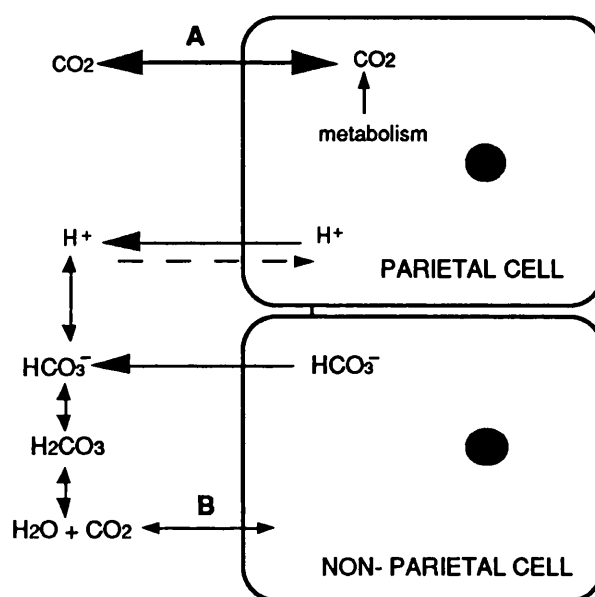
One method for assessing the adequacy of mesenteric perfusion is to monitor mucosal pH or hydrogen ion concentration. Although increased production of lactic acid is commonly thought to be the cause of tissue acidosis during ischaemia, recent data suggest that excessive hydrolysis of nucleoside phosphates is the predominant mechanism responsible for the accumulation of hydrogen ions within cells during intracellular hypoxia (Gores et al 1989, Hochachka and Mommsen 1983). According to the calculation made by Gores et al, hydrolysis of adenosine triphosphate (ATP) and adenosine 5'-diphosphate (ADP) can account for about half of the observed decrease in intracellular pH in an in vitro model of cellular hypoxia (Gores et al 1989). The remainder of the protons may come from the hydrolysis of organic esters. In ischaemic myocardium, measurements of intracellular pH correlate with tissue levels of ATP. Measurements of gut intramucosal pH may determine the adequacy of gut perfusion and indirectly be an indicator of tissue ATP levels.

Measuring gut pHi directly with tissue microelectrodes is cumbersome and does not have clinical applicability. The indirect method called tonometry is based upon the work originally performed by Bergofsky in 1964 (Bergofsky 1964) and later by Dawson et al in 1965 (Dawson et al 1965). These investigators showed that the pCO₂ in the lumen of a hollow viscus could be measured by determining the pCO₂ in the intraluminal fluid. Subsequently, Kivisaari and Niinikoski showed that average tissue CO₂ and O₂ tensions could be determined by measuring the pCO₂ and pO₂ in saline contained within a balloon fashioned out of material that was highly permeable to these gases (Kivisaari and Niinikoski 1973).

The development of tonometry was further extended by Fiddian-Green et al, who suggested that pHi could be calculated tonometrically if it was assumed that the tissue bicarbonate and arterial blood bicarbonate were similar, permitting the substitution of

this value into the Henderson-Hasselbach equation (Fiddian-Green et al 1982). This method also depends upon the assumption that the $p\text{CO}_2$ in the lumen of the bowel equilibrates with the bowel wall and in part on the knowledge that the $p\text{CO}_2$ within the cellular cytosol is linearly related to the $p\text{CO}_2$ in the extracellular environment (Roos and Boron 1981).

Fig 9: Factors determining intraluminal $p\text{CO}_2$



However, the intraluminal $p\text{CO}_2$ is determined both by the diffusion of carbon dioxide from the mucosal cells as a result of metabolism (Fig 9, marked A) and secondly as a result of the production of CO_2 from the secretion of acid and bicarbonate (Fig 9, marked B). In dogs the equilibration of intraluminal CO_2 with intracellular and blood CO_2 occurred within 20 minutes if a neutral or alkaline pH was obtained. When CO_2 was produced from secreted bicarbonate and hydrogen ions, back diffusion of CO_2 occurred relatively slowly and gastric intraluminal CO_2 was higher than intracellular and blood CO_2 . In the canine stomach the half-life for the equilibration of CO_2 was 18 to 22 minutes.

The effect of secreted HCO_3^- was not addressed by Fiddian-Green, during the validation of gastric tonometry in dogs. Fiddian-Green et al studied nine animals

using a gastric tonometer (hollow viscus tonometry) and a glass tissue microprobe. The stomach was filled with saline, HCl or a combination of HCl and NaHCO₃. A correlation of $r=0.79$ was found between tonometrically measured pHi and microprobe determined pHi, but paired data for animals given HCl/HCO₃ combination was not examined. The correlation from these animals may have been expected to show a much poorer correlation since $t_{1/2}$ for the back diffusion of CO₂ is 18 to 22 minutes.

Intramural acidosis may occur by two mechanisms, hypoperfusion and the back diffusion of CO₂. Although there is a barrier for this back diffusion, if acid secretion is high enough, CO₂ may diffuse into the mucosal cells to alter intracellular pH, thus affecting the sensitivity of any determined measurements.

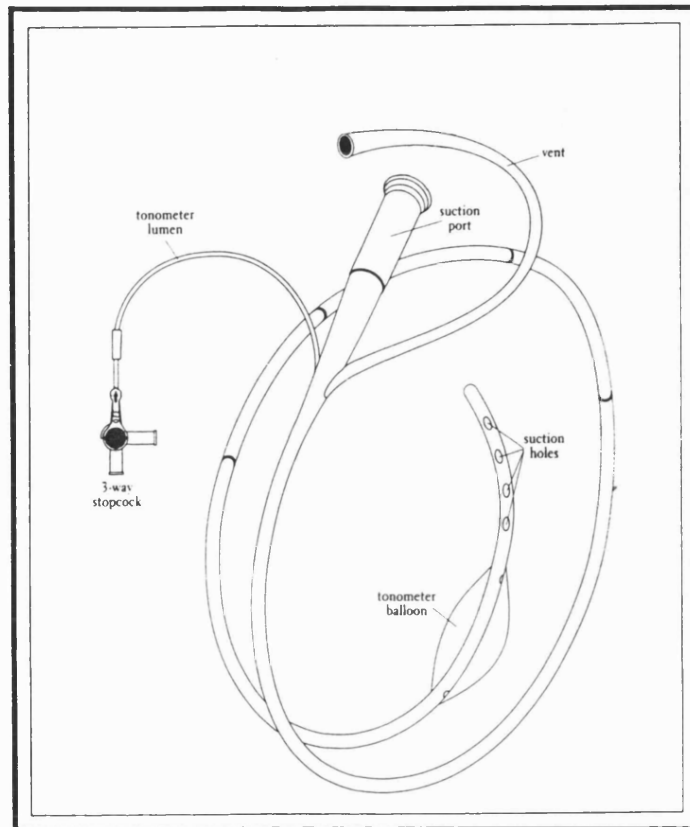
This problem was addressed by Heard et al, in a study investigating the effect of gastric acidity on pHi as determined by gastric tonometry in healthy volunteers. Individuals were randomized to receive ranitidine or no H₂-blocker therapy 12 hours prior to measurement of gastric pHi (Heard et al 1991). Those who had received ranitidine therapy had a higher gastric pH (6.5 vs 1.5, $p=0.0004$) and a lower intraluminal pCO₂ (5.6 vs 7.2 kPa, $p=0.012$) compared to controls. The calculated pHi was thus higher in the ranitidine treated group (7.39 vs 7.30, $p=0.02$) than the control group. Therefore gastric tonometry can result in erroneously low pHi values in patients who have a low gastric intraluminal pH.

Methodology for the determination of pHi

Tonometers are now commercially available (Tonometrics, Worcester, MA, USA), which are referred to as 'tonomitors' by the manufacturing company. One is available for determination of gastric pHi and the second for sigmoid colonic pHi. Although

each is slightly different in size and shape, they share a similar design. The gastric tonometer has three channels (Fig 10).

Fig 10: The gastric tonometer



One acts as gastric vent, the second channel can be used as a gastric sump, and the third channel connects to the silicone balloon which lies at the end of the gastric tonometer. Prior to insertion, 4 ml of normal saline is injected into the balloon to de-air the balloon and prime the dead space of the injection port and channel with saline.

The gastric tonometer is sited following induction of general anaesthesia in the anaesthetic room or in sedated ventilated patients in intensive care unit. The position can be confirmed by passing a measured length of the tonometer per orum, which for each patient equates to the distance between the incisors and the left costal margin.

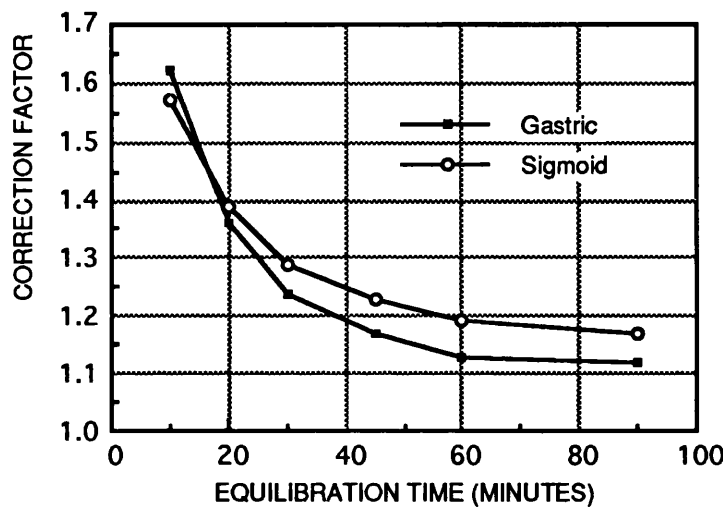
The sigmoid tonometer has only one port which leads to the silicone balloon. This catheter is passed by attaching a string to the end of the tonometer. The string is then threaded through the lumen of the sigmoidoscope so that the tonometer lies flush on its outer surface when tension is applied to both the string and tonometer. With the patient in the left lateral decubitus position, with hips and knees flexed, a sigmoidoscopy is performed. When the sigmoid colon is reached, the sigmoidoscope is gently withdrawn over the string, so as leave the sigmoid tonometer in position.

The tonometers are primed with exactly 2.5 ml of normal saline and the infusion time noted. After a given time 1 ml of saline is withdrawn; this is the sampling time. The equilibration period is therefore the sampling time minus the infusion time. The saline sample is taken to a blood gas analyser for the determination of the $p\text{CO}_2$. At the same time as the tonometer is sampled, arterial blood is also sampled for determination of arterial $[\text{HCO}_3^-]$.

The Steady State Adjusted $pCO_{2(ss)}$

The degree of equilibration of CO_2 in gastric mucosal cells with the saline of the tonometer is dependent upon the time of equilibration. The company (Tonometrics, Worcester, MA, USA), provides correction curves for each tonometer, which have been determined from in vitro studies at $37^\circ C$ (Fig 11).

Fig 11: Correction Factors for Gastric & Sigmoid Tonometers at $37^\circ C$



The differences in the correction factors between the gastric and sigmoid tonometers are due to differences in the area of the balloon membrane. The steady state $pCO_{2(ss)}$ is calculated as measured pCO_2 (mm Hg) x correction factor. This correction factor is governed by the duration of the equilibration period. The pHi is then determined by using the Henderson-Hasselbach equation:

$$pHi = 6.1 + \log_{10} \left[\frac{[HCO_3^-]}{pCO_{2(ss)} \times 0.03} \right]$$

Where 6.1 represents the pKa for the HCO_3^- / CO_2 system in plasma at $37^\circ C$, $[HCO_3^-]$ is the arterial bicarbonate concentration (mmol/ l), and $pCO_{2(ss)}$ is the steady state adjusted pCO_2 for the tonometer sample and 0.03 is the solubility factor for CO_2 in plasma at $37^\circ C$ (mmol l^{-1} mm Hg $^{-1}$).

The Effect of Temperature on the rate of Equilibration of CO₂

The equilibration factors which are available have been determined at 37°C, however, during CPB hypothermia to 28°C is often undertaken, which may influence the rate of diffusion of CO₂ from the mucosal cells to the gut lumen.

The rate of diffusion of a gas is governed by Graham's law, where ρ is the density of the gas.

$$\text{Rate of gas diffusion} \propto \frac{1}{\sqrt{\rho}} \quad (\text{Graham's Law})$$

The density of a gas is its mass per unit volume. The volume that one mole of gas occupies at any given pressure is governed by its temperature ($pV = RT$, Boyle's and Charles's laws, where p = pressure, V = volume, R = gas constant and T = temperature), which can be explained by the kinetic energy theory. By heating a gas, the mean kinetic energy of the molecules increases and the volume occupied by the gas increases. Therefore, cooling a given mass of gas causes a reduction in its volume, but increases its density. The rate of diffusion for any gas of fixed mass would be less at lower temperatures.

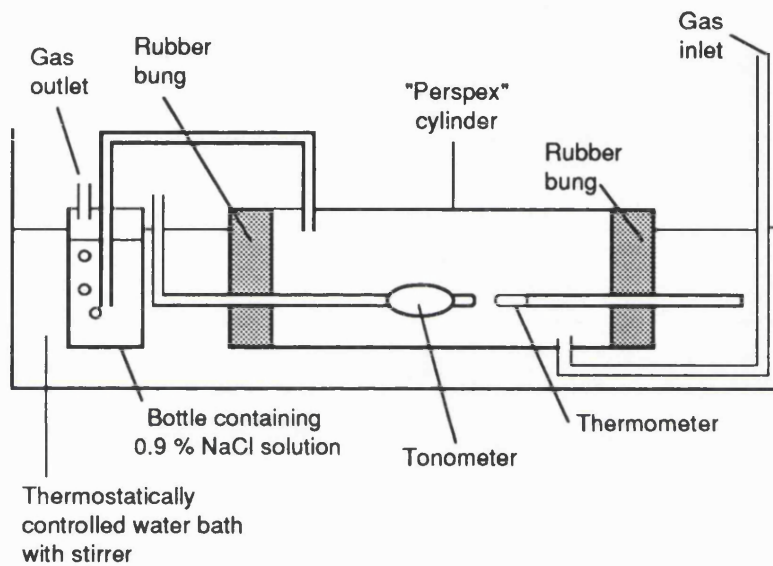
To investigate the influence of temperature on the rate of equilibration in vitro, two models were designed and manufactured. The temperature adjusted correction factors were subsequently employed in a clinical study to investigate the influence of hypothermic CPB on temperature corrected and uncorrected gastric pHi and arterial pH.

Materials & Methods

In Vitro Study 1: The determination of correction factors for the gastric tonometer

Two designs for the in vitro validation of the tonometer were evaluated. The first design examined the equilibration of the tonometer with a gas interface (Fig 12). A mixture of CO₂ and O₂ flowed over the surface of the tonometer within the perspex chamber.

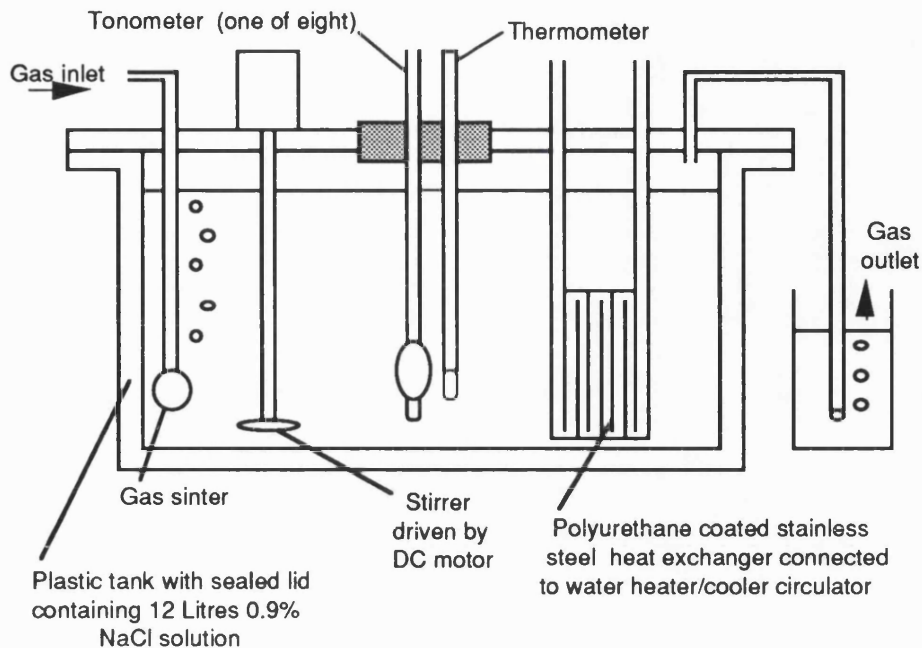
Fig 12: In vitro model for validation of the gastric tonometer: gas interface



However, this system was not stable, because the partial pressure of the CO₂ exerted within the chamber was dependent upon the flow of the gas mixture, which was very difficult to control at a precise rate. This system was therefore abandoned for the examination of the equilibration of CO₂ in a liquid interface with the tonometer (Fig 13). The latter in vitro system was very stable at any given temperature and less dependent upon the gas flow rate.

A glass tank holding 14 litres capacity was used as the in vitro testing chamber (Fig 13). The tank was filled with 12 litres of normal saline.

Fig 13: In vitro validation of the gastric tonometer: liquid interface



A gas-tight perspex lid was fitted, with holes drilled to permit the insertion of:

1. Eight gastric tonometers
2. Tubing for the insufflation of CO₂: O₂ mixture (5%:95%) which connected to a submerged sinter at the bottom of the saline filled tank.
3. A second tube for the escape of gas which was above the fluid in the tank, was connected to a second chamber filled with normal saline. The exhaust gas bubbled through the saline of this second chamber.
4. A thermometer
5. A stirrer to equilibrate gas in the saline in the tank.
6. A heater/ cooler calorifier

The whole system was warmed to the desired temperature and the gas flow adjusted until the pCO₂ in the saline of the tank approximated to 4.7 to 5.3 kPa. Saline of the tank could be measured by aspirating through one of the channels of the gastric tonometer. The whole system was then allowed to equilibrate for a further 90 minutes.

The tonometers were de-aired and primed with sterile 0.9% saline. The tonometer saline pCO₂ was measured for different periods of equilibrations and temperature. To determine the correction factor, the pCO₂ of the saline in the tank and tonometer was determined simultaneously. All saline pCO₂'s were measured using an auto-calibrating ABL4 blood gas analyser (Radiometer, Copenhagen, Denmark).

Four temperatures were investigated (18, 28, 37 & 40°C). At each temperature the equilibration times, 10, 20, 60 and 90 minutes were evaluated. For each equilibration time the reading was repeated 8 times to determine a mean and standard error for the measurements. The tank saline pCO₂ was measured at the start and end of the equilibration period, to ensure stability of the system. Similarly, the pCO₂ of the saline in the sterile bag used to prime the gastric tonometers was also measured before each run to ensure that priming saline pCO₂ did not differ significantly between runs.

In Vitro Study 2: The Stability of CO₂ in saline

Because this study necessitated the sampling from 8 tonometers, it was important to determine if a delay in sample analysis contributed to error in the pCO₂ determined, since the blood gas analyser could only cope with one sample at a time. Normally, arterial blood gas syringes are capped and stored on ice until analysed to minimise diffusion of gases from the blood and reduce cellular metabolism. To investigate if this precaution was adequate for CO₂ in saline, an experiment was also undertaken to examine for the loss of CO₂ from the saline samples with respect to time.

To assess CO₂ stability in saline, identical samples were analysed immediately and after 1 and 2 hours from capped and uncapped syringes left on ice or at room temperature.

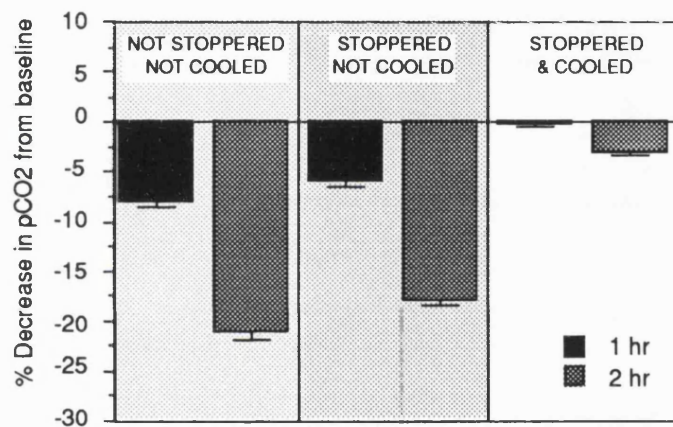
Statistical Analysis

All values are expressed as means \pm standard error of the mean. The Mann-Whitney U test was employed to examine for differences between groups. A non-parametric test was employed because the small sample size ($n=8$) in each group does not allow the frequency distribution of the data to be established.

Results

(a) The stability of CO₂ in saline

Fig 14: The stability of CO₂ in saline



The results are expressed graphically above in Fig 14 and demonstrate that the CO₂ diffuses out from the saline in a plastic syringe if the syringe is not cooled or capped. Even with cooling on ice and capping the saline, the sample should not be left for more than 2 hours since there is a fall in the pCO₂ of the saline by 3.06 ± 0.28 % from baseline values.

In view of these results, the saline tonometer samples were all measured within 10 minutes of sampling times. This was achieved by staggering the priming of the tonometers in the in vitro tank so that samples were harvested with enough time between samples to enable analysis by the blood gas analyser.

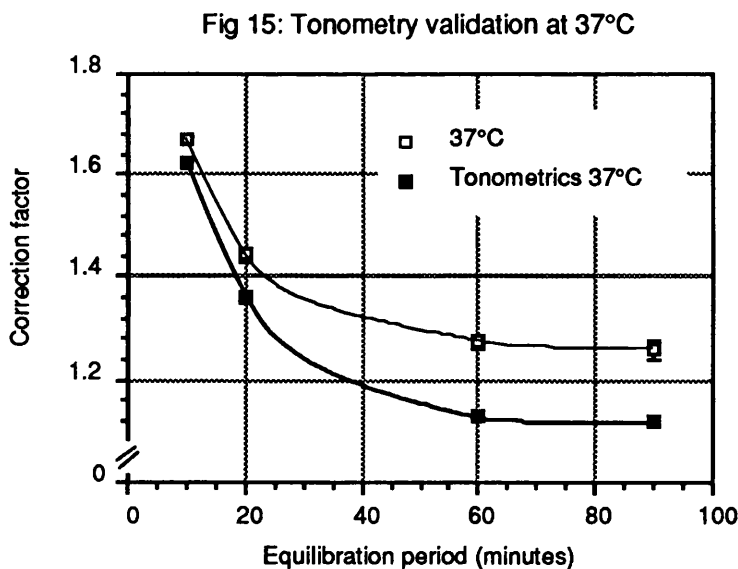
(b) The influence of temperature on the rate of equilibration of CO₂

The results are presented in the table as well as the graphs below, because the small standard errors are not easily depicted graphically.

Tab 6: The influence of temperature on the equilibration of CO₂ with the gastric tonometer

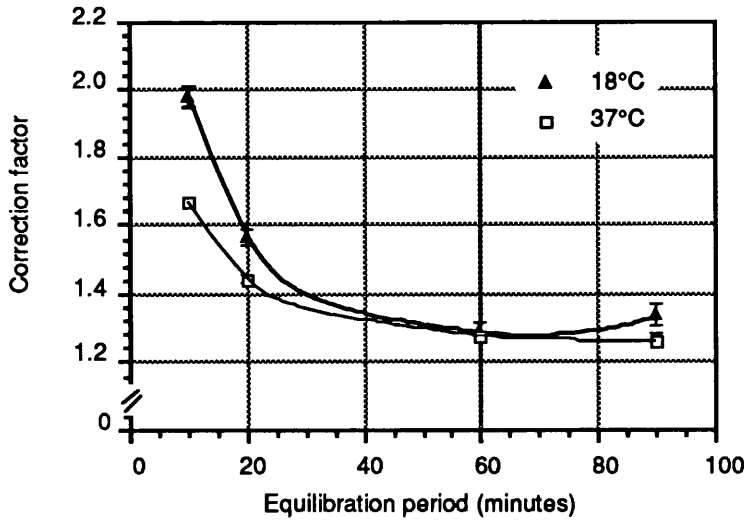
CORRECTION FACTOR				
TEMPERATURE				
EQUIL. TIME	18°C	28°C	37°C	40°C
10	1.98 ± .03	1.74 ± .03	1.67 ± .01	1.67 ± .02
20	1.57 ± .02	1.52 ± .02	1.44 ± .01	1.49 ± .02
60	1.29 ± .02	1.25 ± .01	1.28 ± .01	1.33 ± .01
90	1.33 ± .03	1.26 ± .01	1.26 ± .01	1.31 ± .01

In comparison to the correction factors described by the company, the correction factor was higher at 37°C for each equilibration time period investigated (Fig 15).



At 18°C the correction factor was higher for shorter equilibration times than for longer equilibration times (Fig 16).

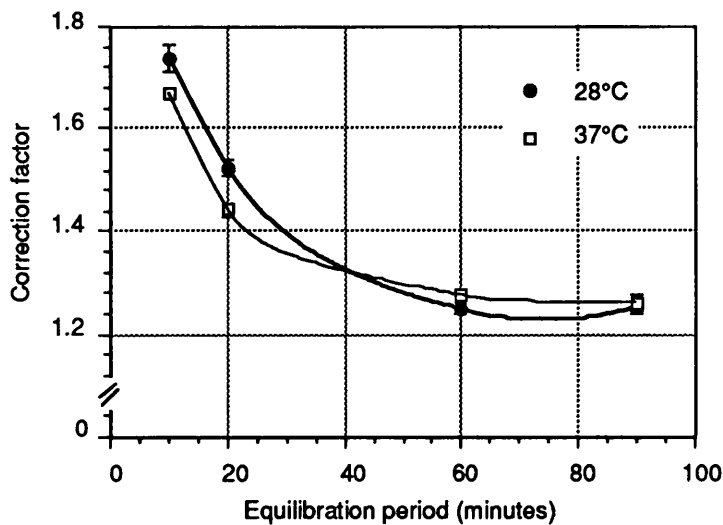
Fig 16: Tonometry validation for 18°C



For an equilibration time of 10 minutes, the correction factor was 1.98 ± 0.03 at 18°C versus 1.67 ± 0.01 at 37°C ($p=0.0008$). Similarly, at 20 minutes, the correction factor was 1.57 ± 0.02 at 18°C versus 1.44 at 37°C ($p=0.0008$). This difference was lost for longer equilibration times; for 60 minutes the correction factor reduced to 1.29 ± 0.02 at 18°C versus 1.28 ± 0.01 ($p=0.59$). However at 90 minutes of equilibration the correction factor was lower at 18°C (1.33 ± 0.03) than at 37°C (1.26 ± 0.02), $p=0.036$.

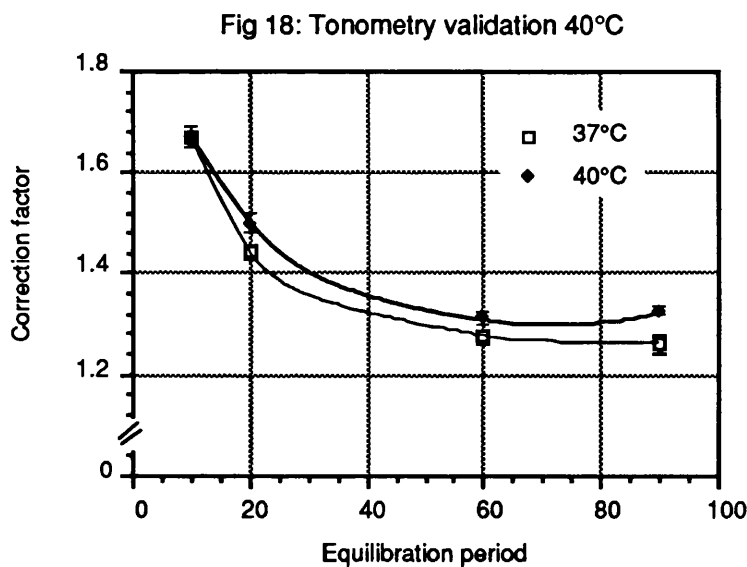
A similar trend was found at 28°C and is illustrated in Fig 17.

Fig 17: Tonometry validation 37 versus 28°C



At longer equilibration times (60 & 90 minutes), there was less difference between the correction factors determined at 28 and 37°C. At 60 minutes, for 28°C the correction factor was 1.25 ± 0.01 compared to 1.28 ± 0.01 at 37°C ($p=0.4$). Similarly for 90 minutes, the correction factor was 1.26 ± 0.01 at 28°C and 1.26 ± 0.02 at 37°C ($p=0.9$). For shorter equilibration times, the difference became apparent. For 10 and 20 minutes of equilibration at 28°C, the correction factors were 1.74 ± 0.03 and 1.52 ± 0.02 compared to 1.67 ± 0.01 and 1.44 ± 0.01 at 37°C respectively ($p=0.046$ and $p=0.009$).

At a temperature representative of pyrexia (40°C), the correction factors were similar to normothermic (37°C) temperature, but slightly greater for longer equilibration times (Fig 18).

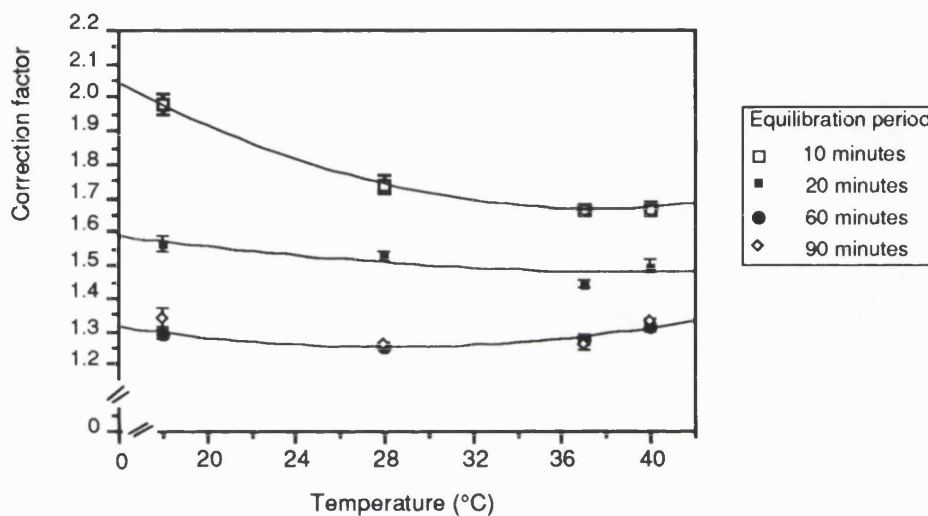


At 10 and 20 minutes of equilibration for 40°C the correction factors were 1.67 ± 0.02 and 1.49 ± 0.02 compared to 1.67 and 1.44 ± 0.01 at 37°C respectively ($p=0.60$ and $p=0.059$) respectively. For equilibration periods of 60 and 90 minutes the correction factors were 1.31 ± 0.01 and 1.33 ± 0.01 at 40°C versus 1.28 ± 0.01 and 1.26 ± 0.02 at 37°C ($p=0.021$ and $p=0.009$).

Discussion

The correction factor for the gastric tonometer is dependent upon both the temperature and the equilibration period. At shorter equilibration periods, cooling makes a larger difference to the correction factor. This difference is lost when an equilibration of 60 minutes or longer is employed, which is illustrated in Fig 19 below.

Fig 19: The effect of temperature on the equilibration of tonometer pCO₂



Furthermore, the results of the Tonometrics Inc (Worcester, MA, USA) were not reproducible in this system at 37°C. At 37°C, after 90 minutes of equilibration, the correction factor was 1.28, suggesting that 78.1% of the CO₂ had equilibrated with the saline of the tonometer, compared to 1.14 determined by Tonometrics Inc (Worcester, MA, USA), which equates to 87.7% equilibration of CO₂. This can only be explained by inaccuracies in the presented experimental protocol or by those determined by the company. The pCO₂ of the tank saline was extremely stable throughout the study period. For example, when assessing at 37°C, for the 32 samples measured from the saline of tank, the pCO₂ was 4.71 ± 0.04 kPa. This represents samples taken over a 48 hour period. If there were minor fluctuations in the tank pCO₂ this may have influenced the pCO₂ of the saline in the tonometer. However, a

ratio of tank/ tonometer pCO₂ is employed to calculate the correction factor for each reading, thus reducing this source of error.

It may be predicted from the gas Laws that at lower temperatures, the time required for diffusion would be longer at any given pressure. However, at 40°C the correction factor was significantly greater than at 37°C for equilibration periods of 60 and 90 minutes (p=0.021 and p=0.009 respectively). This cannot be explained by the higher rate of diffusion which would be expected at 40°C compared to 37°C. A similar tank pCO₂ was employed at 40°C (4.8 ± 0.03 kPa) as was used for 37°C (4.71 ± 0.04 kPa), so the concentration gradients between the tank and the tonometer saline would also have been very similar. The influence of temperature on the permeability of the silicone membrane to CO₂ is not known, although this may account for the differences observed.

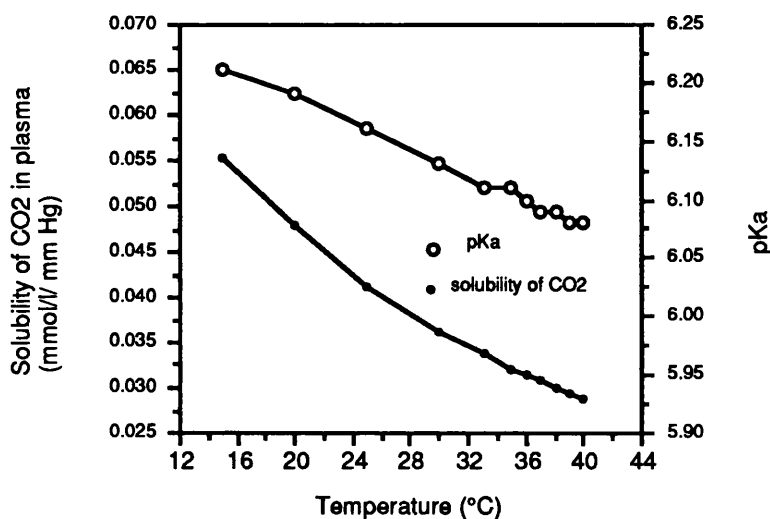
Temperature influences not only the rate of diffusion of CO₂, but also its solubility in plasma, which increases as the body is cooled. Furthermore, the pKa of the HCO₃⁻/CO₂ buffer system increases with temperature. These changes in the solubility of CO₂ in plasma and the pKa with temperature were investigated by Severinghaus et al in 1956 (Severinghaus et al 1956); data from that report is depicted below in Fig 20.

A linear relationship exists for the solubility of CO₂ in saline (mmol/ mm Hg), which may be described by the equation:

$$y = 6.87 \times 10^{-2} - 1.03 \times 10^{-3} x$$

Where y is the solubility coefficient for CO₂ and x is the body temperature. At 37°C the solubility coefficient approximates to 0.0306, but at 28°C it increases to 0.0399.

Fig 20: The influence of body temperature on the solubility of carbon dioxide in plasma and the pKa of the HCO₃/CO₂ system from Severinghaus et al 1956



The relationship between the pKa and body temperature may also be described by the linear equation:

$$y_2 = 6.29 - 5.42 \times 10^{-3} x$$

Where y_2 is the pKa and x is the body temperature. At 37°C, the pKa is approximately 6.090, but at 28°C it increases to 6.139.

The Henderson-Hasselbach equation employed to determine the pH_i must also be adjusted for these changes in CO₂ solubility and the pKa with temperature. At 28°C, the equation becomes:

$$\text{pHi} = 6.138 + \log_{10} \left[\frac{[\text{HCO}_3^-]}{\text{pCO}_2(\text{ss}) \times 0.0399} \right]$$

From the presented data the correction factor required to calculate the pCO_{2(ss)} would alter from 1.44 at 37°C to 1.52 at 28°C for an equilibration period of 20 minutes.

In summary, the in vitro model has found that temperature may become an important source of error in the determination of the gastric pH_i, particularly for short equilibration times. This may result in an over-estimate of the pH_i during the hypothermic phase of CPB. However, adjusting the Henderson-Hasselbach equation and the tonometer correction factors for temperature may improve the accuracy of the

tonometric methodology when investigating hypothermic subjects. The blood gas analyser employed to measure $p\text{CO}_2$ *in saline* may be a potentially greater source of error. When comparing the $p\text{CO}_2$ measured by the CIBA-Corning (CIBA-Corning Diagnostics Ltd, Halstead, Essex, UK) blood gas analyser and the Radiometer ABL4 analyser (Radiometer, Copenhagen, Denmark), a 40% lower value was obtained for the PCO_2 when employing the CIBA-Corning machine (unreported observations). Therefore all *in vitro* and *in vivo* studies have been conducted with the Radiometer ABL4 analyser.

The Effect of Cardiopulmonary Bypass on Gastric & Colonic Mucosal Perfusion: Tonometric Assessment

Post-operative GI sequelae may be related to changes in perfusion of the gut mucosa during and immediately following CPB. To assess the influence of hypothermic non-pulsatile CPB on the perfusion of the gastric and colonic mucosae, gastric and sigmoid tonometry was employed as an indicator of the adequacy of mucosal perfusion. These changes in the gastric and mucosal pHi were related to the arterial pH (pHa). The influence of temperature on gastric pHi was evaluated using the in vitro correction factors determined in the previous study (pages 74-84).

Materials & Methods

Ten patients who were undergoing elective CABG (n=9) and valve replacement (n=1) were recruited for this study. Patients who were known to have diabetes, hypertension, renal failure or a previous history of abdominal surgery were excluded. One patient underwent redo-CABG, one an aortic homograft valve replacement and the remainder were first time CABG.

These studies were approved by the Hillingdon Health Authority Ethics Committee & the Hammersmith Hospital Ethics Research Committee. All patients gave informed written consent.

Tonometer Positioning & Sampling Protocol

The gastric tonometer was sited following intubation and induction of general anaesthesia. Position was confirmed by aspiration of gastric secretions and the insufflation of air. The sigmoid tonometer was sited in the sigmoid colon under direct vision using a rigid sigmoidoscope. In 2 of the 10 patients selected for the study the sigmoidoscope could not be negotiated beyond the rectum and the study was abandoned in these 2 patients.

The gastric and sigmoid tonometers were primed with 0.9% saline by the method previously described and an equilibration period of 20 minutes was allowed before re-priming for the next sample. At the same time as a saline tonometer sample was taken an arterial blood sample was also taken for immediate analysis using the ABL4 blood gas analyser (Radiometer, Copenhagen, Denmark). All samples were read at 37°C, after adjustment of the steady state pCO₂ for temperature, and the Henderson-Hasselbach formula was used to determine the pHi.

Both sigmoid and gastric tonometers were removed before the patient left the operating theatre.

Peri-operative management

Pre-operative medication consisted of intramuscular papavaretum with scopolamine. Ranitidine was administered in two divided oral doses, 300 mg the night before surgery and 300 mg on the morning of surgery.

Anaesthesia was induced with thiopental and maintained with fentanyl, and isoflurane. Muscle relaxation was provided by pancuronium. Ventilation was controlled to maintain carbon dioxide tension at 4.7 to 5.3 kPa and arterial oxygen tension greater than 13.3 kPa.

During the operation, radial artery pressure, nasopharyngeal temperature and cardiac output from the pump-oxygenator during CPB were recorded continuously via an analogue to digital converter onto the hard disc of a computer for subsequent analysis. All cardiac procedures were undertaken via a median sternotomy. Cardiopulmonary bypass was achieved using a hollow fibre membrane oxygenator (Maxima, Medtronic, Watford, UK) without arterial line filtration using non-pulsatile flow and core cooling to 28°C. During CPB non-pulsatile flow from the pump-oxygenator was maintained at 2.4 l/min/m² at 37°C, with graded reductions down to 1.7 l/min/m² at 28°C. Mean arterial pressure (MAP) was maintained during CPB at 50-60 mm Hg and acid base balance was managed using the alpha stat methodology. During CPB

the haematocrit was maintained above 0.2, with the addition of packed red blood cells as required. The packed cell volume (PCV) was determined every ten minutes during CPB.

Myocardial preservation was achieved by using cold (10°C) crystalloid cardioplegia (St Thomas's) with topical myocardial cooling using iced saline solution.

Statistical & Data Analysis

Wilcoxon signed-rank test and Spearman's rank-order correlation analysis were employed in data analysis. Results are expressed as mean ± standard error of mean.

The systemic vascular resistance index was determined using the formula below and expressed as systemic vascular resistance units (SVRU):

$$SVRI = \frac{MAP - CVP}{CI}$$

MAP: mean arterial pressure
CVP: central venous pressure
CI: cardiac index

Where cardiac index is the cardiac output per metre square of surface body area (determined using standard nomogram incorporating weight and height of the patient).

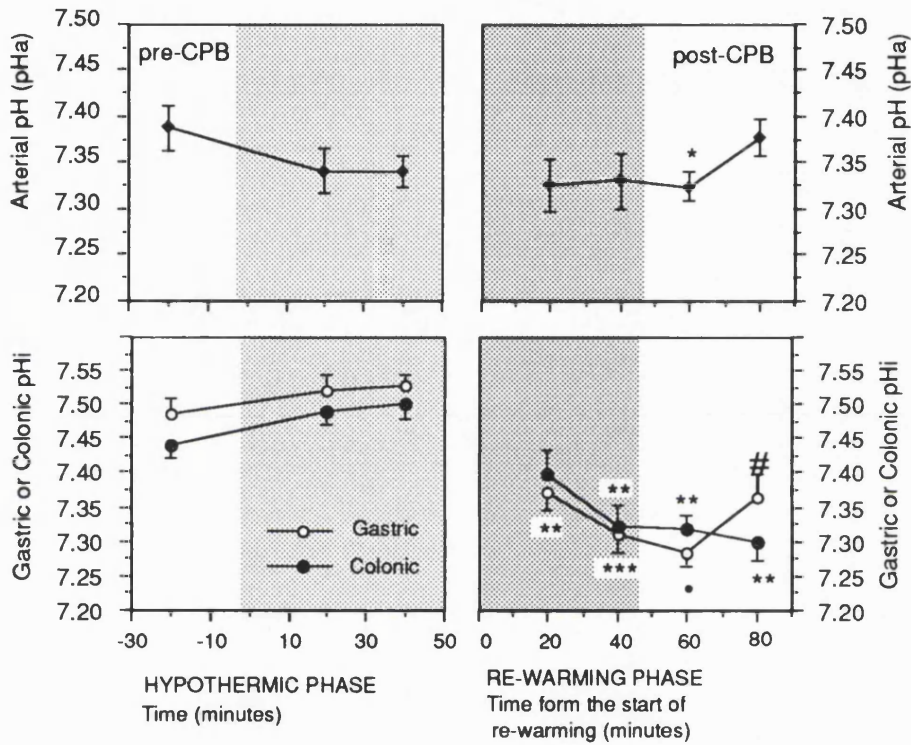
In view of the findings of the previous study examining the influence of temperature on the correction factor, the pHi was determined using the correction for pKa, solubility of CO₂ in plasma at the relevant temperature and also employing the correction factor and Henderson-Hasselbach equation provided by Tonometrics Inc (Worcester, MA, USA), which is for 37°C.

Similarly the arterial pH was corrected for temperature with the pHi, or left uncorrected at 37°C with the pHi.

Results

The mean age of the patients were 59.1 ± 4.5 (n=8), the CPB time was 128.4 ± 17.5 minutes; mean cross-clamp time was 54.8 ± 7.4 minutes and 3.6 ± 0.9 (range 2-5 grafts) grafts per patient were performed. Because the length of CPB time varied

Fig 21: Gastric & colonic pHi and pHa, uncorrected for temperature



Comparing with pre-CPB values

• p=0.011 * p=0.017, ** p=0.02, *** p=0.028, # p=0.046

with the patient, CPB was divided into hypothermic and re-warming phases to allow comparisons to be made between patients for the different phases of CPB.

Temperature uncorrected pHa, gastric & colonic pHi

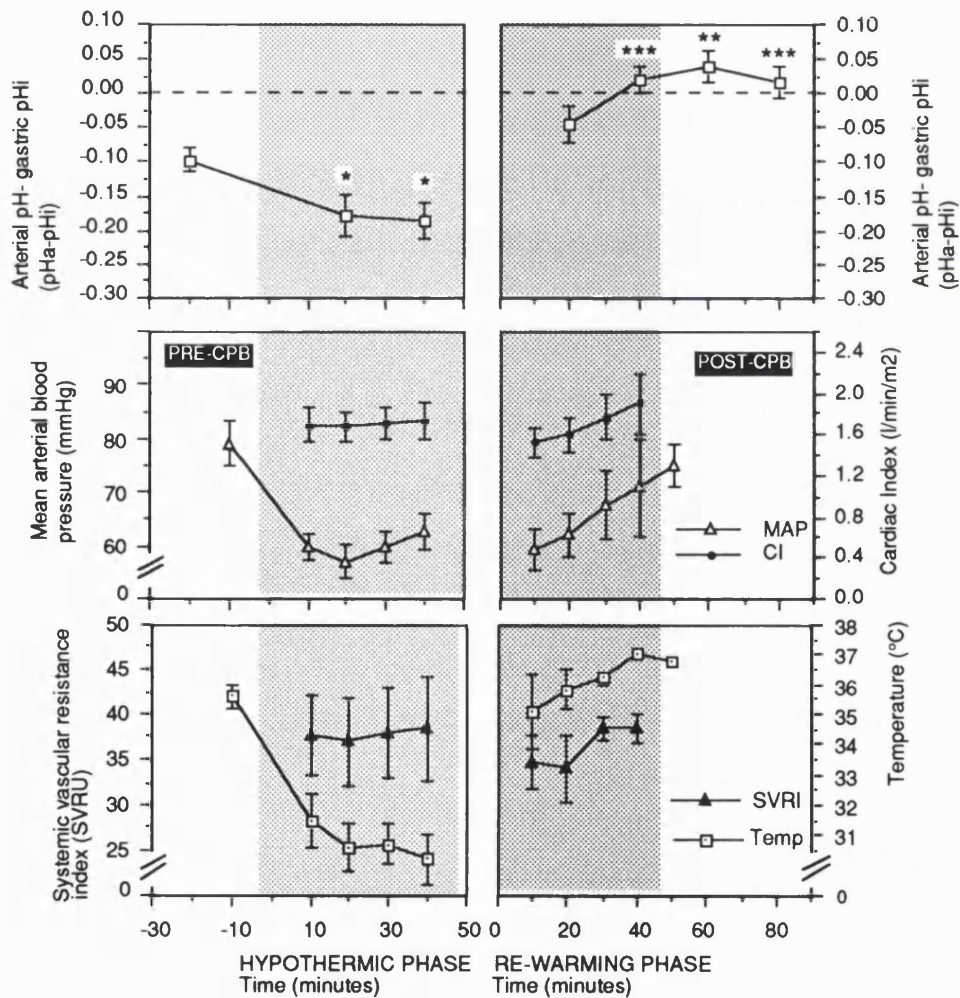
The arterial pH, gastric and colonic pHi did not change significantly during the hypothermic phase of non-pulsatile CPB (Fig 21). The difference between the arterial

pH (pHa) and the gastric pHi (pHa-pHi) increased from a pre-CPB value of -0.097 to -0.186 (p=0.011) after 40 minutes of hypothermic CPB (Fig 21).

During the re-warming phase of CPB, although both pHa, gastric and colonic pHi fell (Fig 21); the pHa/ pHi difference became positive, due a fall in the pHi. This is illustrated for the arterial pHa and gastric pHi in Fig 22.

Forty minutes after the start of re-warming, despite increases in the CI and MAP, the gastric pHi fell from 7.49 ± 0.02 before CPB to 7.31 ± 0.03 (p=0.028), whilst the colonic pHi fell from 7.44 ± 0.02 before CPB to 7.32 ± 0.02 (p=0.02) (Fig 21).

Fig 22: Change in pHa-pHi difference & haemodynamic parameters

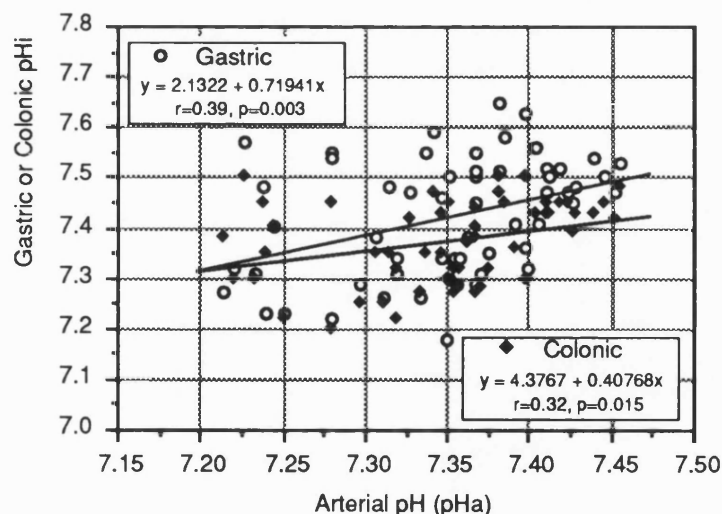


Compared to pre-CPB values
 * p=0.011, ** p=0.017, *** p=0.028

There were no significant differences in the gastric and colonic pHi throughout the study period. Forty minutes after the end of CPB, the gastric and colonic pHi remained depressed below pre-CPB values at 7.36 ± 0.04 ($p=0.046$) and 7.30 ± 0.03 ($p=0.02$) respectively. The gastric pHa-pHi difference (Fig 22) which was -0.097 before CPB, decreased to 0.016 ($p=0.028$) forty minutes post-CPB. This was due in large part to a recovery in the arterial pH towards normal values, whilst both colonic and gastric pHi remained depressed and significantly below pre-operative values (Fig 21). The relationship of these changes to haemodynamic parameters is summarised in Fig 22.

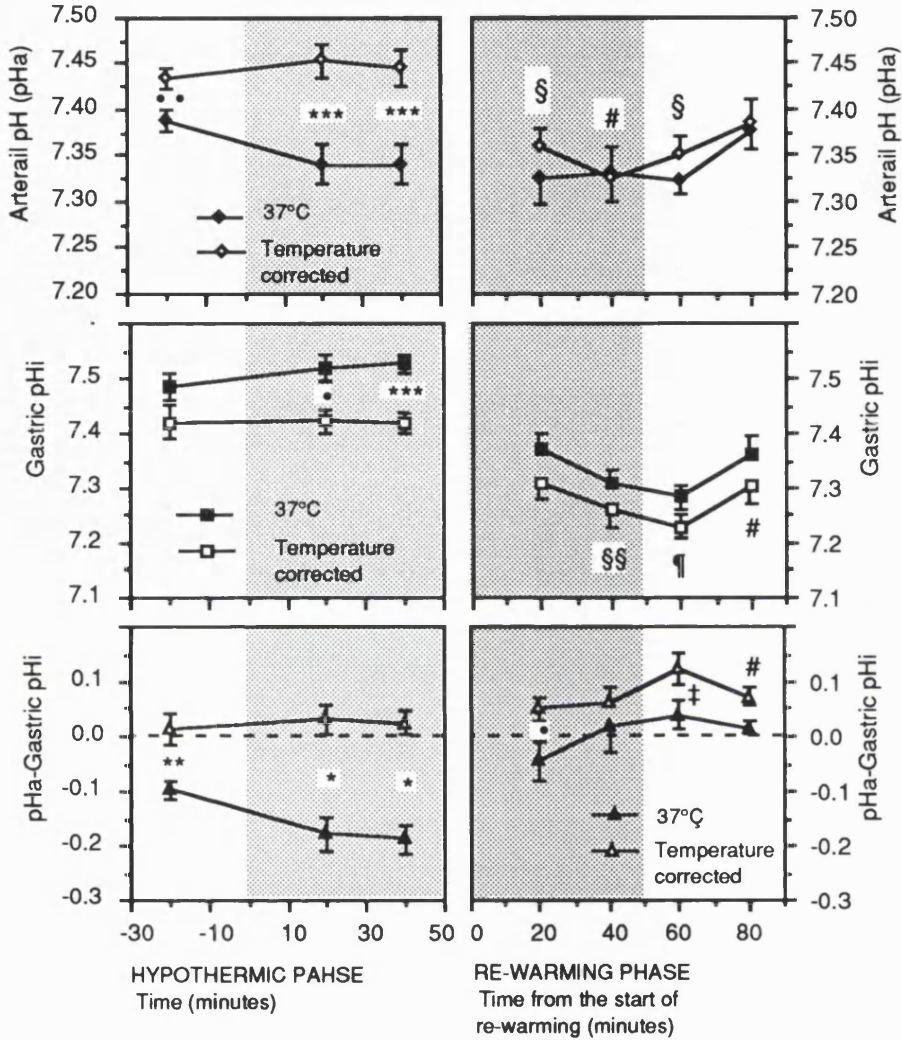
To examine for the relationship between the pHa and pHi a scatterogram was made of the individual pHa and gastric and colonic pHi values and simple regression analysis performed (Fig 23). Both gastric ($r=0.39$, $p=0.003$) and colonic ($r=0.32$, $p=0.015$) showed a linear relationship with pHa, but the changes in gastric intramucosal pH correlated more strongly with the pHa than the colonic pHi.

Fig 23: The correlation with gastric and colonic pHi (uncorrected for temperature) and the pHa (uncorrected for temperature)



Gastric pHi & pHa corrected for temperature

Fig 24: The effect of temperature correcting the pHa & gastric pHi



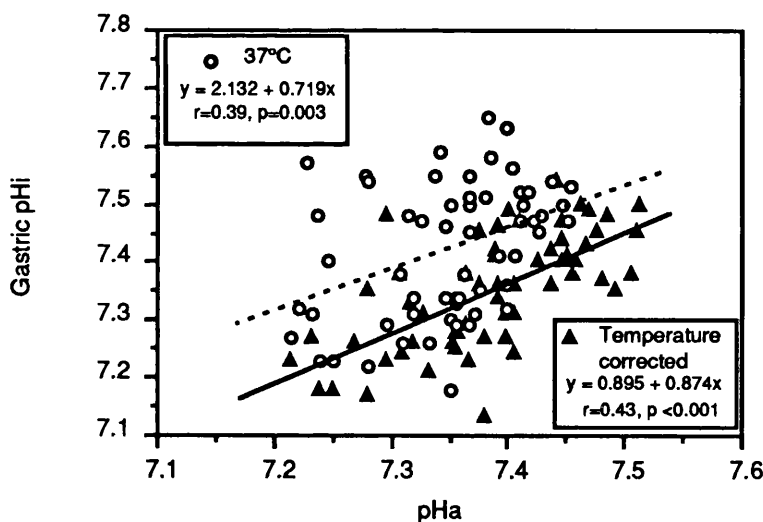
Comparing temperature corrected and uncorrected values
 • p=0.014, ** p=0.036, * p=0.001, ** p=0.004, *** p=0.006,
 ‡ p=0.008
 Compared to pre-CPB values; only p values for temperature corrected values illustrated
 ¶ p=0.012, § p=0.025, §§ p=0.028, # p=0.046,

There was no significant increase in the temperature corrected gastric pHi (pHit) during the hypothermic period, which was 7.42 ± 0.031 pre-CPB and 7.42 ± 0.02 after 40 minutes of CPB. However, it was significantly below the temperature uncorrected gastric pHi, pre-CPB, 20 and 40 minutes of hypothermic CPB (p=0.036,

0.006 and 0.006 respectively) (Fig 24). In the re-warming period, these differences were lost because the patients had been re-warmed to a normothermic temperature.

The gastric pH_{it} followed the temperature corrected pH_a (pH_{at}) closely during the hypothermic phase of CPB. Thus the pH_{at}-pH_{it} difference was small compared to the pH_a-pH_i difference (Fig 24). After 40 minutes of the hypothermic phase, the pH_{at}-pH_{it} was 0.026 ± 0.02 compared to -0.186 ± 0.03 for the pH_a-pH_i difference ($p=0.006$). Both the pH_{at}-pH_{it} and the pH_a-pH_i differences became positive in the post-CPB period *indicating the tendency towards intramucosal acidosis*. Twenty minutes post-CPB the pH_{at}-pH_{it} difference was 0.123 ± 0.03 compared to 0.04 ± 0.02 for pH_a-pH_i ($p=0.008$).

Fig 25: Temperature corrected & uncorrected gastric pH_i and the correlation with temperature corrected & uncorrected pH_a



The pH_{it} correlated with the pH_{at} more closely ($r=0.43$, $p<0.001$), than the temperature uncorrected pH_i and pH_a ($r=0.39$, $p=0.003$) (Fig 25).

Post-operative Morbidity & Mortality

Three patients developed post-operative complications. One patient who had undergone aortic homograft replacement required re-sternotomy for bleeding, two other patients developed post-operative sternal wound infections. In one of these patients mediastinitis was also diagnosed and he underwent re-wiring of the sternum, but died 6 days after his initial operation (CABG). Post-mortem showed that he had died from an acute myocardial infarction secondary to coronary graft thrombosis. The lowest gastric or colonic pHi recorded during the study period in these 3 patients was 7.18, 7.17 and 7.31 respectively (mean 7.22 ± 0.05), compared to 7.26 ± 0.01 for the 5 patients who were free from post-operative complications ($p=0.65$, Mann-Whitney U test).

Discussion

This study has found that despite the maintenance of MAP and CI within a generally acceptable range during hypothermic non-pulsatile CPB, gastric and colonic intramucosal acidosis developed during the re-warming and immediate post-CPB phases. Previous work has confirmed that tonometry can non-invasively monitor the adequacy of visceral perfusion and that peripheral assessments of tissue perfusion are inappropriate in the context of altered haemodynamics in the immediate post-CPB period (Kuttila et al 1990). These include increased metabolic requirements associated with re-warming, which are not met by increases in the cardiac output or enhanced oxygen extraction (Joachimsson et al 1987).

The normal range for gastric intramucosal pH in human volunteers has been determined to be 7.38 ± 0.03 (mean \pm standard deviation), with values below 7.32 being representative of intramucosal acidosis or mucosal hypoxia (Heard et al 1991). The gastric mucosal pHi (temperature uncorrected) fell to 7.31 ± 0.03 and 7.29 ± 0.02 , 40 minutes after re-warming during CPB and 20 minutes after the end of CPB respectively, before recovering. The pHit values were lower than the pHi values throughout the study period and were more closely related to the pHat. A similar fall in the colonic pHi was found at the same time points (7.32 ± 0.03 and 7.32 ± 0.02 respectively). Correcting the pHi values for changes in body temperature would appear to be appropriate since without this correction the pHi tends towards alkalosis and the pHa-pHi difference increases and becomes more negative. Landow et al documented this increase in the pHi and suggested that it may be due to an inadequacy in mucosal perfusion, so that the arterial $[\text{HCO}_3^-]$ no longer relates to the tissue $[\text{HCO}_3^-]$ (Landow et al 1991). If mucosal hypoperfusion does occur with hypothermic CPB, even with a reduction in the metabolic rate, the pHi may be expected to be equal to or less than pre-CPB values. In this study, no significant increase in the temperature uncorrected pHi was observed during the hypothermic phase of CPB, but the trend was an increase in the pHi and the pHa-pHi difference.

This pattern was quite distinct from the temperature corrected values, where the pH_{it} during hypothermic CPB correlated more closely with the pre-CPB value without significant alteration in the pH_{at} - pH_{it} difference. If the in vitro values determined for various temperatures are valid, then it may be concluded from this study that during hypothermic non-pulsatile CPB, mucosal perfusion appears to be adequate to meet metabolic demands. However, this does not invalidate the hypothesis that tissue $[HCO_3^-]$ may be lower than arterial $[HCO_3^-]$, which would lower the pH_{it} values further, suggesting that the true pH_i is still overestimated under these conditions.

Fiddian-Green, who studied 85 cardiac surgical patients for a further 5 hours in the intensive care unit (ICU) post-CPB, found that 49% of patients developed episodes of intramucosal acidosis (Fiddian-Green and Baker 1987). Moreover, the presence and duration of the acidosis was highly predictive of outcome post-operatively (Fiddian-Green and Baker 1987). In the group of patients who developed abnormal gastric pH_i ($n=42$) post-CPB, 9.4% had life-threatening complications, of whom five died (5.9%). No complications or deaths were recorded in the 43 patients who did not develop abnormal measurements of stomach wall pH (Fiddian-Green and Baker 1987). Similarly, colonic intramucosal acidosis has been found to a predictor of post-operative ischaemic colitis in patients undergoing abdominal aortic operations (Fiddian-Green et al 1986). None of the patients in the presented series developed gastrointestinal complications, but three patients developed severe post-operative complications, resulting in the death of one of these patients. Although not statistically significant ($p=0.65$), the patients who developed post-operative complications had a lower mean pH_i for colon/stomach than those who did not develop complications post-operatively.

Gastric and colonic tonometry correlated well with the pH_a , although the gastric pH_i showed a stronger correlation than the colonic pH_i (Fig 23). Colonic tonometry was difficult to perform in all the patients. In 2 of the 10 (20%) patients, the

sigmoidoscope could not be passed beyond the rectum due to faecal loading. However, since both gastric and sigmoid pHi moved in parallel with changes in haemodynamics, then the need for colonic tonometry may be obviated in future studies for providing qualitative data. Similar parallel quantitative changes were found in a pig endotoxic model where gastric, small and large bowel tonometry, although quantitatively different in each region, changed in the same direction with systemic parameters (Montgomery et al 1990). Montgomery et al also found that mucosal pHi changed before there were significant alterations in the blood pressure or cardiac index in the swine endotoxic model, suggesting that bowel tonometry may be a sensitive and early indicator of the inadequacy of oxygen delivery ($\dot{D}O_2$). This may explain the increasing disparity between pHa and pHi in the re-warming and post-CPB period (Fig 22 & 24). Monitoring arterial blood gases does not provide information as regards regional tissue oxygenation and in particular splanchnic oxygen utilisation.

Although in this study alterations in splanchnic blood flow were not monitored, Hampton et al reported a 19% reduction in liver blood flow during hypothermic non-pulsatile CPB (Hampton et al 1989). Furthermore, Rowell and Johnson found that hypothermia reduced splanchnic blood flow in healthy volunteers (Rowell and Johnson 1984). Hormonal mediators released in response to non-pulsatile CPB such as angiotensin II, which is a potent mesenteric vasoconstrictor, may further compromise splanchnic perfusion (Richardson and Withrington 1977, Taylor et al 1979). The relationship between splanchnic oxygen consumption ($\dot{V}O_2$) and $\dot{D}O_2$ during the different phases of CPB and the post-CPB period is currently unknown, but the inadequacy of splanchnic $\dot{D}O_2$ in the re-warming and immediate post-CPB phases may underpin the development of intramucosal acidosis.

Chapter 4

The Effect of CPB on Mucosal Blood Flow: *Laser Doppler Flowmetry*

Introduction & Aims

Tonometer provides an overall index of the state of mucosal oxygenation. This is governed by a number of parameters such as the cardiac index, tissue extraction of oxygen, tissue metabolic demands and oxygen delivery, which are in a constant state of flux. The delivery of oxygen is dependent upon tissue blood flow and the patency of the micro-circulation. To investigate the relationship between tissue oxygenation, as determined by tonometry and tissue blood flow during CPB, laser Doppler flowmetry was used.

Background

There are a number of techniques which are available for the determination of gut blood flow but none has direct clinical applicability. Laser Doppler Flowmetry (LDF), unlike any other techniques, allows for the continuous non-invasive evaluation of tissue blood flow.

The technique is based upon the Doppler principle, which states that an electromagnetic wave which is either emitted or reflected from a moving object will undergo a shift in frequency relative to a stationary observer. This shift in frequency is proportional to the velocity of the moving object. This principle has been applied to the ultrasonic measurement of peripheral vascular blood flow in large blood vessels. However, ultrasound cannot be employed for the determination of blood flow in the microcirculation because of its long wavelength.

Laser light can be generated which is of a particular wavelength, enabling it to be transmitted down optic fibres and to be employed for laser Doppler flowmetry. Red laser light is suitable for this purpose because it is reflected by haemoglobin, whilst green (argon laser) is absorbed by haemoglobin. The longer wavelengths also become unsuitable because they can be absorbed by water contained within the optic fibre. The quality and quantity of light reflected back by the tissue is measured either by the same fibre or by a separate fibre. Thus a LDF probe can consist of one fibre or a number of fibres. The light is reflected from both the non-moving tissue (reference beam) and moving red cells (Doppler shifted beam). The spectrum of collected frequencies is collected by a photodetector and measured indirectly by a process called optical heterodyning. The output of the photodetector is a spectrum of frequencies. This is analysed electronically utilizing the root-mean-square band width of the power spectrum of the Doppler shifted frequencies to compute a signal number which represents the flow.

Stern suggested the employment of these principles for the determination of microcirculatory flow in vivo (Stern 1975), but pointed out that they were 'based on idealised assumptions and should only act as a guide to the interpretation of empirical data'. These assumptions concern changes in vascular geometry with changes in blood flow, non-laminar blood flow, the spinning motion of red cells, and the angle of incidence of laser light upon randomly oriented capillaries. Despite these concerns, the signal processing method provides results which would fit a theoretically idealised model (Stern et al 1977).

The laser Doppler monitor provides a measure of tissue blood flow which is expressed in arbitrary flux units; this is the product of mean red cell concentration in the volume of tissue interrogated by the laser light and their mean velocity.

Validation of Laser Doppler Flowmetry

Laser Doppler flow has been validated both in vitro by measurement of liquid flows through small capillary tubes using a number of vehicles including polystyrene beads and human blood (Stern et al 1977, Watkins and Holloway 1978). In vivo evaluation of LDF in skin and renal cortex has found a linear correlation between xenon washout and radioactive microsphere techniques respectively (Stern et al 1979, Stern et al 1977).

As regards the gut, LDF validation in a feline model has found a linear correlation between blood flow determined by an electromagnetic flow probe and the LDF. However, this relationship only appears to hold true for flow rates < 50 ml/min/100 g (Ahn et al 1986). Blood flow to the human small bowel determined by ^{85}Kr elimination technique during abdominal procedures was estimated to be 38 ± 4 ml/min/100 g, with 75% of the flow distributed to the mucosa/ submucosa (Hulten et al 1976). Similarly LDF measurements made during laparotomies in 48 patients (Ahn et al 1986). Total intestinal blood flow was measured by isolating a segment of bowel on one supplying artery. The vein draining the isolated segment was cannulated and the venous outflow collected in a graduated cylinder. Total blood flow averaged 38 ± 15 ml/min/100g for the jejunum and 30 ± 13 ml/min/100g for the ileum (Ahn et al 1986). A linear relationship was found between total gut blood flow and the laser Doppler signal when TBF was <50 ml/min/100g, indicating that human intestinal blood flow is within the limits of accuracy idealised by Ahn et al (Ahn et al 1986).

Further support for the validity of LDF was provided by an ingenious probe which could simultaneously measure mucosal blood flow with hydrogen (H_2) clearance and LDF; again a linear relationship was found between the two techniques (Kiel et al 1985). Kveitys et al also found a linear relationship between H_2 clearance, LDF and microsphere estimation of cat jejunal blood flow. Although H_2 clearance appeared to overestimate total gut blood flow, LDF correlated well with both total and

mucosal/submucosal blood flow measured by microspheres (Kvietys et al 1985). Ahn et al compared the ^{85}Kr elimination technique with laser Doppler estimation of feline gut blood flow and found a linear relationship (Ahn et al 1985).

Additional evaluation of LDF in liver tissue has found that the LDF technique also has the advantage of recording rapid changes in blood flow. In a rat liver model, Almond and Wheatley found that hepatic nerve stimulation resulted in a $51 \pm 14\%$ reduction in total blood flow, which resulted in $12 \pm 10\%$ fall in LDF flow and a $14 \pm 10\%$ fall in ^{85}Kr clearance determined blood flow (Almond and Wheatley 1992).

However, accurate quantification of tissue blood flow using LDF may not be valid since the laser Doppler signal depends upon the inherent optical property of tissue to reflect light. Therefore differences in the laser Doppler signal for any given flow may be expected to differ not only between tissue, but between individuals for the same tissue and even for different areas within the same tissue. This problem was encountered by Almond & Wheatley in the quantification of the laser Doppler signal in the rat liver model. Although a linear correlation was established between total blood flow and the LDF for each animal, the coefficient of variation of the slopes of the regression lines was 31% for different animals (Almond and Wheatley 1992).

Reproducibility of Laser Doppler Measurements

The variation in repeated measurements of LDF blood flow may be expressed in different ways. Recently, repeated measures analysis of variance with a single factor design was undertaken to evaluate the precision of laser Doppler flowmetry from different tissues. The length of the 95% confidence interval was determined using the standard formula for a t confidence interval (Line et al 1992). Paired readings provided an unacceptable precision estimate in the tissues examined (skin, gastric mucosa and pig kidney), but performing more than four repeated measures enhanced the certainty of the perfusion estimate. However, comparisons could not be made between tissues, because different probes were used for determining blood flow (Line et al 1992).

Other studies describing the temporal and spatial variation in LDF have employed the coefficient of variation as a quantitative assessment. Kvernebo et al found that in the gastric mucosa the spatial variation was low and reproducibility of the LDF measurements consistent (Kvernebo et al 1986). The coefficient of variation may be expected to vary not only with the site under investigation but also the technique and equipment employed to evaluate the LDF blood flow. Tenland et al found the coefficient of variation to be 6% for LDF readings made from a stable emulsion (Tenland et al 1983), whilst Allen et al found the coefficient of variation to be 16.9% when using endoscopic LDF for measuring colonic mucosal blood flow (Allen et al 1987). Because biological systems are in a continuous state of flux, variability of this magnitude has been reported for other blood flow assessment techniques, such as isotope clearance (12.9%) (Forrester et al 1980) and 15-60% coefficient of variation for radiolabelled microspheres (Brown et al 1974, Neutze et al 1968).

The spatial variations in LDF assessment from tissues is due to differences in the vascular architecture for any given area of the tissue. The geometry of the Doppler probes determine the depth and thus the volume of tissue under measurement. With

increases in the measurement volume, the variation in the number of illuminated capillaries may be expected to increase due to vascular heterogeneity. A multiprobe which integrates the signal from several adjacent volumes of tissue may reduce this error. This has been demonstrated by Salerud and Nilsson (Salerud and Nilsson 1986), using a probe which can simultaneously analyse seven volumes of adjacent tissue simultaneously, reducing the spatial variation by the square root of the number of scattering volumes compared to standard probes.

Probe Design & the depth of laser Doppler measurement

The sampling depth of the back scattered photons is determined by the wavelength of the sampling laser light, the optical properties of tissue and by the geometry of the laser Doppler probe. To model the complex passage and back scattering of light that occurs when it passes through tissues, the Monte Carlo simulation has been employed (Wilson and Adam 1983). This is a statistical computer simulation of the photon pathways through tissues, provided the absorption, scattering coefficients and density function of the scattering angle for any given tissue are known. All three functions are also dependent upon the wavelength of the incident laser light. Jakobsson & Nilsson employed this model to predict the variation in sampling depth for different tissues and different probe geometries (Jakobsson and Nilsson 1991). For a single fibre probe with an optical fibre diameter of 500 μm the calculated sampling depth was 90, 110 and 360 μm for liver, skin and brain respectively, for a laser light with a 633 nm wavelength. The model predicts that a laser light source with a longer wavelength would have a greater sampling depth (Jakobsson and Nilsson 1991). Increasing the diameter of the optical fibres and the fibre separation of the probes also increased the sampling depth.

A single-fibre probe can effectively be regarded as a probe with no fibre separation, which minimises the measuring volume for a particular fibre diameter. This relies on the principle that light can be transmitted in both directions down the optic fibre, analogous to looking out or through a glass window.

However, because of the heterogeneity in LDF probe designs which are currently available, this has led to confusion over the depth and thus the volume of tissue that any given probe can measure. This is particularly important in the wall of the gut where blood distribution varies with the layer of the gut wall and changes with modulations in arterial and venous pressures (Kampp and Lundgren 1968, Lundgren and Svanvik 1973). This has created confusion in the literature as to whether the LDF

device measures mucosal blood flow or total gut wall blood flow. Shepherd and Riedel who designed their own LDF machine and probe reported the measurement of mucosal blood flow in the canine model (Shepherd and Riedel 1982), whilst Ahn et al using a Swedish designed laser Doppler flowmeter found that the tissue penetration of their probes and device was over 6 mm which resulted in the evaluation of not mucosal but total blood flow (Ahn et al 1986, Ahn et al 1985). Since these early reports, the importance of optic fibre diameter and separation has become acknowledged (Johansson et al 1991). In the selection of probes tested, Johansson et al found that the greatest depth penetration was achieved using a LDF probe with a fibre core diameter of 700 μ m and fibre separation of 700 μ m and least penetration with probes having a fibre core diameter of only 120 μ m (Johansson et al 1991). Interestingly, the probe employed by Ahn et al was of the 700 μ m configuration (Ahn et al 1986, Ahn et al 1985), but unfortunately information about the fibre core diameter was not provided by Shepherd and Riedel (Shepherd and Riedel 1982).

Laser Doppler Flowmetry System designed for these Studies

1. Laser Probe Design

Access to the mucosa of the bowel wall has been easy since the advent of endoscopy. Endoscopic measurement of mucosal LDF has been previously reported from both the stomach and colon (Allen et al 1987, Kvernebo et al 1986).

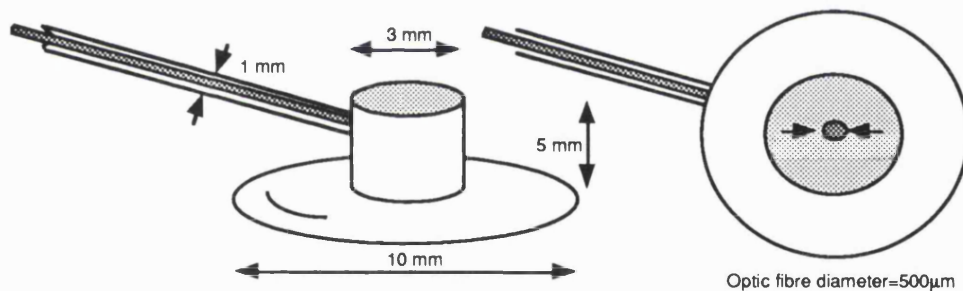
Fig 26: Photograph illustrating the single fibre laser Doppler probe



In conjunction with a British company (Moor Instruments Ltd, Axminster, Devon) an endoscopic laser Doppler probe was designed which could be applied to the gut and bronchial mucosae (Fig 26).

A single-fibre laser Doppler probe was developed because the probe measuring depth could be easily manipulated by altering the diameter of the fibre core; a smaller diameter fibre will emit a laser beam with greater power per unit area, which has deeper tissue penetration. This had to be balanced against the need to produce a probe with a small enough diameter to enable it to be readily passed down the biopsy channel of the endoscope. The probe design and geometry is illustrated in Fig 26 & 27.

Fig 27 : Geometry of single fibre endoscopic laser Doppler probe



The probe is re-usable and can be easily sterilised by soaking in 2% glutaraldehyde for 20 minutes. It is passed retrogradely up the biopsy channel of the endoscope and advanced under direct vision onto the gastric mucosa. The optical coupling of the probe and mucosa can be monitored by the direct current (DC) reading of the laser Doppler machine which falls when optical coupling is lost by peristaltic or other movements.

2. The Laser Doppler Monitor

A Moor Instruments MBF3 blood flow monitor (Moor Instruments Ltd, Axminster, Devon) was employed for all laser Doppler flow measurements. This device utilizes a solid state laser diode as the laser light at between 780 and 810 nm, with a maximum accessible power of 2 mW (MDF3 and MDF3D Manual, Moor Instruments Ltd, Axminster, Devon). Before use the probes are calibrated using a standard solution of polystyrene microspheres. This generates a flux signal using the thermal (Brownian) motion of the microspheres and is therefore temperature dependent. The probes are calibrated at between 20 and 22°C. By calibration to a fixed standard at a defined temperature, readings made by different probes become comparable.

The rate at which readings are recorded (display rate) and the frequency at which the flux signal is filtered (time constant) can be adjusted. All readings from the gut mucosa were made at 1 Hz with a time constant of 3 seconds.

Tissue Haematocrit and the Laser Doppler Signal

Introduction

Although a flow-dependent output can be generated from the power and frequency distribution of the Doppler-shifted signals, the output depends upon both the velocity and number of blood cells within the small sample volume (Bonner and Nossal 1982). In vitro models of the microcirculation have found a linear relationship between the mean particle velocity and the LDF, but have not established the influence of haematocrit on the LDF output. The systemic arterial haematocrit does not reflect the tissue haematocrit which is referred to as the red cell volume fraction (RBCVF). It is the RBCVF which will determine the scattering of laser light of tissue together with the velocity of red blood cells (RBC). Although the normal haematocrit may be 40-45% the RBCVF for skin has been estimated to be <0.5% and for the liver to be 4-8% (Almond and Wheatley 1992).

The haematocrit of the gut mucosa has been estimated using radiolabelled ^{51}Cr and ^{125}I -labelled human serum albumin. Jodal and Lundgren found that for the small intestine the mucosal haematocrit was 50-60% of the arterial haematocrit whilst the muscularis haematocrit was 90% of arterial haematocrit (Lodal and Lundgren 1970). A similar reduction in mucosal haematocrit was not found for the colon. These regional differences in mucosal haematocrit were explained by the small intestinal vascular architecture, with vessels supplying the mucosa branching at right angles from submucosal vessels which may promote plasma skimming (Lodal and Lundgren 1970). Furthermore, the small intestinal mucosal haematocrit was influenced also by the perfusion pressure with increases in mucosal haematocrit when perfusion pressure was reduced (Lodal and Lundgren 1970).

Because the laser Doppler flux signal is a product of both the concentration per unit volume of cells and their mean velocity, then the flux signal may be expected to increase when the erythrocyte concentration increases in the face of a constant velocity. To determine the relationship between blood haematocrit and the laser Doppler flux signal, *in vitro* experiments were undertaken in tubes and freshly excised canine jejunum.

Materials & Methods

In vitro Model

Freshly venesected heparinised blood from sheep was employed for the experiments. Prior to the experiments the probe was calibrated using 2 μm latex spheres as previously described. A single fibre (diameter = 500 μm) was employed to assess laser Doppler blood flow. The blood was diluted with saline to provide variable haematocrit from 2 to 27 %. The blood solutions were pumped through a polyethylene tube with an internal diameter of 1.67 mm (Portex, Hythe, Kent) using a Perfusor precision syringe pump (Braun, Melsingen, Germany) at velocities of 0-0.5 mm/s). The laser Doppler probe was fixed in a stand and applied perpendicular to the longitudinal axis of the tube. A piece of fresh sheep gut was placed behind the tube and acted as a static back scatter, and the tube wall served to scatter the beam. The LDF signal was allowed to settle at each new velocity for 30 seconds before recordings were made for 30 seconds.

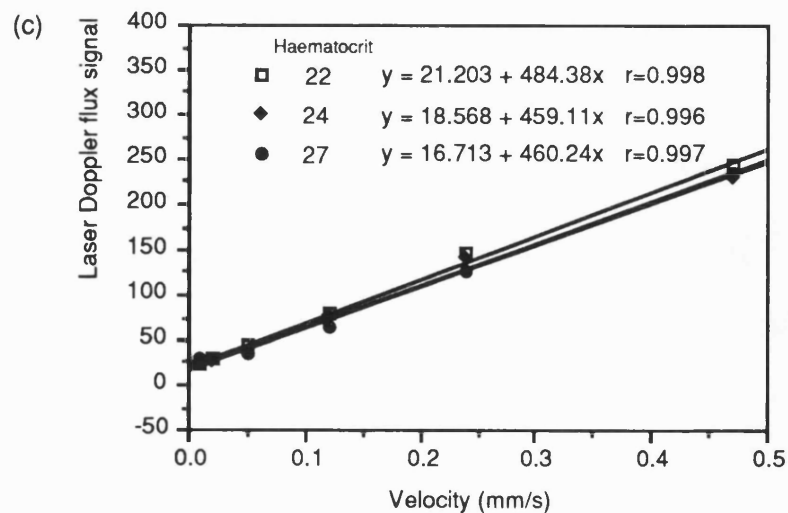
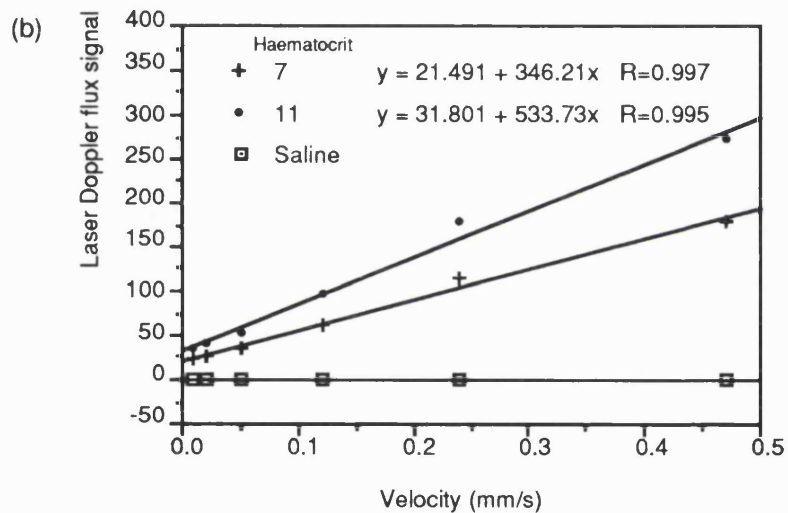
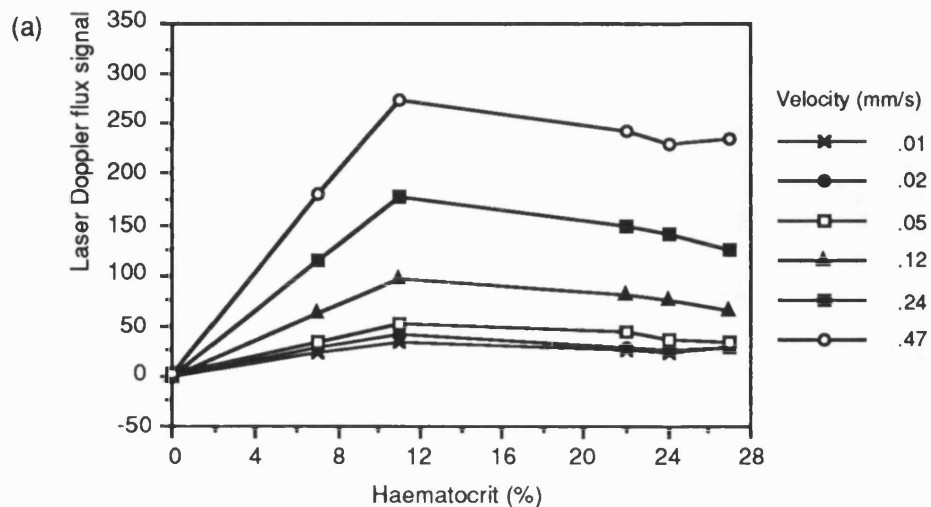
In vitro perfusion of dog jejunum

The study was done by harvesting segments of jejunum from dogs (n=5), following intubation and induction of general anaesthesia. A laparotomy was performed. A loop of proximal jejunum was isolated on its vascular pedicle. The animal was heparinised and the supplying artery cannulated with an 18 gauge polyethylene catheter (Portex, Hythe Kent). A segment was excised and flushed with warm heparinised oxygenated blood (95% O₂ and 5% CO₂). The arterial cannula was flushed with warm normal saline and attached to a Perfusor precision syringe pump (Braun, Melsingen, Germany). The laser Doppler probe was allowed to rest on the jejunal mucosa so as to avoid any effects of pressure. The jejunal segment was then perfused randomly with flows between 0 to 10 ml/ min with blood ranging in haematocrit from 2 to 46%. At each new flow rate the LDF signal was allowed to stabilise and the readings were taken for 30 seconds. Each was repeated four times. Between each change in haematocrit, the bowel was perfused with warm saline (37°C) until the venous effluent became clear. At the end of the experiment the bowel segment was weighed to determine the flow rate in ml/min/100 g.

Results*In vitro: Sheep Blood*

The results are illustrated in Fig 28a. Sheep haematocrit normally runs at a lower value than man or dog because the cells are microcytic (23-48 fl) compared to man (80-95 fl). At a low (7% and 11%) packed cell volume (PCV) a linear response was found in the LDF signal with increases in the flow rate (Fig 28b). At a high PCV (22%, 24% and 27%), although a linear relationship between velocity and LDF signal was maintained ($r=0.99$), there was no significant increase in the LDF due to increase in the haematocrit alone (Fig 28c).

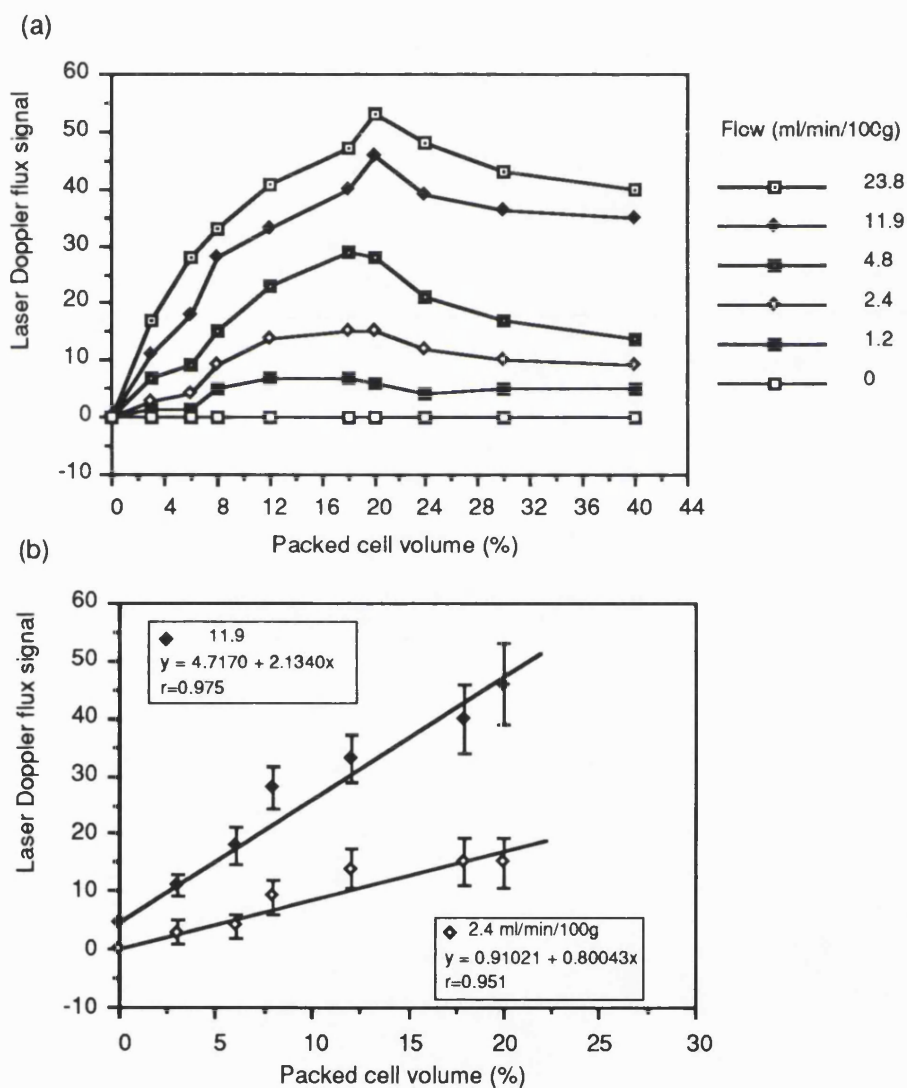
Fig 28: The effect of haemodilution on laser Doppler signal using sheep blood



In vitro perfusion of Dog Jejunum

The perfusion of dog jejunum at variable flow rates and haematocrit produced results very similar to in vitro flow of dog blood in tubes (Fig 29). For any given flow rate (Fig 29), the LDF signal was linear for increasing haematocrit up to a haematocrit of 20%; for clarity this is illustrated for two flows in Fig 29b. Thereafter, the LDF signal declined as the flow rate increased for any given haematocrit.

Fig 29: The effect of haemodilution on laser Doppler signal in an isolated dog jejunal loop



Discussion

The influence of the LDF signal and its relationship to the haematocrit is complex from the results of these *in vitro* experiments. *In vivo*, the laser Doppler probe will be interrogating tissue which will have flow moving in a number of random directions and not just in one direction as in the flow measured in a single length of tubing. With limitations in mind, a linear relationship in the LDF signal was found using sheep blood at velocities up to 0.5 mm/s, which is within the physiological range. The velocity of red blood cells in most tissues is 0.2-0.5 mm/s. Therefore at physiological RBC velocities, the LDF flowmeter may be expected to elicit a linear response. However, increases in the haematocrit did not produce a linear increase in the LDF signal (Fig 28a); at a haematocrit of >12% for sheep blood there was a tendency to underestimate the flow by the laser Doppler monitor.

A similar result was obtained using *in vitro* perfusion of dog jejunum. At high haematocrit (>20%), no significant increase in the LDF signal was observed. This non-linear relationship for the laser Doppler signal at high PCV is predicted by theory and may be due to multiple scattering effects within the measurement volume and Doppler shifts beyond the upper frequency limits of the instrument (Bonner and Nossal 1982). This occurs despite the fact that a linearising circuit is incorporated into the laser Doppler monitor in an attempt to minimise this problem.

The *in vitro* perfusion of dog jejunum showed a linear response to increases in blood flow up to a haematocrit of 20%; thereafter, no significant increase in the LDF signal was observed. Blood, unlike plasma or saline does not behave like a Newtonian fluid in that its viscosity alters with the shear rate (flow). Furthermore, blood unlike plasma or saline also changes its viscosity with alterations in the haematocrit. At high shear rates the blood viscosity is reduced and becomes constant like a Newtonian fluid, but blood viscosity also increases with an increase in the haematocrit. From Poiseuille's Law, the flow is inversely related to the viscosity, so as the viscosity of blood

increases the flow rate decreases. This may explain the reduction in LDF in experiments using dog blood with in vitro perfusion of bowel segments, where the LDF signal was reduced when the PCV was >20% for any given flow rate, but the flow may have also been underestimated due to limitations of the laser Doppler monitor.

During CPB, the haematocrit decreases from approximately 40% to 20-25% with haemodilution. Under these circumstances, the Laser Doppler signal from the bowel mucosa may be expected to increase, if all other parameters, such as temperature, mesenteric blood flow, cardiac index and mean arterial pressure remained constant. Unfortunately, these conditions do not exist clinically. Blood viscosity may fall with reductions in the haematocrit, but hypothermia and reductions in the shear rate due to reductions in the cardiac index during CPB tend to both increase the blood viscosity. Furthermore, the systemic haematocrit cannot be used as a guide to mucosal haematocrit when blood pressure and flow are changing; mucosal haematocrit increases with reductions in the perfusion pressure and blood flow (Lodal and Lundgren 1970).

For the purposes of making qualitative non-invasive assessments of mucosal blood flow it is perhaps more accurate not to attempt to make corrections of the LDF for changes in the haematocrit, but more valid to evaluate the LDF signal when the systemic haematocrit remains fairly constant. This situation occurs following the institution of CPB. Although the haematocrit is reduced to approximately 25%, it usually remains fairly constant thereafter, until the patient has been in the intensive care unit for 4 to 5 hours and begins to haemoconcentrate. This provides the opportunity to study laser Doppler changes in the gut mucosa on a background of a fairly constant haematocrit.

In summary, in vitro experiments have found that there is a non-linear relationship for LDF and haematocrit when the PCV is >20%, with a tendency for the LDF device to underestimate the total flow. However within the physiological ranges of RBC velocity, a linear relationship exists between LDF signal and blood flow for any given haematocrit.

The Effect of Hypothermic Non-pulsatile CPB on Gastric Mucosal Perfusion: A tonometric & laser Doppler assessment

Introduction

In the study examining the change in colonic and gastric pHi during and following CPB (Chapter 3), there was a tendency towards mucosal acidosis at a time when haemodynamic parameters (blood pressure and cardiac index) were returning towards physiological levels. This may have been due to impaired oxygen delivery as a consequence of reduced mucosal blood flow or diminished oxygen carrying ability of blood as a result of haemodilution by the pump-oxygenator priming fluid. To examine the effect of CPB on gastric mucosal blood flow and pHi, a combined study was undertaken using the techniques of gastric tonometry and laser Doppler flowmetry. The relationship between intramucosal pH and mucosal blood flow during the different phases of CPB and their relationship to haemodynamic and arterial blood gas measurements was also examined.

Materials & Methods

Fourteen patients who were undergoing elective CABG were recruited for this study. Patients who were known to have diabetes, hypertension, renal failure or previous history of abdominal surgery were excluded.

These studies were approved by the Hammersmith Hospital Research Ethics Committee and all patients gave informed written consent.

Tonometer Positioning & Sampling Protocol

The gastric tonometer (Tonometrics, Worcester, MA, USA) was sited by the method described in Chapter 3 and was removed before the patient left the operating theatre.

All pCO₂ values were determined using a ABL4 blood gas analyser (Radiometer, Copenhagen, Denmark). The pHi values were calculated using the correction factors provided by the company (Tonometrics, Inc, Worcester, MA, USA), and correction factors determined by the in vitro experiments described in Chapter 3. Similarly, when recording the pHa from the blood gas analyser, temperature corrected and values at 37°C were recorded. The temperature uncorrected (at 37°C) pHi and pHa together with temperature corrected pHit and pHat were employed to determine the pHa-pHi and pHit-pHat differences respectively.

Laser Doppler Flowmetry

Following induction of general anaesthesia, the mucosal laser Doppler probe was sited endoscopically on the antral gastric mucosa using an Olympus PQ 20 gastroscope. A single fibre probe with a diameter of 500 µm was employed for all the experiments after calibration of the probe by the method previously described.

Before CPB, continuous recordings were made from the same area of mucosa for two and five minutes to assess for the variability in laser Doppler flow readings. Data were recorded via an analogue to digital converter (MacLab 8) onto a computer for analysis (Macintosh IISi, Apple Macintosh Computers). During CPB and in the post-CPB period, recordings were made for 2 minutes and mean laser Doppler values determined. The laser Doppler monitor recorded at 1 Hz, so that an average of 120 laser Doppler values were averaged for each recording period.

The laser Doppler probe was removed from the stomach before the patient left the operating theatre. The probe was sterilised for use in the next patient by leaving it soaked for 20 minutes in 20% formaldehyde solution and then washed with normal saline.

Haemodynamic & Arterial Blood Gas measurements

The output from the pump-oxygenator recorded during CPB, mean arterial blood pressure (MAP), jugular venous pressure (JVP) and nasopharyngeal temperature was recorded continuously before during and after CPB onto the hard disc of a Mac IIsi computer. The SVRI was derived for the CPB period from the cardiac index (CI), JVP and the MAP.

Arterial blood gases were taken at the same time as the tonometric samples and the arterial pCO₂, pO₂, pH and bicarbonate concentration recorded. The packed cell volume or haematocrit was determined every ten minutes before, during and after bypass for the study period.

Peri-operative management

Pre-operative medication consisted of intramuscular papavaretum with scopolamine. Ranitidine was administered in two divided oral doses, 300 mg the night before surgery and 300 mg on the morning of surgery.

Anaesthesia was induced with thiopental and maintained with fentanyl, and isoflurane. Muscle relaxation was provided by pancuronium. Ventilation was controlled to maintain CO₂ tension at 4.7 to 5.3 kPa and arterial oxygen tension greater than 13.3 kPa.

All cardiac procedures were undertaken via a median sternotomy. Cardiopulmonary bypass was achieved using a bubble oxygenator (Bard, H 1700, Crawley, Sussex) with arterial line filtration (Pall EC plus 40µm screen arterial line filter, Pall Corporation, Portsmouth, Hants) using non-pulsatile flow with core cooling to 28°C. During CPB non-pulsatile flow from the pump-oxygenator (Sarns, Strathclyde, Scotland) was maintained at 2.4 l/min/m² at 37°C, with graded reductions down to 1.7 l/min/m² at 28°C. Mean arterial pressure was maintained during CPB at 50-60 mm Hg and acid base balance was managed using the alpha stat methodology. During

CPB the packed cell volume (PCV) was maintained above 20%, with the addition of packed red blood cells as required.

Myocardial preservation was achieved by using cold (10°C) crystalloid cardioplegia (St Thomas') with topical myocardial cooling using iced saline solution.

Re-warming was commenced during construction of the final coronary anastomosis, generally ten minutes prior to removal of the aortic X-clamp.

Statistical & Data Analysis

Paired Student's t test and least squares regression analysis was employed in data analysis. Results are expressed as mean \pm standard error of mean; p values are two tailed. Except for the SVRI and CI, results are compared with pre-CPB values, which for tonometry and arterial blood gases are for 20 minutes pre-CPB and all other values are 10 minutes pre-CPB. The SVRI and CI comparisons are for baseline values after 10 minutes of CPB. Laser Doppler flux readings averaged over 2 minutes, ten minutes pre-CPB for each patient were taken as the baseline values; all subsequent measures are expressed as a percentage of baseline.

Results

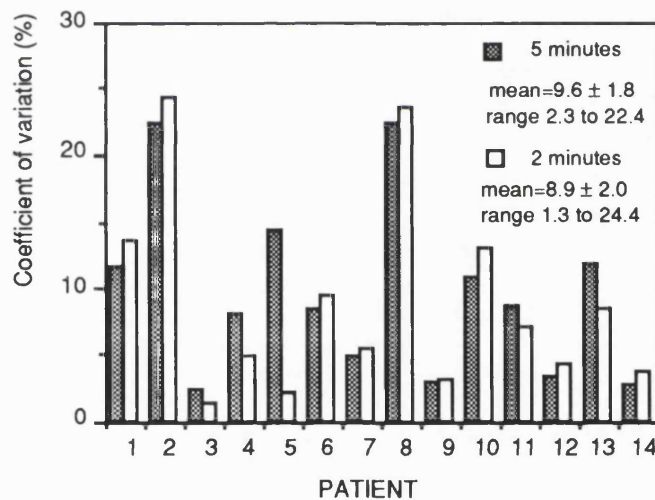
Patient data

The mean age for the patients was 63.1 ± 1.6 years (Range 53 - 73 years). The mean cross-clamp time was 49.2 ± 2.1 minutes and the mean CPB time was 81.6 ± 3.0 minutes. The lowest mean temperature achieved during CPB was 28.3 ± 0.6 °C.

Laser Doppler variability with time of recording

The laser Doppler flux readings had a coefficient of variation of 8.9 ± 2.0 % (range 1.3 to 24.4) for a two minute recording and 9.6 ± 2.4 % (Range 2.2 to 22.4) for 5 minutes of continuous recording ($p=0.83$) (Fig 30).

Fig 30: The variability of endoscopic gastric laser Doppler blood flow measurements



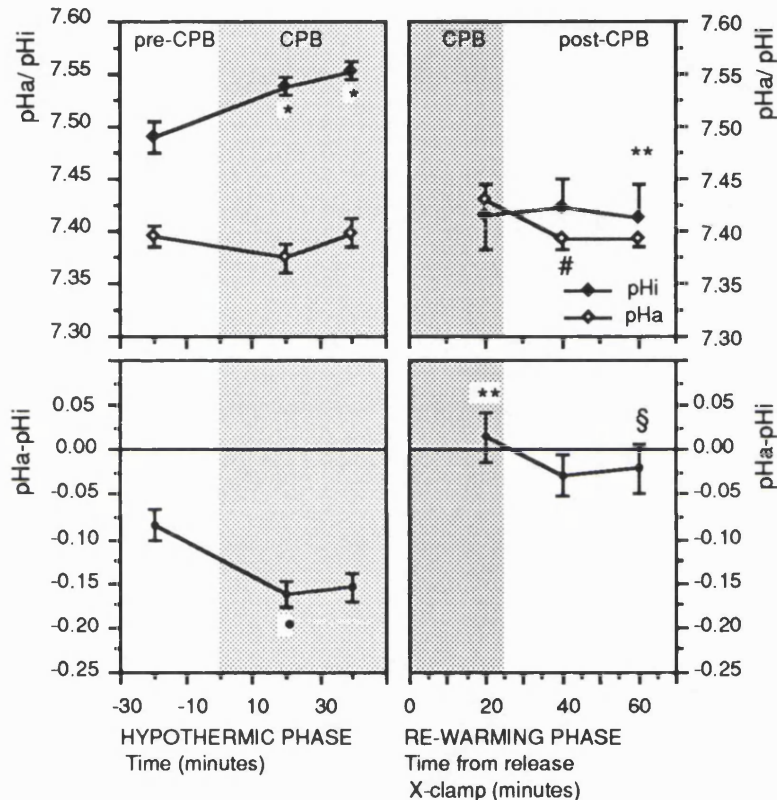
pHi, pHa & pHa-pHi (temperature uncorrected)

The pHi 20 minutes before CPB was 7.48 ± 0.01 and increased to 7.55 ± 0.01 after 40 minutes of hypothermic bypass ($p<0.001$) (Fig 31). During the same period, the pHa was unchanged from pre-CPB at 7.40 ± 0.01 to 7.4 ± 0.01 ($p=0.71$). The pHa-pHi increased from -0.084 pre-CPB to -0.154 after 40 minutes of CPB ($p=0.07$).

Following re-warming, and 20 minutes after the release of the aortic cross-clamp, the gastric pHi fell to 7.41 ± 0.03 ($p=0.07$) and continued to decline in the post-CPB period to 7.37 ± 0.01 forty minutes after the end of CPB ($p=0.033$). The pHa was

7.43 ± 0.01 ($p=0.02$) 20 minutes after cross clamp release and fell to 7.39 ± 0.01 ($p=0.16$) 40 minutes after the end of CPB. The pHa-pHi difference became positive at 0.014 20 minutes after cross-clamp release ($p=0.033$) but by 40 minutes post-CPB the pHa-pHi difference had been reduced to -0.02 ($p=0.046$, Fig 31).

Fig 31: Relationship between pHa & pHi for patients undergoing hypothermic non-pulsatile CPB, with bubble oxygenation



All p values are for comparisons made with pre-CPB values

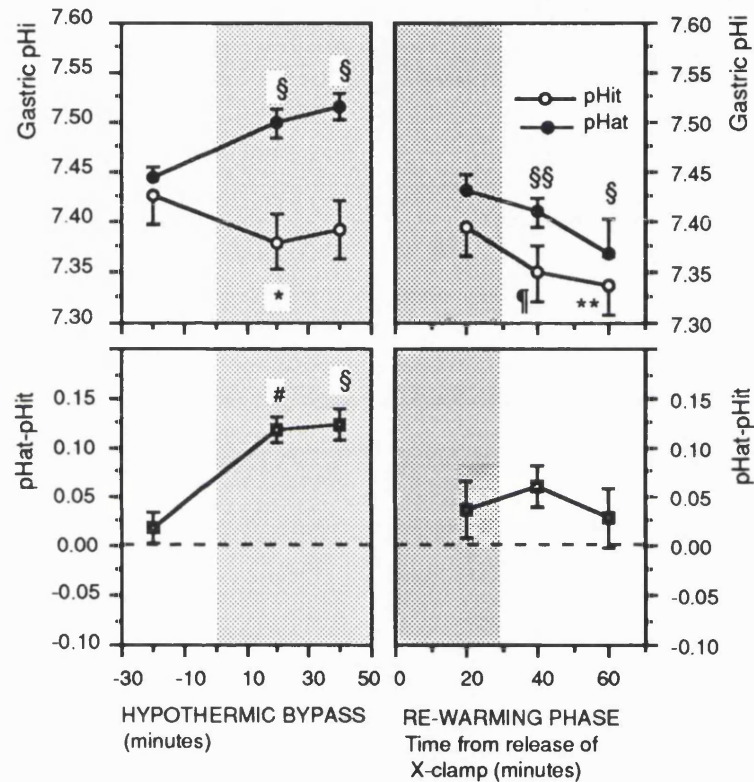
* $p < 0.001$, ** $p = 0.034$, § $p = 0.045$, # $p = 0.021$, • $p = 0.003$

pHit, pHat & pHat-pHit (temperature corrected)

Unlike the temperature uncorrected values, the pHit was below the pHat throughout the study period including the hypothermic phase of CPB (Fig 32), when the temperature uncorrected gastric pHi increased (Fig 31). Twenty minutes into the hypothermic phase of CPB, the pHit had declined to 7.38 ± 0.017 from a pre-CPB value of 7.43 ± 0.019 ($p=0.018$), but subsequently recovered after 40 minutes of hypothermic CPB to 7.39 ± 0.018 ($p=0.14$). The intramucosal pH declined in the post

X-clamp release period and 40 minutes after the end of CPB the pHit was 7.34 ± 0.03 ($p=0.004$). The pHat-pHit difference remained positive throughout the study period, with the greatest difference recorded after 40 minutes of hypothermic CPB at 0.12 ± 0.02 compared to pre-CPB value of 0.02 ± 0.02 ($p=0.002$).

Fig 32: Relationship between temperature corrected pHa (pHat) & pHi (pHit) for patients undergoing hypothermic non-pulsatile CPB, with bubble oxygenation

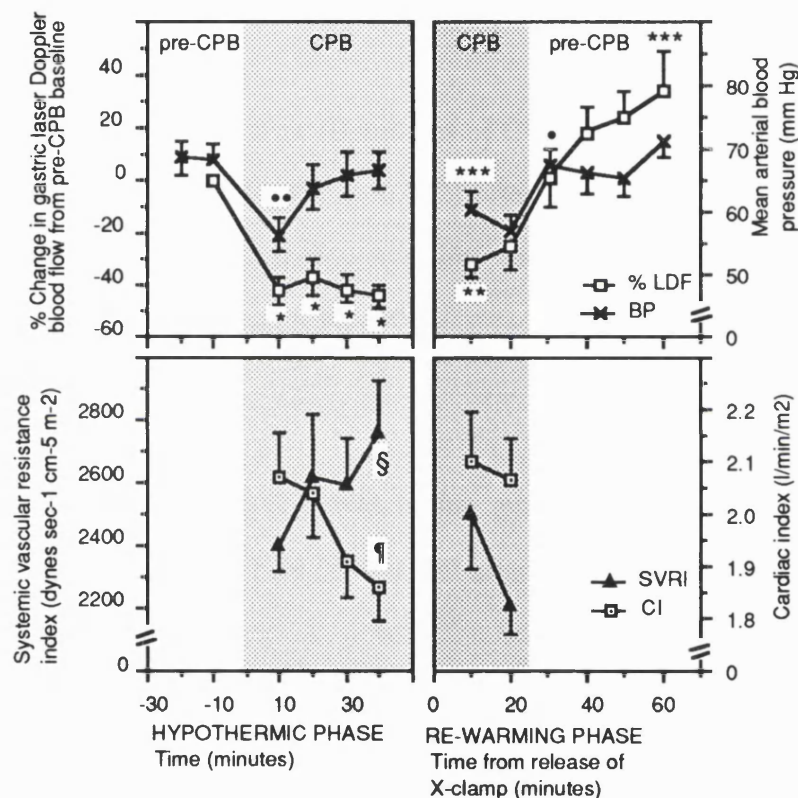


Compared to pre-CPB values
 # $p=0.001$, § $p=0.002$, §§ $p=0.003$, ** $p=0.004$,
 ¶ $p=0.007$, * $p=0.018$,

Laser Doppler Flowmetry

The gastric laser Doppler blood flow showed a progressive fall to -44.3 ± 4.3 % of pre-CPB baseline values after 40 minutes of hypothermic non-pulsatile CPB ($p < 0.001$). Twenty minutes after the release of the aortic cross-clamp the LDF had increased to -31.6 ± 5.8 but was still significantly below pre-CPB values ($p < 0.001$).

Fig 33: The relationship of laser Doppler gastric blood flow to haemodynamic parameters



P values, when compared to pre-CPB values

* $p < 0.001$, * $p = 0.007$, ** $p = 0.008$, ** $p = 0.014$,
*** $p = 0.047$

P values, when compared to the start of CPB

§ $p = 0.002$, ¶ $p = 0.017$

A progressive increase in LDF blood flow was observed with re-warming and after the discontinuation of CPB. Twenty minutes after CPB the flow had returned to pre-CPB values at $+19.1 \pm 10.3$ % ($p = 0.088$), but 40 minutes post-CPB the LDF blood flow was above pre-CPB values at $+33.8 \pm 14.8$ % ($p = 0.041$) (Fig 33).

Haemodynamic Parameters

The mean arterial blood pressure (MAP) was maintained at 50-70 mm Hg throughout CPB. The MAP declined to 56.4 ± 2.6 mm Hg ten minutes after the institution of CPB ($p=0.008$), but increased progressively to 66.0 ± 3.6 mm Hg ($p=0.39$) after 40 minutes of hypothermic CPB. Re-warming and aortic X-clamp release were associated with a reduction in MAP to 57.1 ± 2.4 mm Hg ($p=0.007$), 20 minutes after cross clamp release (Fig 33). The MAP recovered in the post-CPB period to 71.1 ± 2.5 mm Hg ($p=0.84$), 40 minutes post-CPB.

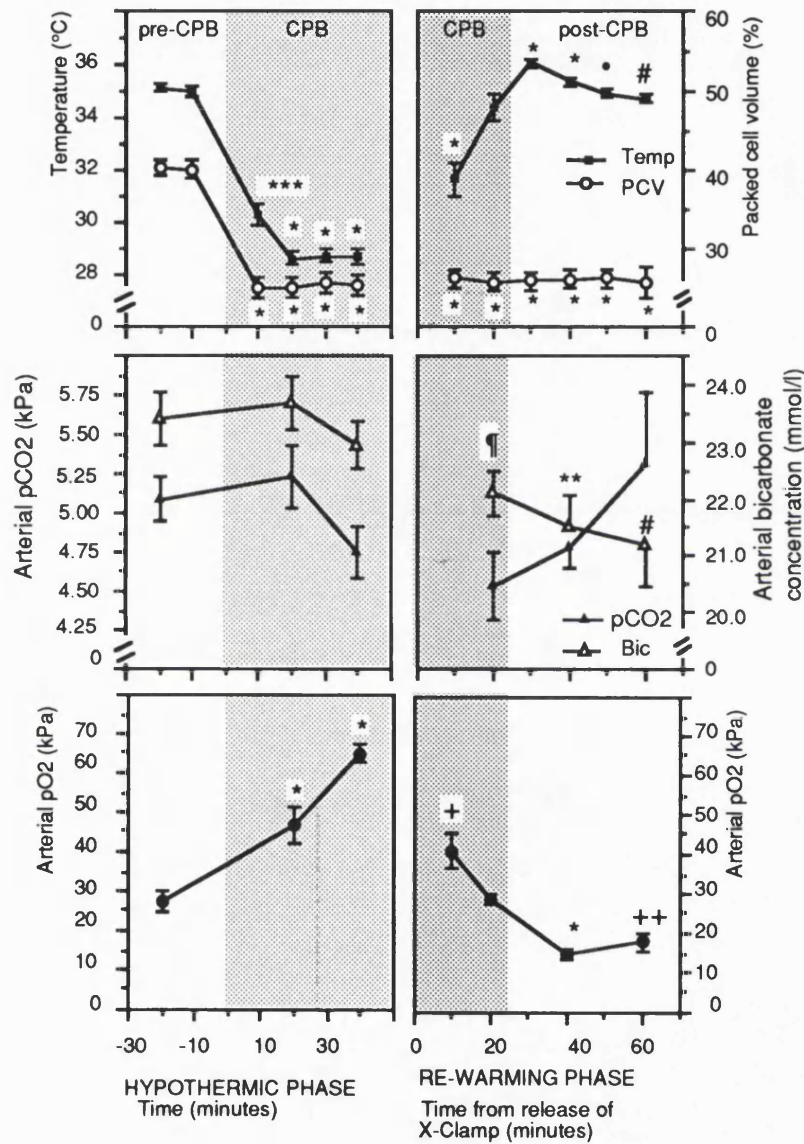
The SVRI was 29.9 ± 0.9 SVRU after 10 minutes of CPB, but with core cooling the SVRI increased to 34.5 ± 2.02 SVRU ($p=0.002$) after 40 minutes of hypothermic CPB. Release of the aortic X-clamp and re-warming were associated with a reduction in the SVRI to 27.6 ± 1.1 SVRU ($p=0.18$), 20 minutes post cross clamp release (Fig 33).

The CI was reduced with core cooling, which after ten minutes of CPB was 2.07 ± 0.08 l min⁻¹ m⁻², but decreased to 1.86 l min⁻¹ m⁻² after 40 minutes of CPB ($p=0.02$), when the core temperature had been reduced to 28.7 ± 0.26 °C ($p<0.001$). Twenty minutes after cross clamp release, the temperature had increased to 35.1 ± 0.53 °C ($p=0.08$) and the CI had risen to 2.06 ± 0.08 l min⁻¹ m⁻² ($p=0.96$) (Fig 33).

LDF & Packed Cell Volume

The PCV fell from a pre-CPB value of 40.2 ± 1.0 to 25.3 ± 1.4 % ($p<0.001$), 40 minutes after the institution of bypass. During this time the LDF blood flow declined to $-44.3 \pm 4.3\%$, but in the rewarming phase of CPB and in the immediate post-CPB the PCV remained constant despite significant alterations in the LDF gastric blood flow. The PCV was $26.2 \pm 1.2\%$, 20 minutes after release of the aortic cross-clamp and $25.7 \pm 1.97\%$, 40 minutes post-CPB (Fig 34).

Fig 34: Changes in arterial blood gases, temperature & haematocrit during non-pulsatile CPB with bubble oxygenation



All p values are for comparisons with pre-CPB values
 *p<0.001, **p=0.003, ***p=0.004, ¶ p=0.006 *p=0.001, # p=0.018
 +p=0.03, ++p=0.04

Arterial pO₂, pCO₂ & bicarbonate

The changes in arterial pCO₂ are illustrated in Fig 34; due to the standard error of the data, statistical significance was not achieved for values during CPB, when compared to the pre-CPB pCO₂. The pO₂ was 27.4 ± 2.4 kPa, 20 minutes pre-CPB, and increased to 64.8 ± 2.5 kPa (p<0.001), 40 minutes after hypothermic CPB, but decreased in the re-warming period to 28.7 ± 1.4 kPa (p=0.69), 20 minutes after release of the cross clamp (Fig 34). The pO₂ continued to fall in the post-CPB period, but recovered to 17.8 ± 2.4 kPa, 40 minutes post-CPB, which was still below pre-CPB values (p=0.039).

Arterial bicarbonate concentration, declined progressively throughout the study period and 40 minutes post-CPB was 21.2 ± 0.74 mmol l⁻¹, compared to 23.4 ± 0.45 mmol l⁻¹ before CPB (p=0.01) (Fig 34), which coincided with a reduction in the pHa from 7.40 ± 0.01 to 7.39 ± 0.01 (p=0.16).

Correlation of LDF with haemodynamic and arterial blood gas parameters

A significant correlation was identified between LDF and cardiac index, core temperature and the arterial pO₂, as indicated in Fig 35-37.

Fig 35: Correlation between gastric laser Doppler flow & the cardiac index

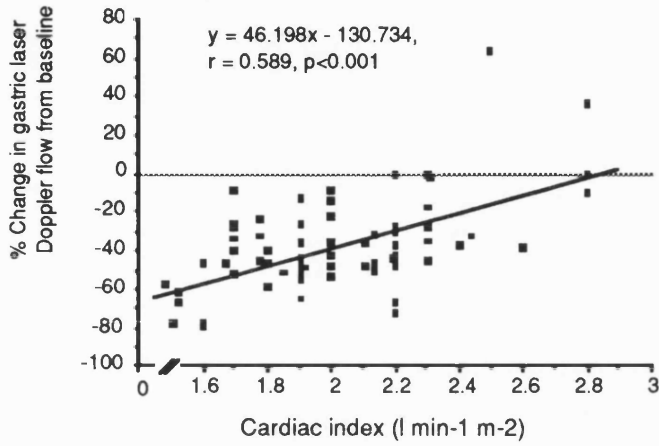


Fig 36: Correlation between gastric laser Doppler blood flow & core temperature

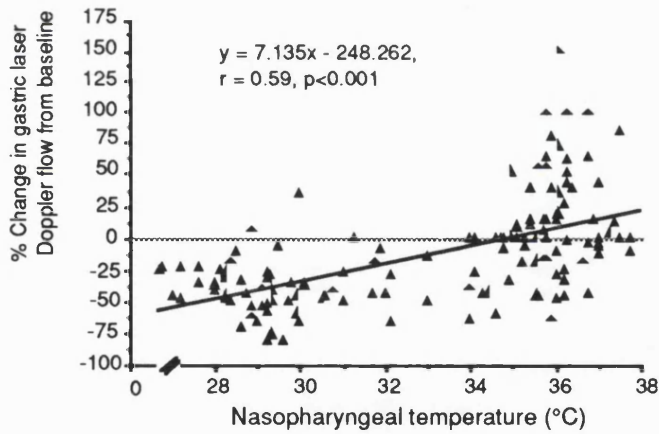
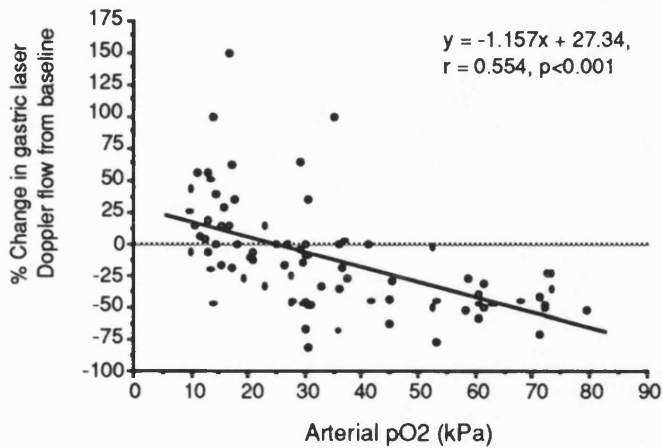


Fig 37: Correlation between gastric laser Doppler blood flow & arterial pO₂



No correlation was found between LDF and MAP, pCO₂ and SVRI (Fig 38-40).

Fig 38: Correlation between mean arterial blood pressure & gastric laser Doppler blood flow

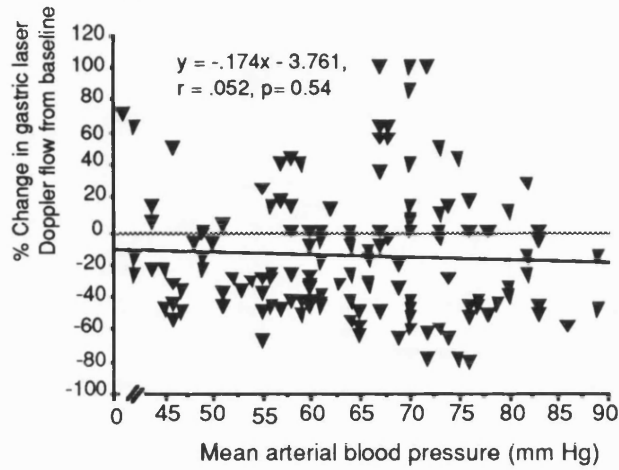


Fig 39: Correlation between gastric laser Doppler flow and the SVRI

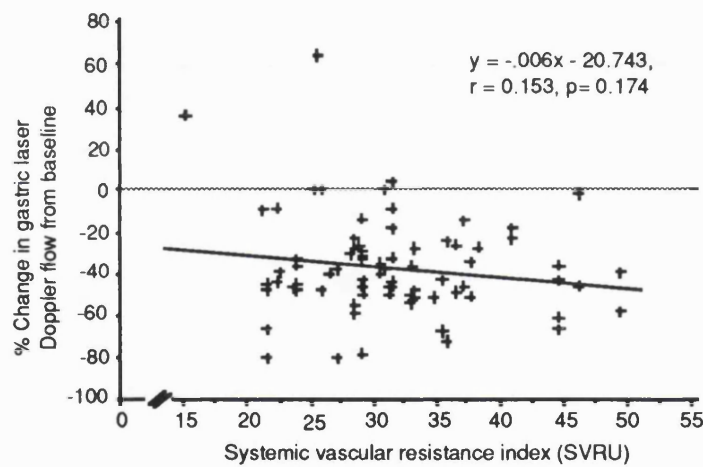
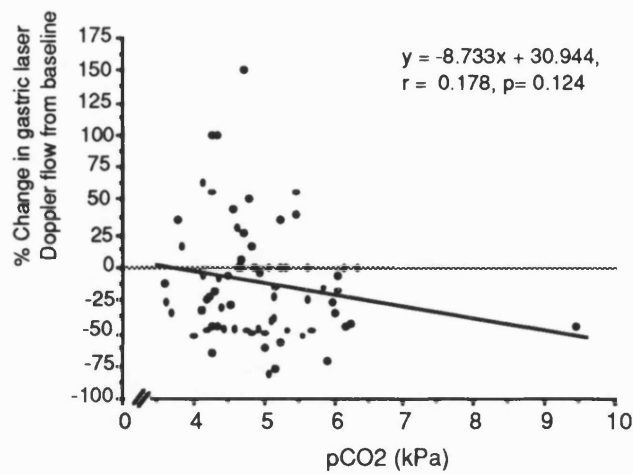


Fig 40: Correlation of laser Doppler gastric blood flow with pCO₂



Discussion

This study has found that hypothermic CPB results in an increase in the gastric pHi, which was previously noted in the combined study using gastric and colonic tonometry (Chapter 3). This was associated with progressive reductions in the gastric LDF. The increase in the pHi was evident only if no correction was made for temperature variations during the study period. However, using the in vitro correction factors determined by the earlier study (Chapter 3), a significant reduction in the pHit compared to pre-CPB values was found. In addition, the methodology may still underestimate the pHi. Antonsson et al found in an animal model that mechanical occlusion of the superior mesenteric artery (SMA) caused a reduction in the pHi as measured by tonometry, but the tonometer overestimated the pHi when compared to a tissue microelectrode (Antonsson et al 1990). When intramucosal acidosis was induced by endotoxaemia then tonometric pHi correlated closely with microelectrode pHi (Antonsson et al 1990). They suggested that it may be invalid to assume that tissue and arterial bicarbonate concentrations are the same at times of reduced arterial inflow (Antonsson et al 1990). This would imply that tissue bicarbonate concentration is reduced at times of tissue hypoperfusion as a consequence of cellular hypoperfusion and that a bicarbonate gradient is built up between the tissue and arterial blood, which is only abolished when normal tissue perfusion is restored. If these findings are extrapolated to the pHit values determined in this study, then the intramucosal pH would be even lower during hypothermic CPB. This would suggest that during this period the supply of oxygen is insufficient to meet gut mucosal tissue demands, despite core cooling and reductions in the metabolic rate.

The variability in the LDF with time was not significantly different, between 2 and 5 minutes of recording, although a longer recording may have been expected to reduce the coefficient of variation. This probably reflects the dynamic nature of circulatory

haemodynamics, which makes longer recordings of laser Doppler flow more susceptible to haemodynamic changes during the recording period.

Despite the maintenance of adequate haemodynamic parameters during CPB (MAP, CI & SVRI), gastric laser Doppler blood flow declined progressively during the hypothermic phase. This may in part have been due to a reduction in the PCV, which fell from approximately 40% to 25%, with the institution of bypass, but in the re-warming phase, when LDF was increasing, the haematocrit remained largely unchanged. Further evidence that reductions in LDF are not due to haemodilution alone, has come from a porcine model of CPB, in which the PCV was kept constant on the institution of CPB with blood transfusions. Significant reductions in ileal mucosal LDF flow were found during bypass, which were associated with an increase in the superior mesenteric arterial (SMA) blood flow (Cox et al 1992).

A simple linear correlation was established between LDF and the CI during CPB. Normally the gut receives approximately 25% of the cardiac output, but redistribution of blood flow occurs to other tissue beds at times of shock or haemodynamic stress. Rowell and Johnson, found that hypothermia in normal human volunteers reduced splanchnic blood flow (Rowell and Johnson 1984), a finding which has been supported by a positive linear relationship that was found in our study for core temperature and LDF ($r=0.59$, $p<0.001$). A similar relationship was not found for the MAP within the range 50-70 mm Hg assessed during this study period. This may be due to the autoregulatory ability of the mesenteric vasculature.

The mesenteric vasculature may be considered to consist of three primary parallel circuits serving the muscularis propria, serosa and mucosa. Each circuit is composed of five series-coupled components. Sequentially, they are the arterioles, pre-capillary sphincters, capillaries, post-capillary sphincters and the venous capacitance vessels

(Folkow 1967). The resistance arterioles primarily govern vascular resistance and vasodilate in response to reductions in MAP. This is both a direct myogenic response to a reduction in MAP and a metabolic response to the accumulation of local metabolites such as adenosine (Reilly and Bulkley 1993). In addition to autoregulation of total blood flow, re-distribution of blood flow occurs within the gut wall (Lundgren 1967), so with increasing reductions in MAP blood flow becomes prioritised to the villi at the expense of the deeper layers of the gut wall. This would make teleological sense, since the mucosa is the most metabolically active part of the gut wall. However, in circulatory shock these autoregulatory mechanisms are overridden, with local and systemic vasoconstrictors acting at the level of the resistance arterioles to reduce mesenteric perfusion (Reilly and Bulkley 1993). The state of autoregulatory mechanisms during CPB and in particular the effects of hypothermia are unknown, but hypoperfusion may occur as a result of increases in arteriolar and pre-capillary sphincter tone by the action of systemic vasoconstrictors. Adrenergic stimuli act predominantly upon the post-capillary capacitance vessels to autotransfuse the individual and improve cardiac output by increasing preload of the heart (Gershon and Erde 1981).

Haemodynamic modulations undoubtedly contribute to mucosal hypoperfusion which was found during CPB, but other mechanisms may also play a significant role. Cardiopulmonary bypass may be considered to be a form of controlled circulatory shock. One of the major consequences of CPB is a progressive increase in systemic vascular resistance, particularly if non-pulsatile perfusion is used during the extracorporeal perfusion period (Taylor et al 1980). The loss of pulsatility in the renal arteries together with a reduction in MAP during CPB is associated with increased renin release (Many et al 1967). The end-product of the renin-angiotensin axis is the production of angiotensin II. The secretion of this hormone may be ameliorated by the use of angiotensin-converting enzyme inhibitors or by the employment of

pulsatile perfusion (Taylor et al 1979, Taylor et al 1980). Angiotensin II is a highly selective mesenteric vasoconstrictor with high affinity receptors for this peptide hormone on splanchnic vascular smooth muscle (Gunther et al 1980), and much of the increase in the SVRI during CPB may be mediated via mesenteric vasoconstriction. Evidence for the importance of this hormone in the development of non-occlusive mesenteric ischaemia has been found in animal models of cardiogenic shock. Animals subjected to cardiogenic shock by pericardial tamponade sustained severe mesenteric vasoconstriction. This response was unaffected by sympathetic denervation but abolished by ablation of the renin-angiotensin axis (Bailey et al 1987).

Angiotensin II is not the only candidate that may mediate mucosal hypoperfusion during bypass. CPB results in 'a whole body inflammatory response' secondary to the bioincompatibility of the extracorporeal circuit. Products of this inflammatory response also have vasoactive properties which may be important at the pre-capillary or tissue level. Thromboxanes (A₂ and B₂) are both released during CPB. Thromboxanes can vasoconstrict small and large blood vessels in nearly all tissue beds, and some evidence exists that less thromboxane production occurs with pulsatile flow (Watkins et al 1982). In endotoxic pigs cyclo-oxygenase inhibitors improved mesenteric perfusion and ameliorated ileal intramucosal acidosis (Fink et al 1989). Leukotrienes, products of lipoxygenase pathway of arachidonic acid metabolism are also potent mesenteric vasoconstrictors (Bayorh et al 1985, Feigen 1983) and leukotriene production increases following CPB [S Allen 1993, personal communication]. Pre-treatment with LY171883, a leukotriene antagonist, improves mesenteric blood flow in endotoxic pigs (Cohn et al 1990).

Whatever the underlying mechanisms involved in the regulation of mucosal perfusion, the ultimate aim is the maintenance of adequate supplies of nutrients,

particularly oxygen to the mucosal cells. Gut tissue pO_2 is dependent upon $\dot{V}O_2$, haemoglobin concentration, the percentage of haemoglobin saturated with oxygen in arterial blood, the affinity of haemoglobin for oxygen, cardiac output and the distribution of perfusion. Tissue oxygenation is in a dynamic state of flux and alters with the balance between $\dot{V}O_2$ and $\dot{D}O_2$. During steady state conditions, $\dot{V}O_2$ is independent of $\dot{D}O_2$. Only when delivery is compromised by hypoxia/ischaemia does $\dot{V}O_2$ become dependent upon $\dot{D}O_2$. In some pathological states such as sepsis (Haupt et al 1985) and adult respiratory distress syndrome (Danek et al 1980) $\dot{V}O_2$ is supply dependent, i.e, graded reductions in $\dot{D}O_2$ and cause similar reductions in $\dot{V}O_2$, which may be due to an underlying defect in tissue oxygen extraction. The ability of the gut to withstand hypoxic stress is far greater than ischaemic stress. In a dog model, reducing $\dot{D}O_2$ to <60% by hypoxia alone caused a reduction in gut $\dot{V}O_2$. When $\dot{D}O_2$ was compromised by both hypoxia and ischaemia, $\dot{D}O_2$ needed to be decreased to only 51% before a reduction in the $\dot{V}O_2$ was observed (Grum et al 1984). The point at which reductions in $\dot{D}O_2$ result in decreases in $\dot{V}O_2$ is known as the critical $\dot{D}O_2$ value. Furthermore, the critical $\dot{D}O_2$ value is higher for gut tissue than non-gut tissue, which is reflected by the gut having lower maximal oxygen extraction fractions than other tissues (Nelson et al 1987).

The intestine matches $\dot{V}O_2$ with $\dot{D}O_2$ by two distinct mechanisms. The first is based on the metabolic demand theory of local vasoregulation. Microvascular smooth muscle tone (pre-capillary sphincters) respond to local tissue metabolites in particular a low pO_2 and vasodilate to increase the number of perfused capillaries. This latter mechanism is independent of nervous and humoral influences (Granger and Nyhof 1982). This may explain the negative linear correlation that was found for laser Doppler gastric blood flow and arterial pO_2 ($r=0.55$, $p<0.001$, Fig 37). This is the main mechanism which operates with moderate reductions in $\dot{D}O_2$. The increase in perfused gut capillaries enables a greater fraction of the supplied oxygen to be

extracted. Only with greater reductions in the $\dot{D}O_2$ does arteriolar vasodilation also occur to increase blood flow.

The inadequacy of tissue oxygen causes a reduction in energy stores, as mitochondrial oxidative phosphorylation can no longer continue and the cell shifts to anaerobic glycolysis in an attempt to maintain cellular ATP levels. Continued glycolysis in the face of reduced $\dot{D}O_2$ results in the accumulation of lactic acid and hydrogen ions. Intracellular acidosis, as determined by tonometry may therefore be a good indicator of tissue oxygenation. Poole et al found in a canine model that graded reductions in intestinal blood flow resulted in linear reductions in the gut pHi (Poole et al 1987). In the present study, gastric mucosal pHi declined slightly during the initial phase of hypothermic CPB before recovering after 40 minutes of hypothermic CPB. As previously discussed, this may still be an overestimate of the true pHi if tissue $[HCO_3^-]$ is less than the arterial $[HCO_3^-]$ during periods of mucosal hypoperfusion. During this period, reductions in $\dot{D}O_2$ secondary to reductions in haematocrit and CI, may not be entirely offset by a decrease in the metabolic rate with core cooling. However, after release of the aortic cross-clamp in the re-warming period and the immediate post-CPB phase, there was a tendency towards intramucosal acidosis, despite increases in gastric laser Doppler blood flow. This suggests that during this period there is a disparity between $\dot{V}O_2$ and $\dot{D}O_2$, with net ATP hydrolysis and the accumulation of H^+ ions. Several explanations may account for this fall in gastric pHi. Firstly, with a return of mucosal blood flow towards pre-CPB levels, tissue $[HCO_3^-]$ will approximate more closely to arterial $[HCO_3^-]$, thus reducing the pHi. Secondly re-warming results in an increase in the metabolic rate, but during this period the oxygen carrying ability of the blood remains limited, since haematocrit is only 25% (mucosal haematocrit may be only 50% of this value (Jodal and Lundgren 1970)), and the cardiac index is still well below the physiological level of 3.0-3.2 l/min/m² (Taylor 1990). This assumes that re-warming returns tissue metabolism to

pre-CPB levels for any given temperature, which may be untrue. The generalised inflammatory response, with damage to cellular membranes may increase the metabolic demand of cells. Energy requirements of the gut epithelium, damaged by CPB, may be increased from membrane pumps to preserve intracellular homeostasis. Under these circumstances normal levels of $\dot{V}O_2$ would be inadequate to prevent net degradation of ATP. Endotoxaemia during CPB may be contributory to this phenomenon. Endotoxaemia during CPB increases soon after cross-clamp release (Jansen et al 1992), coinciding with a reduction in pHi , and lipopolysaccharide is known to increase the rate of metabolism and can uncouple mitochondrial phosphorylation (Tavakoli and Mela 1982). Further evidence for the presence of anaerobic metabolism has been found in a skeletal muscle biopsy study of patients. This latter study, in which skeletal muscle biopsies were obtained after hypothermic bypass, found a persisting oxygen deficit in the tissues, exhaustion of the cell's functional reserve and an activation of anaerobic metabolism (Del Canale et al 1990).

In this study gut or whole body oxygen utilisation was not formally assessed, but the steep fall in the pO_2 in the re-warming phase of bypass when oxygenation was kept constant suggests that oxygen extraction and therefore oxygen utilisation increases during this period. Even if mixed venous oxygen from a central vein or indeed the portal vein had been sampled, it would only be able to provide qualitative data like tonometry as to the state of gut mucosal oxygenation. In part, this may be due to the counter-current exchange of oxygen which may progressively reduce villus pO_2 from the base to the tip (Kampp et al 1968). Thus mixed venous sampling would not indicate villus tip oxygenation because of the villus arterio-venous oxygen shunt. These findings may have implications for the normal physiological functions of the gut in the post-operative period, in particular its function as a barrier to luminal toxins.

Chapter 5

The Effect of CPB on Small Bowel Saccharide Transport & Permeability

Introduction & Aims

Previous (Chapters 3 & 4) found that CPB results in the development of intramucosal acidosis in the re-warming and immediate post-CPB periods. Laser Doppler flowmetry found that mucosal hypoperfusion occurs during the hypothermic phase of CPB with a return/ increased blood flow in the post-CPB period. The implications of these findings on gut barrier and absorptive function are unknown.

The aims of the studies presented in this Chapter were to investigate the effect of non-pulsatile hypothermic CPB on the absorption of monosaccharides and gut permeability.

Background

The function of the gut is not only the absorption of nutrients, electrolytes and water, but to maintain a barrier preventing the translocation of bacteria and the egress of microbial products from the lumen into the portal circulation. The concept of a “leaky gut” was first proposed in 1955 by Jacob Fine following observation on animal models of experimental shock (Schweinburg and Fine 1955), which may have relevance to modern cardiopulmonary bypass. Fine suggested that bacterial derived endotoxins (lipopolysaccharides) could traverse the mucosal barrier in the setting of impaired host resistance. Support for this hypothesis was derived from the observations that (a) the administration of enteral non-absorbable antibiotics improved survival in experimental shock, (b) circulating concentration of lipopolysaccharides (LPS) was higher in the experimental shock animals compared to a control animal group, and (c) the lethality of exogenously administered endotoxin was markedly increased by haemorrhage.

Two years later, Lillehei provided further support for Fine’s thesis, when he showed that perfusing the gut with blood from a normotensive animal led to a marked reduction in the mortality of dogs in haemorrhagic shock; similar protection could not be achieved by perfusing the liver or the brain (Lillehei 1957). Intestinal ischaemia could therefore be acting in some crucial manner in the development of shock and death in these animals.

Alterations in Intestinal Permeability in Health & Disease

The assessment of alterations in permeability in various disease states cannot be made without establishing the limits of a normal range. The indigenous normal populations of East and West Africa, the Middle East, Indonesia, Papua New Guinea, India & Thailand have normally high permeability values (Bjarnason et al 1988), which have been related to the high incidence of tropical enteropathy in some of these areas (Ukabam et al 1986). This is in contrast to low normal values for whites in England, the continent, America and Australia (Bjarnason et al 1988).

A number of diseases are associated with increased gut permeability. These include Crohn's and coeliac disease (Hollander 1988, Shorter et al 1972), schizophrenia (Sugerman et al 1982), the spondylarthropathies (Mielants et al 1988) and rheumatoid arthritis (Zaphiropoulos 1986). In some of these disorders the increased permeation of gut macromolecules may be important in the pathogenesis of other disease processes, eliciting an immune response by induced molecular mimicry. A luminal macromolecule may have the amino acid homology with a structural protein so that the immunological response to the macromolecule is also directed at the host tissue (Yu 1988).

In the context of gut hypoperfusion and/or ischaemia, gut mucosal damage may be manifest as an increase in permeation of both bacterial products as well the translocation of bacteria. Patients who are in the intensive care unit are at particular risk since they may possess factors which in experimental animals have been shown to promote bacterial translocation, such as impaired cell-mediated immunity (Owens and Berg 1980), bacterial overgrowth or superinfection due to antibiotic therapy (Berg 1981), haemorrhage (Baker et al 1988) and endotoxaemia (Deitch et al 1987). Patients undergoing open-heart surgery share some of these risk factors. Cardiopulmonary bypass has been associated with impaired cell mediated immunity (Ohri 1993). There is a relative lymphopaenia following CPB in the first 24 to 48

hours (Roth et al 1980). The helper to suppressor T cell ratio is reduced (Hisatomi et al 1989), and Leroux et al have reported a depression of the proliferative response to mitogen following open heart surgery (Leroux et al 1986). This combined with the immunodepressive actions of anaesthetic agents (Salo 1978) and blood transfusions (Ottino et al 1987), may put the patient undergoing CPB at particular risk from any rises in gut permeation.

The Assessment of Intestinal Permeability

Background

The development of techniques for assessing the intestinal barrier function has a short history following the introduction of non-metabolised di and tri-saccharides (oligosaccharides) as test substances in 1974 (Menzies 1974). The physical property of permeability relates to that property of membrane which enables passage by unmediated diffusion (Csaky 1984 p.51).

Diffusion implies bidirectional or random movement of molecules, with net transfer from a region of higher concentration to one of lower concentration as described in Fick's Law (Fick 1855). The rate of diffusion can be measured and is dependent on the temperature, viscosity of solvent and size of the diffusing molecule.

Permeability can be determined by several methods and expressed as a reflection coefficient or by measurement of "permeation" which is a solute flux rate or velocity of transport, across a defined area of membrane over a given time with a defined concentration and pressure gradient (Fordtran et al 1965). Under these control conditions the permeability can be expressed as a constant derived from Fick's equation (Fick 1855). However, where the conformation of the membrane is uncertain as is the case for most biological tissue, the permeability constant is substituted by a diffusion constant.

The transfer of a solute across the human small intestine can occur:

(a) by *simple diffusion*, which does not show saturation kinetics and will be referred to as *permeation*. The permeation of a marker substance across a membrane is an indicator of the permeability of the membrane;

(b) by a *carrier mediated* process which will be referred to as *absorption*. This may be either active (requiring energy) or passive, but both mechanisms have a limited capacity which can be saturated (Lehniger 1976 p.779). However, absorption is an indicator of the function of a biological membrane to aid the transfer of electrolytes and nutrients (Powell 1981).

The basis of Techniques for Assessing Permeability

Fordtran et al, in 1965 described a test for measuring human permeability by perfusing intestinal segments with hyperosmolar solutions of solutes with graded molecular weights (Fordtran et al 1965). The principle of the technique was that a non-permeating solute would draw a fixed amount of water into the lumen while the solute capable of permeating would exert less osmotic effect indicated by correspondingly less water movement. Their calculations, which assumed mannitol (radius 0.4 nm) was non-absorbable, indicated decreasing effective pore size in the more distal parts of the small intestine; the average pore radius being less than half the jejunal pore radius. Later, other solutes such as L-xylose, erythritol and urea were employed, which indicated that the permeation of the small intestinal mucosa to molecules with a radius less than 0.4 nm was substantial.

In 1973, Loehry et al using a number of substances with a molecular weight of 60 to 80,000 demonstrated that there was a relationship between the molecular weight of a substance and its blood to lumen clearance (Fordtran et al 1965).

Unlike the controlled in vitro situation when physical parameters can be closely regulated, the in vivo assessment of gut permeability cannot be made with the same degree of precision. However, with this limitation in mind, an estimate can be made by measuring the urine excretion of orally administered test substances (Cooper 1984, Menzies 1974). Oligosaccharides were the first substances to be used for this purpose, including lactulose, melibiose, raffinose, stachyose and cellobiose. They have subsequently been used in conjunction with a monosaccharide such as mannitol or L-rhamnose for reasons outlined below. Though sugars are widely employed the various polymers of polyethylene glycol and ⁵¹chromium labelled ethylenediamine-tetraacetate (⁵¹Cr EDTA) have also been utilised but are less suitable for the accurate assessment of gut permeability when employed in isolation.

Characteristics of Probe Molecules used for the Assessment of Gut Permeability

Lactulose (4-O-β-D-galactopyranosyl-D-fructose) approaches the criteria of the ideal test substance with urinary recoveries approaching 100% following intravenous injection (Maxton et al 1986, Menzies 1974). It is normally excreted in very small amounts in the urine following oral ingestion and it is therefore necessary for analytical purposes to administer 5.0g of this disaccharide. This quantity is sufficient to retard the permeation of L-rhamnose (6 deoxy L-mannose) by at least 30% because of its osmotic retention effect on water in the intestinal lumen (Maxton et al 1989). A converse effect can be obtained when large doses of rhamnose are administered, but because a sufficient amount appears in the urine following the oral dose of 1 g, larger doses can be avoided. Approximately 73% of L-rhamnose is excreted in the urine during 24 hours following intravenous injection (Maxton et al 1986).

The extent to which the test substances reflect alteration in macromolecular permeability is still undergoing investigation, but evidence is emerging that a correlation does exist. Davin et al found that a significant correlation exists between intestinal permeability to ⁵¹Cr EDTA and IgA immune complex plasma levels in patients with IgA nephropathies (Davin et al 1988). Similarly the permeation of N-formyl-methonyl-leucyl-¹²⁵I-tyrosine, a synthetic analogue of the bacterial chemotactic peptide N-formyl-methonyl-leucyl-phenyl-alanine, correlated significantly with intestinal permeability to ⁵¹Cr EDTA (Ferry et al 1989). However, increased permeability of test markers may not be paralleled by increased permeation of macromolecules since their passage is also influenced by concentration, degradation by digestive enzymes, local and systemic immune factors.

Studies have shown that the permeation of the test substance is influenced by the osmolality of the test solution. For lactulose there is little effect in healthy adults until the osmolality of the test solution is >1500 mosmol/l, when the urinary excretion of lactulose increases. This is known as hyperosmotic stress, a phenomenon not

observed with the monosaccharides such as L-rhamnose even at osmolalities of 3600 mosmol/l (Menzies 1974). The permeability ratio increases (lactulose/ L-rhamnose or ^{51}Cr EDTA/ L-rhamnose ratio), which from the principle of differential absorption cannot be explained by pre or post-mucosal factors. Morphological evidence has been forthcoming using light microscopy to explain this phenomenon with evidence of subepithelial blebs with loss of nuclear regularity, loss of contour of the gut epithelial cells with eventual stripping off; all changes being more pronounced at the villus cell tips (Cooper et al 1978). However, Madara et al have found an increase in transepithelial resistance indicating a reduction in paracellular permeability after in vitro exposure of guinea pig ileum to hyperosmolar mannitol (Madara 1983).

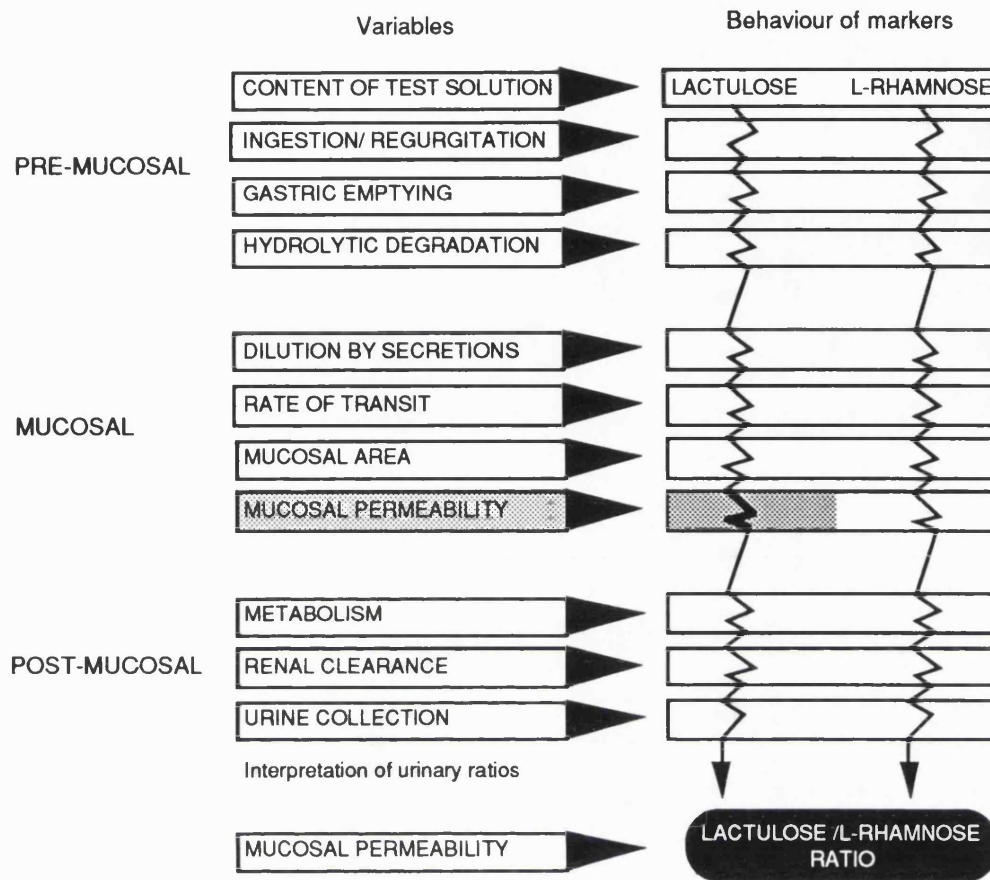
Translating these findings to practical application, it is important to administer isosmolar solution to the gut lumen if permeation changes are not to be induced by the test solution itself.

The Principle of Differential Urine Excretion of Saccharides

The amount of a test substance excreted in the urine following oral ingestion is dependent on pre-mucosal, mucosal and post-mucosal factors (Fig 41). In the illustrated system the urinary excretion of the test substances is dependent upon a number of factors. However, if all these factors equally affect both substances then an increase in the permeation of the mucosa to macromolecules such as lactulose will become evident as an increase in the differential urine excretion ratio of lactulose/ L-rhamnose, an index of gut permeability.

Earlier studies employed only one marker (^{51}Cr EDTA) and a increase in the urine excretion was attributed to increased intestinal permeability. It is clear that changes in other factors, such as the glomerular filtration rate, could also account for these changes. As a result of these shortcomings, the principle of differential saccharide permeation (oligosaccharide/ monosaccharide) following oral administration has evolved. This also has the added advantage of avoiding radioactive tracers.

Fig 41: The Principle of differential absorption



Although a disaccharide and monosaccharides are employed together, other probes may also be used for a similar purpose. However, the precise reasons for alteration in the excretion ratio may vary in each condition under investigation. For example, an increase in the lactulose permeation causing a increase in the ratio (as illustrated in Fig 41) has implications for the integrity of the mucosal barrier and potentially for the transport of other macromolecules. Equally the ratio may increase due to a reduction in the permeation of the monosaccharide (L-rhamnose) which is largely determined by the small intestinal surface area. Although it is uncertain where the bulk of the monosaccharides traverse the intestine it is unusual to find reduction in permeation of these probes without a significant degree of villus atrophy (Bjarnason et al 1986, Cooper 1984).

The Pathway of Lactulose & L-rhamnose Permeation

The Three Permeation Pathway

In a study performed by Maxton et al, healthy volunteers ingested a combination of polyethylene glycol 400 (PEG 400), lactulose, ^{51}Cr EDTA and L-rhamnose in isosmolar (300 mosmol/l) or hyperosmolar (1500 and 2300 mosmol/l) solutions. The response to the detergent cetrimide was also assessed (Maxton et al 1986). There was a significant correlation between the permeation of lactulose and ^{51}Cr EDTA ($r=0.98$; $p<0.001$), but not PEG 400 or L-rhamnose (Maxton et al 1986). The efficiency of PEG 400 urinary excretion was similar to D-xylose which has a passive carrier mediated transport. The data suggested that lactulose and ^{51}Cr EDTA share the same low capacity permeation pathway, which is also in part shared by the smaller molecule L-rhamnose. However, unlike the other markers, L-rhamnose permeation was unaffected by either hyperosmotic stress or by cetrimide, whose permeation was 40 times greater than either lactulose or ^{51}Cr EDTA. This suggested that another small pore or high capacity pathway existed for the permeation of L-rhamnose. This small pore pathway is thought to consist of aqueous channels in the enterocyte membrane and is therefore part of the transcellular pathway (Lieb and Stein 1969).

It is generally accepted that larger molecules such as lactulose and ^{51}Cr EDTA cannot pass through cells and their permeation takes a paracellular route or through areas of cell extrusion at the villus tips. To explain the high rate of permeation of PEG 400 (approximately 100 times that of lactulose), despite sharing a similar molecular weight and radius to lactulose, it has been suggested that it permeates through a separate pathway, namely the lipophilic domains of the brush border (Bjarnason et al 1986, Cooper 1984).

This model helps to explain the changes which occur in the permeation of these markers in disease states such as coeliac disease (Bjarnason et al 1986, Menzies 1974). In this disease when villus atrophy occurs, there is an increase in the

permeation of lactulose and decrease in the permeation of L-rhamnose. The former may be explained by an increase in large pore permeability and the latter in the loss of absorptive surface area, which is characteristic of coeliac disease, and therefore the small pore pathway. This has been supported by morphological freeze-fracture studies which have found a reduction in villus tight junction strand numbers and depth, which relates inversely to intercellular permeability (Madara and Trier 1980).

Determination of Gut Absorptive function using Monosaccharides

Ideally the test substance should be non-toxic, rapidly excreted in the urine with minimal metabolic losses after intravenous injection and readily assayed in both urine and blood.

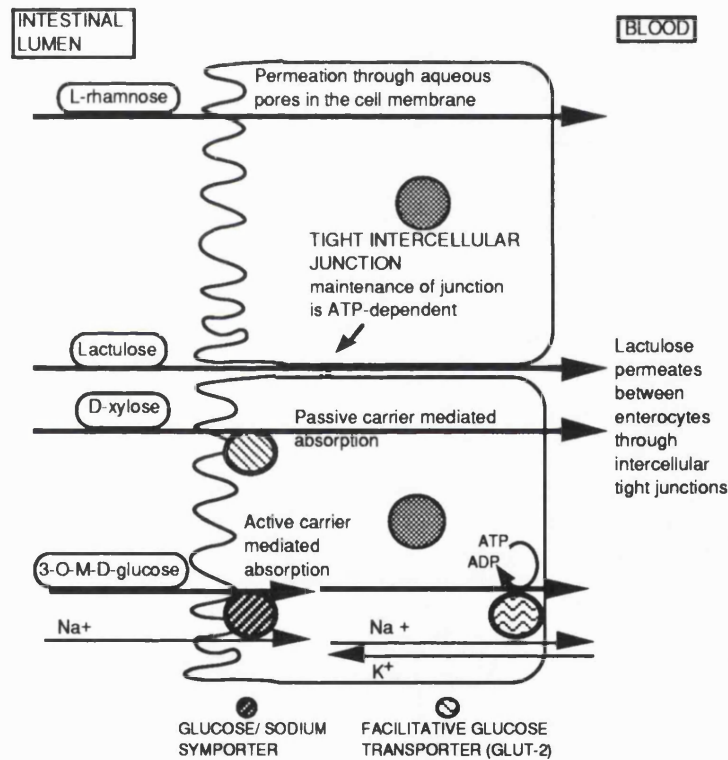
The *D-xylose* absorption test was introduced by Helmer and Fouts in 1937 for the assessment of malabsorption in patients with pernicious anaemia (Helmer and Fouts 1937). It does not fulfil the requirements of the ideal test substance because it undergoes metabolism and may therefore be influenced by metabolic or endocrine disorders such as myxoedema (Broitman et al 1964). Approximately 50% of an administered dose is excreted unchanged in the urine by patients with normal renal function (Craig et al 1983). Five percent is eliminated unchanged in the bile, 15% is metabolised to CO₂ and another 15% converted to D-threitol (Huguenin et al 1978, Pitkanen 1977, Segal and Foley 1959). Non-renal clearance is also reduced in renal failure, by mechanisms which are not understood (Craig et al 1983).

The absorption of D-xylose takes place in the jejunum and upper ileum with 90% of the test dose being absorbed in the first 100 cm of small intestine, with little absorption in the distal ileum, unlike glucose or 3-O-methyl-D-glucose (3-O-m-D-glucose) (Fordtran et al 1962). The absorption of D-xylose from the gut lumen is similar to glucose in that it stimulates sodium ion transport and is dependent upon the presence of sodium ions (Csaky and Lassen 1964). Although transport of D-xylose is competitively inhibited by glucose, it is unaffected by the inhibition of glucose transport, suggesting a different mode of transport from glucose (Heyman et al 1981). *The putative mode of transport for D-xylose is postulated to be a passive carrier mediated process.* Menzies found the rate ratios for absorption of D-xylose/glucose and D-xylose/ D-arabinose to be 0.25 and 2.75 respectively; glucose is transported by

an active carrier mediated transport and arabinose by an unmediated process (Menzies et al 1979).

3-O-m-D-glucose on the other hand is a synthetic sugar, ie it is not metabolised, with 90% urinary recovery of an enteral dose within 48 hours (Fordtran et al 1962). The small bowel has such a large reservoir of absorptive capacity for this hexose that it decreases its sensitivity as a marker of intestinal disease and it therefore has not found favour as a test substance for malabsorption (Fordtran et al 1962).

Fig 42: The mechanisms of absorption & permeation for 3-O-M-D-glucose, D-xylose, L-rhamnose & lactulose from the small intestinal lumen to the blood



However, this property can be employed to advantage in a combined (D-xylose and 3-O-m-D-glucose) saccharide absorption and excretion study because if all the other factors that can influence urinary excretion of a saccharide are shared by the test substances then the effect of metabolism can be assessed when given together, since

3-O-m-D-glucose is not metabolised, whilst D-xylose does undergo significant post-absorption metabolism.

The carrier for 3-O-M-D-glucose is shared with glucose since the presence of both sugars inhibit each other's absorption in both animals and Man (Fordtran et al 1962). The absorption of glucose from the gut lumen is linked to the transport of Na⁺, the carrier responsible is known as the Na⁺-glucose symporter or cotransporter and the DNA encoding for this protein has been described (Hediger et al 1989). This carrier protein annotated as GLUT-2, actively transports glucose from the lumen of the intestine against its concentration gradient by coupling glucose uptake with that of Na⁺, which is transported down its concentration gradient (Fig 42). The Na⁺ gradient is maintained by the active transport of Na⁺ across the basolateral (antiluminal) surface of the brush border cells by membrane bound Na⁺-K⁺-ATPase (Bell et al 1990).

The Effect of CPB on Gut Permeability & Function

The aims of this study were:

(1) to determine the effect of hypothermic non-pulsatile bypass on gut permeability (barrier function).

(2) to determine the effect of hypothermic non-pulsatile bypass on small bowel absorptive function,

(3) to assess differences in gut permeability with respect to the cardiac procedure (valve replacement versus coronary artery bypass grafting),

(4) to relate changes in gut mucosal perfusion with alterations in gut barrier and absorptive functions.

Materials & Methods

Forty one patients undergoing elective coronary artery bypass grafting (CABG) and/or valve surgery were studied. Patients with a history of diabetes, renal disease, hypertension, connective tissue disorders, previous history of abdominal disease or surgery or patients treated with corticosteroids were excluded from this study.

The distribution of operations amongst the 41 studied patients was CABG 68% (n=28), valve 27% (n=11), valve and CABG 5% (n=2). Fifteen percent (n=6) of patients had redo operations.

These studies were approved by the Hammersmith Research Ethics and Hillingdon Health Authority Ethics Committees. All patients gave informed written consent.

Peri-operative management

Pre-operative medication consisted of intramuscular papavaretum with scopolamine. Anaesthesia was induced with thiopental and maintained with fentanyl, and isoflurane. Muscle relaxation was provided by pancuronium. Ventilation was controlled to maintain carbon dioxide tension at 4.5 to 5.5 kPa and arterial oxygen tension greater than 13.5 kPa.

During the operation, radial artery pressure, nasopharyngeal temperature and cardiac output from the pump-oxygenator during CPB were recorded continuously via a analogue to digital converter onto the hard disc of a computer for subsequent analysis. All cardiac procedures were undertaken via a median sternotomy. CPB was achieved using a hollow fibre membrane oxygenator (Maxima, Medtronic, Watford, UK) without arterial line filtration using non-pulsatile flow with core cooling to 28°C. During CPB non-pulsatile flow from the pump-oxygenator was maintained at 2.4 l/min/m² at 37°C, with graded reductions down to 1.7 l/min/m² at 28°C. Mean arterial pressure (MAP) was maintained during CPB at 50-60 mm Hg and acid base balance was managed using the alpha stat methodology. During CPB the haematocrit (PCV) was maintained above 20%, with the addition of packed red blood cells as required. The packed cell volume (PCV) was determined every ten minutes during CPB.

Myocardial preservation was achieved by using cold (10°C) crystalloid cardioplegia (St Thomas') with topical myocardial cooling using iced saline solution.

Following extubation of the endotracheal tube, patients were commenced on oral fluids. Twenty four hours post-operatively, the majority of patients were on a light oral diet and routine post-operative medications which included frusemide (40 mg od), amiloride (10 mg od), digoxin (0.25 mg od) and aspirin (300 mg od). Prophylactic intravenous antibiotics (cefuroxime 750 mg tds and flucloxacillin 500 mg qds) were discontinued after removal of the jugular venous line at 24-48 hours post-operatively.

During the post-operative period, patients had routine haematological investigations and any gastrointestinal or other system complications were recorded.

Assessment of Gastric Mucosal Blood Flow

In 10 patients undergoing CABG a laser Doppler probe (Model MBFD3, Moor Instruments, Axminster, UK) was sited on the mucosa of the body of the stomach following induction of general anaesthesia. The probe is made of a single laser fibre attached to a polyurethane disc (Fig 26 & 27). The optic fibre was passed retrogradely up the biopsy channel of a flexible gastroscope (PQ 20, Olympus, UK) allowing accurate positioning of the polyurethane disc on the gastric mucosa. Details of the laser Doppler flowmetry methodology are provided in Chapter 4.

During CPB the gastric laser Doppler blood flow was recorded continuously onto the hard disc of a computer for data analysis, and mean readings determined over one minute for any given period during CPB.

Small Intestinal Saccharide Studies

The test involves an overnight fast followed by the ingestion of the 4 saccharides (3-O-m-D-glucose, D-xylose, lactulose and L-rhamnose) in a standardized solution. A 5 hour urine collection was made and the sample preserved in merthiolate to prevent bacterial degradation. Each patient underwent 3 studies. The first study was 2 days prior to CPB, the second commenced within 3 hrs following the end of CPB and the third was conducted on the 5th post-operative day. For each study a 100 ml test solution (240 mosmol/l) was administered orally or enterally followed by a 5 hr urine collection for saccharide analysis (some authors have suggested that a complete urine collection is not necessary (Cobden et al 1978); a complete collection has the advantage of providing information about the individual permeation of the markers not evident on analysis of the ratio alone). The immediate post-CPB study was

conducted by instilling the test solution via a nasogastric tube which remained clamped for the duration of the study period.

The solution contained 3-O-m-D-glucose (0.2g), D-xylose (0.5g), L-rhamnose (1.0g) and lactulose (5.0g). A 20 ml sample of the 5 hr urine collection was then stored with methiorlate in a refrigerator at 10-15°C until analysed.

Analysis of Test Substances

The analysis of test substances was performed by Dr Somasundaram and Dr Menzies, Department of Chemical pathology, UMDS, St Thomas' Hospital, London.

A modified thin layer chromatographic technique was used which has been developed and modified by Menzies et al (Menzies et al 1978, Noone et al 1986). This involves measurement of peak heights by scanning densitometry and incorporates raffinose and arabinose as disaccharide and monosaccharide internal standards to correct application errors. Saccharide separations are achieved by multiple development, disaccharides (lactulose) on a three-quarter (915 x 20 cm) plate of F1500 plastic-backed silica gel (Scliecher & Schull, Dassel, Germany). Three consecutive ascending runs with two different solvents (A) butan-1-ol: ethanol: acetic acid: water, 60:30:10:10 and (B) ethyl acetate: butan-1-ol: pyridine: acetic acid: water, 70: 5: 15: 10: 10 by volume in sequence ABA are required for the disaccharides (each rise 13.5 cm), whilst monosaccharide (L-rhamnose, D-xylose and 3-O-m-D-glucose) separation is obtained using three ascending runs with solvent B (each rise 8.5 cm). The layers are dried for at least 30 minutes between runs and then for 4 hours to remove pyridine before performing a 4-aminobenzoic/ phosphoric acid colour reaction at 120-130°C for 10 minutes. After location the layers are kept refrigerated in polyethylene envelopes and exposure to light minimised during scanning. Peak heights are measured and corrected to a constant internal standard value, and test concentration then read by interpolation from standard concentration curves for each saccharide derived from the same chromatogram.

The technique is accurate and sensitive with recovery being >90% with a minimum level of detection of <0.1 mmol/l. The precision coefficient of variation without replication is 3-8% (Menzies et al 1978, Noone et al 1986).

Statistical Analysis

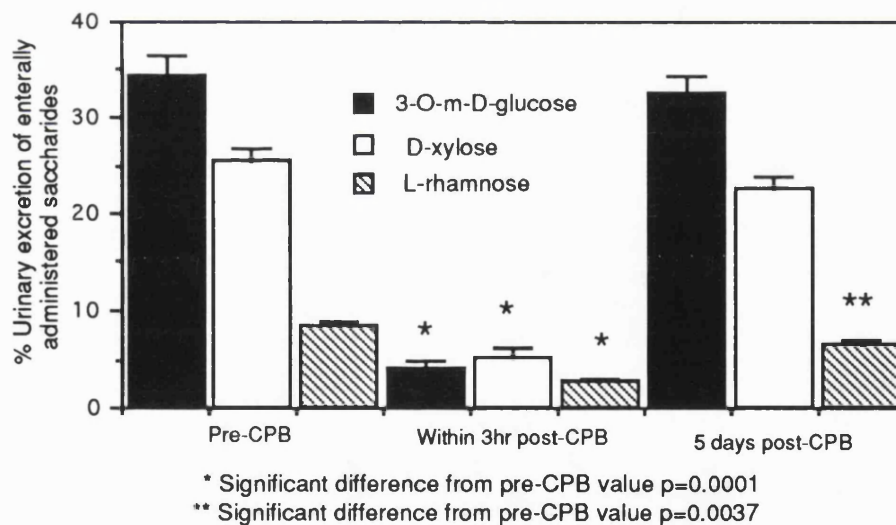
All results are expressed as mean \pm standard error. The paired Student's t test was used to examine for differences in the sequential determination of saccharides or laser Doppler flowmetry. The unpaired Students t test was employed to examine for differences between different groups; two tailed values are expressed for the probability. Multiple regression analysis was used to examine the relationship of post-CPB permeability with patient and perfusion parameters.

Results

Saccharide studies

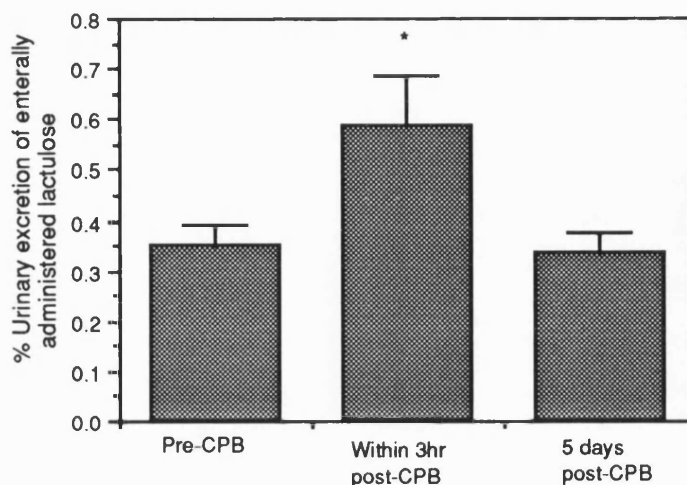
In 41 patients studied with saccharides, the mean age was 59.0 ± 2.9 years (Range 40-75 years), the mean CPB time was 100.9 ± 6.5 minutes, cross clamp time was 54.2 ± 4.5 minutes and the mean core cooling was $29.0 \pm 0.3^\circ\text{C}$. Following CPB (n=41) there was a dramatic decrease in urinary excretion of 3-O-m-D-glucose from $34.0 \pm 2.2\%$ to $5.2 \pm 6.1\%$ ($p < 0.0001$) and D-xylose from $25.4 \pm 1.4\%$ to $4.1 \pm 0.8\%$ ($p < 0.0001$). There was a significant but less marked reduction of L-rhamnose from $8.3 \pm 0.6\%$ to $2.6 \pm 0.4\%$ ($p < 0.0001$), which remained depressed at $6.6 \pm 0.5\%$ even 5 days post-operatively ($p = 0.0037$, Fig 43).

Fig 43: The active & passive carrier & transcellular transport of saccharides undergoing non-pulsatile hypothermic CPB (n=41)



The permeation of lactulose increased following CPB from $0.35 \pm 0.04\%$ to $0.59 \pm 0.1\%$ ($p = 0.018$, Fig 44).

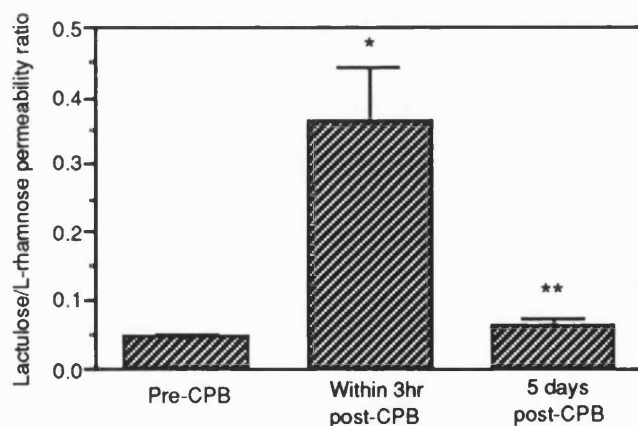
Fig 44: Cardiopulmonary bypass induced rise in lactulose permeation (n=41)



* Significant difference from pre bypass value $p=0.018$

Therefore the lactulose/ L-rhamnose permeability ratio, a specific index of gut permeability, rose from 0.045 ± 0.04 to 0.36 ± 0.08 (Normal range <0.08 ; $p=0.004$, Fig 45).

Fig 45: The effect of CPB on gut permeability (n=41)



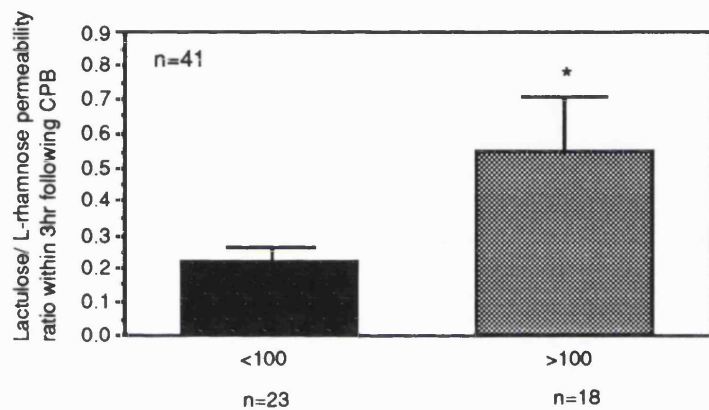
* significant difference from pre-CPB value $p=0.0004$

**significant difference from pre-CPB value $p=0.0241$

On the fifth post-operative day the lactulose/L-rhamnose urine excretion ratio significantly reduced to 0.062 ± 0.01 due to depressed L-rhamnose permeation, but was still greater than pre-CPB levels ($p=0.024$).

Patients were sub-divided into those patients with a CPB time of 100 minutes or longer (n=18) and those with a CPB time of less than 100 minutes (n=23), to assess the effect of CPB time on the transport of saccharides. Analysis found no significant difference between the groups for 3-O-m-D-glucose and D-xylose absorption or for the permeation of L-rhamnose. The lactulose/ L-rhamnose permeability ratio was greater in the long CPB time group, 0.54 ± 0.17 versus 0.22 ± 0.04 ($p=0.049$), immediately following CPB (Fig 46).

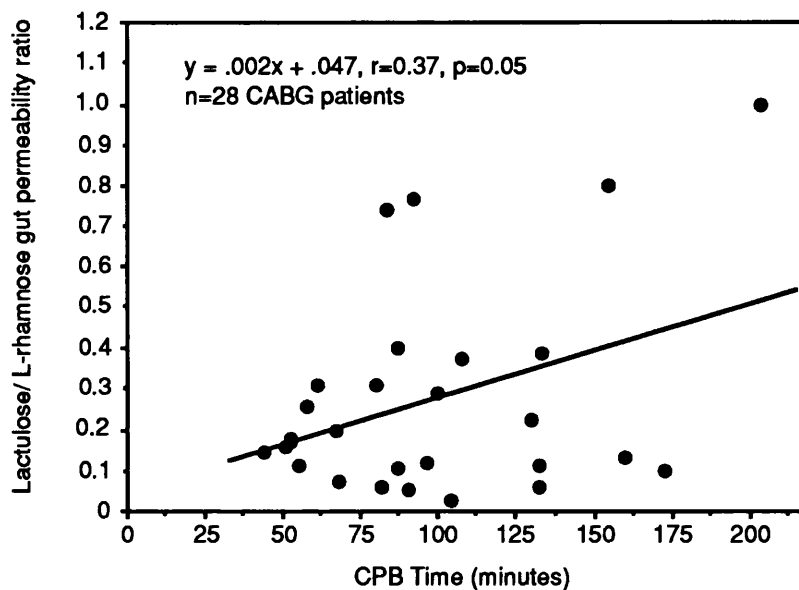
Fig 46: The effect of CPB time on the gut permeability ratio



* Indicates a significant difference between the two groups $p=0.049$

A significant correlation was found for gut permeability and the duration of the cardiopulmonary bypass ($r=0.37$, $p=0.05$). To exclude any differences that may have been present between closed and open cardiac procedures, only CABG patients (n=28) have been included in this analysis (Fig 47).

Fig 47: The influence of CPB time on gut permeability



Analysis of all the patients (valve and CABG) using multiple linear regression analysis was performed to reduce biases and attribute relative risk and identify other factors that may have contributed to post-CPB permeability as a result of age, the consumption of pre-operative aspirin, the type of operation and the x-clamp time. The total CPB time was the best predictor of an increased post-CPB gut permeability ($p=0.05$, Tab 7).

Tab 7: Multiple linear regression analysis examining patient & operation factors influencing immediate post-CPB gut permeability

Beta Coefficient Table					
Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
Intercept	-0.443				
Age	-0.001	0.008	-0.029	0.160	0.874
Aspirin	0.081	0.227	0.064	0.359	0.722
Operation	0.174	0.205	0.148	0.849	0.403
X-Clamp time	0.001	0.004	0.020	0.111	0.912
CPB time	0.005	0.002	0.379	2.041	0.051

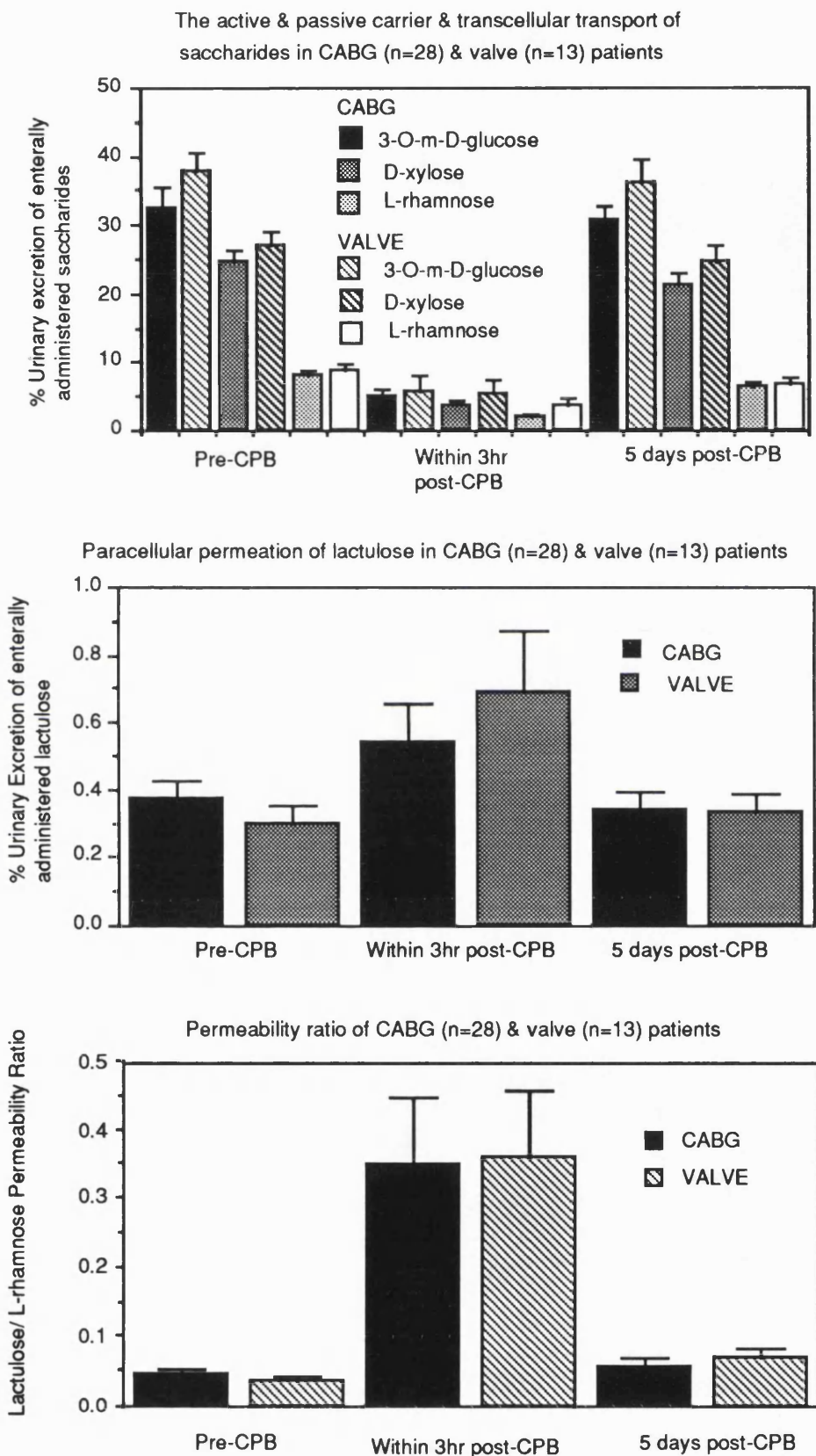
Analysis of the study population was undertaken for differences which may have been due to open (valve) or closed (CABG) procedures; no significant differences were

found in the perfusion times (Tab 8) or in the absorption or permeation of the 4 test substances (Fig 48).

Tab 8: Comparison of CABG (n=28) & valve (n=13) patients.

	CABG	VALVE	P value
Age (yr)	59.6±2.3	55.5±3.0	0.30
CPB Temp (°C)	29.3±0.3	28.4±0.4	0.11
CPB Time (min)	97.7±7.9	107.8±12	0.47
Cross-clamp time (min)	55.5±5.8	51.2±6.3	0.67

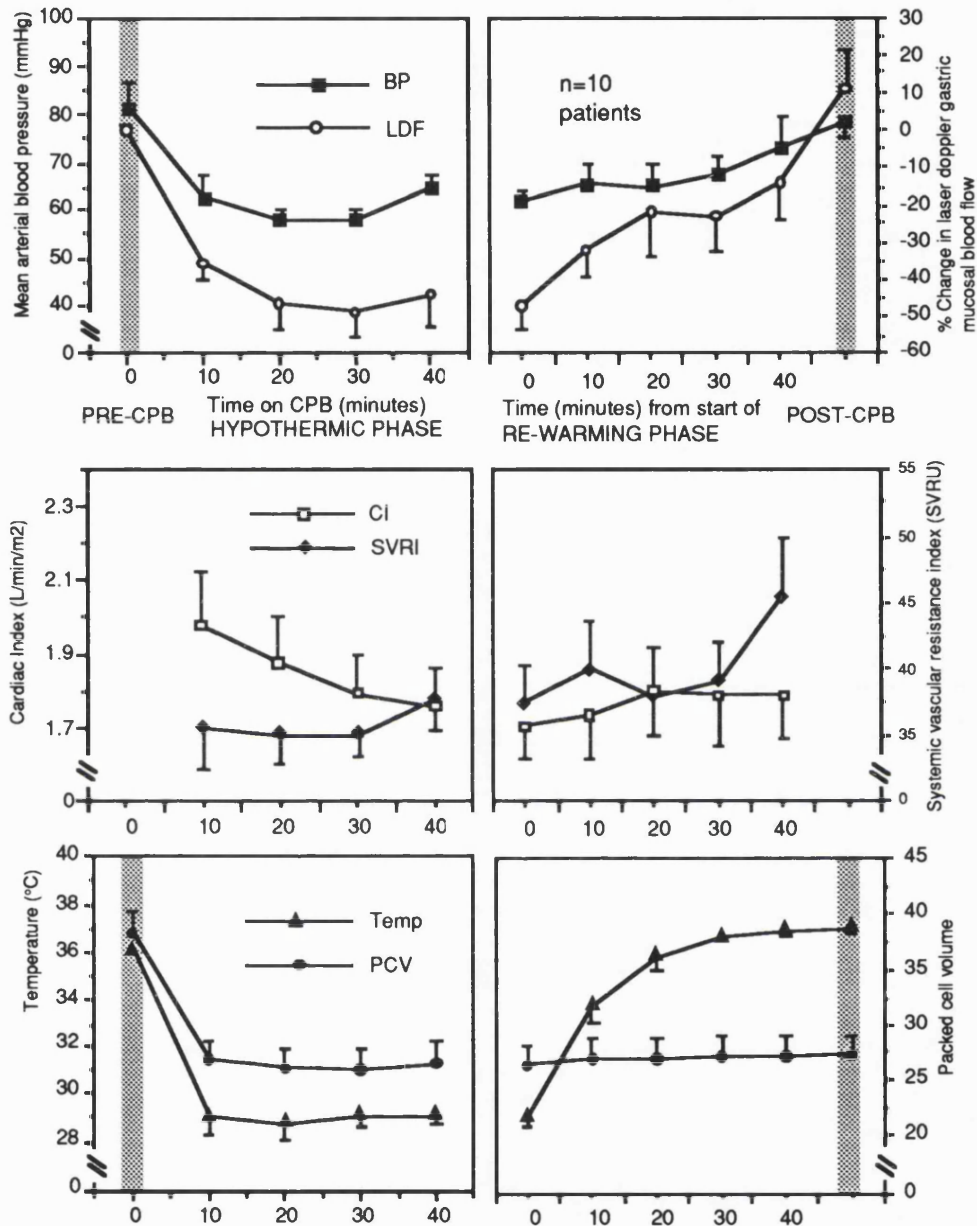
Fig 48: Saccharide studies for patients undergoing CABG or valve operations



Haemodynamic studies

The mean age was 61.8 ± 3.4 years (range 40-75 years), mean CPB time was 113.8 ± 6.9 minutes, cross clamp time was 41.8 ± 3.9 minutes. The mean core cooling was $29.1 \pm 1.4^\circ\text{C}$ (n=10). The changes in gastric mucosal flow are illustrated in Fig 49. In the 10 patients undergoing CABG in whom LDF studies were performed a $48.7 \pm 7.0\%$ reduction in mucosal LDF was found 30 minutes after the institution of CPB ($p=0.0001$). During the re-warming phase of CPB there was a return of the LDF towards normal values, and in the immediate post-CPB period this was above pre-CPB values $11.0 \pm 9.8\%$ ($p=0.28$). The PCV fell on the institution of CPB from $38.1 \pm 1.9\%$ to $26.4 \pm 1.9\%$ but remained at approximately 27% during the re-warming phases of CPB when LDF gastric blood flow was increasing.

Fig 49: Haemodynamic & gastric mucosal laser Doppler flow alterations during cardiopulmonary bypass (n=10) in patients undergoing CABG



Morbidity & Mortality

Two patients (4.8%) died following surgery; one patient developed ventricular fibrillation and cardiac arrest following CABG and a second patient developed low cardiac output syndrome following mitral and tricuspid valve replacement. Autopsies on these patients were not performed.

Excluding the above 2 patients who died, 18 other patients (46.2%) developed 26 non-fatal complications excluding arrhythmias. The incidence of arrhythmias was 25.6% (10 out of 39 patients). Six patients (15.6%) developed gastrointestinal complications as defined in Chapter 2 (three reversible hepatic dysfunction with elevated bilirubin and alkaline phosphatase, one oesophagitis, one patient had diarrhoea and one patient persistent nausea and vomiting with hyperamylasaemia but with a normal pancreatic ultrasound and normal renal function). Nine patients had intrathoracic complications (6 patients with pleural effusions, 3 with significant pulmonary atelectasis requiring protracted antibiotic therapy and physiotherapy).

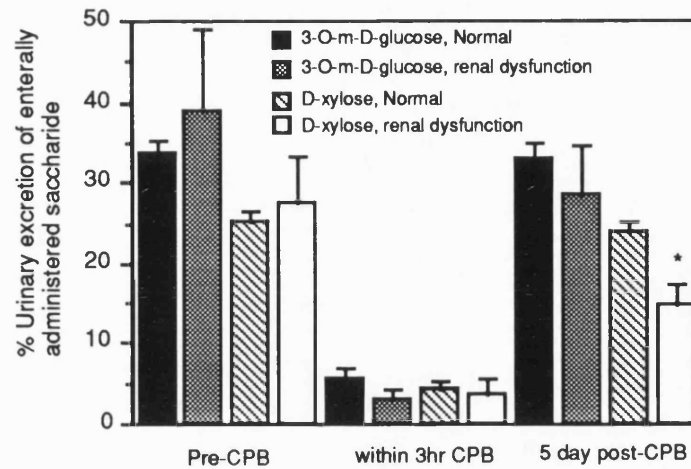
Six patients developed reversible renal dysfunction as defined in Chapter 2 (one of these patients also developed reversible post-CPB jaundice). Renal clearance was not formally assessed in this study but serum creatinine values were noted for patients during each phase of their saccharide permeation tests and is presented in Tab 9 .

Tab 9 : Changes in serum creatinine in patients following CPB

	Pre-CPB	Within 3hr post--CPB	Day 5 post-CPB
All patients (n=41)	95.4±2.7	91.5±3.9	96.6±3.7
Renal dysfunction (n=6)	117±4.0	126.7±11.9	127±12.2
p value	0.0007	0.0001	0.0005
Without renal impairment (n=35)	91.8±2.7	85.6±3.2	91.6±3.2

Patients with renal dysfunction had reduced urinary excretion of 3-O-m-D-glucose, D-xylose, L-rhamnose and lactulose compared to patients without renal impairment, but this did not reach statistical significance except for the day 5 post-CPB study for D-xylose (Fig 50).

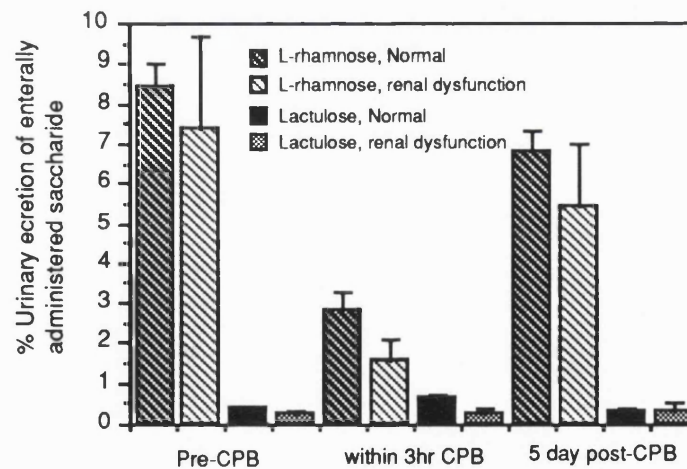
Fig 50: 3-O-m-D-glucose & D-xylose absorption: normal (n=35) versus patients with post-CPB renal dysfunction (n=6)



* Indicates a significant difference between patients with normal and abnormal renal function $p=0.008$

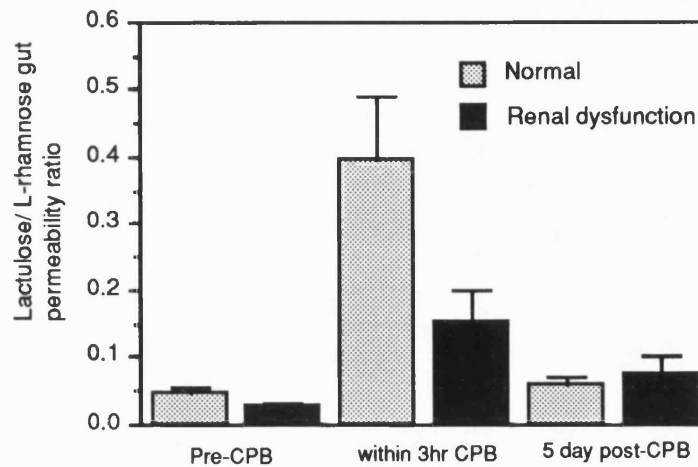
Patients without renal impairment had D-xylose excretion of $23.8 \pm 1.3\%$ versus 14.7 ± 2.7 for patients with renal dysfunction ($p=0.008$).

Fig 51: L-rhamnose & lactulose urinary excretion in normal (n=35) and patients with renal dysfunction (n=6)



The permeability ratio increased following CPB in both groups (Fig 52). The permeability ratio was 0.152 ± 0.049 and 0.398 ± 0.094 for patients with and without renal impairment respectively ($p=0.29$). The reduced permeability ratio in the renal dysfunction group may have in part been a result of reduced CPB time of 73.7 ± 9.8 minutes compared with 106 ± 7.2 minutes for patients without renal impairment ($p=0.08$).

Fig 52: Change in permeability ratio in normal (n=35) and patients with renal dysfunction (n=6)



Pre-operative Drug Therapy & Gut Permeation

To examine for possible influence of pre-operative drug therapy, the CABG group (n=28) was examined since it formed a homogenous group in this regard. Nearly all the patients were on a combination of a β -blocker, calcium channel blocker and aspirin (n=26). In two patients only was an angiotensin converting enzyme inhibitor also employed due to low ejection fraction (<25%) as determined by angiography.

Without exception, all patients who underwent CABG were administered aspirin pre-operatively; the dose varied from 75 to 300 mg daily and was dependent upon the cardiological referral. Patients who were still consuming aspirin up to the time (within 2 days) of their operation (n=9) were compared to patients who had discontinued aspirin consumption for more than 7 days pre-operatively (n=19).

No significant differences were found for the absorption of 3-O-m-D-glucose or D-xylose, or for the permeation of L-rhamnose or Lactulose (Figs 53, 54 & 55). Similarly the permeability ratio was not significantly different between the groups pre or post-CPB.

Fig 53: The influence of pre-operative aspirin consumption on saccharide absorption

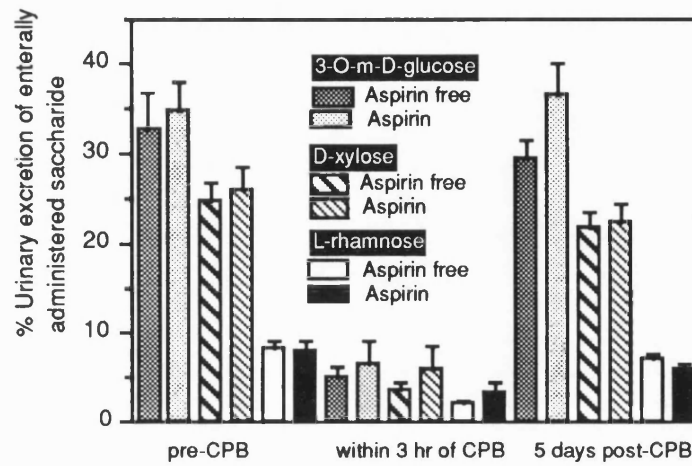


Fig 54: The effect of pre-operative aspirin consumption on lactulose permeation

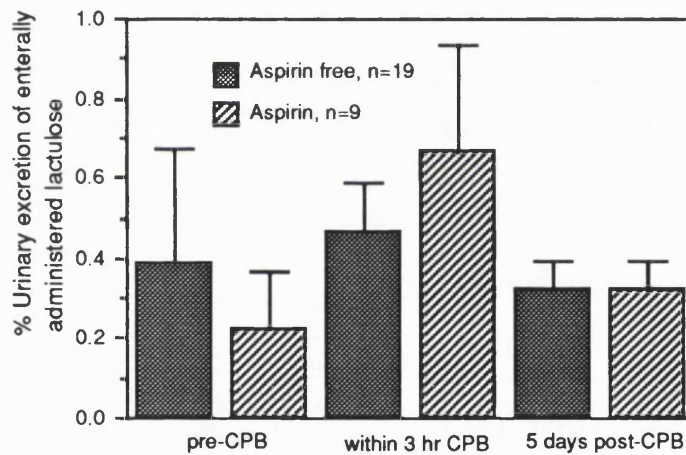
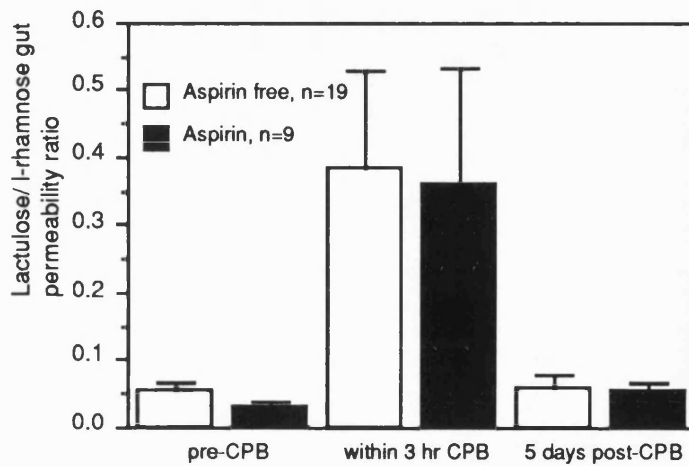


Fig 55: The effect of pre-operative aspirin consumption on the gut permeability ratio



Discussion

This study has found that coincident with profound reductions in mucosal blood flow, significant but reversible impairment was demonstrated in both active (3-O-m-D-glucose) and passive (D-xylose) carrier mediated absorption of saccharides in the small bowel. This may have implications for patients who require nutritional support in the immediate post-CPB period, since saccharides administered via an enteral route may be malabsorbed initially. More importantly, this temporary malabsorption, may influence the pharmacokinetics of orally administered drugs in the immediate post-operative period.

The chronic consumption of non-steroidal anti-inflammatory drugs (NSAIDs) has been associated with the development of NSAID enteropathy (Bjarnason et al 1987). This occurs in approximately 70% of patients on long-term NSAID therapy who develop asymptomatic small intestinal inflammation (Bjarnason et al 1987). This is often associated with ileal dysfunction, blood and protein loss and the occasional patient may develop unique diaphragm-like intestinal strictures (Bjarnason et al 1988, Bjarnason et al 1987). The pathogenesis is uncertain but the increase in gut permeability may be related to reduced mucosal prostaglandins, since the increased permeation induced by NSAIDs can be related to their potency in inhibiting the cyclo-oxygenase enzyme (Bjarnason et al 1986). Although the majority of patients who underwent CABG (n=19) were taking aspirin pre-operatively, no significant differences were found in the transport of the individual saccharides or the gut permeability ratio pre or post-CPB, compared to those patients who were not consuming aspirin.

An increase in gut permeability was demonstrated by the rise in the lactulose/ L-rhamnose permeability ratio. Lactulose is a non-hydrolysable di-saccharide which permeates or diffuses through intercellular tight junctions, whilst the monosaccharide L-rhamnose has been postulated to move passively through aqueous pores in the cell

membrane (Fig 42) (Menzies 1984). The maintenance of the integrity of tight intercellular junctions between enterocytes is a dynamic process, actively controlled by ATP dependent intracellular mechanisms and cytoskeleton (Madara 1983, Madara et al 1986). During episodes of hypoperfusion the integrity of these junctions and therefore of the mucosal barrier may become impaired. The distribution of the number of tight junctions is not constant along the villus. At sites of cell extrusion, which are located at the villus tip, the cell to cell adhesion is at its weakest and tight junctions are corresponding fewer in number (Marcial et al 1984). The mechanism of reduced monosaccharide and increased di-saccharide permeation across the intestine is uncertain. It presumably occurs in response to intestinal ischaemia and it is the villus tip which is most prone to ischaemic damage during periods of hypoperfusion. Within the villus there is a counter-current exchange mechanism, which progressively reduces the capillary pO₂ from the villus base to the tip (Lundgren and Haglund 1978). The changes in permeability ratio cannot be explained by any alteration in pre-(gastric emptying and dilution, small intestinal transit and dilution) or post-mucosal (blood flow, volume of distribution of saccharides or renal clearance) factors as this would affect both the saccharides (lactulose and L-rhamnose) equally. Although the absorption of monosaccharides was reduced in patients who had post-CPB renal dysfunction, there was no difference between this group of patients in the immediate post-CPB studies and the majority in whom no renal impairment was apparent (Fig 50 & 51). The immediate post-CPB reduction of carrier mediated transport may have been due hypoxic injury of the villus enterocytes which may have affected the turnover of saccharide carriers. Similar reductions in carrier mediated transport were found by Molyneux et al in a study of 12 patients with falciparum malaria, who also had demonstrable reductions in liver blood flow (Molyneux et al 1989). In the latter study transcellular transport was also impaired and thought to be a result of splanchnic ischaemia. However, the premucosal factor of gastric emptying may also have contributed to the reduced absorption of these saccharides. Anaesthesia and CPB in combination may impair gastric emptying post-operatively thus reducing the

delivery of the test solution into the small bowel and therefore the total dose available for absorption.

The possible consequences of increased intestinal permeability following CPB is uncertain. Increased intestinal permeability to lactulose and ⁵¹Cr EDTA correlates with increased permeation of macromolecules (Ramage et al 1988) and chemotactic bacterial peptides in animals (Ferry et al 1989). It is therefore possible that it is directly responsible for the endotoxaemia which has been documented in patients undergoing CPB (Andersen et al 1987, Rocke et al 1987).

In summary, a increase in gut permeability coinciding with reduced mucosal blood flow was found in patients undergoing CPB. These findings, which may have implications for the development of CPB-related endotoxaemia, prompted a study designed to investigate the relationship between endotoxaemia and changes in gut permeability (Chapter 7).

Chapter 6

The Effect Pulsatile Perfusion & Oxygenator Type on Gut Saccharide Transport & Permeability

Introduction & Aims

The mechanisms of splanchnic injury during and following CPB are diverse and may be governed by patient and CPB perfusion factors. To examine the influence of perfusion parameters, patients were randomized to receive either pulsatile or non-pulsatile flow, bubble or membrane oxygenation during CPB.

Bubble oxygenators have been associated with greater activation of complement and the generation of microemboli, which may influence the degree of tissue injury during and following CPB. A hollow fibre membrane oxygenator was employed to achieve satisfactory pulsatile flow, since sheet membrane oxygenators tend to attenuate the pulsatility of the flow generated by the pump-oxygenator.

The aims of this study were:

- (1) to evaluate the differences in gastric mucosal perfusion and post-CPB gut permeability with the type of perfusion employed during CPB.
- (2) to determine the relationship between gut mucosal injury determined by tonometry and the injury determined as gut permeability.
- (3) to assess if these different methods of evaluating gut damage were predictive of post-operative outcome.

Materials & Methods

Twenty-eight patients were randomized to one of four groups:

- (i) bubble oxygenator pulsatile flow (BP), n=7,
- (ii) bubble oxygenator non-pulsatile flow (BNP), n=7,
- (ii) membrane oxygenator pulsatile flow (MP), n=7,
- (iv) membrane oxygenator, non-pulsatile flow (MNP), n=7.

Patients with a history of diabetes, renal disease, hypertension, connective tissue disorders, previous history of abdominal disease or surgery or patients treated with corticosteroids were excluded from this study.

These studies were approved by the Hammersmith Hospital Research Ethics Committee. All patients gave written informed consent.

Peri-operative management

Pre-operative medication consisted of intramuscular papavaretum with scopolamine. Patients received 300 mg ranitidine on the night before surgery and a further 300 mg orally on the morning of surgery at 0600 hrs; anaesthetic induction began at approximately 0800 hrs. Anaesthesia was induced with thiopental and maintained with fentanyl, and enflurane. Muscle relaxation was provided by pancuronium. Ventilation was controlled to maintain carbon dioxide tension at 4.7 to 5.3 kPa and arterial oxygen tension greater than 13.3 kPa.

During the operation, radial artery pressure, nasopharyngeal temperature and cardiac output from the pump-oxygenator during CPB were recorded continuously via a analogue to digital converter onto the hard disc of a computer for subsequent analysis. All cardiac procedures were undertaken via a median sternotomy.

CPB was achieved using a bubble (Harvey 1700, Bard, Crawley, Sussex) or hollow fibre membrane oxygenator (Harvey 5700, Bard, Crawley, Sussex) with arterial line filtration (Pall EC plus 40µm screen arterial line filter, Pall Corporation, Portsmouth, Hants) using pulsatile or non-pulsatile flow, delivered by a Sarns pump-oxygenator (Sarns, Strathclyde, Scotland).

The pump-oxygenator was primed with 2 litres of Hartmanns solution with 25 mmol of bicarbonate. If pulsatile flow was employed, pulsatility was maintained throughout the CPB period including following release of the aortic cross clamp (X-clamp). Core cooling was undertaken in all cases to 28°C. During CPB, flow from the pump-oxygenator was maintained at 2.4 l/min/m² at 37°C, with graded reductions down to 1.7 l/min/m² at 28°C. MAP was maintained during CPB at 50-70 mm Hg. During CPB, if the MAP was >70 mm Hg for more than 5 minutes or if the MAP was >80 mm Hg an infusion of glyceryl trinitrate was commenced. If the MAP pressure was <50 mm Hg for more than 5 minutes or the MAP was <35 mm Hg, then an intravenous bolus of methoxamine was administered.

Acid base balance was managed using the alpha stat methodology. No bicarbonate was administered unless the base excess was greater than -10 mmol l⁻¹. Potassium was maintained at 4.0-5.5 mmol l⁻¹. During CPB the haematocrit was maintained above 20%, with the addition of packed red blood cells as required. The packed cell volume (PCV) was determined every ten minutes during CPB.

Myocardial preservation was achieved by using cold (10°C) crystalloid cardioplegia (St Thomas') with topical myocardial cooling using iced saline solution.

Following removal of the endotracheal tube, patients were commenced on oral fluids. Twenty four hours post-operatively, the majority of patients were on a light oral diet

and routine post-operative medications which included frusemide (40 mg od), amiloride (10 mg od), atenolol (50 mg od) and aspirin (300 mg od). Prophylactic intravenous antibiotics (cefuroxime 750 mg tds and flucloxacillin 500 mg qds) were discontinued after removal of the jugular venous line at 24-48 hours post-operatively.

During the post-operative period, patients had routine haematological investigations and any gastrointestinal or other system complications were recorded. Data were collated from ICU and ward charts for ventilation, ICU and total hospital times.

Gastric Tonometry & Laser Doppler Flowmetry

A gastric tonometer and laser Doppler flow probe was positioned in the stomach following induction of general anaesthesia as previously described (Chapter 3) and removed before the patient left theatre.

Saccharide Transport Studies

Patients underwent three saccharide studies 2 days before surgery, within three hours post-CPB and 5 days post-operatively as previously described. In addition the creatinine clearance during the study period was determined using urine and plasma creatinine levels. The urinary excretion of saccharides were then expressed as both actual and values standardised to a clearance of 70 ml/min/m².

Statistical Analysis

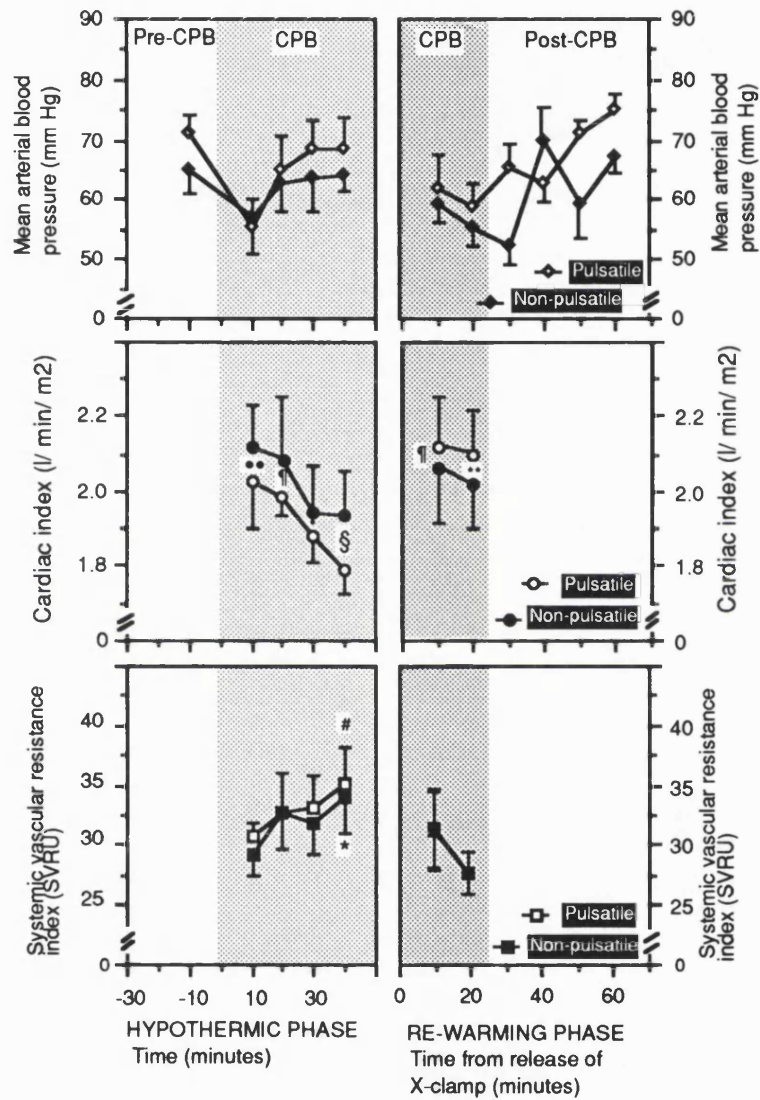
The Mann-Whitney U test was employed for comparisons between groups of continuous variables; the Chi-square test with Fisher's correction for comparison of groups with quantal results (two tailed p values were determined) and the Wilcoxon signed-rank test for comparison of paired samples. The Spearman rank-order regression analysis was used for evaluating the correlation between two parameters. Results are expressed as the mean \pm standard error.

Results*Haemodynamic parameters (Fig 56 & 57)*

In all four groups (BP, BNP, MP & MNP), similar MAP were achieved during and following CPB. Patients who had undergone pulsatile perfusion (BP & MP) had significantly higher MAP 10 minutes post-CPB than non-pulsatile patients (BNP & MNP). This difference continued for a further 10 minutes for membrane patients, but not for patients who had received bubble oxygenation.

The SVRI measurements were very similar in all 4 groups, although 20 minutes after the start of hypothermic CPB; the SVRI was higher for MP than the MNP patients ($p=0.024$). Following release of the aortic X-clamp and re-warming of the patients, there was a substantial fall in the MAP in all the groups (Fig 56 & 57).

Fig 56: Bubble oxygenation and the influence of pulsatile and non-pulsatile perfusion on haemodynamic parameters during hypothermic CPB



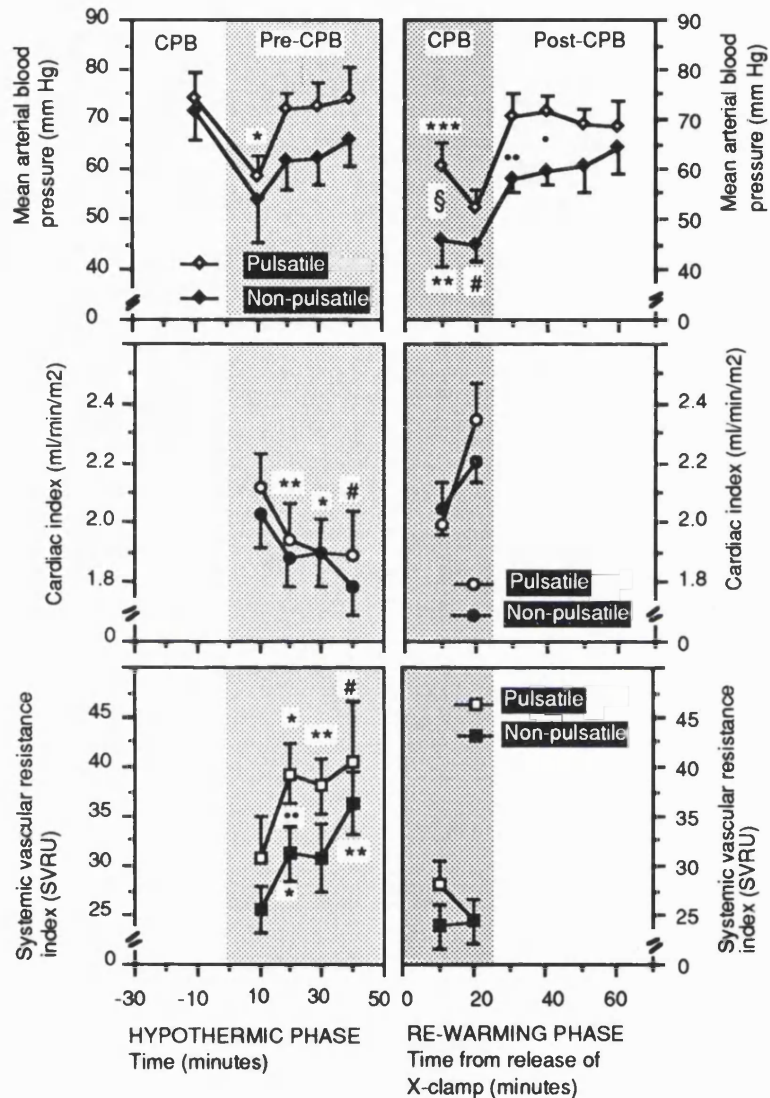
P values for comparisons with 10 minutes after the start of CPB for SVRI and CI

* p=0.018, # p=0.043

Comparing pulsatile and non-pulsatile groups

• p=0.009, § p=0.018, ** p=0.024, ¶ p=0.028, ** p=0.044

Fig 57: Membrane oxygenation and the influence of pulsatile and non-pulsatile perfusion on haemodynamic parameters during hypothermic CPB



P values for comparisons with pre-CPB values for blood pressure, and with 10 minutes after the start of CPB for SVRI and CI

* p=0.018, ** p=0.028, *** p=0.034, # p=0.043

Comparing pulsatile and non-pulsatile groups

• p=0.018, § p=0.024, ** p=0.028

This difference was most pronounced in patients having membrane oxygenation; 10 minutes after X-clamp release, the MP group had a MAP of 60.4 ± 4.8 versus 45.9 ± 3.1 mm Hg ($p=0.024$) for MNP patients. This difference in MAP between pulsatile and non-pulsatile groups was continued into the post-CPB period with BNP patients achieving the lowest post-CPB MAP at 59.3 ± 5.6 mm Hg, 30 minutes post-CPB.

The SVRI fell in all 4 groups during the post X-clamp release period towards or below levels at the start of hypothermic CPB.

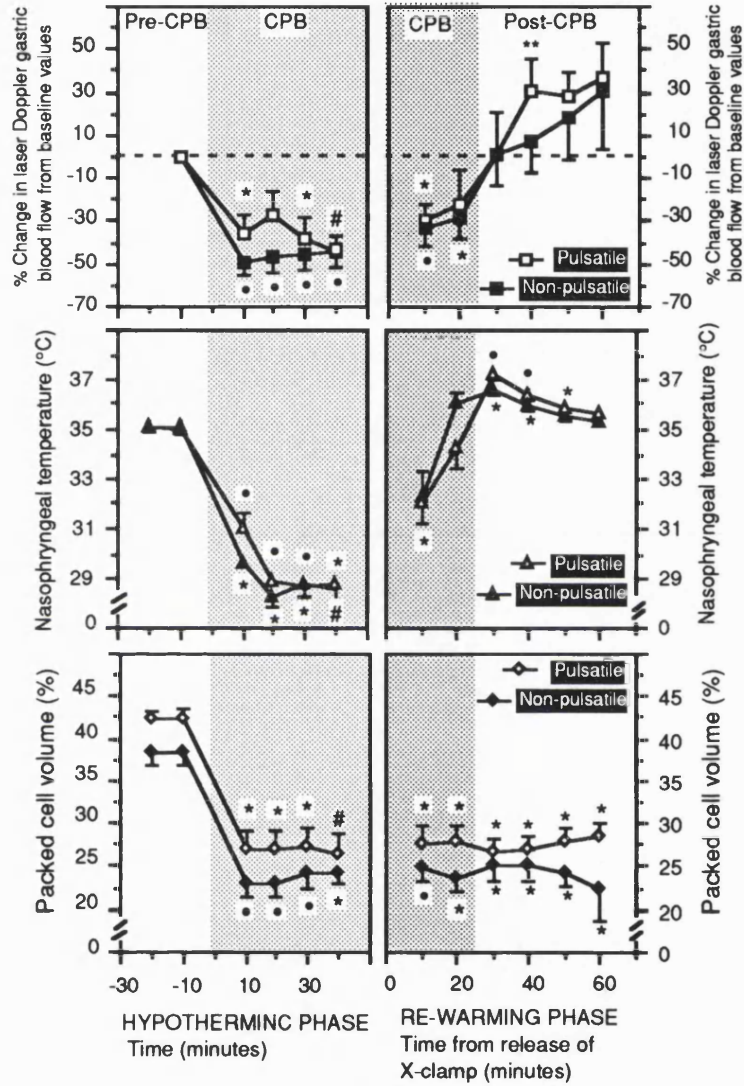
Gastric Laser Doppler Flowmetry (Tab 10, Fig 58 &59)

Gastric LDF values for BP, BNP, MP and MNP groups are provided in Table 10. No significant differences were observed for the type of oxygenation or flow. During the re-warming phase of CPB, despite no significant change in the haematocrit in any of the groups, the LDF returned towards the pre-CPB values. Twenty minutes following release of the aortic X-clamp, the LDF was -27.9 ± 9.2 , -22.6 ± 16.0 , 17.7 ± 11.1 and 19.1 ± 8.6 below pre-CPB values for BP, BNP, MP and MNP groups ($p=0.028$, 0.18 , 0.068 and 0.17 respectively). In the post-CPB period, the gastric LDF returned to pre-CPB values. For the MP group, forty minutes post-CPB the flow was significantly above pre-CPB values ($p=0.028$) (Tab 10 & Fig 59).

Tab 10: The changes in gastric laser Doppler flow in the 4 groups, expressed as a % of pre-CPB baseline values.

	CPB +10 min	CPB +40 min	20 min post X-clamp release	40 min post-CPB
BP	-35.3 ± 8.3	-44.2 ± 5.0	-22.6 ± 15.9	36.3 ± 16.5
BNP	-49.4 ± 5.8	-44.4 ± 7.0	-27.9 ± 9.2	31.0 ± 27.5
MP	-32.3 ± 7.7	-43.1 ± 7.4	-19.1 ± 8.6	32.1 ± 11.2
MNP	-40.1 ± 5.2	-43.0 ± 3.4	-17.7 ± 11.1	34.3 ± 15.9

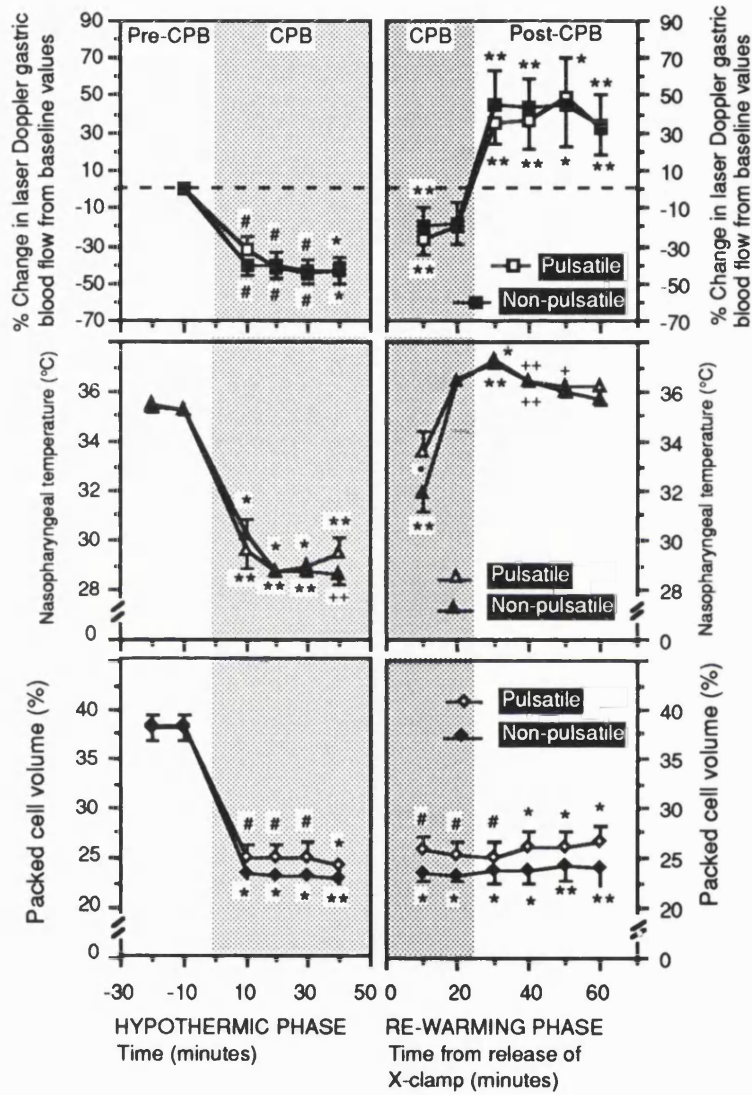
Fig 58: Bubble oxygenation and the influence of pulsatile and non-pulsatile perfusion on gastric laser Doppler blood flow.



Compared with pre-CPB values

* p=0.018, # p=0.028, ** p=0.034, # p=0.043

Fig 59: Membrane oxygenation and the influence of pulsatile and non-pulsatile perfusion on gastric laser Doppler blood flow.



Compared to pre-CPB values

p=0.012, * p=0.018, ** p=0.028, + p=0.035, ++ p=0.042

Comparing pulsatile and non-pulsatile groups

• p=0.049

Gastric pHi and arterial pH (pHa)- Temperature uncorrected (Fig 60 & 61)

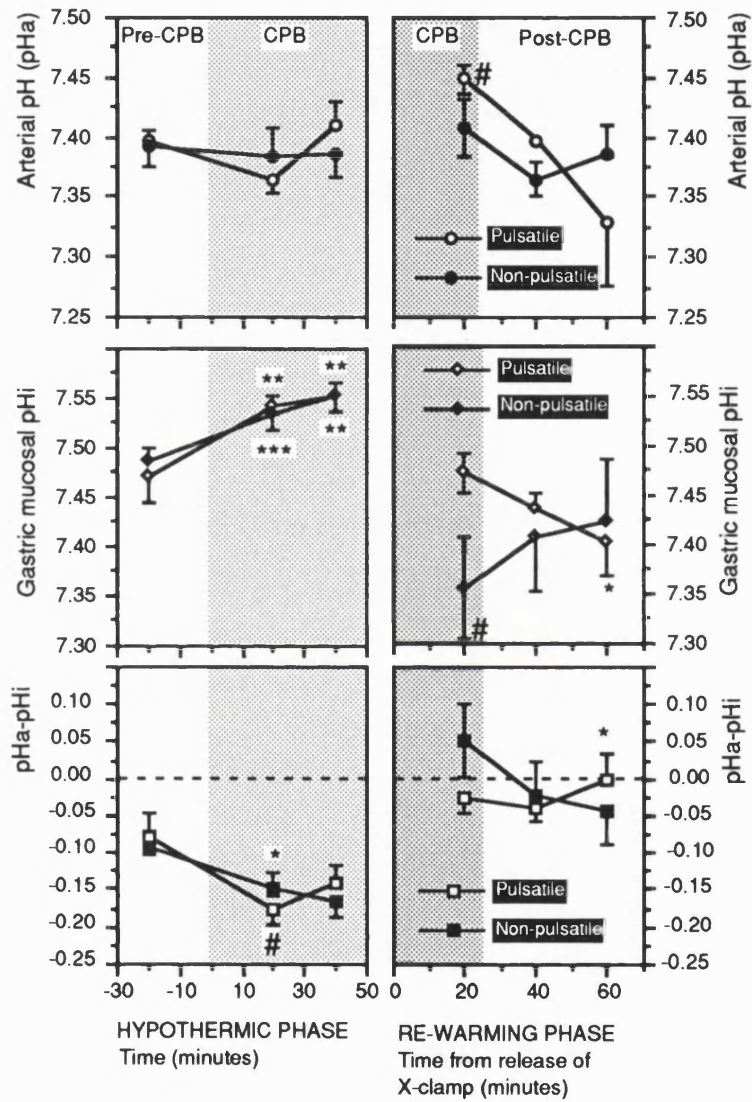
The pHa did not change significantly with hypothermic phase of CPB for BP and BNP groups, but the pHa increased from pre-CPB values of 7.41 ± 0.021 and 7.38 ± 0.18 for MP and MNP groups to 7.45 ± 0.024 and 7.49 ± 0.036 ($p=0.075$ and $p=0.018$ respectively) after 40 minutes of hypothermic CPB.

During this period, the gastric pHi increased from 7.47 ± 0.03 , 7.49 ± 0.01 , 7.44 ± 0.017 and 7.43 ± 0.031 pre-CPB for BP, BNP, MP and MNP groups to 7.55 ± 0.01 , 7.55 ± 0.01 , 7.54 ± 0.03 and 7.57 ± 0.04 ($p=0.028$, 0.14 , 0.018 and 0.075 respectively). The pHa-pHi difference increased during this hypothermic phase in all four groups (Fig 60 & 61).

During the re-warming phase, although there was little change in the pHa, in all 4 groups the gastric pHi fell towards or below pre-CPB levels. This trend was most prominent in the BNP group, in whom the pHi fell 20 minutes after release of the aortic X-clamp to 7.36 ± 0.05 ($p=0.046$), compared to 7.47 ± 0.02 , 7.48 ± 0.02 and 7.47 ± 0.03 for BP, MP and MNP groups respectively. This fall in the pHi was reflected in the pHa-pHi difference which returned towards pre-CPB values (Fig 60 & 61).

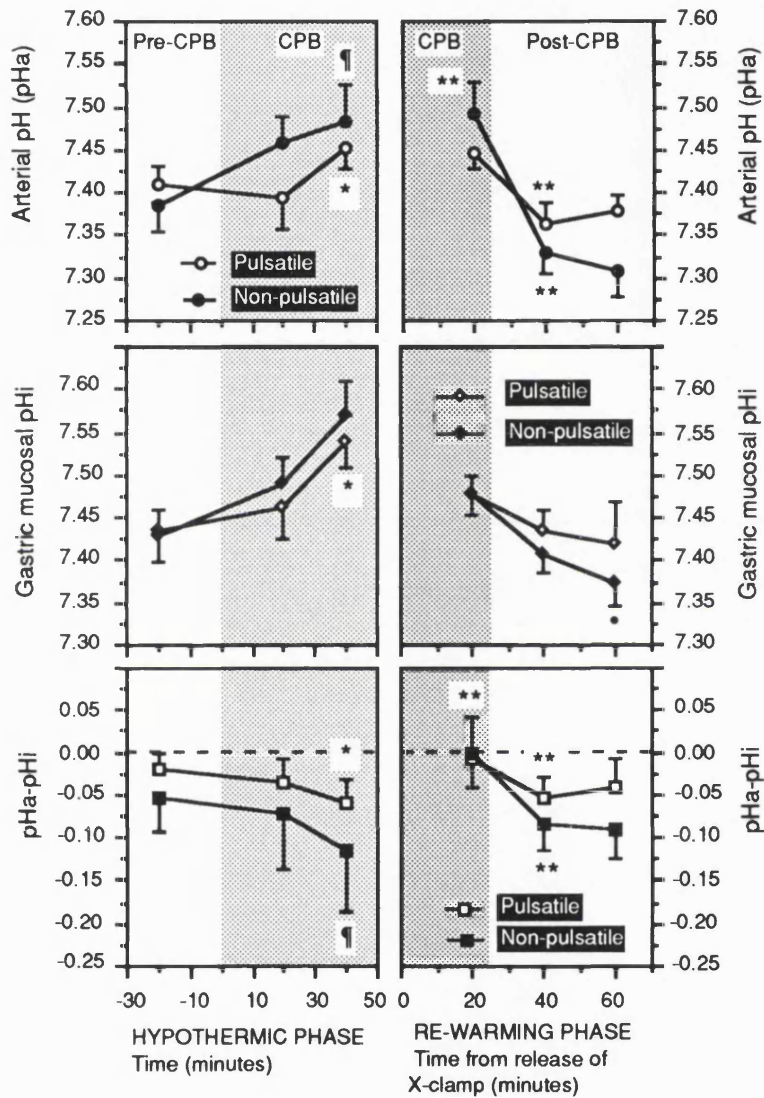
In the post-CPB period, the pHi continued its decline in all groups, although the BNP group, which had the largest fall in pHi post aortic X-clamp release had evidence of resolution of intramucosal acidosis, increasing from $7.36 \pm .05$ (20 minutes post aortic X-clamp release) to 7.42 ± 0.06 , 40 minutes post-CPB (Fig 60). Forty minutes post-CPB the pHi was 7.40 ± 0.03 , 7.42 ± 0.05 and 7.37 ± 0.03 for BP, MP and MNP groups ($p=0.043$, 0.89 and 0.059 respectively).

Fig 60: Bubble oxygenation & changes in arterial pH (pHa), gastric intramucosal pH (pHi) with pulsatile and non-pulsatile CPB.



All p values are for comparisons made with pre-CPB values
 # p=0.046, * p=0.043, *** p=0.027, ** p=0.017

Fig 61: Membrane oxygenation & changes in arterial pH (pHa), gastric intramucosal pH (pHi) with pulsatile & non-pulsatile CPB.



All p values are for comparisons made with pre-CPB values
 * p=0.018, ** p=0.028, ¶ p=0.042, ° p=0.05

Temperature Corrected pHi (pHit) & pHa (pHat)

Unlike the uncorrected values for pHi, the pHit showed no significant increase during the hypothermic phase of CPB, as described in Chapter 3. The pHit declined after 20 minutes of CPB from pre-CPB values of 7.40 ± 0.02 , 7.45 ± 0.03 , 7.43 ± 0.04 and 7.37 ± 0.03 for BP, BNP, MP and MNP groups to 7.36 ± 0.02 , 7.40 ± 0.02 , 7.33 ± 0.04 and 7.34 ± 0.03 ($p=0.13$, 0.07 , 0.02 and 0.17 respectively) (Fig 62 & 63). A similar trend in the pHit was found following release of the aortic X-clamp as in the uncorrected values. However, unlike the values uncorrected for temperature, the pHat-pHit difference was positive during the hypothermic phase of CPB.

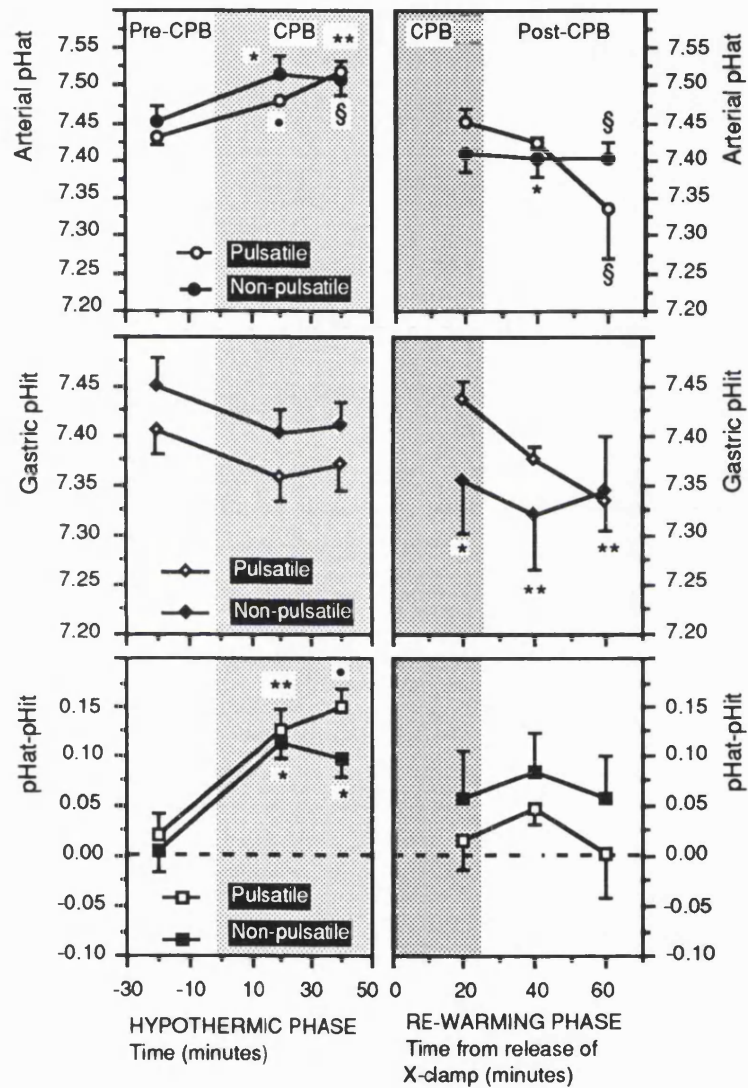
Pulsatile versus Non-pulsatile Perfusion: Gastric pHit

Data were also analysed by dividing patients into pulsatile and non-pulsatile groups, regardless of the type of oxygenation. The data are presented in table 11. There were no significant differences before CPB, or during the hypothermic phase of CPB. Following release of the aortic X-clamp, pulsatile patients maintained a higher pHit ($p=0.14$) and the pHat-pHit difference was smaller than non-pulsatile patients ($p=0.12$) (Tab 11). There were no significant differences between the groups in the immediate post-CPB period, although pulsatile patients had a higher mean pHit and a smaller pHat-pHit difference.

Tab 11: Gastric intramucosal acidosis during & following hypothermic CPB: the effect of pulsatile and non-pulsatile perfusion.

		20 min pre-CPB	CPB 20 min	CPB 40 min	20 min post X-Clamp release	20 min post-CPB	40 min post-CPB
Gastric pHit	Pulsatile	7.42 ± 0.02	7.35 ± 0.02	7.39 ± 0.02	7.45 ± 0.02	7.38 ± 0.02	7.35 ± 0.03
	p value	0.77	0.48	0.36	0.14	0.30	0.54
	Non-pulsatile	7.41 ± 0.02	7.37 ± 0.02	7.41 ± 0.02	7.39 ± 0.03	7.33 ± 0.03	7.33 ± 0.03
pHat-pHit	Pulsatile	0.05 ± 0.04	0.14 ± 0.02	0.14 ± 0.02	0.01 ± 0.02	0.01 ± 0.03	0.01 ± 0.03
	p value	0.77	0.68	0.14	0.12	0.52	0.82
	Non-pulsatile	$.01 \pm .02$	0.14 ± 0.03	0.10 ± 0.03	0.03 ± 0.03	0.03 ± 0.03	0.03 ± 0.03

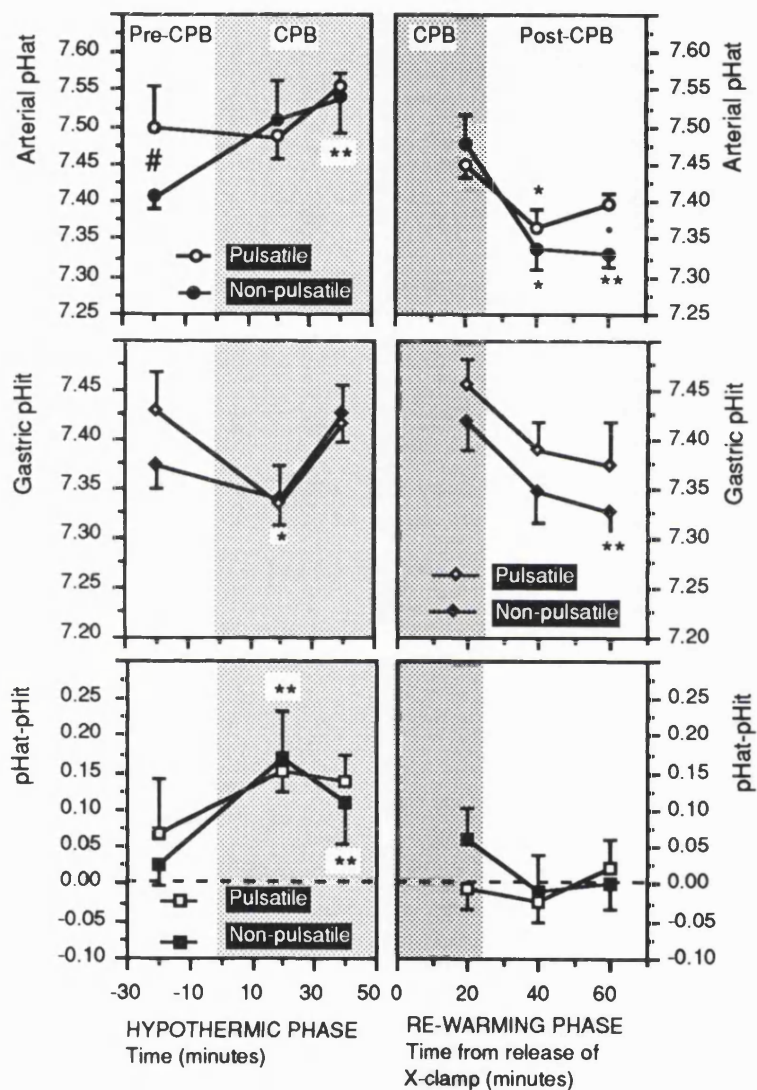
Fig 62: Bubble oxygenation & changes in temperature corrected arterial pH (pHat) and gastric intramucosal pH (pHit) with pulsatile & non-pulsatile CPB.



All p values are for comparisons made with pre-CPB values

* p=0.018, ** p=0.028, § p=0.042, • p=0.046

Fig 63: Membrane oxygenation & changes in temperature corrected arterial pH (pHat), and gastric intramucosal pH (pHit) with pulsatile & non-pulsatile CPB.



p values for comparisons made with pre-CPB values
 * p=0.018, ** p=0.028

Comparing pulsatile & non-pulsatile groups
 # p=0.047, • p=0.018

Arterial Blood Gases (Fig 64 & 65)

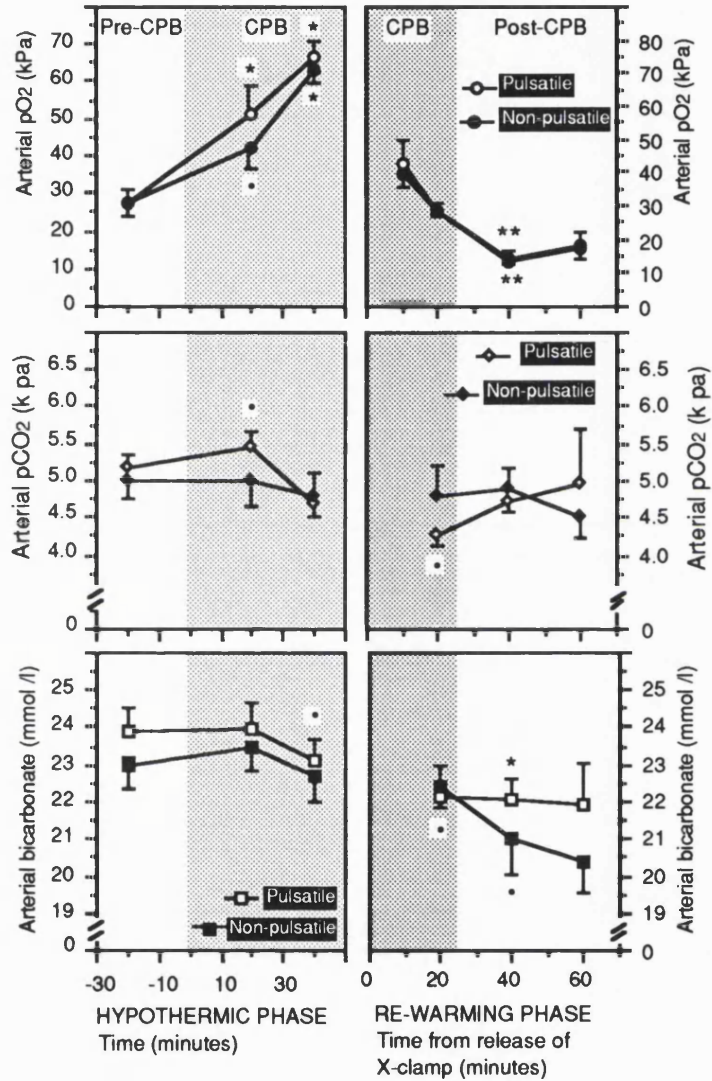
Arterial oxygenation followed a similar pattern in all 4 groups. At the end of the hypothermic phase the pO₂ was 66.6 ± 4.0, 63.1 ± 3.2, 47.9 ± 4.1 and 54.9 ± 1.4 kPa for BP, BNP, MP and MNP groups, compared to pre-CPB values of 27.6 ± 3.6, 27.1 ± 3.6, 21.3 ± 3.9 and 25.3 ± 2.1 kPa (p=0.028, 0.028, 0.028 and 0.043 respectively).

Although α -stat pH methodology was employed in all 4 groups, the arterial pCO₂ was lower during the hypothermic phase of CPB in the membrane MNP group. After 40 minutes of CPB, the MNP group had a pCO₂ of 3.5 ± 0.28 kPa compared to 4.4 ± 0.30 kPa for MNP patients (p=0.048).

As regards the arterial [HCO₃⁻], this was lower in the MNP at 20.2 ± 0.82 mmol/l compared to 22.4 ± 0.51 mmol/l for the MP group (p=0.083) at 40 minutes of hypothermic CPB. Similarly at the same time the arterial [HCO₃⁻] was lower for BNP patients at 22.7 ± 0.71 mmol/l compared to 23.2 ± 0.53 for BP patients, although the difference was less apparent for the bubble oxygenator group (p=0.95).

During the re-warming phase of CPB, there was a profound fall in the pO₂, although the oxygen flow rate was kept constant to the pump-oxygenator. Twenty minutes after X-clamp release it was 28.7 ± 2.0, 28.7 ± 2.1, 32.1 ± 4.6 and 29.7 ± 2.8 kPa for BP, BNP, MP and MNP groups respectively. The post-CPB period coincided with further de-saturation of arterial blood, which 20 minutes after the end of CPB had fallen to 14.5 ± 2.2, 13.4 ± 0.9 and 17.2 ± 2.5 kPa for BP, BNP and MNP groups (p=0.018, 0.018, 0.018 respectively), but for the MP group the pO₂ was still 20.8 ± 2.7 (p=1.0).

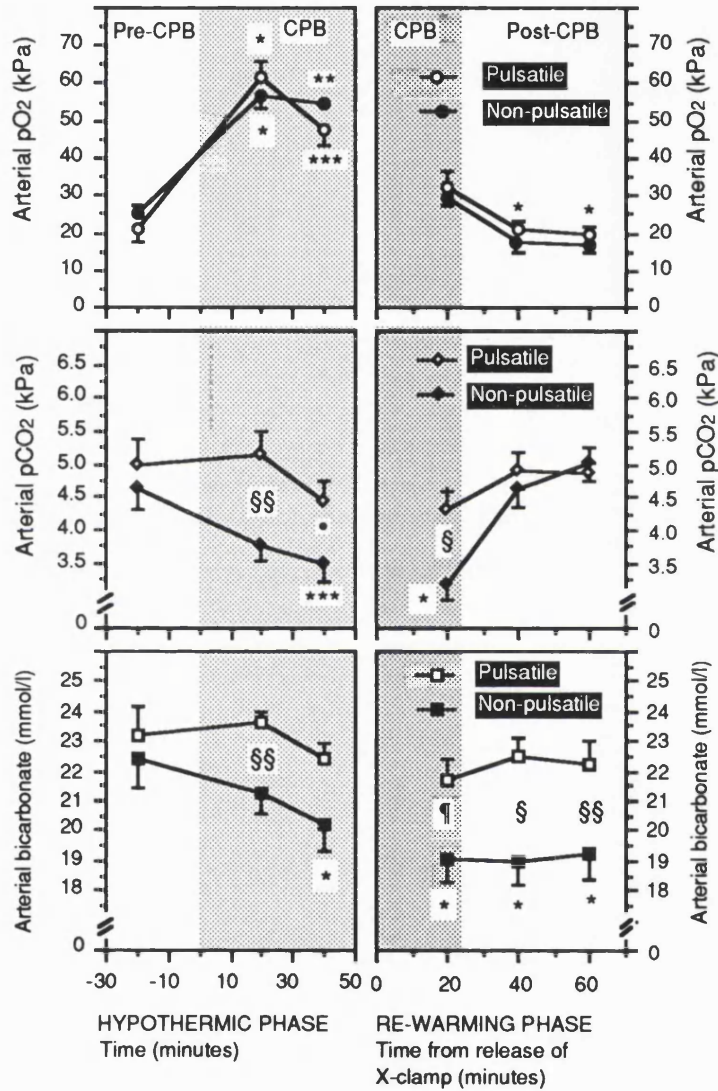
Fig 64: Bubble oxygenation and the influence of pulsatile & non-pulsatile perfusion on arterial pO₂, pCO₂ and bicarbonate concentration.



All p values are for comparisons made with pre-CPB values

• p=0.043, * p=0.028, ** p=0.018

Fig 65: Membrane oxygenation and the influence of pulsatile & non-pulsatile perfusion on arterial pO₂, pCO₂ and bicarbonate concentration.



Comparison with pre-CPB values
 * p=0.018, ** p=0.028, *** p=0.043

Comparing pulsatile and non-pulsatile groups
 § p=0.01, §§ p=0.02, ¶ p=.036, • p=0.049

The re-warming period was associated with reductions in the arterial $[\text{HCO}_3^-]$ in all groups, despite differences in oxygenation and pulsatility (Fig 64 & 65). This fall was most pronounced in the membrane oxygenator patients 20 minutes after aortic X-clamp release; for MP the arterial $[\text{HCO}_3^-]$ was 21.7 ± 0.69 mmol/l and for MNP it was 19.0 ± 0.77 mmol/l ($p=0.036$). This difference continued into the post-CPB period. Forty minutes after the end of CPB the arterial $[\text{HCO}_3^-]$ was 21.9 ± 1.1 , 20.4 ± 0.84 , 22.2 ± 0.46 and 19.2 ± 0.86 mmol/l for BP, BNP, MP and MNP groups ($p=0.08$, 0.14 , 0.21 and 0.018 , respectively compared to pre-CPB concentrations).

Saccharide Transport

To illustrate the influence of creatinine clearance on the excretion of individual monosaccharides and lactulose, all the results, standardised and unstandardised are shown in Tab 12.

Tab 12: Urinary excretion of monosaccharides & lactulose, with & without standardization to a the creatinine clearance of 70 ml/min/m²

Pre-CPB	3-O-m-D-glucose	D-xylose	L-rhamnose	Lactulose
BP	34.47 ± 4.71	22.38 ± 3.18	6.65 ± 1.18	0.16 ± 0.02
BNP	34.58 ± 5.91	22.59 ± 4.33	5.88 ± 1.03	0.25 ± 0.05
MP	31.30 ± 4.89*	21.99 ± 3.60*	8.67 ± 1.62	0.22 ± 0.04
MNP	40.96 ± 1.89	31.30 ± 2.04	8.31 ± 1.40	0.24 ± 0.03
Standardised				
BP	71.55 ± 6.69	45.52 ± 2.75	13.94 ± 1.92	0.37 ± 0.06
BNP	65.03 ± 7.95	42.33 ± 6.62	11.57 ± 2.51	0.52 ± 0.14
MP	59.39 ± 6.71	42.80 ± 3.75	17.74 ± 2.10	0.39 ± 0.06
MNP	79.87 ± 10.23	58.91 ± 5.80	15.44 ± 2.24	0.44 ± 0.03
CPB				
BP	5.53 ± 0.99	3.49 ± 0.81	1.05 ± 0.07	0.21 ± 0.10
BNP	8.03 ± 2.15	3.63 ± 0.67	2.76 ± 0.90	0.56 ± 0.39
MP	10.19 ± 2.01	6.19 ± 0.97	1.56 ± 0.20	0.30 ± 0.08
MNP	6.03 ± 1.32	3.93 ± 0.63	2.06 ± 0.62	0.70 ± 0.32
Standardised				
BP	16.31 ± 5.12	8.82 ± 1.32	3.45 ± 0.95	0.95 ± 0.76
BNP	20.97 ± 6.25	7.70 ± 1.83	6.59 ± 1.81	1.56 ± 1.22
MP	19.01 ± 4.28	11.83 ± 2.28	2.91 ± 0.44	0.52 ± 0.13
MNP	17.99 ± 5.14	10.93 ± 1.85	3.46 ± 1.95	1.86 ± 0.91
5 days Post-CPB				
BP	23.94 ± 7.01	14.66 ± 4.96	3.25 ± 1.03	0.12 ± 0.03
BNP	31.92 ± 8.14	20.68 ± 3.97	5.53 ± 1.05	0.42 ± 0.18
MP	27.2 ± 4.51	19.44 ± 3.68	7.00 ± 1.60	0.25 ± 0.05
MNP	28.8 ± 6.34	20.77 ± 5.16	5.23 ± 1.96	0.44 ± 0.11
Standardised				
BP	68.8 ± 19.90	38.39 ± 7.37	9.71 ± 2.80	0.34 ± 0.09
BNP	61.33 ± 9.54	35.78 ± 7.94	11.68 ± 2.04	1.04 ± 0.56
MP	53.99 ± 3.35	40.08 ± 4.34	13.85 ± 2.62	0.48 ± 0.08
MNP	61.3 ± 14.74	45.25 ± 12.25	8.44 ± 3.44	1.00 ± 0.31

Comparing pulsatile versus non-pulsatile groups

* p=0.035

Standardisation for renal clearance did not change the statistical significance, when comparing pulsatile versus non-pulsatile groups, of the unstandardised values except

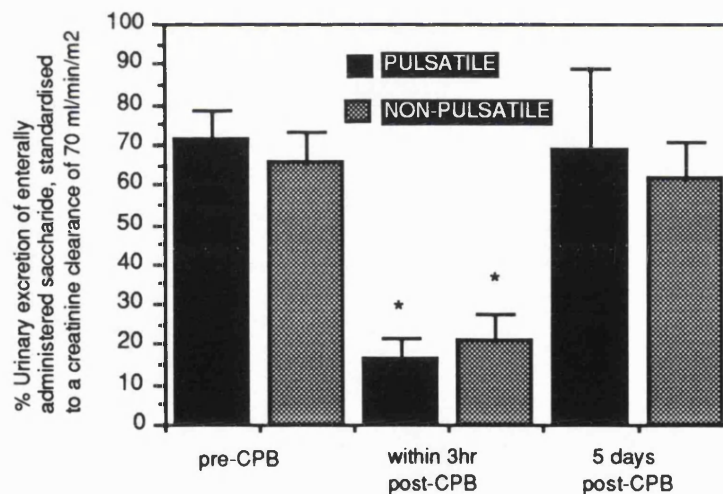
for the membrane group of patients for the pre-CPB urinary excretions of 3-O-m-D-glucose and D-xylose (Tab 12).

Standardised excretion values (to a creatinine clearance of 70 ml/min/m²) and the gut permeability ratio are presented below to illustrate the changes from pre-CPB to post-CPB values in the 4 groups investigated.

(a) 3-O-m-D-glucose absorption (Fig 66 & 67)

3-O-m-D-glucose transport fell from 71.6 ± 6.7, 65.0 ± 8.0, 59.4 ± 6.7 and 79.8 ± 10.2 % pre-CPB to 16.3 ± 5.1, 21.0 ± 6.2, 19.0 ± 4.3 and 18.0 ± 5.1 % immediately post-CPB for BP, BNP, MP and MNP groups (p=0.028, 0.028, 0.028 and 0.018 respectively).

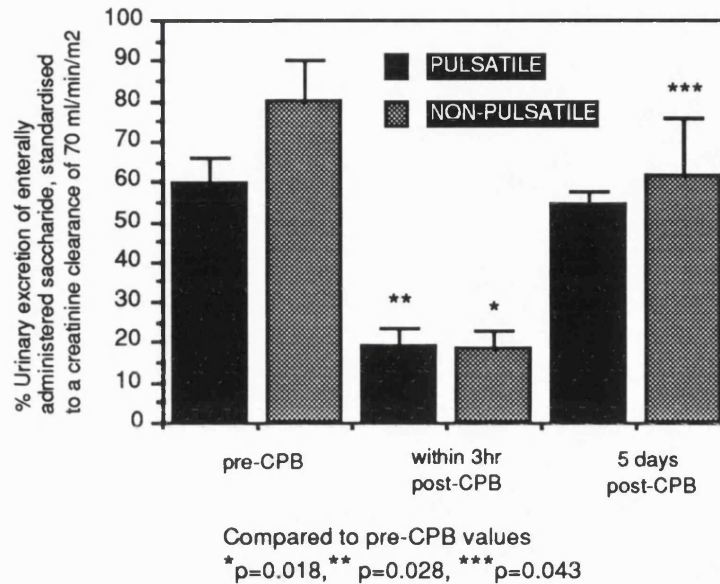
Fig 66: Bubble oxygenation, the influence of pulsatile and non-pulsatile perfusion on the transport of 3-O-m-D-glucose, standardised for alterations in the ceatinine clearance.



* p=0.028, compared to pre-CPB value

Patients who received non-pulsatile flow in the membrane oxygenator group had a significant reduction in 3-O-m-D-glucose absorption even 5 days after CPB. In these patients (MNP), the excretion of 3-O-m-D-glucose was 61.3 ± 14.7 % at 5 days compared to 79.8 ± 10.2 % pre-operatively (p=0.043). There was no significant difference between pulsatile and non-pulsatile patients.

Fig 67: Membrane oxygenation; the influence of pulsatile and non-pulsatile perfusion on the transport of 3-O-m-D-glucose, standardised for alterations in the creatinine clearance



(b) D-xylose absorption (Fig 68 & 69)

The passive carrier mediated absorption of D-xylose was reduced in all 4 groups. Immediately post-CPB the D-xylose excretion fell from 45.5 ± 2.7 , 42.3 ± 6.6 , 42.8 ± 3.7 and 58.9 ± 5.8 % pre-CPB to 8.8 ± 1.3 , 7.7 ± 1.8 , 11.8 ± 2.3 and 10.9 ± 1.8 (for BP, BNP, MP and MNP groups ($p=0.028$, 0.028 , 0.028 and 0.018 respectively)).

Similar to 3-O-m-D-glucose absorption, patients who were in the MNP group had impaired D-xylose absorption 5 days after the end of CPB at 45.3 ± 12.3 compared to 58.9 ± 5.8 % pre-operatively ($p=0.043$). There was no significant difference between MNP and MP groups.

Fig 68: Bubble oxygenation: the influence of pulsatile and non-pulsatile perfusion on the transport of D-xylose, standardised for alterations in the creatinine clearance

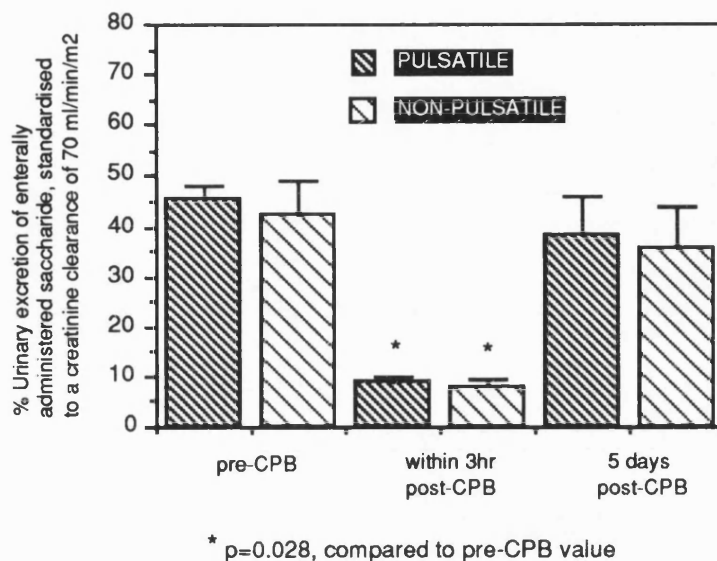
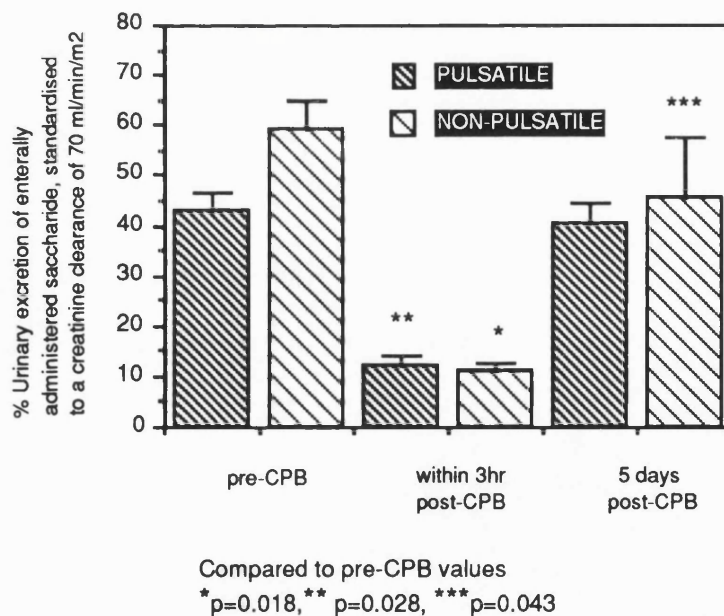


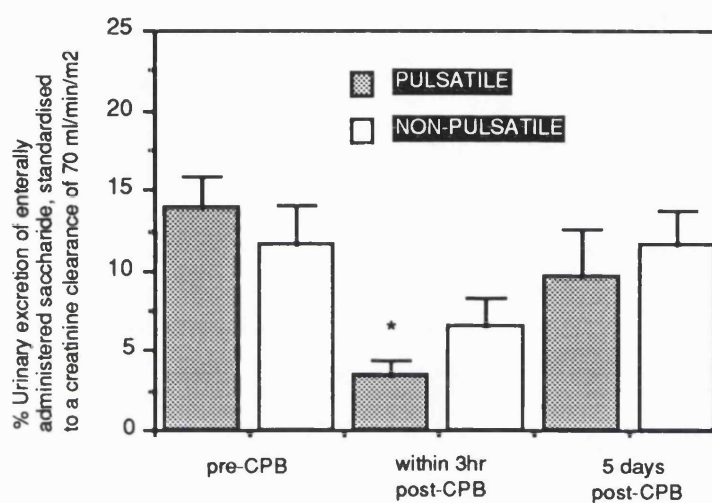
Fig 69: Membrane oxygenation: the influence of pulsatile and non-pulsatile perfusion on the transport of D-xylose, standardised for alterations in the creatinine clearance



(c) L-rhamnose permeation (Fig 70 & 71)

The permeation of L-rhamnose was reduced in all 4 groups immediately post-CPB with no significant differences between the groups. There were no significant differences between the 4 groups for the day 5 post-CPB urinary excretion for L-rhamnose.

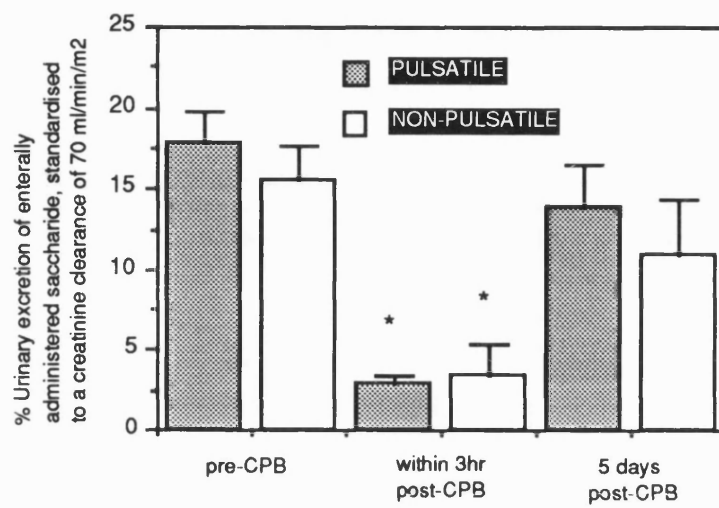
Fig 70: Bubble oxygenation: the influence of pulsatile and non-pulsatile perfusion on the transport of L-rhamnose, standardised for alterations in the creatinine clearance



* $p=0.028$, compared to pre-CPB value

The permeation of L-rhamnose was reduced in all groups (Fig 70 & 71) except for the BNP group. In these patients the L-rhamnose excretion was reduced by 43% from 11.6 ± 2.5 pre-CPB to 6.6 ± 1.8 post-CPB but did not reach statistical significance ($p=0.075$).

Fig 71: Membrane oxygenation: the influence of pulsatile and non-pulsatile perfusion on the transport of L-rhamnose, standardised for changes in the creatinine clearance.



* p=0.028, compared to pre-CPB value

(d) Lactulose permeation (Fig 72 & 73)

Unlike the monosaccharides, the permeation of lactulose (disaccharide) was increased in all 4 groups following CPB. The most significant increase was observed in patients who had received non-pulsatile flow, either bubble or membrane oxygenation.

Fig 72: Bubble oxygenation: the influence of pulsatile and non-pulsatile perfusion on the permeation of the disaccharide lactulose, standardised for alterations in the creatinine clearance

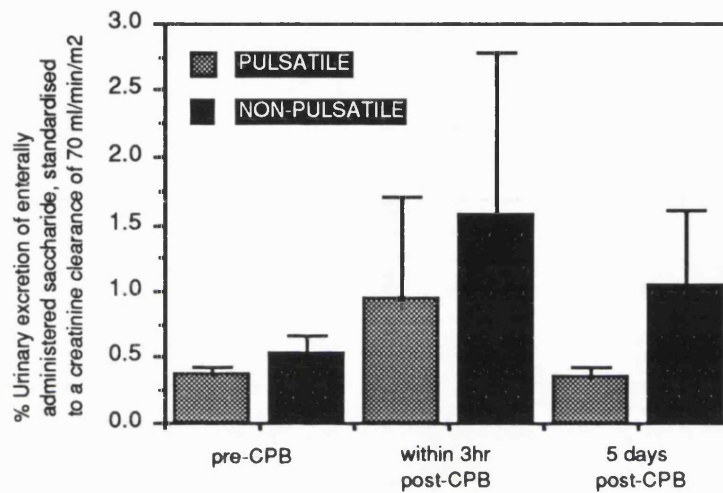
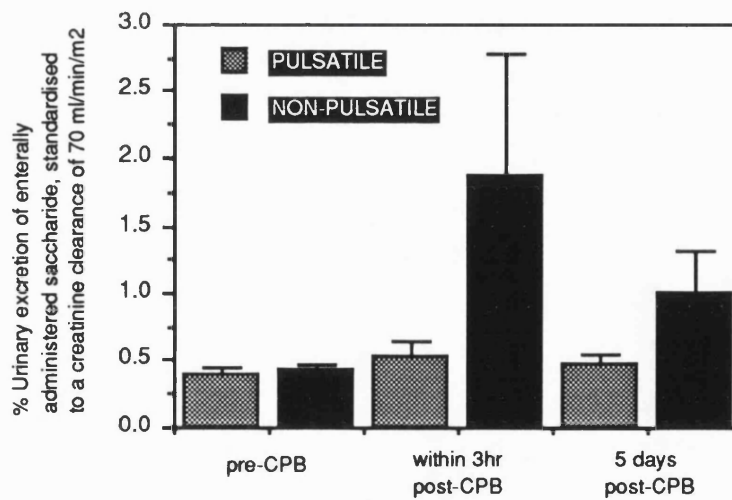


Fig 73: Membrane oxygenation: the influence of pulsatile & non-pulsatile perfusion on the permeation of lactulose, standardised for alterations in the creatinine clearance



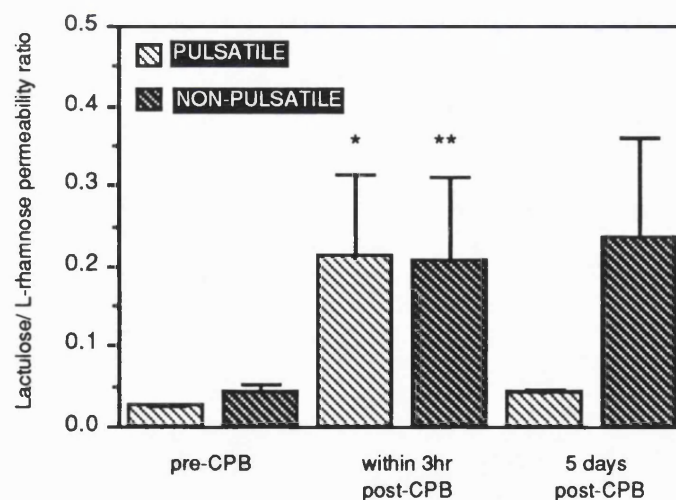
Immediately post-CPB, the standardised urinary excretion values were $0.97 \pm 0.76\%$, $1.56 \pm 1.2\%$, $0.52 \pm 0.13\%$ and $1.86 \pm 0.91\%$ compared to pre-CPB values of $0.37 \pm$

0.06%, $0.52 \pm 0.14\%$, $0.39 \pm 0.06\%$ and $0.44 \pm 0.03\%$ for BP, BNP, MP and MNP groups ($p=0.46$, 0.35 , 0.46 and 0.18 respectively). This trend was continued into the post-operative period, although none of the values was significantly different from pre-CPB values.

(e) Gut Permeability (lactulose/ L-rhamnose ratio) (Fig 74 & 75)

All groups underwent substantial increases in gut permeability post-CPB, which was not statistically different between groups. There was a sustained increase in gut permeability in patients who had received pulsatile perfusion. Five days post-CPB the gut permeability ratio was 0.04 ± 0.01 , 0.24 ± 0.12 , 0.04 ± 0.01 and 0.10 ± 0.04 for BP, BNP, MP and MNP groups ($p=0.043$, 0.5 , 0.25 and 0.027 respectively).

Fig 74: Bubble oxygenation: the effect of pulsatile & non-pulsatile perfusion on gut permeability

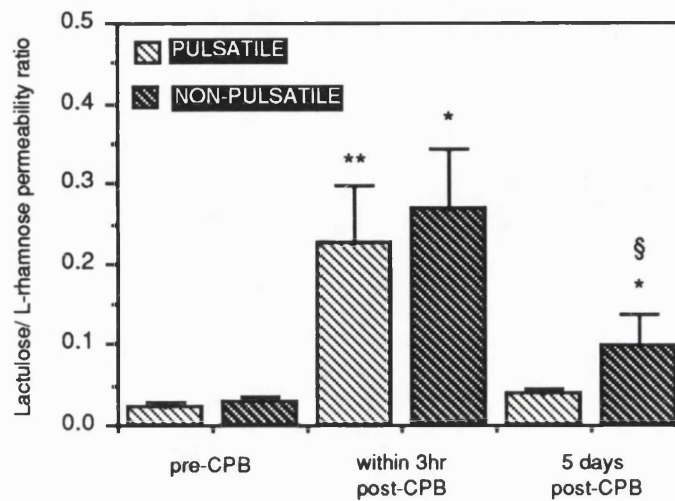


* Indicates $p=0.028$, compared to pre-CPB values

** Indicates $p=0.018$, compared to pre-CPB values

There was no difference between the pulsatile bubble and membrane groups ($p=0.41$), or between non-pulsatile bubble and membrane groups ($p=0.75$).

Fig 75: Membrane oxygenation: the effect of pulsatile & non-pulsatile perfusion on gut permeability



* Indicates $p=0.028$, ** $p=0.018$ compared to pre-CPB values

§ Indicates $p=0.015$, comparing pulsatile and non-pulsatile groups

However, patients who underwent membrane non-pulsatile perfusion had a greater permeability ratio 5 days post-CPB than BP group ($p=0.044$) or MP group ($p=0.025$).

No significant differences were found for the absorption of saccharides or the permeability ratio when patients were grouped into either bubble or membrane groups, regardless of the nature of the flow delivered during CPB.

Post-operative Morbidity(a) Intensive Care Data (Tab 13 & 14)

Patients who received non-pulsatile flow tended to have longer ventilatory times and total intensive care unit (ICU) time, but this did not reach statistical significance. The difference was most apparent when comparing MP versus BNP groups (Tab 15), despite no difference in aortic X-clamp ($p=0.80$) or total CPB ($p=0.80$) times. The advantage of the pulsatile group may have been due to the slightly younger age of this group of patients (57.0 ± 2.3 versus 64.0 ± 0.24 years, $p=0.08$). Patients who received MP flow had a ventilation time of 9.4 ± 1.4 compared to 14.6 ± 3.2 hours for BNP patients ($p=0.12$). Similarly the ICU time was 15.7 ± 1.4 hours for MP compared to 20.1 ± 3.0 hours for BNP patients ($p=0.57$).

Tab 13: Patient data, bubble oxygenation

	Pulsatile	Non-pulsatile	P value
Age (yr)	62.3 ± 2.3	64.0 ± 2.4	0.94
X-clamp time (min)	46.5 ± 2.6	51.9 ± 3.2	0.28
CPB time (min)	79.3 ± 4.5	84.0 ± 4.1	0.75
Ventilation time (hr)	12.5 ± 1.2	14.6 ± 3.2	0.58
Intensive care unit time (hr)	17.3 ± 1.02	20.1 ± 3.9	0.77
Hospital stay (days)	11.5 ± 6.3	8.1 ± 0.34	0.16
Total drain loss (ml)	1175.0 ± 262.0	840.7 ± 127.4	0.16

Total hospital stay was shorter for MP patients than MNP (7.9 ± 0.4 versus 10.7 ± 1.7 days), but this did not reach statistical significance ($p=0.31$). The reverse was true for the bubble oxygenator group (11.5 ± 6.3 versus 8.1 ± 0.34 days, $p=0.16$), because two patients in the BP group developed respiratory infections requiring antibiotic and physiotherapy treatment.

Tab 14: Patient data, membrane oxygenation

	Pulsatile	Non-pulsatile	P value
Age (yr)	57.0 ± 2.33	61.3 ± 2.4	0.22
X-clamp time (min)	43.0 ± 7.4	60.7 ± 6.3	0.44
CPB time (min)	86.6 ± 8.7	93.3 ± 8.7	0.52
Ventilation time (hr)	9.4 ± 1.4	12.7 ± 2.7	0.31
Intensive care unit time (hr)	15.7 ± 1.4	20.7 ± 4.4	0.46
Hospital stay (days)	7.9 ± 0.4	10.7 ± 1.7	0.31
Total drain loss (ml)	1016.4 ± 143.2	966.7 ± 166.9	0.67

Comparing MP versus BNP groups, a shorter ventilation time was observed for MP patients ($p=0.12$) (Tab 15). However, MP patients were younger than BNP patients, 57.0 ± 2.33 versus 64.0 ± 2.4 years respectively ($p=0.08$), which may have accounted for reduced ventilation and ICU times, although both groups had similar CPB and aortic X-clamp times.

Tab 15: Patient data, membrane pulsatile versus bubble non-pulsatile groups.

	Membrane Pulsatile	Bubble Non-pulsatile	P value
Age (yr)	57.0 ± 2.33	64.0 ± 2.4	0.08
X-clamp time (min)	43.0 ± 7.4	51.9 ± 3.2	0.80
CPB time (min)	86.6 ± 8.7	84.0 ± 4.1	0.80
Ventilation time (hr)	9.4 ± 1.4	14.6 ± 3.2	0.12
Intensive care unit time (hr)	15.7 ± 1.4	20.1 ± 3.9	0.57
Hospital stay (days)	7.9 ± 0.4	8.1 ± 0.34	0.41
Total drain loss (ml)	1016.4 ± 143.2	840.7 ± 127.4	0.67

Dividing the patients into pulsatile and non-pulsatile groups revealed no significant differences for the ventilation time (pulsatile 10.8 ± 1.0 hr versus non-pulsatile $13.7 \pm$

2.1 hr, $p=0.48$), total ICU time (pulsatile 16.4 ± 0.9 days versus non-pulsatile 20.4 ± 2.8 days, $p=0.67$) or the hospital time (pulsatile 9.5 ± 1.3 days versus non-pulsatile 9.3 ± 0.8 days, $p=0.91$).

(b) Post-operative Morbidity

Although H₂-antagonist (ranitidine) was administered to all the patients duodenal ulcers developed in two patients, which were confirmed by endoscopy. Both these patients received non-pulsatile flow and were in the membrane oxygenator group ($p=0.46$); neither had a history or symptoms of pre-operative gastrointestinal disorder. The incidence of other post-operative complications is recorded in tables 16 & 17.

Tab 16: Bubble oxygenation, post-operative complications

	Pulsatile	Non-pulsatile	P value
Gastrointestinal	0	0	-
Central nervous system	0	0	-
Renal	0	0	-
Respiratory	2 infection	1 infection	0.80
Inotrope support	0	0	-

Tab 17: Membrane oxygenation, post-operative complications

	Pulsatile	Non-pulsatile	P value
Gastrointestinal	0	2 duodenal ulcers x 2	0.46
Central nervous system	0	0	-
Renal	1	0	0.99
Respiratory	1 infection	1 infection	-
Inotrope support	0	1	0.99

Gastric pHi, GI permeability & post-operative morbidity

The two patients who developed duodenal ulcers, the lowest gastric pHit recorded was 7.32 ± 0.03 , compared to 7.33 ± 0.02 for the remaining 26 patients who did not develop GI complications ($p=0.66$).

The predictive value of a low pHit (<7.35) following aortic X-clamp release was assessed using multiple linear regression analysis. This analysis uses the *method of least squares* to obtain the regression equation. However, it has the advantage that both continuous and non-continuous (categorical) explanatory variables may be analysed. In the presented analyses the categorical variables are pulsatile/non-pulsatile perfusion, bubble/membrane oxygenation and a gastric pHit $>$ or $<$ 7.35. For the purposes of analysis a binary code has been allocated to these variables so that pulsatile flow, membrane oxygenation and gastric pHit >7.35 are coded 0, whilst non-pulsatile flow, bubble oxygenation and pHit <7.35 are coded as 1.

As regards ventilation time and ICU time a low gastric pHit (<7.35) did not predict a protracted stay in ICU or a prolonged ventilation time. However, as regards total time spent in hospital, patients who developed a low gastric pHit (< 7.35) had a longer total hospital stay ($p=0.29$), although the age of the patient was the best predictor of the length of hospitalization ($p=0.20$) (Tab 18).

Tab 18: Multiple linear regression model: factors predicting the length of hospital stay

Beta Coefficient Table					
Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	0.877				
Age	0.201	0.151	0.351	1.329	0.204
X-C time	0.216	0.189	0.788	1.147	0.270
CPB time	-0.169	0.148	-0.76	1.143	0.271
Oxygenator (B/M)	0.833	2.145	0.107	0.388	0.703
Flow (P/NP)	0.983	2.138	0.127	0.484	0.649
Post-CPB Perm	-2.050	5.168	-0.107	0.397	0.697
Gastric pHit <7.35	-2.230	2.050	-0.284	1.087	0.294

Intramuscular acidosis (pHit <7.35) was also the best predictor of the duration of ventilation (p=0.05, Tab 19) and the total time spent in ICU (p=0.07, Tab 20).

Tab 19: Multiple linear regression model: factors predicting the duration of ventilation

Beta Coefficient Table					
Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	16.138				
Age	0.032	0.203	0.038	0.160	0.875
X-C time	-0.168	0.257	-0.392	0.652	0.524
CPB time	0.085	0.204	0.245	0.418	0.682
Oxygenator (B/M)	-2.733	2.974	-0.227	0.919	0.372
Flow (P/NP)	-5.217	2.978	-0.432	1.751	0.099
Post-CPB Perm	-5.702	7.199	-0.188	0.792	0.440
Gastric pHit <7.35	5.804	2.752	0.480	2.109	0.051

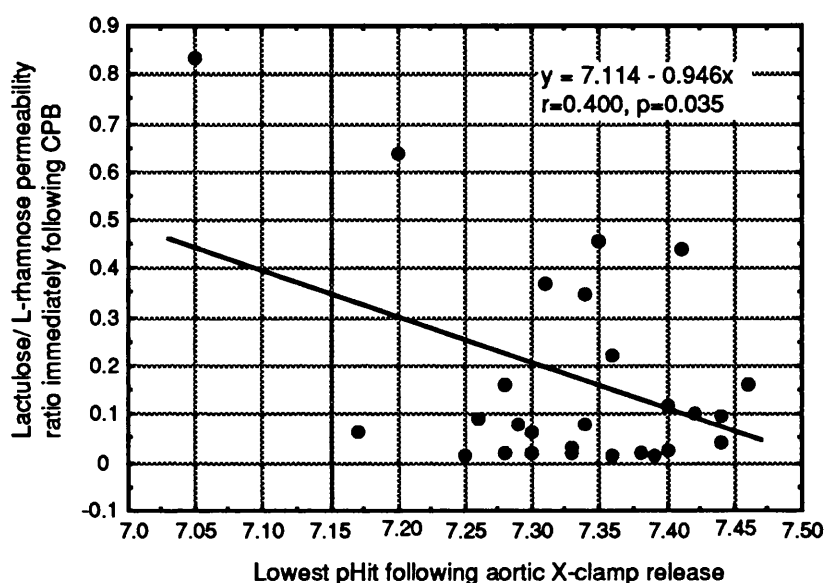
Tab 20: Multiple linear regression model: factors predicting the length ICU stay

Beta Coefficient Table					
Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	12.036				
Age	0.201	0.252	0.190	0.799	0.436
X-Clamp time	0.037	0.320	0.070	0.116	0.909
CPB time	-0.127	0.254	-0.296	0.500	0.624
Oxygenator (B/M)	0.435	3.696	0.029	0.118	0.908
Flow (P/NP)	-4.437	3.702	-0.298	1.196	0.248
Post-CPB Perm	-2.440	8.948	-0.065	0.273	0.789
Gastric pHit <7.35	6.837	3.420	0.445	1.941	0.070

Gastric pHit & Gut Permeability

Patients who had a lower gastric pHit (<7.35) following aortic X-clamp release had a greater rise in gut permeability. A linear regression analysis (Spearman rank-order), revealed an inverse linear relationship between gut permeability and the lowest gastric pHit recorded following release of the aortic X-clamp ($r=0.40$, $p=0.035$, Fig 76).

Fig 76: The relationship between gastric mucosal oxygenation (pHit) and post-CPB gut permeability



This relationship between intramucosal acidosis and the development of increased post-CPB permeability was supported by multiple linear regression analysis. Analysed in conjunction with patient and perfusion variables, the best predictor of intramucosal acidosis (pHit <7.35) was the post-CPB gut permeability ratio ($p=0.08$, Tab 21).

Tab 21: Multiple linear regression model: factors predicting gastric intramucosal acidosis

Beta Coefficient Table					
Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	7.02				
Age	0.004	0.003	0.258	1.249	0.228
X-Clamp time	0.00008	0.004	0.010	0.018	0.988
CPB time	-0.00008	0.003	-0.013	0.023	0.982
Oxygenator (B/M)	0.038	0.037	0.215	1.019	0.320
Flow (P/NP)	0.041	0.037	0.229	1.113	0.278
Post-CPB Perm	-0.154	0.084	-0.364	1.830	0.082

Pulsatile flow & Post-CPB Gut Permeability

The importance of pulsatile flow in preventing an increase in gut permeability was analysed. Using this model, pulsatile flow was the most important patient or perfusion variable examined for predicting a normal post-CPB gut permeability ($p=0.21$, Tab 22).

Tab 22: Multiple linear regression model: factors predicting an increase in the immediate post- CPB gut permeability ratio.

Beta Coefficient Table					
Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	0.159				
AGE	0.004	0.007	0.120	0.544	0.592
X-C TIME	-0.004	0.009	-0.244	0.406	0.689
CPB TIME	0.002	0.007	0.164	0.281	0.781
OXYGENATOR	-0.015	0.095	-0.037	0.163	0.872
FLOW	-0.115	0.090	-0.274	1.285	0.212

On the basis of these findings, changes in post-CPB gut permeability were analysed by dividing the patients up into either pulsatile ($n=14$) or non-pulsatile ($n=14$) flow groups, regardless of the type of oxygenator used during CPB. Although no difference was identified in the permeability ratio immediately post-CPB ($p=0.8$), patients who had received non-pulsatile flow had a substantially greater gut permeability 5 days post-CPB ($p=0.007$, Tab 23).

Tab 23: The effect of pulsatile (n=14) and non-pulsatile (n=14) perfusion on immediate post-CPB gut permeability and recovery 5 days post-CPB.

PERMEABILITY RATIO POST-CPB		
	Within 3 hr	5 days
PULSATILE	0.23 ± 0.06	0.04 ± 0.01
p value	0.801	0.007
NON-PULSATILE	0.24 ± 0.06	0.17 ± 0.07

Normal lactulose/ L-rhamnose gut permeability ratio is <0.04

This difference in the permeability ratio at 5 days post-CPB was due to increased permeation of lactulose (pulsatile $0.40 \pm 0.06\%$ versus non-pulsatile $1.02 \pm 0.30\%$, $p=0.07$), whilst the permeation of L-rhamnose was similar for the two groups (pulsatile $11.58 \pm 1.86\%$ versus non-pulsatile $11.31 \pm 1.91\%$, $p=0.89$).

Discussion

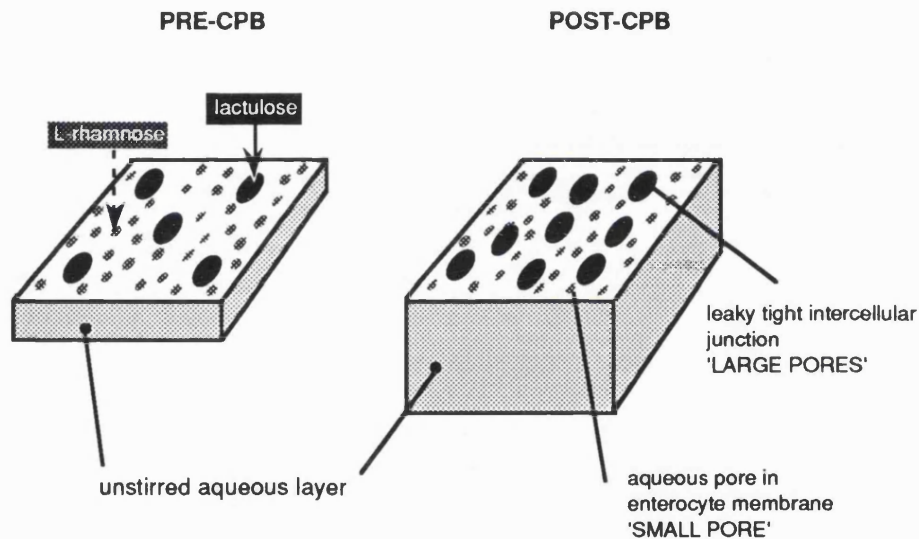
This study has demonstrated that renal clearance may influence the excretion of the individual saccharides, but it does not challenge the significant alterations in the transport of gut saccharides previously found in Chapter 5 nor influence the gut permeability (lactulose/ L-rhamnose ratio). No differences were apparent immediately following CPB for patients who received pulsatile or non-pulsatile perfusion and membrane or bubble oxygenation for the transport of 3-O-m-D-glucose and D-xylose transport. However, in the membrane oxygenator study, patients who had received non-pulsatile perfusion had impaired transport of both 3-O-m-D-glucose and D-xylose 5 days post-CPB, whilst pulsatile patients had recovered to pre-CPB levels. These monosaccharides are transported by active and passive carrier mediated mechanisms respectively (Menziez et al 1979) and are dependent upon a supply of carrier protein and ATP. This finding suggests that in these patients the injury sustained during CPB had not completely resolved 5 days post-operatively, although at this stage all the patients were on an enteral diet.

The permeation of L-rhamnose is not ATP dependent; it moves through aqueous pores in the cellular membrane by simple diffusion (Lieb and Stein 1969), but also shares, by virtue of its size, the pathway of lactulose transport which is via a paracellular or intercellular route. There was no difference in the BNP group for L-rhamnose transport despite differences for 3-O-m-D-glucose and D-xylose in the immediate post-CPB study. Similarly, although there was a significant reduction of L-rhamnose transport for the MNP group, the permeation of L-rhamnose was greater than for the MP group. This coincided with the non-pulsatile patients having the highest post-CPB lactulose permeation in either membrane or bubble groups. Nevertheless, all patients had an overall reduction in L-rhamnose transport, albeit to a variable extent. This reduction in the transport of L-rhamnose continued into the post-operative period, although it was not statistically significant on the day 5 studies. The

influence of CPB on L-rhamnose permeation may indicate another form of gut injury since its transport is not directly linked to ATP availability.

The gut mucosa may be represented diagrammatically as in Fig 77, with the total area of the mucosal surface covered in large (intercellular tight junctions) and small pores (intra-membrane aqueous pores with a molecular radius of 0.4 nm). Following CPB the total area available for the transport of L-rhamnose or lactulose has not changed because no gut was resected.

Fig 77: The gut mucosa as a single membrane : alterations in small and large pore number and the unstirred aqueous layer following CPB



The total amount which has been delivered to the gut may be reduced if there is impaired gastric emptying. However, this latter phenomenon is unlikely to occur 5 days after surgery when patients are on a normal diet and the majority are about to be discharged home the following, if not the same day. L-rhamnose moves through aqueous pores within the cell membrane referred to as 'small pores'. A reduction in transport may therefore be a result of a reduction in the effective number of pores available for permeation or an increase in the unstirred layer through which L-rhamnose has to move by simple diffusion down its concentration gradient. An

increase in the unstirred water layer, formed by the combination of the extracellular and intracellular water, may retard its transport. At present, there is no evidence to suggest that the number or configuration of small pores changes with CPB, but it is well documented that capillary permeability increases post-CPB (Smith et al 1987). There is an increase in the extracellular fluid compartment, with patients tending to retain sodium and water, in a time-dependent manner with the duration of the total CPB time (Cleland et al 1966). This, combined with a tendency towards mucosal hypoxia in the re-warming phase and immediate post-CPB periods, may lead to cellular oedema and an increase in the intracellular water. Both these factors may contribute to the increase in water retention within the layers of the gut wall.

The extracellular water content within the gut has been measured as an increase in interstitial fluid pressure. Following CPB, interstitial pressure was greatest in the myocardium, intermediate in the stomach and the least in the muscle (Utley 1990). However, the intracellular component may be less important, if the studies on skeletal muscle biopsies can be extrapolated to the splanchnic bed. Del Canale et al, examined changes in intra and extracellular water content in skeletal muscle biopsies of patients undergoing low and normal flow hypothermic non-pulsatile CPB (Del Canale et al 1990). In these biopsies there was no significant increase post-CPB in intracellular water in either group, although there was expansion of the extracellular fluid compartment (Del Canale et al 1990). Nevertheless, the gut undergoes some of the most dramatic increases in tissue water content following injury because it has a largely unrestricted space within the abdomen to expand (Utley 1990). It may therefore be postulated that the reduction in passive L-rhamnose transport is a manifestation of the increase in the unstirred layer, which may be both intra and extracellular. However, less reduction in L-rhamnose transport may have been expected for pulsatile patients, but non-pulsatile perfusion by virtue of its action on intercellular tight junctions, may permit the permeation of both lactulose and rhamnose through these large pores. In animal models, fluid requirements and

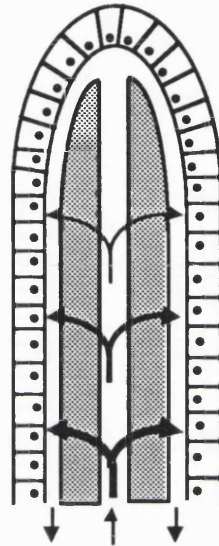
pulmonary oedema have been less for pulsatile than for non-pulsatile perfusion, but these differences only became apparent after three hours of CPB (Dunn et al 1974).

The increase in lactulose permeation may be explained by an increase in the 'large pores' (Fig 77), but in reality this equates to increased leakiness of the intercellular tight junctions. The maintenance of these tight junctions is controlled by ATP dependent intracellular mechanisms and cytoskeleton (Madara 1983, Madara et al 1986). Therefore reductions in enterocyte ATP would tend to decrease absorptive capacity (3-O-m-D-glucose) but also decrease gut barrier function. The 'leakiness' of the villus is not constant along its length; at sites of cell extrusion, which lie near the villus tip, inter-cell adhesion is weaker and therefore the permeability to macromolecules greater (Gumbier 1987, Marcial et al 1984).

This study has also found that pulsatile flow was the most important patient or CPB perfusion factor examined for predicting a normal post-CPB gut permeability ($p=0.004$). Furthermore, patients who had received pulsatile perfusion recovered their gut permeability ratio by 5 days post-CPB, whilst non-pulsatile patients had a significantly higher permeability ratio ($p=0.007$). These findings are the most important evidence for the benefits of pulsatile flow in the maintenance of adequate gut oxygenation/perfusion during CPB. In large part, these differences in the permeability ratio were due to the disparity between pulsatile and non-pulsatile flow groups for the permeation of lactulose, particularly 5 days post-CPB, when L-rhamnose excretion was almost identical in the two groups.

These differences may be explained by the villus arterio-venous shunting of oxygen (Fig 78) (Lundgren 1967). This predicts that villus tip hypoxia will occur first, which may explain why the tips are the first to show signs of histological damage following episodes of ischaemia or ischaemia/reperfusion injury.

Fig 78: The villus countercurrent exchange of oxygen



Non-pulsatile perfusion may be sufficient to maintain the pO_2 of the majority of the villus to a similar extent as pulsatile perfusion, but pulsatile perfusion may have advantages in maintaining flow and oxygenation to the villus tips.

Support for this hypothesis that damage to the villus tip may cause an increase in gut permeability has been produced by studies examining the effect of cetrimide and hypertonicity on the permeation of test markers. Both of the latter stresses have been correlated with increases in gut permeability and would be expected to damage the villus tip the most (Laker and Menzies 1977, Maxton et al 1986). The villus crypts are also sites of increased permeability (Gumbier 1987, Marcial et al 1984), but because they are situated at the base of villi, they may be expected to be less prone to hypoxic damage.

The absorptive capacity is found along the entire length of the villus. No significant differences were demonstrable between pulsatile and non-pulsatile patients immediately following CPB, although at 5 days MNP patients had impaired transport of 3-O-m-D-glucose and D-xylose absorption. A similar pattern may have been expected for the bubble oxygenator group, but the advantage of pulsatile flow may have been lost by microcirculatory occlusions in these patients. Bubble oxygenators have been shown to produce more retinal micro-occlusions than membrane oxygenators (Blauth et al 1990), and a similar pattern may occur for tissues of the splanchnic bed.

The alteration in haemodynamic parameters was not significantly different in the 4 patient groups. Despite pulsatile perfusion, the SVRI was similar to non-pulsatile patients, which may have diminished the differences between pulsatile and non-pulsatile groups. However, pulsatile patients tended to maintain a higher MAP immediately post-CPB. The benefits of pulsatile perfusion cannot be assessed at a macrovascular level, since ultimately the benefits lie in capillary patency and the adequacy of tissue oxygenation (Matsumoto et al 1968, Shepard and Kirklin 1969).

Pulsatile perfusion has been found to maintain tissue oxygen consumption, whilst non-pulsatile flow results in reduced oxygen $\dot{V}O_2$ (Shepard and Kirklin 1969). Ordinarily, tissue $\dot{V}O_2$ is independent of $\dot{D}O_2$, until it reaches a critical level; below this threshold, $\dot{V}O_2$ decreases with delivery (Grum et al 1984). This implies that $\dot{D}O_2$ is impaired during non-pulsatile perfusion. During hypothermia, cellular protection may be achieved by a reduction in metabolic rate, thus matching delivery with tissue requirements. De Canale et al found that in skeletal muscle post-CPB, with non-pulsatile flow, cellular ATP content was maintained (Del Canale et al 1990). However, the cellular phosphocreatine level decreased, suggesting a reduction in cellular oxidative capacity. The authors postulated that this was secondary to impaired oxygen delivery during CPB, possibly a consequence of arterio-venous

shunting of tissue (Del Canale et al 1990). Unfortunately, a comparison with pulsatile flow was not made.

Despite reductions in gut wall blood flow, as measured by LDF, overall oxygenation of gut tissue may be adequate during the hypothermic phase, since p_{Hit} , p_{Hat} and arterial pO_2 are slightly below or above pre-CPB levels. However, these findings do not preclude the development of an oxygen debt during this period, which may be greater for patients undergoing non-pulsatile CPB. Re-warming and aortic X-clamp release is associated with substantial changes in mucosal oxygenation (p_{Hi}), systemic oxygenation (pO_2), p_{Ha} and arterial $[HCO_3^-]$. The return of LDF towards pre-CPB appears to be closely linked to the cardiac index, which both increase with re-warming during CPB. Despite return of gut wall blood flow towards normal, intramucosal acidosis develops, with a fall in the pO_2 and arterial bicarbonate, which is most pronounced in non-pulsatile patients.

During the re-warming and immediate post-CPB phases there may be a mis-match between $\dot{V}O_2$ and $\dot{D}O_2$. This would elegantly illustrate the concept of 'relative ischaemia' i.e. gut oxygen demand is not met by oxygen delivery. If present, this phenomenon may be particularly acute for patients receiving non-pulsatile flow. Return of pulsatile flow for these patients may stimulate nitric oxide synthesis and release, enabling the increased blood flow to be distributed to areas which were poorly perfused. Pulsatile perfusion by its increased shear-stress on the endothelium (Melkumyants and Balashov 1990), may maintain nitric oxide production and tissue flow during the hypothermic phase better than non-pulsatile perfusion. Re-warming is associated with increases in whole body $\dot{V}O_2$, above pre-CPB levels at the same temperature. The increase in demand in the gut may not be met, since oxygen carrying ability remains limited by the constant haematocrit and cardiac index, which is still below physiological levels. If the small bowel mucosa undergoes a similar

tendency towards hypoxia as the gastric mucosa, then gut cellular injury probably occurs during this period of reperfusion.

The development of mucosal injury may have important consequences for post-operative cardiac function. In animal models, ischaemic mucosal injury has been implicated in the pathogenesis of myocardial infarction (Haglund 1988). This can be prevented in animal models by maintaining gut oxygenation by intestinal insufflation of oxygen (Fiddian-Green 1989 p.349), or by preventing myocardial exposure to the blood released into the portal circulation or reperfusion of the gut (Haglund et al 1984 p.305). Clinically, the development of intramucosal acidosis on the day of cardiac surgery has been found to be highly predictive of death from low cardiac output syndrome or acute myocardial infarction (Fiddian-Green et al 1986). In the presented study, the gastric pHi was not predictive of post-operative morbidity, although the two patients who developed post-CPB duodenal ulcers had a lower pHi than the remaining patients. In large part, this may be due to the limited duration of the study period post-CPB, which was until the patients left the operating theatre, approximately one hour post-CPB. The predictive studies of Fiddian-Green et al continued observation of patients with gastric tonometry into the ICU for a total of 5 hours post-CPB (Fiddian-Green et al 1986).

In summary, this study has found that patients undergoing pulsatile perfusion tend to maintain gut mucosal oxygenation (pHi), pHa and arterial $[\text{HCO}_3^-]$; whilst non-pulsatile flow patients are more pre-disposed to the development of mucosal hypoxia in the re-warming and post-CPB period. Patients who received membrane oxygenation and pulsatile perfusion maintain gut saccharide transport and barrier function better than patients receiving non-pulsatile flow or bubble oxygenation. Pulsatile flow was the most important perfusion factor examined for preventing an increase in gut permeability. Moreover, pulsatile CPB patients recovered their gut permeability faster than non-pulsatile patients. These differences in permeability were

a result of increased permeation of the gut wall in non-pulsatile patients for the disaccharide lactulose. Both patients that developed post-operative GI complications had received non-pulsatile perfusion and had lower mean gastric pHit than those patients without GI complications. A significant correlation was present between gastric pHit and gut permeability; intramucosal acidosis (pHit <7.35) or hypoxia was associated with a higher post-CPB gut permeability. Furthermore, a low gastric pHit (<7.35) was the best predictor of a protracted ventilation time and ICU stay.

These findings may become more important for patients undergoing protracted CPB, since a temporal relationship between CPB time and gut permeability has been previously established (Chapter 5).

Chapter 7

Endotoxaemia & Cytokine production during Cardiopulmonary Bypass

Introduction & Aims

The clinical studies reported in Chapters 5 & 6, have found evidence of mucosal hypoxia with impairment of barrier function as measured by the gut permeability ratio. The relationship of gut permeability to endotoxaemia is unknown. However, before this relationship can be examined the sensitivity of the Limulus amoebocyte lysate (LAL) assay in the context of CPB needs to be addressed, since heparin can inhibit the assay and crystalloid haemodilution can enhance assay sensitivity.

The aims of the studies in this Chapter were:

- (1) to study the effect of crystalloid plasma dilution and heparinisation on LAL assay sensitivity and to develop modifications of the assay to limit these sources of error when investigating patients undergoing CPB,
- (2) to study the relationship between endotoxaemia and TNF α generation,
- (3) to examine the relationship between endotoxaemia during and following CPB and post-CPB gut permeability, gastric intramucosal acidosis and post-operative morbidity.

Background

The concept of the 'whole body inflammatory response' during CPB was introduced by Kirklin et al in 1983 (Kirklin et al 1983). Kirklin et al found that contact activation of complement was due to material dependent blood activation. The activation of complement was later shown to activate neutrophils, which were the mediators of tissue injury. Contact activation of Hageman factor (factor XII) also occurs during CPB with activation of the kinin, fibrinolytic and clotting systems, with resultant platelet and neutrophil activation (Royston 1990). These two systems (complement and Hageman factor) are the main components of the material or contact dependent generation of inflammatory mediators.

However, activation of complement may occur independent of contact activation. This has been observed following release of the aortic X-clamp during CPB and evidenced by increased levels of tissue plasminogen activator (tPA) (Wildevuur 1988). The mechanism of this activation may be via a material independent source, since this activation occurs soon after release of the aortic X-clamp. The activating substance(s) may be released from tissues following return of pulsatile blood flow.

In 1987, Andersen et al, proposed that endotoxin may in part account for the systemic inflammatory response of CPB and reported the presence of circulating endotoxin in patients undergoing CPB (Andersen et al 1987). All patients in this latter study were negative for endotoxin prior to the study and had no signs of infection. Using non-pulsatile flow and a bubble oxygenator, a substantial increase in the endotoxin levels was observed (median 64-95 ng/l). Rocke et al, in 1987, found an increase in endotoxin levels during CPB, which peaked 10-15 minutes after release of the aortic X-clamp. Peak endotoxin concentrations correlated with both the duration of the total CPB time and the duration of the aortic X-clamp time (Rocke et al 1987). None of the patients in these studies had any signs of post-CPB complications (Andersen et al 1987, Rocke et al 1987). However, patients were in NYHA Class I and II; the

significance of CPB-related endotoxaemia may become apparent in patients with more marginal myocardial function (NYHA III and IV). Patients in a poor pre-operative clinical condition may also have impaired immune responses (Hisatomi et al 1989), which may limit their ability to mount a satisfactory response to an endotoxin/bacteraemic challenge.

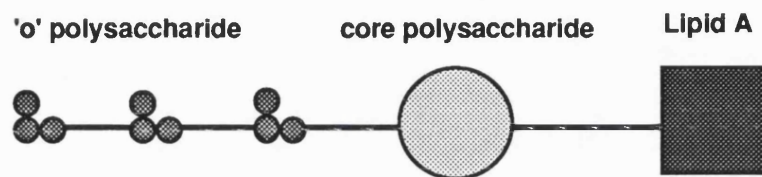
The contribution of endotoxaemia during CPB to post-operative morbidity and mortality is unknown. Following CPB, patients develop pyrexia of non-infective origin (Freeman and Gould 1985), marked peripheral vasoconstriction, hypotension and normal or high cardiac output with a low vascular resistance. When these clinical signs become severe and are associated with renal insufficiency, bleeding diathesis or neurological sequelae, they are collectively termed the 'post-perfusion syndrome' (PPS). The majority of patients can be managed by limited fluid support, but in a minority a severe form of PPS develops. This is evident as increasing fluid requirements as a consequence of underlying increased capillary permeability, with deteriorating lung function from non-cardiogenic oedema (Maggart and Stewart 1987). This may progress to full blown ARDS, which occurs in approximately 1.7 % of patients post-CPB (Fowler et al 1983).

Two studies have demonstrated that the administration of endotoxin may result in the onset of PPS in animals (Hinshaw et al 1981) or early clinical features of septic shock in normal human volunteers (Suffredini et al 1989). The mechanisms involved are complex and are discussed below, but ultimately endothelial cells are damaged, with the lung as one of the first target organs for this injury (Brigham and Meyrick 1986).

The Endotoxin Molecule & putative routes of absorption

Although exotoxins produced by some bacteria can produce septic shock, the majority of cases are a consequence of gram-negative bacteria and the lipopolysaccharide component of the outer bacterial membrane. The outermost part of the endotoxin molecule consists of a series of oligosaccharides that are structurally and antigenically diverse and which are responsible for the O serotype.

Fig 79: The structure of endotoxin



Internal to the 'O' side chains are the core polysaccharides, which are similar in different strains of gram negative bacteria. The lipid A moiety is bound to the core polysaccharides and is highly conserved and responsible for mediating the toxicity of endotoxin (Fig 79). This information may allow the future development of lipid A analogues that can block the toxic effects of endotoxin or act as endotoxin antagonists.

Organisms which are normally resident in large bowel of man shed their outer walls into the gut lumen during their growth and endotoxin is therefore found in large quantities in the colon of man (1-10 mg free endotoxin/ ml free faeces). When this endotoxin is confined to the gut lumen no untoward effects are observed. Ingestion of 150 mg of Boivin-extracted *E. coli* endotoxin by human volunteers did not induce adverse systemic reactions (Emody et al 1974), whereas endotoxin given intravenously (4 ng/kg) may produce symptoms of sepsis (Michie et al 1988). This suggests that under normal physiological circumstances significant portal endotoxaemia does not occur. This has been confirmed by collecting blood from portal and systemic blood in patients without bowel disease (Brearily et al 1985),

whilst those with bowel pathology had portal endotoxaemia (Jacob et al 1977). Circulating endotoxin is partly bound to high density lipoprotein (HDL) and is cleared by liver macrophages, where it persists for some time. After partial degradation, endotoxin fragments are excreted in the bile and eliminated in the stool.

However, the portal vein is not the only splanchnic export route for endotoxin leaving the gut lumen. Superior mesenteric arterial occlusion in rabbits induces endotoxaemia without portal endotoxaemia (Cuevas and Fine 1972) and portal vein occlusion in rats does not prevent the development of systemic endotoxaemia (Olcay et al 1974). The lymphatic system is now acknowledged as an important draining route for endotoxin. Substantial recovery of radioactive endotoxin from the thoracic duct was made following intraperitoneal injection in dogs (Daniele et al 1970). In a rat model, after induction of peritonitis by caecal perforation, endotoxin levels of 38 ng/l were found in the thoracic duct, which were 400-500 times the concentration of portal vein endotoxin levels (Olofsson et al 1986). In these experiments, systemic endotoxaemia was not an early phenomenon, but went in parallel with increasing endotoxin concentrations in the thoracic lymph duct. If account is taken for the higher flow rate of the portal vein compared to the lymphatic system and that in these animal experiments, the increase in systemic endotoxin levels correlated closely with thoracic duct endotoxin concentrations, then the liver would appear to inactivate portally absorbed endotoxin, whilst the thoracic duct is the major transport route of endotoxin from the peritoneal cavity into the systemic circulation. In the context of CPB, macrophage function is impaired (Subramanian et al 1968) and this may predispose patients undergoing CPB to systemic endotoxaemia. However the contribution of the lymphatic system in the transport of endotoxin from the gut has not been studied in detail and the influence of CPB on this transport is unknown.

Uptake of Endotoxin in Disease

The absorption of endotoxin can be precipitated by colonoscopy and biopsy (Kelley et al 1985), although it may be argued that patients who are undergoing such investigation may have underlying colonic pathology which may make them more susceptible to the absorption of endotoxin. Patients with inflammatory bowel disease have provided support to the hypothesis that diseased bowel permits greater permeation of endotoxin. The severity of Crohn's disease has been correlated with the level of systemic endotoxaemia on acute admission, whilst those patients in remission have significantly lower levels of endotoxin (Kruis et al 1984). In this study, although systemic endotoxaemia was absent, patients had anti-lipid A antibodies, suggesting immunization from chronic exposure to endotoxin (Kruis et al 1984). This may have important implications in the evaluation of results from clinical trials aiming to reduce mortality of septic shock by the administration of exogenous monoclonals to endotoxin (Ziegler et al 1991). Patients who have higher circulating levels of the anti-lipid A antibody may have a survival advantage, regardless of the exogenous administration of anti-lipid A monoclonals.

Similarly, children with bowel pathology have been reported to sustain episodes of endotoxaemia. Endotoxaemia was reported in 23 of 47 febrile episodes in 45 children with necrotizing enterocolitis, although only 3 had gram negative bacteraemia. The newborn may be at particular risk, if the barrier to macromolecules has not fully developed. The permeability of the gut to high molecular weight molecules similar in size to endotoxins has been found to be increased in premature infants compared to older children and adults (Scheifele et al 1985). Alterations in blood flow and oxygen delivery during CPB may further compromise gut barrier function in neonates and young children. Endotoxaemia has been recently reported in children undergoing CPB (Andersen et al 1990, Casey et al 1992). Interestingly, children with extremely low cardiac output and poor oxygenation pre-operatively had high levels of circulating endotoxin, which the authors suggested may be due to increased

permeation of gut endotoxin secondary to mesenteric hypoxia (Andersen et al 1990). Support of the latter hypothesis has come from a rat model, in which the absorption of endotoxin following occlusion of the superior mesenteric artery could be reduced by infusion of gaseous oxygen into the bowel lumen (Shute 1977).

Mechanisms of Endotoxin mediated Tissue Injury

Both endotoxin and contact activation by the surface polymers of the extracorporeal circuit can activate complement via the alternate pathway. The anaphylatoxins C3a and C5a that are generated mediate increased capillary permeability, platelet and neutrophil activation and aggregation.

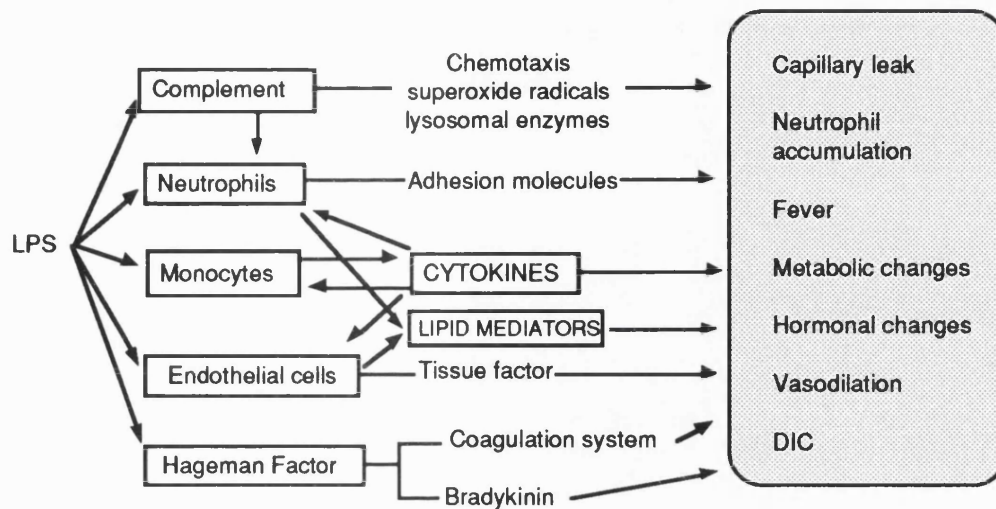
Activated neutrophils have been implicated in the pathogenesis of ARDS, because of the massive neutrophil sequestration which is found in patients with this condition, with pronounced proteolytic and oxidative capacity (Weiland et al 1986). In the canine model, neutrophils incubated in vitro with endotoxin can mediate acute lung injury when re-injected (Welsh et al 1989). The ability of endotoxin to activate neutrophils has been confirmed by isolating neutrophils from patients and exposing them to the same concentrations of endotoxin as that found during CPB (Andersen 1991). However, the absolute concentration of endotoxin may not be important, because endotoxin may act synergistically to activate neutrophils in the presence of other substances. This priming effect has been demonstrated by the ability of trace amounts of endotoxin to produce lung injury in animals whose neutrophils have been exposed to small amounts of a chemotactic peptide (Worthen et al 1987).

Activated neutrophils adhere to each other and to endothelial cells through the interactions of receptors on endothelial cells and ligands on neutrophils. In vitro, endothelial cell receptors mediate injury to endothelium caused by activated neutrophils (Gibbs et al 1990). These receptors are known collectively as adhesion molecules. Two of these adhesion molecules, intercellular adhesion molecule 1

(ICAM-1) and endothelial leucocyte adhesion molecule 1 (ELAM-1), have been shown to mediate lung injury in different settings in animal models (Barton et al 1989, Mulligan et al 1991). The neutrophil ligand for ICAM-1 is the CD18 molecule and antibodies against CD 18 molecule prevent pulmonary oedema in gram negative sepsis in pigs (Walsh et al 1991). The expression of CD 18 and CD 11b, both components of the neutrophil Mac-1 adhesion molecule, has been found to be increased following CPB (AM Gillinov, unpublished work, presented at 29th Meeting, The Society of Thoracic Surgeons, 25-27 January 1993). Furthermore, plasma levels of ICAM-1 were increased when bubble oxygenation was employed during CPB, but not when a membrane oxygenator was used (AM Gillinov, unpublished work, presented at 29th Meeting, The Society of Thoracic Surgeons, 25-27 January 1993). The adhesion of neutrophils is also promoted by the induction of endothelial adhesion molecule expression by endotoxin and cytokines (TNF and IL-1) (Schleimer and Rutledge 1986).

Hageman factor (factor XII) is central not only to contact activation during CPB but can also be activated by endotoxin. Activated factor XII triggers the intrinsic and coagulation pathway through activation of factor XII and endothelial cells and macrophages to produce tissue factor (Fig 80). Unregulated activation of these pathways may lead to disseminated intravascular coagulation (Warr et al 1990). Activated factor XII can also convert pre-kallikrein to kallikrein. Kallikrein in turn cleaves high molecular weight kininogen to release bradykinin, a potent hypotensive agent, which may also increase gut permeability. Intestinal endotoxaemia in rats has been induced by intravenous injection of serotonin and bradykinin (Cuevas and Fine 1973).

Fig 80: The deleterious effects of endotoxin



LPS: Lipopolysaccharide DIC: Disseminated intravascular coagulation

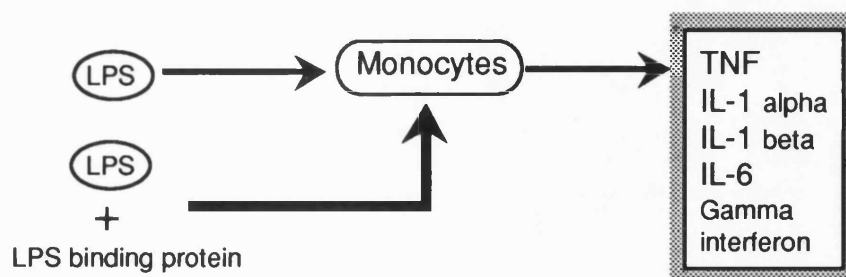
The hypotension of septic shock may be mediated in part by the release of vasoactive amines, but predominantly by the generation of nitric oxide (NO). Endotoxin can induce an isoform of NO synthase (NOS) in phagocytic cells which can aid in the destruction of microbial pathogens (Marletta 1989). However, at higher concentrations of endotoxin, the immunological benefit of NO production is lost by the induction of NOS activity in endothelial cells (Radomski et al 1990) and vascular smooth muscle (Busse and Mulisch 1990). This induction of NOS contributes to the hyporeactivity to adrenergic agonists and to development of septic shock (Wright et al 1992).

There is evidence that some of the effects of endotoxin may also be mediated by lipid mediators such as platelet activating factor (PAF) and arachidonic acid metabolites. In vitro studies suggest that arachidonic acid metabolites exert control over the production of TNF and IL-1. PAF, LTB₄ and C3a are potent stimuli for the production of cytokines (Dinarello et al 1984, Dubois et al 1989, Haeffner-Cavaillon et al 1987), whilst PGE₂ inhibits in vitro TNF and IL-1 production. Injection of PAF can reproduce many of the features of endotoxaemia, which may be inhibited by a selective PAF antagonist (Chang et al 1987). The effect of CPB on PAF is unknown,

but LTB₄ levels peak during CPB following aortic X-clamp release (Gu et al 1992), which is a neutrophil activator. The role of the related peptidoleukotrienes LTC₄/D₄/E₄ in the context of endotoxaemia remains unclear, although in one dog study, haemodynamic stability was achieved using a LTD₄/LTC₄ antagonist following endotoxin injection. Thromboxane A₂ (TxA₂) and its role in endotoxaemia is better defined. TxA₂ causes pulmonary hypertension during endotoxaemia, but is not responsible for increases in pulmonary capillary permeability (Winn et al 1983).

As previously discussed in the introductory chapter (Chapter 1), the monocytic cells have a pivotal role in the mediation of the biological effects of endotoxin. Firstly they can remove and detoxify endotoxin from the blood, although the clearance by the Kupffer cells of the liver may be bypassed by lymphatic transport of endotoxin. Secondly, endotoxin-stimulated monocytes elaborate cytokines such as TNF and IL-1. Several binding sites for endotoxin have been described for endotoxin on the macrophage cell surface. Endotoxin may also interact with macrophage surface receptors after binding to plasma acute-phase protein called lipopolysaccharide-binding protein (LBP), which binds to the lipid A moiety of endotoxin (Fig 81). Endotoxin-LBP complexes are a ligand for the monocyte CD14 receptor and can stimulate TNF production by macrophages at concentrations far below those required by endotoxin stimulation alone (Wright et al 1990).

Fig 81: Endotoxaemia and cytokine production.



LPS: Lipopolysaccharide, TNF: Tumour necrosis factor,
IL-1: Interleukin 1, IL-6: Interleukin 6

Cytokine production during CPB, in particular TNF, IL-1 & IL-6, has been previously discussed in Chapter 1. Both IL-1 and TNF, when administered to animals can induce the characteristics of endotoxic shock. Therein lies their importance, since the modulation of cytokine production and binding to target tissues may provide a therapeutic window for the prevention of endotoxaemia-associated morbidity and mortality.

Endotoxin & Cardiac Dysfunction

Although cardiac output is normal or increased in endotoxaemia, ventricular function is abnormal. Both left and right ventricular ejection fractions are reduced with increased end-diastolic ventricular volumes (Parker et al 1990). Ventricular dilatation has been noted to occur within 48 hours of severe sepsis, with reversal after 5 to 10 days in survivors (Ognibene et al 1988). Myocardial depression in animal models has been linked to the dose of infecting organisms, but both gram-positive bacteria (not releasing endotoxin) and gram-negative organisms can induce similar cardiac dysfunction (Natanson et al 1989). Until recently, myocardial depression was postulated to occur via global myocardial ischaemia. However coronary blood flow in humans with sepsis has been found to be normal or elevated (Cunnion et al 1986). Lefler and Martin postulated over 20 years ago that myocardial depression in shock may be due to a circulating substance (Lefler and Martin 1970). Recent support of their hypothesis has been forthcoming from studies showing depression of rat myocardial contractility in vitro following exposure to serum obtained from humans during the acute phase of septic shock (Parrillo et al 1985). Subsequently, studies have documented that patients with septic shock whose serum contains this myocardial depressant activity have lower ejection fractions, larger end-diastolic volumes, higher pulmonary artery wedge pressures and higher peak blood lactate concentrations than those without such activity (Reilly et al 1989). The myocardial depressant factor(s) has not been identified, but studies suggest it is a water-soluble substance with a molecular weight of 10,000 to 30,000 (Parrillo et al 1985). The

cytokines share these latter characteristics and TNF in vitro produces a concentration-dependent depression of myocardial cell shortening by a calcium-dependent mechanism (Kumar et al 1991).

In summary, endotoxin results in the generation of a large number of mediators and activated inflammatory cells, which after interaction with endothelial cells may cause microcirculatory and cellular dysfunction and ultimately tissue/organ failure.

The Measurement of Endotoxin

Background

Levin and Bang introduced the *Limulus* amoebocyte lysate (LAL) assay for the detection of endotoxin in 1964 (Levin and Bang 1964). This assay is based upon the principle that bacterial polysaccharide specifically activates the clotting cascade in blood of the American horseshoe crab *Limulus polyphemus* and results in the gelation of a lysate of the blood corpuscles (amoebocytes). This reaction is combined with a chromogenic substrate which enables quantification with a spectrophotometer (Zhang et al 1988). The sensitivity of the test has been estimated to be 0.8 ng/l using standard endotoxin with very good correlation ($r=0.98$) using more sophisticated assay techniques, such as gas/ liquid chromatography and mass spectrometry (Brandtzaeg et al 1992).

Recently, the specificity of the LAL assay has been questioned by Kakinuma et al who found that β -glucans can also activate the LAL system, although by a different mechanism (Kakinuma et al 1981). Morita et al isolated two components, one sensitive to endotoxin, named factor C, and the other to β -glucan, named factor G (Morita et al 1981). These findings led some workers to develop another assay system using the Asian horseshoe crab *Tachypleus tridentatus* (TAL), which lacks factor G (Obayashi et al 1985). However, the reactivity of (1-3)-D-glucan with the LAL system is 1000 times lower than with endotoxin (Zhang et al 1988). Furthermore, Soderhall et al studied the reactivity of several (1-3)-D-glucans with LAL and showed that naturally occurring glucans, including zymosan from fungal walls, did not activate the enzyme system in LAL, but derivatized glucan, which is a carboxymethylated curdlan, could activate the clotting enzyme in LAL (Soderhall et al 1985). This derivitised glucan was the same that had been employed in the studies by Kakinuma et al and Morita et al, who showed that this glucan could activate the lysates in the Asian horseshoe crab (TAL) (Kakinuma et al 1981, Morita et al 1981). Soderhall et al therefore concluded that artificially derivitised glucans may activate

the LAL system, but endotoxin-free naturally occurring (1-3)- β -D-glucans were inactive except at high concentrations (Soderhall et al 1985). Thus the LAL test has sufficient sensitivity and specificity to be applied to routine determination of endotoxins during CPB and has been the assay of choice for measurement of endotoxin in recent reports (Andersen et al 1987, Andersen et al 1989, Andersen et al 1990, Rocke et al 1987).

Limitations of the LAL Assay during CPB: In vitro validation

CPB results in profound changes in the haematocrit as a result of haemodilution by the prime of the pump-oxygenator. This may alter the sensitivity of the assay because human plasma contains natural inhibitors (Levin et al 1970). Haemodilution during CPB may therefore result in overestimation of endotoxin levels. Sample dilution and/or heat treatment has been advocated (Levin et al 1970, Cohen and McConnell 1984), but an optimum regimen has not been determined. Furthermore, heparinisation which occurs routinely during CPB, may also interfere with the assay (McConnell and Cohen 1985, Marcum and Levin 1989) and needs to be standardized, if the sensitivity of the assay is to be maintained for samples taken during CPB.

The objectives for this in vitro study were to investigate the effect of haemodilution and heparinisation on the endotoxin assay and to formulate a methodology to reduce sources of error in the assay technique.

Materials & Methods**Reagents and Plasma Preparation**

Endotoxin was measured using a modified form of the LAL chromogenic microassay using commercially available reagents (Coatest Endotoxin, Kabi, Quadrachem, Epsom, Surrey, UK). Pyrogen free equipment and an aseptic technique were used throughout. Blood samples were collected in polystyrene tubes (Falcon, Becton Dickson, Lincoln Park, New Jersey, USA) to which sodium heparin (mucous preservative free, CP Pharmaceuticals Ltd, Wrexham, UK) had been added; blood heparin concentration was 15 to 43 U/ml. Samples were centrifuged immediately at 2000g for 10 minutes at 4°C, the plasma removed and stored at -40°C prior to the assay. Plasma samples were diluted in either pyrogen free water (Antigen Ltd, Roscrea, Ireland) or Hartmanns solution (Baxter Healthcare) and heat treated (75°C for 5 minutes) prior to the assay.

Chromogenic Assay

After vigorous vortex mixing, 50 µl of either sample or diluted standard endotoxin solution (*E. coli* 0111:B4) were pipetted into replicate wells of a flat bottomed ELISA (enzyme linked immunosorbant assay) plate (Nunclon Delta). The plates were equilibrated at 37°C for 15 minutes and 50 µl of LAL (reconstituted in water and preheated) were added. After the initial incubation (15 to 25 minutes) 100 µl of preheated chromogenic substrate (AC-Ile-Glu-Gly-Arg-pNA) in 50 mmol/l Tris buffer; pH 9.0 were added. ELISA plates were agitated automatically for 45 seconds immediately after the addition of both the LAL and the substrate. During the second incubation (less than 30 minutes), plate well absorbance values were measured at 405 nm every 90 seconds using a Titertek Twin reader Plus plate reader with the internal plate incubator adjusted to 37 °C.

Data Analysis & Statistics

Data are presented in one of two ways; either as the mean of the blank subtracted absorbance values (blank = 50 µl of diluted sample + 150 µl water), where the highest subtraction was less than 1.0 Alternatively, data are presented as blank subtracted absorbance change per minute. Values are presented as means ± standard error of the mean and statistical significance determined using the paired Student's test, with two tailed p values.

Protocol

(i) In the absence of plasma, the effect of heparin on the assay was investigated with and without prior heat treatment of the samples (75°C for 5 minutes). Heparin concentrations of 0, 0.3, 3 and 30 U/ml (n=5) were investigated.

(ii) The effect of plasma on the chromogenic assay was investigated. In order to optimise endotoxin recovery, different plasma sample heat treatment regimens were used i.e 60°C for 30 minutes, 75°C for 5 minutes or 100°C for 5 minutes (n=5). The test standards with and without plasma were spiked with 0.6 EU/ml endotoxin.

(iii) An in vitro reconstruction of CPB with respect to blood sample heparin fluctuations was performed in order to establish whether or not the heparin administered to the patient during CPB could compromise the reliability of the chromogenic assay. This was achieved by diluting plasma samples three fold with sterile water prior to heparinisation. Heparin concentrations of 20 and 40 U/ml were employed with complementary test curves performed with 20+3 and 40+ 3 U/ml heparin (n=6). Endotoxin was added at a dose of 0.3 EU/ml.

This experiment was repeated for plasma heparin concentrations of 15, 25 and 35 U/ml with complementary curves for 15+3, 25+ 3 and 35+3 U/ml heparin (n=5). Endotoxin was added at a dose of 0.4 EU/ml and plasma samples diluted three fold with Hartmanns solution prior to heparinisation.

Subsequent modifications were performed to the method were made to overcome this effect.

(iv) The effect on the chromogenic assay of plasma dilution with Hartmanns solution (to the extent which can occur during CPB) was investigated.

(v) The detection limit of the assay was determined. A standard curve of the change in absorbance at 405 nm per minute versus plasma endotoxin concentration (0 to 80 ng/l; 1 ng endotoxin = 12 EU) was plotted using standards consisting of normal heparinised (25 U/ml) plasma spiked with endotoxin and diluted three fold in heparinised Hartmanns solution (n=5), to simulate CPB haemodilution and heparinisation.

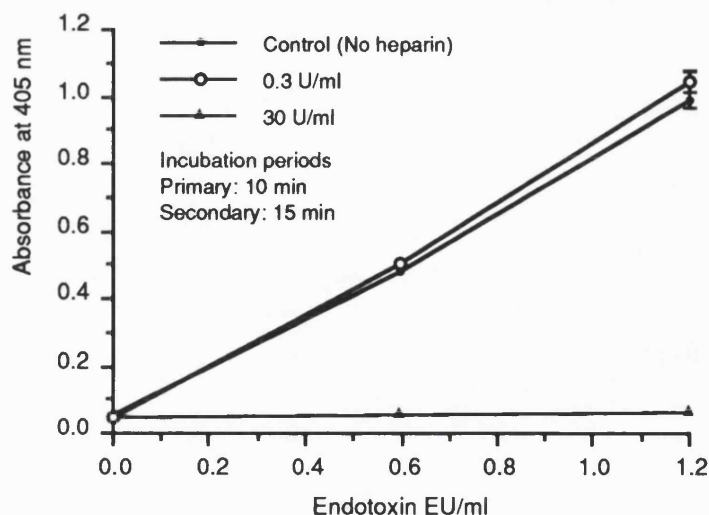
(vi) The chromogenic assay method was modified in order to minimise the detrimental effects of heparin and crystalloid haemodilution and the endotoxin detection limit of the modified assay was determined.

Results

Heparin & the Chromogenic Assay

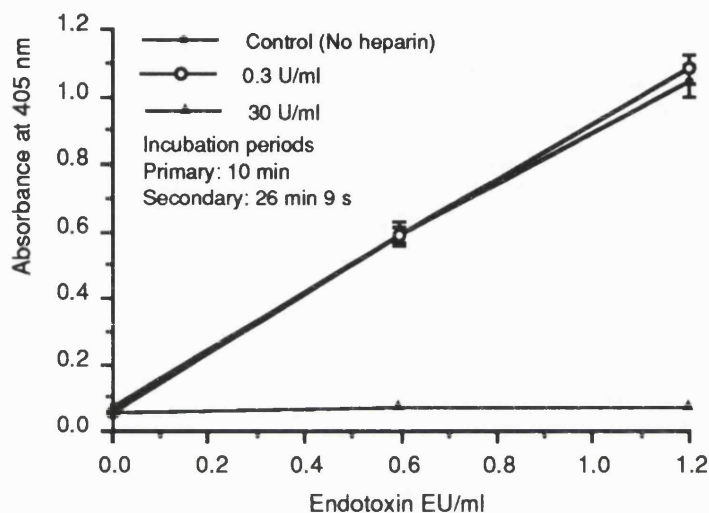
At an endotoxin concentration of 1.2 EU/ml, 0.3 U/ml heparin did not exert a significant effect on the chromogenic assay whereas at a concentration of 30 U/ml it was profoundly inhibitory ($p < 0.001$) (Fig 82).

Fig 82: The effect of heparin on the endotoxin assay



Following heat treatment (75°C for 5 minutes) of control and test standards prior to the assay the effect of heparin on the endotoxin assay was not modified (Fig 83).

Fig 83: The effect of heparin (in the absence of plasma) on the LAL assay with heat treatment of standards at 75°C for 5 minutes



The effect of Plasma & Sample Heat Treatment

In the absence of heat treatment, endotoxin recovery from plasma was substantially reduced (Tab 24). Without plasma, endotoxin recovery was not affected by heat treatment regimens of 60°C for 30 minutes and 75°C for 5 minutes, whereas it was significantly reduced by heating to 100°C for 5 minutes ($p < 0.001$). Endotoxin recovery from plasma was increased significantly by all three heat treatment regimens, but enhanced the most by heating to 75°C for 5 minutes.

Tab 24: Percentage recovery of endotoxin & heat treatment regimens

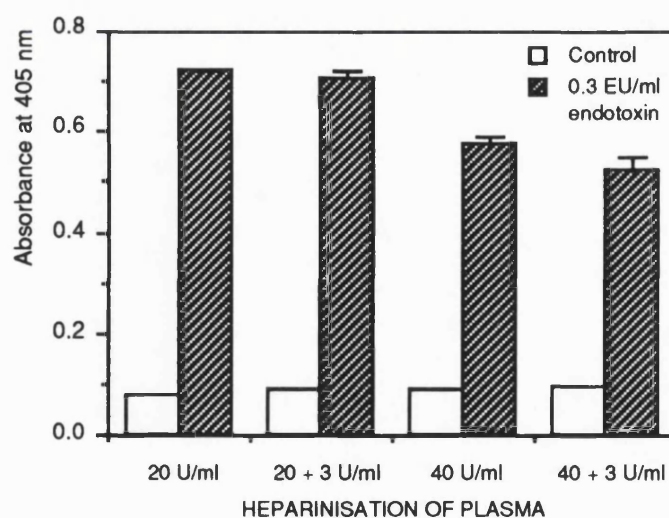
	Without plasma	With plasma
No heat treatment	100	1.19
60°C for 30 minutes	101.6	81.8
75°C for 5 minutes	98.8	117.5
100°C for 5 minutes	1.8	24.2

Standard curves, with and without plasma were plotted over the endotoxin concentration (range 0 to 0.6 EU/ml). The absorbance at 405 nm at 0.6 EU/ml corresponded to 100% recovery. Test standards with and without plasma were spiked with 0.6 EU/ml endotoxin and were subjected to the above heat regimes (n=5). Incubation periods: primary 15 min; secondary 10 min 29 s.

The effect of Plasma Heparin Fluctuations

The effect on the assay of plasma heparinisation with 20 or 23 U/ml was not significantly different (Fig 84), whereas the addition of 3 U/ml of heparin to plasma previously heparinised with 40 U/ml increased the inhibition of the assay but not significantly.

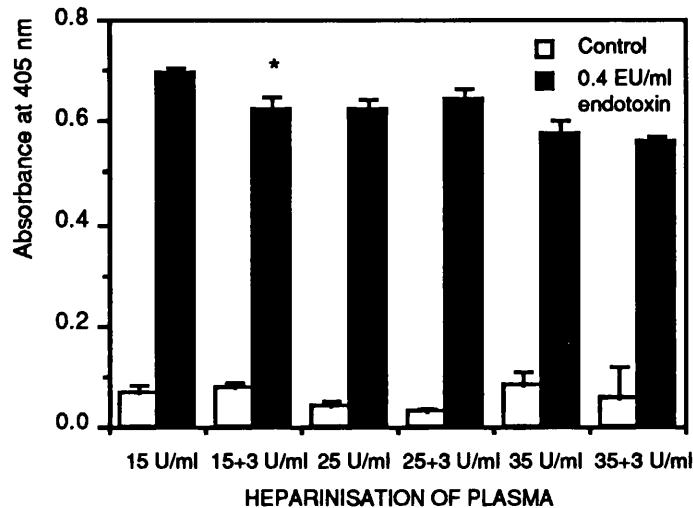
Fig 84: The effect of plasma heparin level fluctuations on the endotoxin chromogenic assay (endotoxin 0-0.3 EU/ml).



Incubation periods; primary: 25 min, secondary: 19 min 5 sec

The results of a similar experiment repeated with control and test heparin concentrations of 15, 15+3, 25, 25+3, 35 and 35+3 U/ml using endotoxin concentration of 0-0.4 EU/ml is illustrated in Fig 85.

Fig 85: The effect of plasma heparin fluctuations of the endotoxin chromogenic assay (endotoxin 0-0.4 EU/ml).



* $p < 0.05$ compared to original plasma heparinisation of 15 U/ml

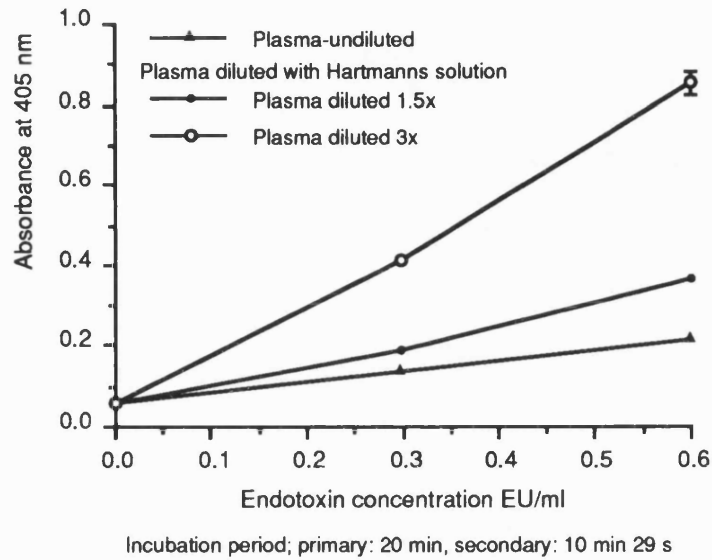
Incubation period; primary: 20 min; secondary: 13 min 37 s

The inhibitory effect of additional heparin was only significant ($p < 0.05$) when the original heparin concentration was 15 U/ml.

The effect of Plasma Haemodilution with Hartmanns Solution

At a constant heparin concentration of 40 U/ml, dilution of plasma caused a progressive enhancement (or loss of inhibition) of the chromogenic assay.

Fig 86: The effect of plasma haemodilution with crystalloid solution on the LAL chromogenic endotoxin assay

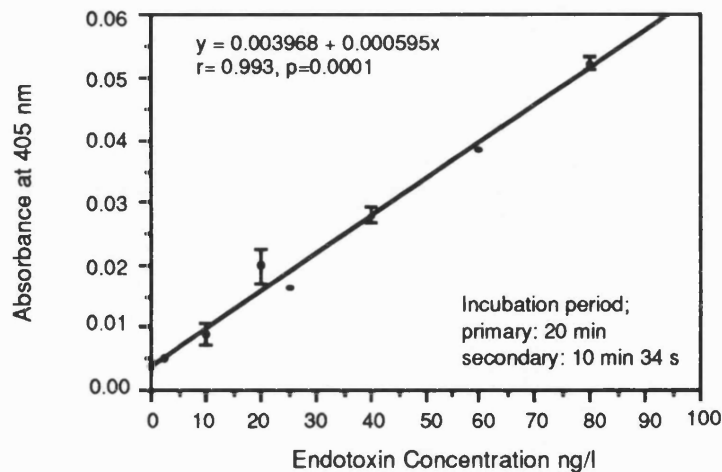


The differences between all three groups at the endotoxin level of 0.3 EU/ml are significant ($p < 0.001$, Fig 86).

The detection limit of the Modified Endotoxin Assay

The detection limit of the modified LAL assay (heparinisation at 25 U/ml of plasma haemodiluted threefold by Hartmanns solution) was 2.5 ng/l (0.03 EU/ml) endotoxin with a coefficient of variation (CV) of 21%; at 10 ng/l the CV was 27% (Fig 87).

Fig 87: The detection limit of the modified LAL chromogenic assay endotoxin assay



Discussion

This study has found that heparin level fluctuations and progressive crystalloid haemodilution may compromise the reliability of the Limulus chromogenic assay. The observation that 30 U/ml heparin causes significant inhibition of the LAL assay confirms previously reported findings; McConnell and Cohen found a 90% reduction in detectable endotoxin at a similar heparin concentration (McConnell and Cohen 1985). Furthermore, the inhibitory effect of 30 U/ml heparin on the assay could not be counteracted by heat treatment.

An alternative approach to counteracting the inhibitory effect of heparin on the assay is to neutralise it in vitro with protamine sulphate. Cohen and McConnell showed that the inhibitory effect of up to 100 U/ml heparin could be neutralised by the addition of protamine sulphate (Cohen and McConnell 1984). However, protamine also has an inhibitory effect on the assay even under conditions when heparin and protamine are stoichiometrically balanced. Alternatively, the effect of heparin level fluctuations on the chromogenic assay may be reduced by sample dilution, but this may also reduce assay sensitivity, if very high dilution factors are used. Another option is to add a large amount of heparin to the blood at the time of collection, to render heparin fluctuations associated with CPB insignificant. The addition of 3 U/ml heparin (a level comparable to plasma level fluctuations during CPB) causes the smallest perturbation to the assay if blood samples are heparinised with 25 U/ml at the time of collection as opposed to 15, 35 or 40 U/ml. The results suggest that if plasma is diluted three-fold, the reliability of the assay may be enhanced if blood is heparinised to a level of 25 U/ml as opposed to a lower or higher value at the time of sampling.

Recent evidence suggests that lipoproteins may be responsible in part for the inhibitory effect of plasma on the LAL assay (Cavaillon et al 1990). This inhibitory action of plasma may be reduced by heat treatment of the samples; the optimum

sample heat treatment regimen of those selected was 75°C for 5 minutes. However, during CPB, because of crystalloid haemodilution, the sensitivity of the LAL assay would be enhanced, with overestimation of the endotoxin concentration. This may be overcome by measuring the haematocrit at the time of blood sampling and making an appropriate correction to a standard haematocrit. This approach enables the assay sensitivity to be increased with respect to haemodilution.

Using the kinetic method, the refined LAL assay has a detection limit of 2.5 ng/l (coefficient of variation = 21%). However, in view of the recognised susceptibility of the assay to interference, a detection limit of 10 ng/l is more appropriate.

Endotoxaemia & TNF α production following CPB: The influence of Oxygenator type & Pulsatile Perfusion

Introduction

The aims of this study were:

- (i) to investigate the relationship between endotoxaemia and TNF α production as a result of CPB.
- (ii) to examine the effect of membrane or bubble oxygenation and pulsatile or non-pulsatile blood flow on endotoxaemia and TNF α production.
- (iii) to relate endotoxaemia with gut permeability ratio and gastric pHi measurements described in Chapter 6.

Materials & Methods

Patients & CPB Protocol

The same patients which were reported in Chapter 6 were also investigated as regards endotoxaemia and TNF α production i.e. four groups randomized into BP (n=7), BNP (n=7), MP (n=7), MNP (n=7).

Endotoxin

The plasma endotoxin was measured using the modified LAL chromogenic assay described in the validation study in the previous section. Corrections for haemodilution were made using the following equation:

$$\text{endotoxin corrected} = \text{endotoxin uncorrected} \times (\text{Hct}_{\text{ind}} - \text{Hct}_{\text{s}} / \text{Hct}_{\text{ind}})$$

where Hct_{ind} is the arterial haematocrit following induction of anaesthesia and Hct_s is the haematocrit of any subsequent arterial blood sample.

TNF α

TNF α was measured using the Predicta enzyme immunoassay (Genzyme Corporation, Cambridge, MA, USA), which has an assay sensitivity of 10 ng/l. This immunoassay contains a 96 well microliter plate pre-coated with monoclonal antibody to TNF α . 100 μ l of patient plasma, standard or control is added to each well. The wells are then washed and a biotin labelled polyclonal antibody to TNF α is added which attaches to biotin in the immune complex on the plate. The wells are washed and a substrate (peroxide) and chromagen (tetramethylbenzidine) are added, producing a blue colour in the presence of peroxidase enzyme. The colour reaction is then stopped by the addition of acid, which changes the colour to yellow. The intensity of the yellow colour is in direct proportion to the amount of TNF α present in the sample, standard or control. The absorbance of each well is read at 450 nm and a standard curve constructed to quantitate TNF α concentrations in the controls and samples. All patient samples and standards were measured in duplicate and the mean value determined.

Although it is recommended by the company that plasma samples be diluted two-fold, preliminary assays found very low levels of TNF α in CPB samples. Therefore, to increase the sensitivity undiluted plasma samples were assayed.

Sampling Protocol

Arterial blood samples were taken into appropriate tubes, and plasma harvested and stored at -40°C until assayed for endotoxin and TNF α . Blood samples were taken from a radial artery line or from the pump-oxygenator during CPB; these were taken at the following time points:

1. Following induction of general anaesthesia.
2. Ten minutes following the institution of CPB.
3. Ten minutes post aortic X-clamp release during CPB.

4. Immediately following the termination of CPB
5. One hour post-CPB
6. Two hours post-CPB.

The crystalloid prime from the pump-oxygenator was also sampled from 6 patients after the circuit had been allowed to circulate for 30 minutes to look for exogenous endotoxin.

Statistical Analysis

Due non-normal distribution of data the Mann-Whitney U test was used to assess for differences between study groups and the Wilcoxon signed-rank test to assess for differences within each group. All values are the mean \pm standard error of the mean. Spearman's rank-order correlation was used to assess for the relationship between endotoxin levels and patient and CPB perfusion parameters.

Results

Endotoxin

(1) The pump-oxygenator prime

The mean endotoxin concentration of pump priming fluid was 8.3 ± 3.2 ng/l (n=6).

(2) Plasma Endotoxin

There were no significant differences between patients receiving bubble or membrane oxygenation. Patients in both groups showed the same increase in endotoxin levels following release of the aortic X-clamp and in the re-warming phase of hypothermic CPB (Fig 88 & 89; the time axis is a non-linear scale).

Fig 88: Endotoxaemia during & following CPB with bubble oxygenation

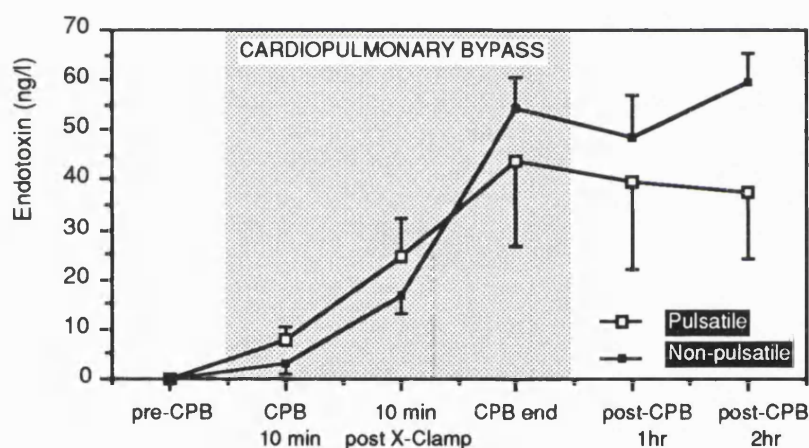
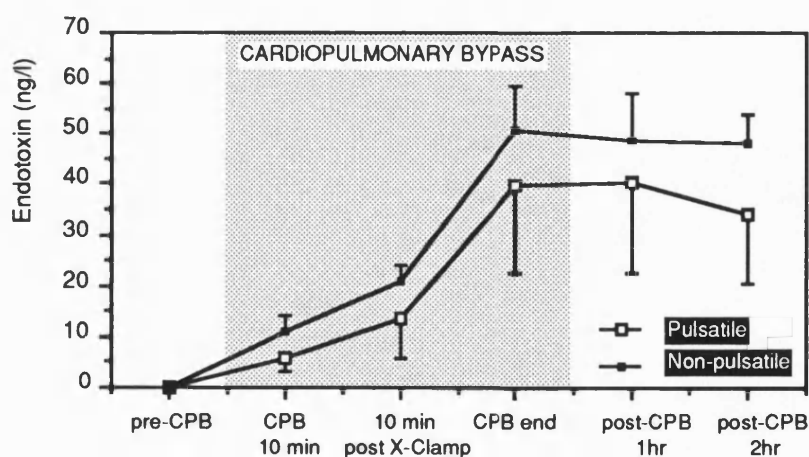
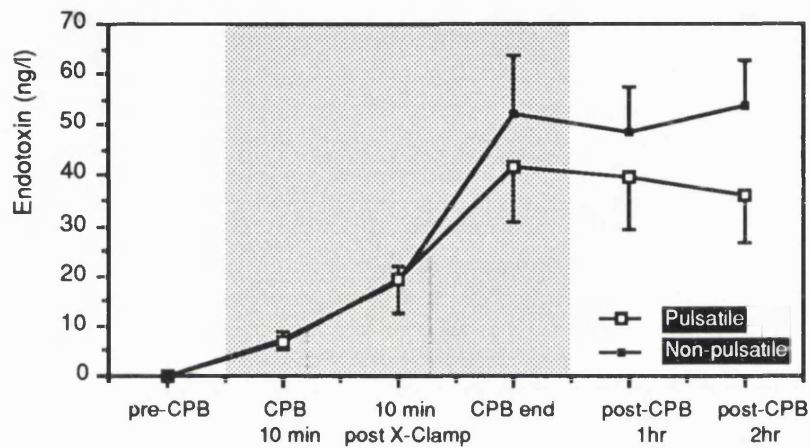


Fig 89: Endotoxaemia during & following CPB with membrane oxygenation



Patients who received pulsatile perfusion maintained lower plasma endotoxin levels in the re-warming and immediate post-CPB periods. This difference between pulsatile and non-pulsatile patients was greatest 2 hours post-CPB (Fig 90; the time axis is a non-linear scale); pulsatile patients had a mean endotoxin of 35.8 ± 8.9 ng/l compared to 53.8 ± 8.9 ng/l for non-pulsatile patients ($p=0.10$).

Fig 90: Endotoxaemia during & following CPB: pulsatile versus non-pulsatile perfusion



TNF α

There were no significant increases in plasma TNF α during CPB or in the post-CPB period. The membrane pulsatile group had the largest increase in TNF α levels, but this was due to one patient, who had elevated TNF α levels pre-CPB (Tab 25).

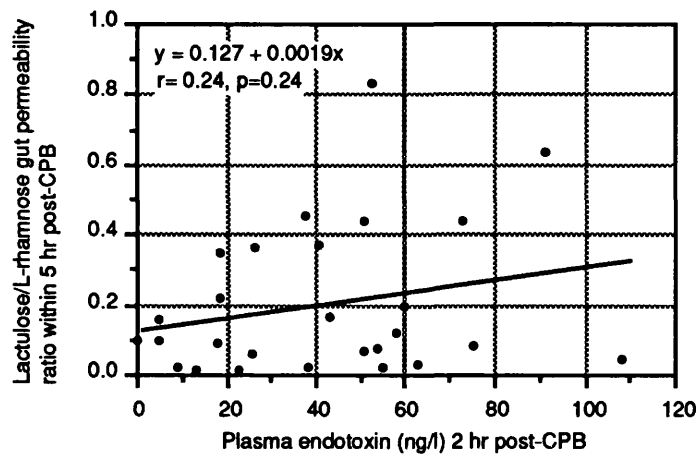
Tab 25: Tumour necrosis factor alpha production (ng/l) during & following CPB

Sample time	BUBBLE		MEMBRANE	
	Pulsatile	Non-pulsatile	Pulsatile	Non-pulsatile
Pre-CPB	0	0.16 \pm 0.16	3.29 \pm 3.10	0
CPB +10 min	0.21 \pm 0.21	0	2.50 \pm 1.90	0
+10 min post aortic X-clamp release	0.47 \pm 0.47	0.68 \pm 0.33	2.70 \pm 2.60	0
CPB end	0.51 \pm 0.37	0.56 \pm 0.36	3.70 \pm 3.20	0
Post-CPB +60 min	0.79 \pm 0.44	1.99 \pm 0.58	5.10 \pm 3.30	1.10 \pm 1.10
Post-CPB +120 min	2.10 \pm 1.30	1.89 \pm 0.64	6.86 \pm 4.70	1.00 \pm 1.00

The Correlation of Endotoxaemia with Patient and Perfusion Parameters

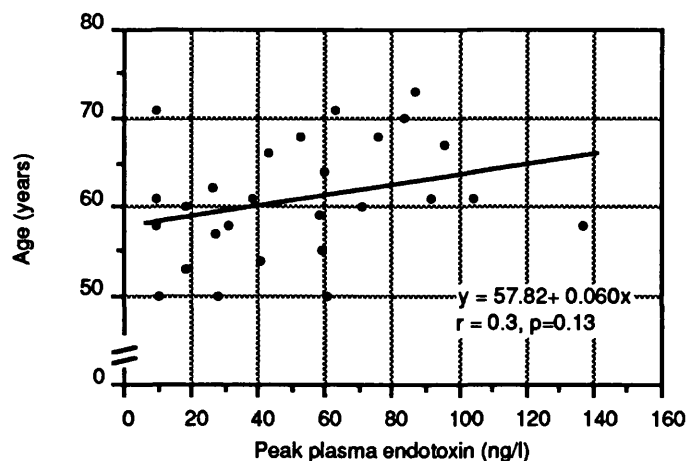
There was no evidence for a linear correlation between the endotoxin plasma levels measured 2 hours post-CPB and the gut permeability, which was assessed between 2-5 hours post-CPB ($r=0.24$, $p=0.24$, Fig 91).

Fig 91: The correlation between post-CPB endotoxaemia & gut permeability



Similarly, the correlation between peak endotoxaemia and the gastric pHit ($r=0.17$, $p=0.39$), the aortic X-clamp time ($r=0.14$, $p=0.48$), the total CPB time ($r=0.04$, $p=0.83$) was poor. The best correlation was found between the age of the patient and peak endotoxaemia ($r=0.30$, $p=0.13$, Fig 92), but this was still non-significant.

Fig 92: The correlation between age & peak endotoxaemia



When analysing patient and perfusion parameters with multiple linear regression, the best predictor for the development of endotoxaemia was the age of the patient ($p=0.15$) and the aortic X-clamp time ($p=0.12$). In this analysis a low gastric pHit was defined as pHit <7.35 (Tab 26).

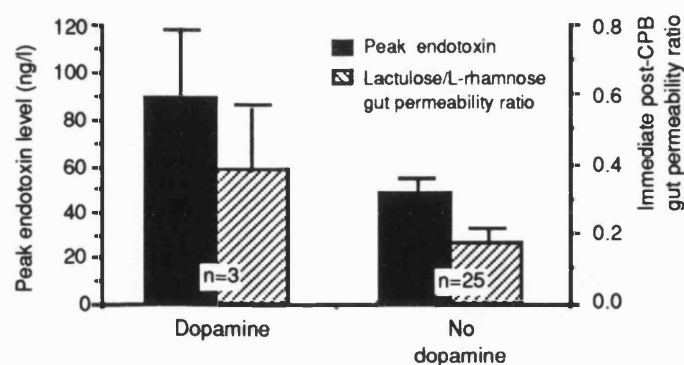
Tab 26: Multiple linear regression analysis: factors determining peak endotoxaemia

Variable:	Beta Coefficient Table				Probability:
	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	
INTERCEPT	-458.113				
Age	1.813	1.224	0.361	1.482	0.157
X-C Time	-2.321	1.436	-0.972	1.617	0.124
CPB Time	1.435	1.107	0.752	1.297	0.212
Oxygenator (B/M)	23.198	15.818	0.343	1.467	0.161
Flow (P/NP)	-2.416	15.88	-0.036	0.152	0.881
Post-CPB Perm	19.57	36.419	0.124	0.537	0.598
Gastric pHit <7.35	49.767	88.697	0.135	0.561	0.582

Post-operative Morbidity & Endotoxaemia

The 3 patients who required inotropic support (dopamine) in the post-CPB period were examined. Although these patients had apparently higher endotoxin levels (88.0 ± 28.5 ng/l versus 47.5 ± 5.9 ng/l, $p=0.12$) and higher immediate post-CPB gut permeability ratio (0.38 ± 0.18 versus 0.17 ± 0.04 , $p=0.19$), these results were not significant (Fig 93).

Fig 93: Peak endotoxaemia, immediate post-CPB gut permeability & post-CPB inotrope requirements.



No correlation was found between peak endotoxaemia and post-operative pyrexia (within 48 hours of CPB), total white cell count or the neutrophil count.

Using multiple linear regression analysis, endotoxaemia was not a good predictor of the total hospital time. However, peak endotoxaemia correlated with the ventilation time ($p=0.04$) and the time spent in ICU ($p=0.03$) by patients (Tab 27 & 28).

Tab 27: Multiple linear regression analysis: the influence of peak endotoxaemia & other patient & perfusion factors on the duration of the ventilation time

Beta Coefficient Table					
Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	7.468				
Age	0.223	0.216	0.254	1.034	0.319
X-Clamp time	-0.397	0.27	-0.933	1.470	0.164
CPB time	0.204	0.203	0.591	1.005	0.332
Oxygenator (B/M)	0.144	3.035	0.012	0.048	0.963
Flow (P/NP)	-6.671	2.996	-0.541	2.227	0.043
Post-CPB Perm	-8.012	6.726	-0.262	1.191	0.253
Gastric pHIt <7.35	7.110	2.737	0.581	2.598	0.021
Peak endotoxaemia	-0.095	0.043	-0.530	2.217	0.044

Tab 28: Multiple linear regression analysis: the influence of peak endotoxaemia & other patient & perfusion parameters on the duration of the time spent in ICU post-CPB

Beta Coefficient Table					
Variable:	Coefficient:	Std. Err.:	Std. Coeff.:	t-Value:	Probability:
INTERCEPT	1.575				
Age	0.430	0.259	0.395	1.659	0.119
X-Clamp time	-0.301	0.324	-0.571	0.928	0.369
CPB time	0.055	0.244	0.129	0.227	0.824
Oxygenator (B/M)	4.377	3.648	0.288	1.200	0.250
Flow (P/NP)	-6.799	3.601	-0.444	1.888	0.080
Post-CPB Perm	-5.308	8.086	-0.140	0.657	0.522
Gastric pHIt <7.35	8.735	3.29	0.575	2.655	0.019
Peak endotoxaemia	-0.127	0.051	-0.571	2.465	0.027

Similarly, non-pulsatile flow also was correlated with a longer ventilation time ($p=0.04$) and ICU stay by patients ($p=0.08$). However, the best predictor for a protracted ventilation ($p=0.02$) and ICU time ($p=0.02$) was a low gastric pHIt (<7.35) (Tab 27 & 28).

Discussion

This study has found that considerable endotoxaemia occurs during CPB. The majority of this endotoxaemia was found to take place following release of the aortic X-clamp and in the re-warming phase of CPB. This would lend support to the hypothesis, that this is due to reperfusion of previously hypoperfused gut tissue, with a washout of endotoxin in the reperfusion period (Jansen et al 1992). A difference between bubble oxygenation and membrane oxygenation was not evident; the difference between the oxygenators may have been attenuated by the use of arterial line filters, which may limit microembolism associated with bubble oxygenators (Blauth et al 1990). However, the difference between pulsatile and non-pulsatile patients was much greater ($p=0.10$), and the data suggest that pulsatile flow may be an important factor in limiting endotoxaemia during CPB. This would again strengthen the notion that endotoxaemia may be a consequence of gut hypoperfusion, since pulsatile flow has been advocated for its ability to maintain microvascular patency and tissue oxygenation (Shepard and Kirklin 1969, Shepard et al 1966).

A strong correlation was not found between gut permeability and peak endotoxaemia. This may be due to differences in the physiochemical properties of the test substances and the endotoxin molecule. The test substances (lactulose & L-rhamnose) are water soluble, whilst endotoxin has both hydrophobic (lipid A moiety) and hydrophilic (polysaccharide groups) domains, which may allow it to permeate both between cells as well as through them. The test is designed to measure the integrity of the enterocyte to enterocyte binding or the competence of the zona occludens. Further support that endotoxin may transgress through enterocytes, as well as between them, is evident from the differences in the size of the endotoxin molecule and lactulose the largest test substance used for measuring gut permeability. Lactulose has a Van der Waals radius of 0.62 nm, but most of the endotoxins released by gut bacteria are substantially larger molecules, with variations in size depending on the bacterial strain. This may explain the presence of endotoxaemia in patients undergoing

pulsatile CPB, some of whom had normal post-CPB gut permeability and the absence of a correlation between gut permeability and endotoxaemia following CPB.

Endotoxin may itself produce rises in gut permeability. A single dose of intravenous endotoxin in human volunteers was found to double gut permeability as determined by urinary lactulose excretion, and was associated with significant rises in plasma noradrenaline and cortisol (O'Dwyer et al 1988). Endotoxin when absorbed from the gut, in the face of impaired cell mediated immunity, may act to further increase its own permeation thus acting in a positive feed back loop to cause more deleterious effects on the cardiovascular system. Patients who had sustained significant thermal trauma (>20% burns of total body surface area) were found to have rises in gut permeability, which was postulated to be a result of cytokine activation in response to thermal injury. However rises in hepatic messenger RNA (mRNA) for TNF were only found when burns injury was associated with microbial invasion (Marano et al 1988). Endotoxin may also modulate gut permeability by altering mesenteric haemodynamics. Navratnam et al evaluated the haemodynamics of the gut in an ovine model of endotoxaemia. They found a 50% reduction in mesenteric blood flow and 100% incidence of bacterial invasion of mesenteric lymph nodes, compared to 15% for sheep not receiving endotoxin (Navaratnam et al 1990). The mechanisms through which endotoxin acts to cause increases in gut permeability are not yet understood, but the generation of oxygen free radical species may be important. In a rat model, pretreatment with allopurinol prevented endotoxin-induced increase in ileal permeability (Deitch et al 1991).

However, the gut is not the only source of endotoxin. Other sources include the crystalloid prime of the pump oxygenator, iced saline used for topical cooling of the heart and intravenous fluids. Human colloid solutions, which have very high levels of endotoxin (Andersen 1991), were not infused into any of the study patients. Nevertheless, endotoxin was detected in the crystalloid prime of the pump-

oxygenator (8.36 ± 3.2 ng/l, n=6). This together with other 'exogenous' endotoxins probably amounts to about 30 ng of endotoxin, which for a 70 kg individual would create a concentration of approximately 6 ng/l. Therefore, it is unlikely that exogenous or 'environmental endotoxin' is responsible for the substantial increase in endotoxin recorded at the end of CPB (mean 47.1 ± 7.8 ng/l, n=28).

In the study conducted by Michie et al on human volunteers, 4 ng/ kg was infused intravenously to investigate the effect of endotoxin on cytokine production (Michie et al 1988). For a 70 kg individual, this would approximate to a systemic concentration of 56 ng/l. In normal human volunteers, an intravenous bolus of purified *E. coli* endotoxin was associated with the production of significant levels of TNF α (270 ± 70 ng/l) 90 minutes after the endotoxin challenge (Michie et al 1988). Associated with this increase in TNF α was the development of chills, headaches, myalgia and nausea, suggesting a cause and effect relationship between endotoxaemia, TNF α production and the onset of a syndrome typical of sepsis. However, although in this study and in those previously reported, comparable levels of endotoxaemia have been found, an association with increased TNF α production has not been a consistent finding (Jansen et al 1992), and no correlation has been established between endotoxaemia during CPB and post-operative pyrexia or leucocytosis. The findings in patients undergoing CPB would be in keeping with critically ill patients with gram-negative sepsis, in whom the presence of TNF α has also been inconsistent (Scuderi et al 1986). Evidence which has been cited to support the role of TNF α in the pathogenesis of endotoxaemia includes the development of pulmonary oedema with lung sequestration of neutrophils following endotoxin challenge (Tracey et al 1986) and C3H/ HeJ mice, which cannot synthesize TNF, are resistant to the deleterious effects of injected endotoxin (Beutler et al 1986).

The disparity between experimental and clinical findings may be due to a number of factors. Firstly, under normal circumstances, biologically active TNF α is present in only small amounts within macrophages, because genes controlling their production are strongly repressed. A stimulus causes increased transcription for mRNA for TNF α , after which macrophages may become refractory to further stimulation (Michie et al 1988). Following release, TNF α has a short half-life (15 to 17 minutes) (Blick et al 1987), which may limit its detection. Furthermore, systemic levels of TNF α may not indicate local release of this cytokine. Local production by endothelium, glial cells and astrocytes may directly influence the neuroendocrine and thermoregulatory centres within the hypothalamus (Lumpkin 1987), to provoke the development of pyrexia. Even with these limitations in mind, the unreproducibility of high TNF α levels in the presence of endotoxaemia may also be due to differences in the potency of endotoxin measured as well as its route of delivery.

In Michie's study, endotoxin was infused directly into the blood stream, which would bypass the important reticuloendothelial barrier presented by the liver, although endotoxins are also transported from the gut via the lymphatic system. Secondly, a bolus was administered, which would be unlike the challenge presented during CPB, when a progressive increase was observed following release of the aortic X-clamp. Finally, the endotoxin administered by Michie et al was purified endotoxin, which was derived from a strain of *E. coli* (Michie et al 1988). Although *E. coli* is found in abundance in the colon of humans, the physicochemical nature of endotoxins absorbed from the shed walls of bacteria may differ in its structure from chemically purified endotoxin. This point has been recently demonstrated by Tesh and Morrison, who found that radiolabelled endotoxin from *E. coli* 0111:B4 released from the bacterial surface in the presence of serum has physicochemical and biological properties different from chemically extracted endotoxin (Tesh and Morrison 1988).

The biological activity of endotoxin, has also been found to be modulated by the binding of endotoxin to serum lipoproteins. Endotoxin-stimulated release of cytokines from monocytes is prevented by binding of endotoxin to these lipoproteins (Flegel et al 1989). Perhaps more importantly in the context of CPB and the presence of the 'whole body inflammatory response', the binding of endotoxin may be altered. Endotoxin binding to lipoproteins, and thus its biological properties, has been found to be modulated by inflammatory sera (Warren et al 1986, Warren et al 1988). The proportion of endotoxin which is biologically active/ inactive during CPB is unknown, but if a high proportion is bound to lipoproteins, it may explain the low level of TNF α detected in this and previous CPB studies.

To establish a clinically significant relationship between endotoxaemia during CPB and post-CPB morbidity/ mortality, three criteria need to be fulfilled. Firstly, an association between CPB and the development of endotoxaemia, secondly the presence of endotoxaemia and the development of post-CPB morbidity and mortality, and finally the ability of blocking or binding molecules to endotoxin or its effector substance(s) to prevent the defined morbidity. At present, from the results of this study and those previously cited, only the first condition has been met i.e the presence of endotoxaemia following CPB. The link with morbidity to date has been weak. In this study and that reported by Andersen et al, post-operative pyrexia was not correlated with the degree of endotoxaemia (Andersen et al 1987). However, this is the first study to report the correlation, using a multiple linear regression model, between peak endotoxaemia and the ventilation and ICU time. The lower levels of endotoxin found in patients receiving pulsatile flow requires confirmation in a larger study, but supports the benefits of pulsatile flow in limiting post-CPB permeability and the degree of intramucosal acidosis reported in Chapter 6.

Although bacterial mediators, namely endotoxin, are postulated as putative mediators of low output syndrome post-CPB, non-bacterial mediators have received less

recognition in the literature. Until the *cause* and *effect* relationship has been established for endotoxin, other bacterial and non-bacterial mediators released from the gut following hypoperfusion/ ischaemia must also be considered as potential candidates for post-CPB morbidity. In this context, endotoxaemia during CPB may be acting simply as a marker of gut damage and other putative substances may be responsible for post-CPB morbidity. In animal models, intestinal ischaemia has been found to result in the release of myocardial depressant substances. Haglund and Lundgren found that myocardial function could be preserved in a cat model if intestinal venous blood was exchange transfused with fresh blood from a donor animal. Following transfusion of the original intestinal venous blood, the animals rapidly developed systemic hypotension, reduced cardiac output, reduced stroke work and reduced myocardial performance (Haglund and Lundgren 1973). In vitro studies applying venous blood from an animal model of gut ischaemia has confirmed the presence of myocardial depressants, as found by the inhibitory action upon rabbit papillary muscle (Lundgren et al 1976). A fraction with a mass of 1500 to 2000 daltons has been identified with Na-K-ATPase inhibitory activity (Lundgren et al 1987).

In summary, this study has found that endotoxaemia occurs during and following CPB, but is not associated with the generation of TNF α . The most likely source is the endogenous store of the GI tract, particularly since considerable gut hypoperfusion has already been found during hypothermic CPB (Chapter 5 & 6). Pulsatile perfusion may be important in limiting gut injury and reducing endotoxaemia. The clinical significance of CPB-induced endotoxaemia remains unproven. No correlation was found between peak endotoxaemia and post-operative pyrexia or leucocytosis. However, peak endotoxaemia was a predictor of protracted ventilation and ICU time, when considered in conjunction with other patient and perfusion factors. Until a stronger specific relationship between endotoxaemia and post-CPB morbidity

becomes established, the clinical significance of CPB-related endotoxaemia must remain in doubt.

Chapter 8

The Adequacy of Gut Oxygen Utilisation in a Canine Model of Cardiopulmonary Bypass

Introduction & Aims

Previous clinical studies reported in this thesis (Chapters 5 & 6) have found impaired gut function and rises in gut permeability following CPB. The tonometric clinical studies (Chapters 3, 4 & 6), found that colonic and gastric intramucosal acidosis occurred in the re-warming phase of CPB following release of the aortic-X-clamp and the immediate post-CPB period. This was despite increases in laser Doppler flow towards or above pre-CPB levels during the same period. These data suggested that gut hypoxia during this period was not due to limited blood flow, but $\dot{D}O_2$ may have been impaired by limited oxygen carrying ability and/or $\dot{V}O_2$ exceeding supply.

The aims of using an animal model were to understand further the pathophysiology of gut injury during CPB by investigating:

- (1) the relationships between gut $\dot{V}O_2$, $\dot{D}O_2$ and blood flow and the effect upon gut tissue oxygenation,
- (2) the changes in gut barrier and absorptive function under more controlled conditions,
- (3) the relationship between the pathophysiology and structural changes in the gut wall.

Materials & Methods

Anaesthesia & Monitoring

Eleven greyhound dogs weighing 19.5 to 29.4 kg (mean 24.6 ± 1.06 kg) were used in this study. Surface area for the calculation of flow during CPB was determined from a standard canine nomogram (Ettinger and Suter 1970 p.209). Dogs were fasted for food but not water for 24 hr prior to experimentation. Anaesthesia was induced with intravenous sodium pentobarbital (20 mg kg^{-1}). Endotracheal intubation was undertaken and general anaesthesia maintained by inhalation of a mixture of nitrous oxide, oxygen and halothane. Femoral cut down was performed and arterial pressure recorded via a strain gauge transducer. The femoral vein was also cannulated for the administration of intravenous maintenance fluids (10 ml/kg/hr). Cardiac monitoring was undertaken using ECG electrodes. Arterial blood gases were performed every 20 minutes to maintain arterial pO_2 , pCO_2 and base excess within normal canine range. Arterial and mesenteric vein samples were sampled at ten minute intervals for 60 minutes prior to CPB, during CPB and post-CPB for determination of haematocrit, haemoglobin, oxygen content. All dogs underwent bladder catheterization for monitoring urine output. Continuous data acquisition was made with a MacLab 8™ (AD Instruments, London, UK) analogue to digital converter onto the hard disc of an Apple Macintosh IIsi computer.

Cardiopulmonary Bypass Protocol

The animal was positioned in a left lateral decubitus position. A right lateral thoracotomy through the 4th interspace was performed in preparation for total cardiopulmonary bypass. A pump-oxygenator (Sarns, Inc, Ann Arbor, Michigan, USA) was employed using a Venotherm bubble oxygenator (Polystan, Copenhagen, Denmark). The pump was primed with Hartmanns solution (2 l) with 25 mmol bicarbonate. Animals were anti-coagulated with 3 mg/ kg heparin prior to insertion of

bypass cannulae. All animals underwent total cardiopulmonary bypass for 90 minutes employing an aortic cannula and single two stage right atrial cannula. Hypothermic bypass with core cooling to 28°C with non-pulsatile flow was undertaken for 45 minutes followed by re-warming to 38°C for 45 minutes. Flow was maintained at 2.4 l/min/m² throughout bypass, without a reduction in flow during cooling. Alpha-stat pH methodology was employed.

Operative Procedures

A midline laparotomy was performed and the superior mesenteric artery located and an electromagnetic probe sited (Gould Statham, Statham Boulevard, Oxnard, California, USA), and secured in position with a detachable gate. A tributary of a small bowel vein was identified and cannulated so the tip of the polyethylene catheter was in the superior mesenteric vein (SMV). The cannula was flushed with heparinised saline and secured via a purse-string suture.

The duodeno-jejunal (DJ) flexure was identified and a 50 cm segment of proximal jejunum isolated with its vascular supply intact. This was achieved by closing the lumen bowel proximally and distally with circumferential silk ties. This proximal isolated segment of jejunum was used to study changes in saccharide absorption and permeation in dogs pre-CPB (n=5) or post-CPB (n=5). The same animals could not be employed to study pre and post-CPB changes during the study period, because the saccharides require at least 48 hours to ensure complete excretion from the body.

At 60 cm from the DJ flexure an anti-mesenteric enterotomy was performed and a sigmoid tonometer, which had been previously de-aired, was inserted (Tonometrics Inc, Worcester, MA, USA). Through the same enterotomy a laser Doppler probe was applied to the mucosa and second probe applied onto the serosa in the same position

as the mucosal probe using a non-constricting suture. Laser Doppler blood flow measurements were made after calibration of the probes as instructed by the manufacturer using the Model MBFD3 (Moor Instruments, Axminster, UK). Continuous LDF serosal and mucosal readings were obtained and recorded onto computer via the MacLab 8™ system.

At 150 cm from the DJ flexure, a full thickness jejunal biopsy was made 60 minutes prior to bypass and a second biopsy was made at 170 cm from the DJ flexure 60 minutes post-CPB. Following completion of all studies, in 6 dogs a segment of jejunum was made ischaemic for 20 minutes by clamping off both the arterial and venous supply, followed by 20 minutes of reperfusion. This gut was excised and used to provide control tissue representing maximal damage, having sustained both CPB and ischemia-reperfusion injury. All tissue was sent for routine histology and neurohistochemical analysis. Analysis of this tissue is described below.

After completion of operative procedure, the bowel was placed back into the abdomen and covered with warm wet packs to limit heat and fluid losses. Following the end of all bowel handling, a 60 minute period for baseline recordings was allowed before commencement of CPB. Post-CPB recordings were made for a further 60 minutes.

Tonometric Analysis

Tonometric sampling was performed by injecting exactly 2.5 ml of fresh saline (not allowed to equilibrate with air) into the tonometer. After 20 minutes of equilibration 1 ml of saline was aspirated and the pCO₂ determined using an ABL4 blood gas analyser (Radiometer, Copenhagen, Denmark). The tonometer readings were adjusted for changes in temperature as previously described (Chapter 3); similarly the arterial pH also adjusted for changes in body temperature.

Saccharide Studies

30 ml of a solution containing the following quantities of saccharide was instilled into the isolated jejunal loop: 0.06 g 3-O-D-M-glucose, 0.15 g D-xylose, 0.3 g L-rhamnose, 1.5 g lactulose, which had an osmolality of 240 mosmol/l. A 3 hour urine collection was made either before CPB (n=5) or after the end of CPB (n=5) for the determination of % urinary excretion of enterally administered saccharides. Urinary saccharide analysis was undertaken by thin layer chromatography as previously described (Chapter 5). Creatinine clearance was determined during the 3 hour collection period by measuring plasma and urinary creatinine levels using a standard formula.

Histology & Neurohistochemical Analysis

(a) Tissue Preparation

Jejunal samples were fixed by immersion in Zamboni's fluid (0.1 mol/l phosphate buffer containing 2% wt/vol paraformaldehyde and 15% vol/vol saturated picric acid) (Stefanini et al 1967). Following thorough washing in 0.01 mol/l phosphate-buffered 0.15 mol/l saline (PBS) containing 15% wt/vol sucrose, cryostat blocks were prepared. Sections of 10µm thickness were cut at -20°C and collected on pol-L-lysine coated glass slides. Sections were treated with 3% vol/vol hydrogen peroxide in methanol for 30 minutes to inactivate endogenous peroxidase. They were then washed in PBS and incubated in normal goat serum (diluted 1:30) for 30 minutes. The serum was drained off and sections were reacted with primary rabbit antibodies diluted in PBS overnight at 4°C. Substances screened with primary antibodies included: protein gene peptide product (PGP), substance P, vasoactive intestinal peptide (VIP), calcitonin gene related peptide (CGRP), somatostatin, endothelin, neural and endothelial nitric oxide synthase (NOS), von Willebrand's factor (vWF), leucocyte function associated antigen (LFA) and intercellular cell adhesion molecule (ICAM). Following thorough rinsing in PBS, the avidin-biotin method was

performed. Enhancement of the peroxidase reaction was achieved using nickel ammonium sulphate and nascent hydrogen peroxide according to the method of Shu et al (Shu et al 1988).

Routine negative control immunostaining included the replacement of the primary antibodies with preimmune serum or omission of one or more of the reagents in the immunostaining procedure. Immunoreactive sites were photographed using a Polyvar (Leica UK Ltd, Milton Keynes, UK) photomicroscope with bright field illumination.

(b) Quantitation

The density of nerve staining for VIP in the tissues was objectively determined using an Unix-based computer program OpenStereo. This program has been developed at the RPMS to facilitate the quantitative investigation of innervation and vascularity from histological sections and reduce operator error (DC Abrams, 1993, personal communication).

Histological images are viewed using a microscope with attached video camera and high resolution monitor (1280 x 900 pixels), which permits the simultaneous display of two images (512 x 512 pixels), when performing thresholding and segmentation of the images. Randomly selected images were captured using OpenStereo and subsequently analysed.

(i) Thresholding

Thresholding is the process whereby the operator and computer interactively set a threshold to allow delineation/ identification of the stained and unstained tissue in the images under study. Images requiring analysis are randomly displayed in duplicate on the computer screen. One reference image allows the user to view this original image with no overlays. The other image, the interactive image, is used to display the

threshold results. The images are then randomly re-presented to the operator for delineation of the reference space. This is the total area *under study* and enables the elimination of tissue areas which may have been captured from a field of view but do not belong in the particular region/ compartment of interest.

(ii) Delineation

The reference space (area of interest under study) is defined by selecting an area marked out using the computer mouse; this is saved as a binary image to the hard disc of the computer. This procedure permits the investigator to analyse the structures under investigation in many compartments by delineation of each compartment.

(iii) Processing

Following completion of thresholding and delineation of each compartment (mucosa, submucosa and muscularis), the computer calculates the number of pixels in the reference space and the number of pixels representing the thresholded area for each image or field of view analysed. This data is then expressed as a ratio for each compartment.

Image Analysis Protocol

Five dogs were randomly selected and 5 sections made for each dog jejunum biopsies before and after CPB. Three fields from three compartments of the gut wall (mucosa, submucosa and muscularis) were randomly captured by the computer for analysis of the density of VIP staining. Thus, for each dog, 45 images were analysed pre-CPB and 45 images post-CPB. In total 225 images were analysed before CPB and 225 post-CPB. Immunohistochemistry for other hormones was not undertaken, since subjective analysis of the stained tissue suggested no significant changes.

Formulae used in this Study

(1) Oxygen content of blood = (Hb x 1.36 x Blood %O₂ saturation) + (pO₂ x 0.003)
ml 100 ml⁻¹

(2) Small bowel oxygen consumption ($\dot{V}O_2$) ml min⁻¹ kg⁻¹ body weight was calculated from the following equation:

$$VO_2 = \frac{SMA \text{ flow} \times (\text{Arterial } O_2 \text{ content} - \text{SMV } O_2 \text{ content})}{\text{body weight}} \times 0.01$$

(3) Small bowel oxygen extraction (%) = $\frac{\dot{V}O_2}{\dot{D}O_2} \times 100$

(4) The systemic vascular resistance index (calculated as systemic vascular resistance units or SVRI) during CPB was calculated from the formula:

$$SVRI = \frac{MAP - CVP}{CI}$$

MAP: mean arterial pressure
CVP: central venous pressure
CI: cardiac index

(5) The Q₁₀ is the decrease in oxygen consumption for a 10°C reduction in core temperature and complies with van't Hoff's Law (Bigelow et al 1950). This was calculated by plotting the oxygen consumption data against the core temperature. This was divided into two phases; from the start of the experiment to the end of the hypothermic phase, and from the start of re-warming and the end of the experiment. Regression line analysis was then performed upon the data and the linear regression equation used to determine the oxygen consumption at 28°C ($\dot{V}O_{2T28}$) and 38°C ($\dot{V}O_{2T38}$) for the two phases of the experiment.

The Q₁₀ is then calculated as:

$$\frac{\dot{V}O_{2T38}}{\dot{V}O_{2T28}}$$

Saccharide urinary excretion is expressed as % of enterally administered dose per 100 cm gut, standardised to a creatinine clearance of 70 ml/min/m².

Care of Animals

Animals received care in compliance with Home Office Regulations, United Kingdom. At the end of the experiment the animals were killed with a lethal dose of intravenous barbiturate.

Statistical Analysis & Data Analysis

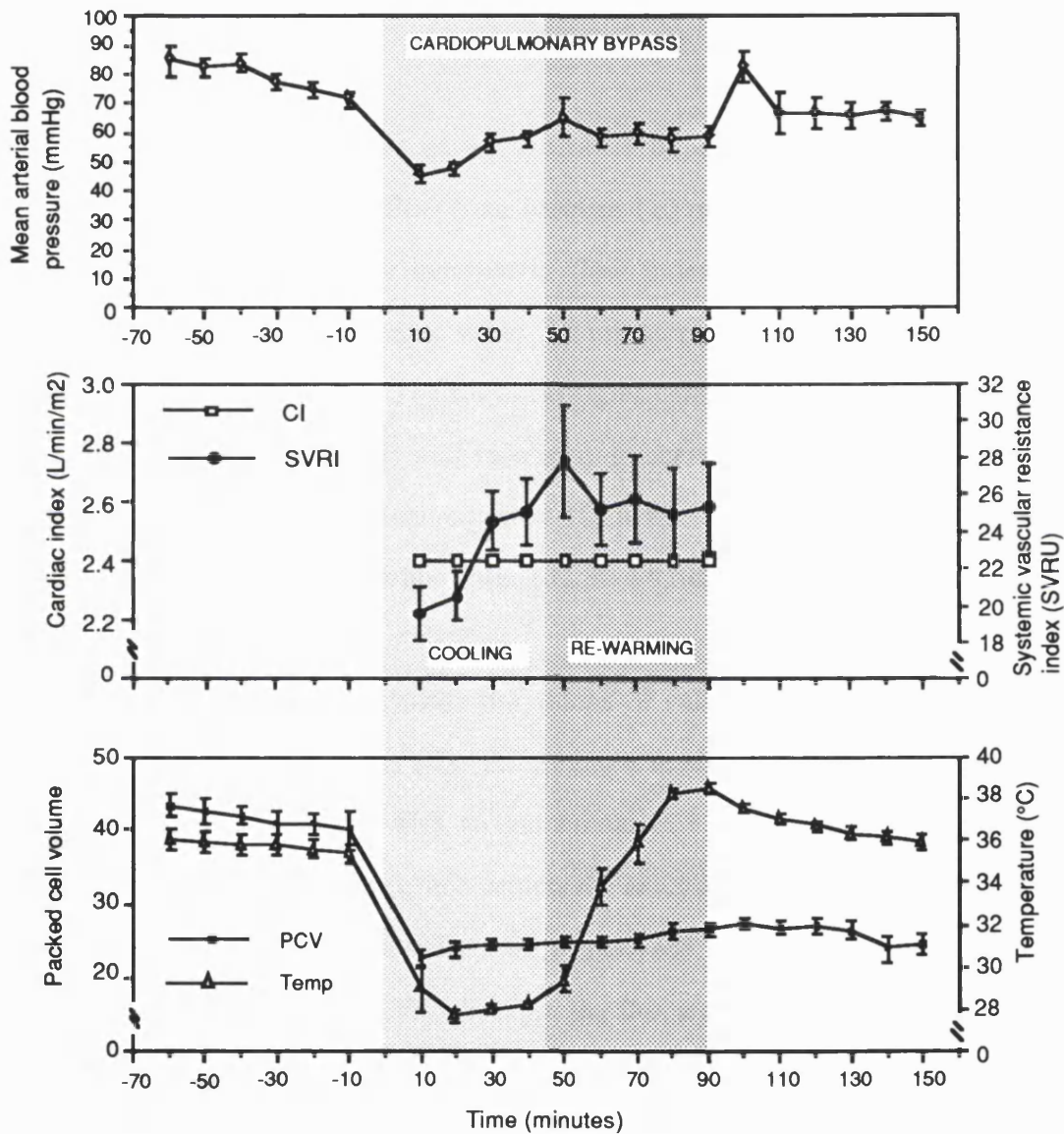
A paired Student's t test was employed to test for differences for paired samples between the baseline period and other periods during the experiment. Values are expressed as means \pm standard errors. To determine differences between the slopes of regression lines for the Q₁₀ before and after CPB a Student's t test was applied. All p-values are two tailed. Details of the statistical methods used for analysis of the vasoactive intestinal peptide stained tissues is provided in the results section of the immunohistochemistry.

Results

Systemic Haemodynamics

The institution of CPB coincided with a fall in mean arterial pressure (MAP) from a baseline of 71.5 ± 2.7 to 46.0 ± 4.7 mm Hg ($p=0.0001$, Fig 94).

Fig 94: Changes in MAP, CI, SVRI, PCV & temperature during the study period.



The cardiac index was kept constant throughout CPB and no vasoactive drugs were employed during the CPB period. A vasoconstrictive response to non-pulsatile hypothermic CPB was observed with the systemic vascular resistance index (SVRI)

increasing from 19.6 ± 1.5 SVRU at the start of the hypothermic phase to 25.0 ± 1.8 SVRU at the end of the hypothermic phase ($p=0.028$, Fig 94). However during re-warming the SVRI was largely unchanged, and after 45 minutes of re-warming it was 25.2 ± 2.5 SVRU.

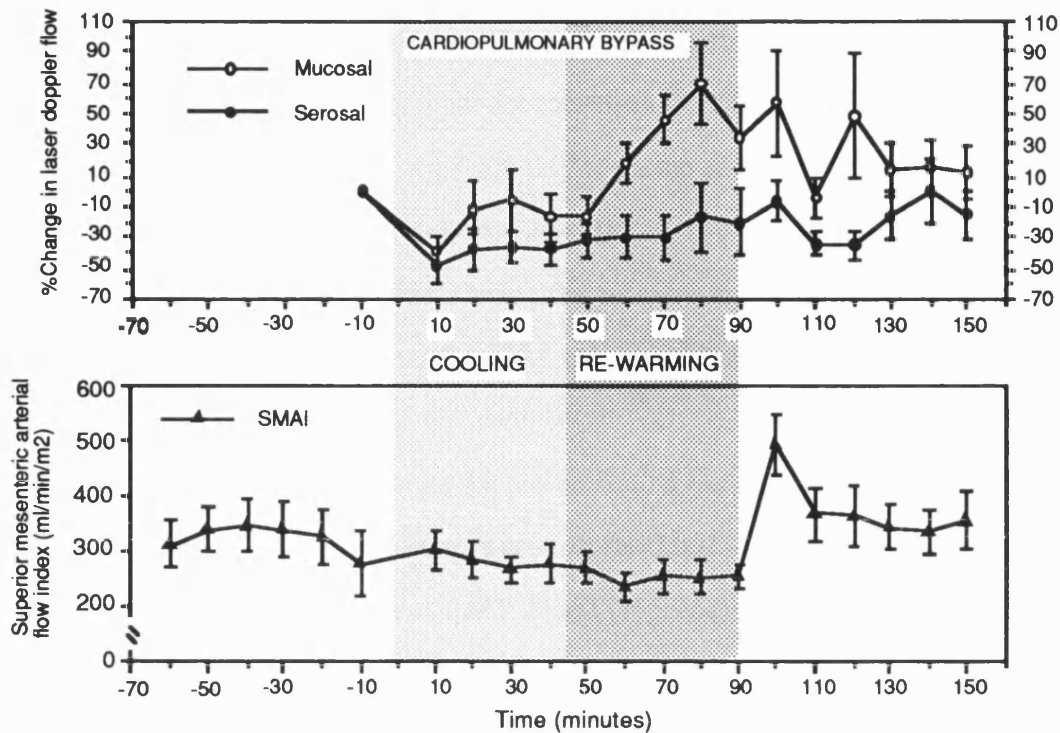
The PCV fell from $40.0 \pm 2.7\%$ pre-CPB to $22.6 \pm 1.2\%$ after ten minutes of CPB ($p=0.0001$); thereafter the PCV remained greater than 22% (Fig 94).

Small Bowel Blood Flow

Following the commencement of non-pulsatile hypothermic CPB, there was no significant alteration in SMA flow from baseline (10 minutes prior to CPB); after 10 minutes of CPB the superior mesenteric flow index (SMAI) was 288.6 ± 34.6 ml/min/m² compared to a baseline value (10 minutes prior to CPB) of 259.3 ± 55.6 ml/min/m² ($p=0.65$) (Fig 95). There was no significant alteration in the SMAI in the remainder of the hypothermic and re-warming phase of CPB (Fig 95); after the termination of CPB the SMAI increased to 463.5 ± 59.9 ml/min/m² ($p=0.02$) with a return of MAP to pre-CPB baseline value (82.3 ± 4.9 mm Hg; $p=0.1$).

Laser Doppler flow (LDF) readings are means of values obtained over a 2 minute interval. The LDF monitor was adjusted so that a reading for flow (flux signal) was recorded once every second (1 Hz), so that a mean of 120 data points were calculated for each reading. Therefore the graph depicts the analysis of 1320 data points for each time point on the graph. All values were analysed as percentage changes from baseline (10 minutes prior to CPB), because the flux signal does not provide absolute values of blood flow.

Fig 95: Alterations in SMA blood flow & gut serosal and mucosal laser Doppler blood flow.



Ten minutes following the institution of CPB both serosal and mucosal blood flow fell by $47.3 \pm 11.4\%$ and $38.2 \pm 9.3\%$ of pre-CPB values ($p=0.006$ and $p=0.008$) respectively (Fig 95). After 20 minutes of CPB, the mucosal blood flow had recovered to $-11.0 \pm 17.3\%$ ($p=0.26$), whilst serosal blood flow remained depressed at $-37.6 \pm 13.4\%$ ($p=0.03$). During the re-warming phase of CPB, there was a dramatic increase in mucosal LDF, which peaked at $+69.8 \pm 15.2\%$ ($p=0.03$) after 35 minutes of re-warming, whilst serosal LDF returned towards pre-CPB values ($-16.4 \pm 21.5\%$; $p=0.24$). At the same period of CPB the SMAI was unchanged at 243.1 ± 30.9 ml/min/m², compared to the baseline value of 295.1 ± 43.9 ml/min/m² ($p=0.14$).

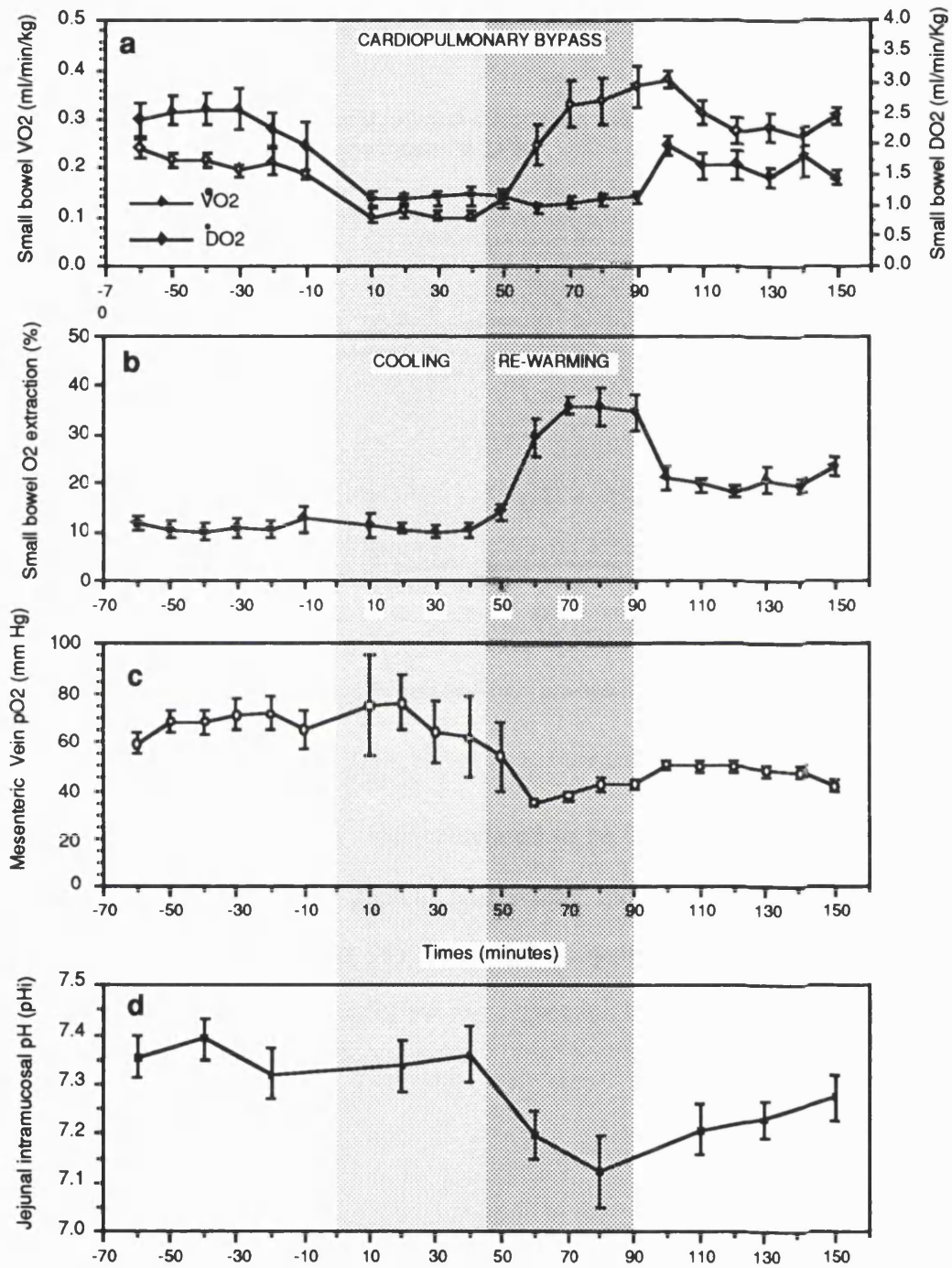
Small Bowel Oxygen Utilisation

Small bowel $\dot{V}O_2$ fell to 0.099 ± 0.014 ml min⁻¹ kg⁻¹ after ten minutes of CPB from a baseline value (10 minutes prior to CPB) of 0.17 ± 0.014 ml min⁻¹ kg⁻¹ ($p=0.005$). $\dot{D}O_2$ also fell during this period with the reduction in PCV, from 1.98 ± 0.39 ml min⁻¹ kg⁻¹ to 1.12 ± 0.14 ml min⁻¹ kg⁻¹ ($p=0.05$). In the re-warming phase (Fig 96a) of CPB, $\dot{V}O_2$ increased progressively, peaking after 45 minutes of re-warming at 0.345 ± 0.136 ml min⁻¹ kg⁻¹ ($p=0.009$). During the re-warming phase of CPB, since there were no significant alterations in SMAI and PCV (Fig 94 & 95), $\dot{D}O_2$ was also largely unchanged. As a result the increase in $\dot{V}O_2$ was met by increased oxygen extraction fraction (Fig 96b), which increased from a baseline value of $12.9 \pm 2.7\%$ to $34.4 \pm 3.8\%$ after 45 minutes of re-warming ($p=0.001$). This was also reflected by increased oxygen desaturation of SMV blood (Fig 96c); the pO_2 fell to 42.6 ± 1.8 mm Hg after 45 minutes of re-warming from a baseline of 65.1 ± 7.2 mm Hg ($p=0.01$); it was even lower after 15 minutes of re-warming at 32.2 ± 1.4 mm Hg ($p=0.002$, Fig 96c).

Intramucosal pH (pHi), an indicator of tissue oxygenation, was unchanged during the hypothermic phase of CPB (Fig 96d & 97), but during the re-warming phase the pHi fell to 7.12 ± 0.07 ($p=0.045$) after 35 minutes of re-warming, suggesting mucosal hypoxia was present. Thereafter, there was a progressive increase in the pHi towards normal values. This is illustrated in Fig 97, which plots the alterations in pHi, SMV pH and arterial pH. The gradient between arterial and mucosal pH becomes most pronounced during the re-warming phase with gradual recovery post-CPB (Fig 97).

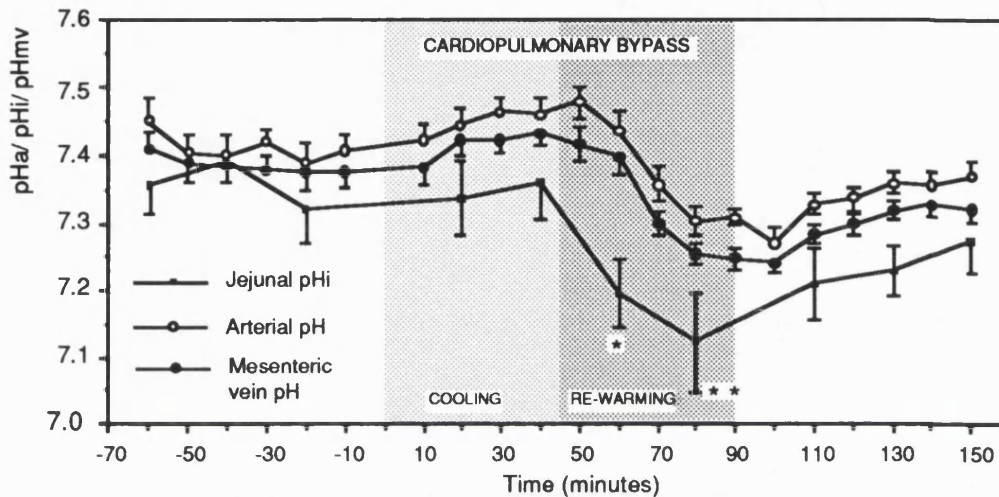
As regards $\dot{V}O_2$ post-CPB, this was elevated even 60 minutes after the end of CPB at 0.293 ± 0.023 ml min⁻¹ kg⁻¹ ($p=0.0005$), although $\dot{D}O_2$ had improved to 1.44 ± 0.12 ml min⁻¹ kg⁻¹ ($p=0.23$, Fig 96a) and O_2 extraction had fallen to $23.5 \pm 1.9\%$ ($p=0.009$, Fig 96b).

Fig 96: Mesenteric oxygen utilisation in a canine model of hypothermic CPB



During the post-CPB period the pH_i gradually returned towards pre-CPB levels and 60 minutes post-CPB it had recovered to 7.27 ± 0.05 but was still below the baseline value of 7.36 ± 0.04 ($p=0.04$, Fig 96d & 97).

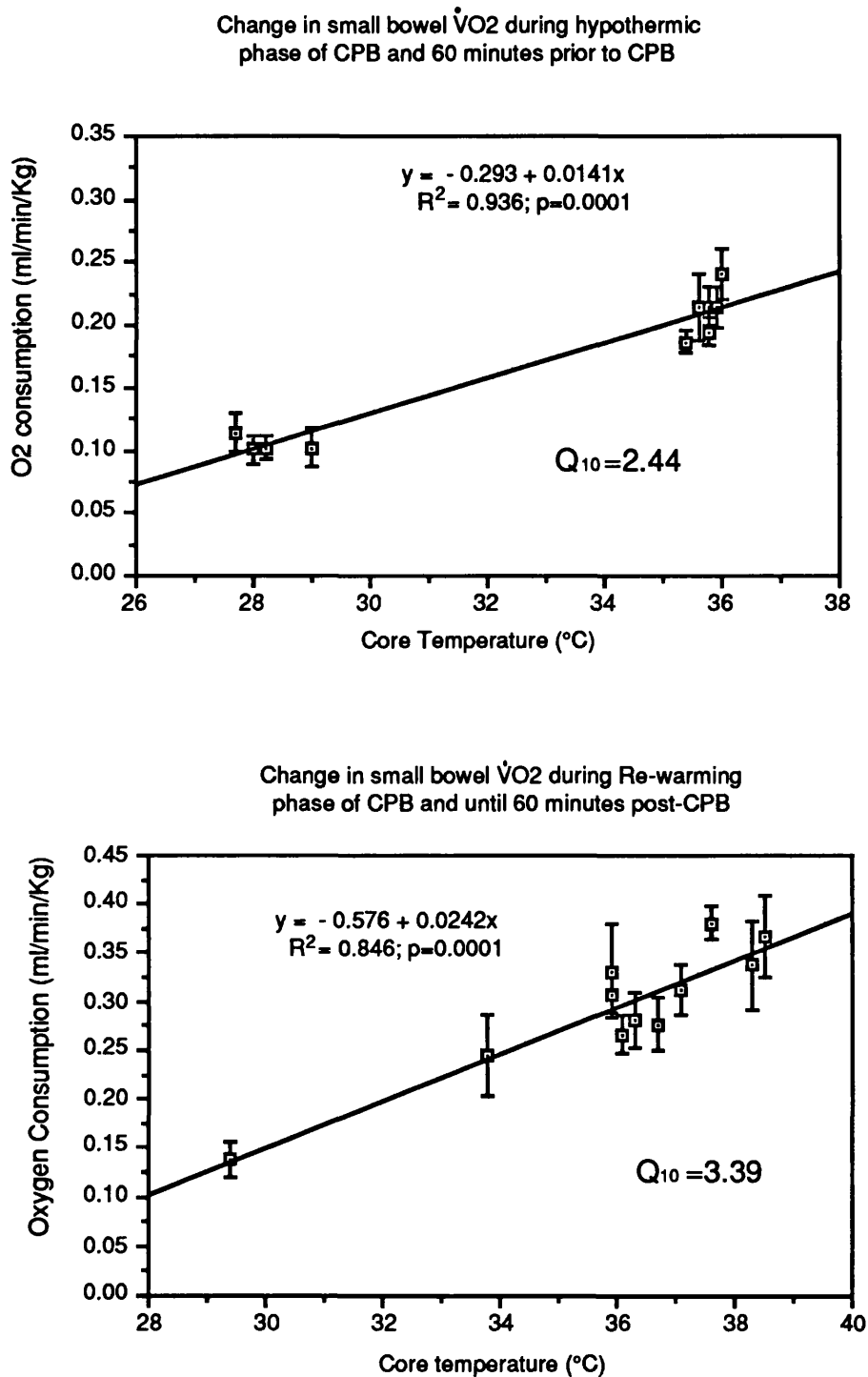
Fig 97: Changes in pHa, jejunal pH_i and mesenteric vein pH (pH_{mv}) during the study period in a canine model of CPB.



Symbols indicate p values (Paired t test) from pre-bypass (20 min pre-CPB) values
 * $p=0.05$ ** $p=0.04$

The Q_{10} for small bowel $\dot{V}O_2$ was calculated to be 2.44 for the first half of the experiment, and 3.39 for the second half which included re-warming and the immediate post-CPB period (Fig 98). Statistical analysis confirmed that there was a significant difference in the slopes of the two regression lines ($p=0.015$, Fig 98).

Fig 98: Changes in small bowel $\dot{V}O_2$ during hypothermic & re-warming phases of CPB



There is a significant difference between the slopes of two regression lines ($p=0.015$)

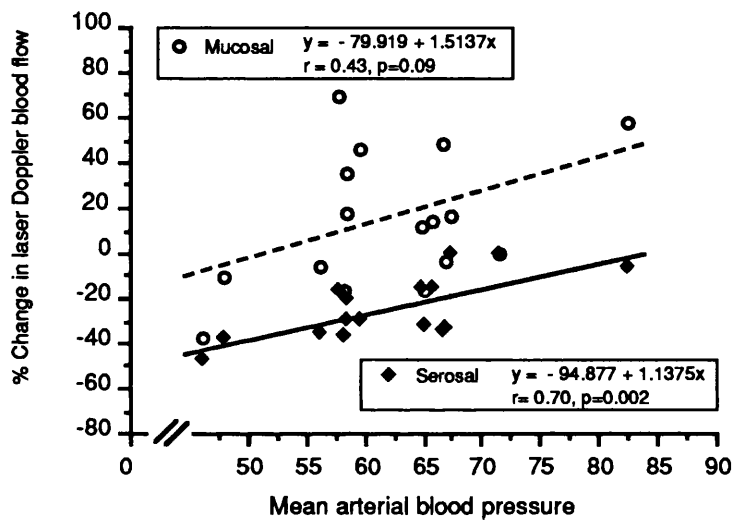
Correlations

Factors controlling mucosal and serosal blood flow

(a) Mean arterial blood pressure.

Mucosal laser Doppler blood flow to the jejunum is independent of changes in MAP in the range 40-85 mm Hg ($r=0.43$, $p=0.09$), whilst serosal blood flow decreases with reductions in MAP ($r=0.70$, $p=0.002$, Fig 99).

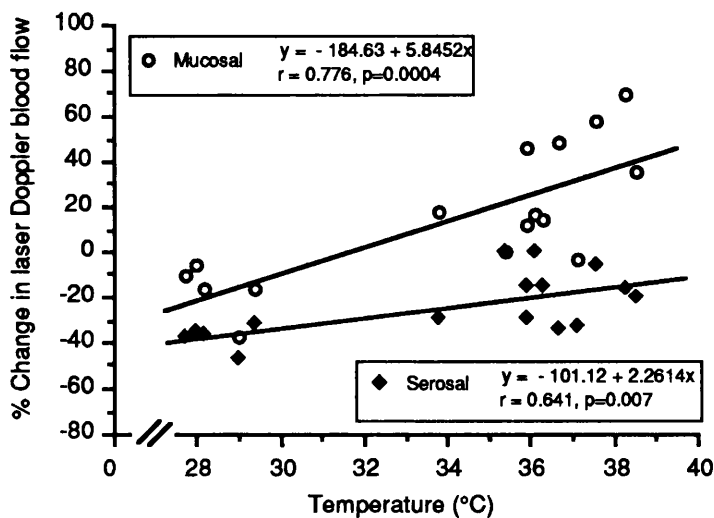
Fig 99: The correlation between serosal & mucosal jejunal laser Doppler blood flow and mean arterial blood pressure.



(b) Temperature

Both mucosal and serosal aspects of the jejunum were affected by cooling. Reductions in laser Doppler blood flow were observed as the temperature was reduced from 38°C to 28°C. The relationship was closer for mucosal ($r=0.78$, $p=0.0004$), than for serosal blood flow ($r=0.64$, $p=0.007$) (Fig 100).

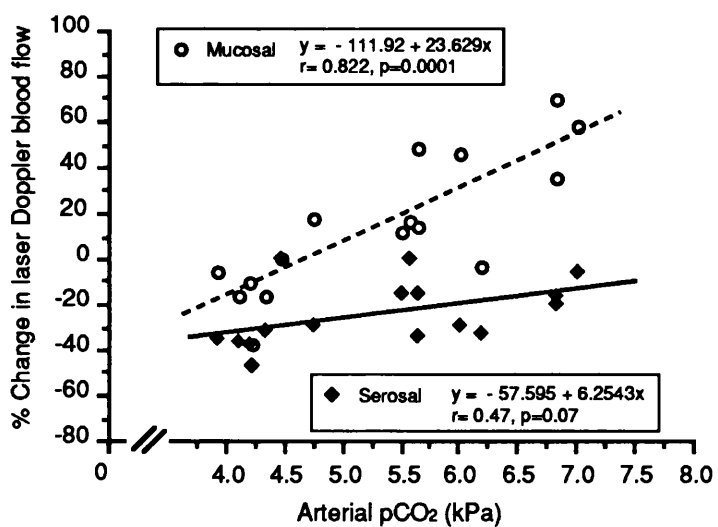
Fig 100: The correlation between serosal & mucosal jejunal laser Doppler blood flow and core temperature.



(c) Arterial pCO₂

Mucosal laser Doppler blood flow was closely related to alterations in the arterial pCO₂ (r=0.82, p=0.0001), whilst serosal blood flow was less influenced by modulations in the arterial pCO₂ (r=0.47, p=0.07, Fig 101).

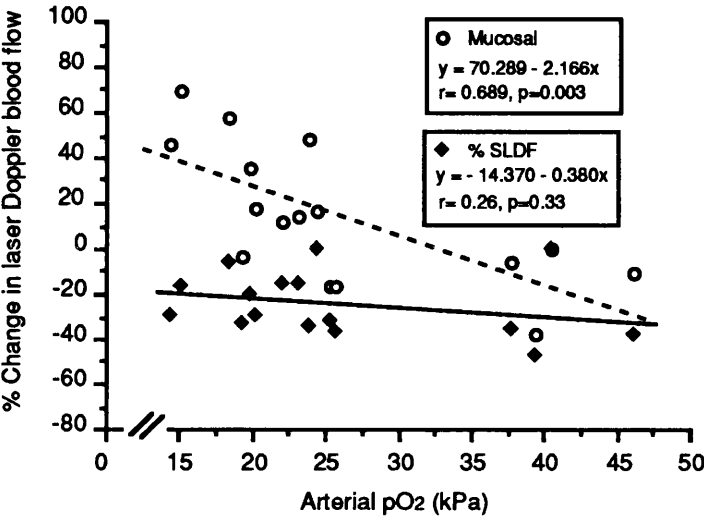
Fig 101: The influence of arterial pCO₂ upon jejunal mucosal and serosal laser Doppler blood flow.



(d) Arterial pO₂

Hyperoxia was associated with a reduction in mucosal blood flow ($r=0.69, p=0.003$), whilst serosal laser Doppler blood flow was independent of the arterial pO₂ ($r=0.26, p=0.33$, Fig 102).

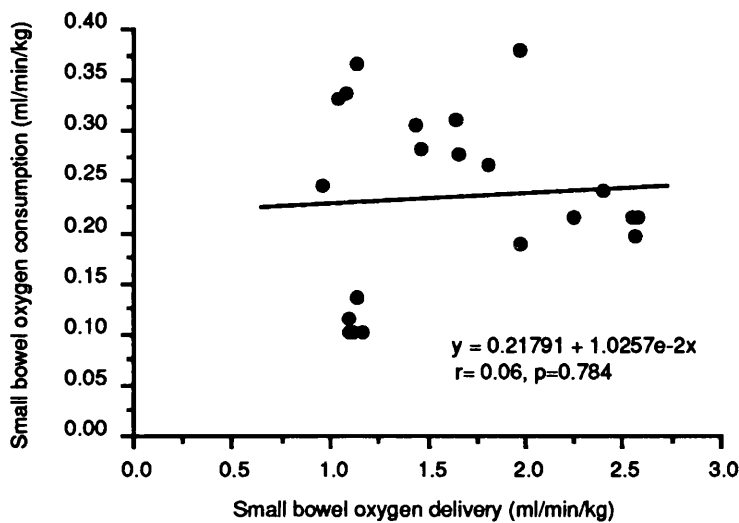
Fig 102: The influence of arterial pO₂ on jejunal mucosal and serosal laser Doppler blood flow.



Small Bowel Oxygen Consumption & Delivery

Oxygen consumption was independent of $\dot{D}O_2$ during the study period, despite alterations in MAP, CI and core temperature during and following CPB ($r=0.06$, $p=0.78$, Fig 103).

Fig 103: Small bowel oxygen consumption is independent of small bowel oxygen delivery.

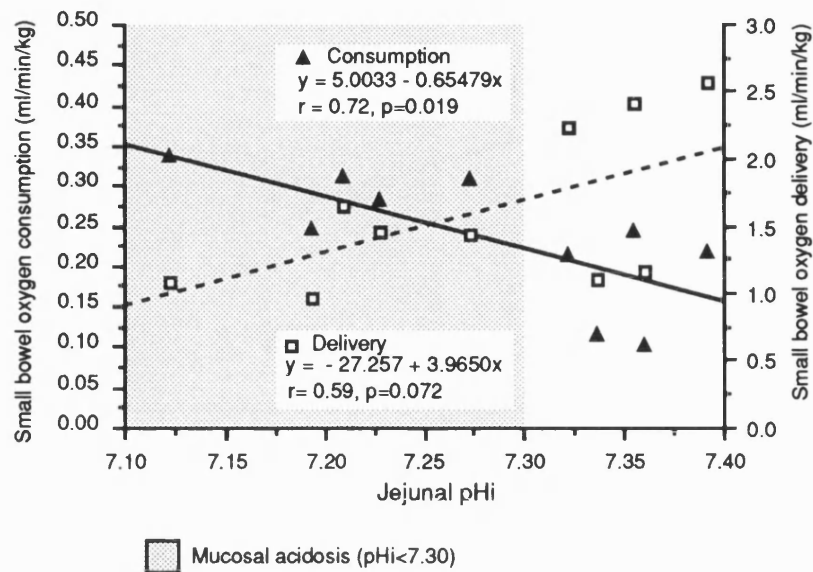


Factors affecting the Jejunal Intramucosal pH

(a) The jejunal pHi & Oxygen consumption & delivery

The jejunal pHi decreased as the $\dot{V}O_2$ increased (Fig 96), but was not met by increased $\dot{D}O_2$. However, because $\dot{V}O_2$ was independent of $\dot{D}O_2$ (Fig 103), the pHi declined with the increase in $\dot{V}O_2$ ($r=0.72$, $p=0.02$), and was not significantly correlated with $\dot{D}O_2$ ($r=0.59$, $p=0.07$, Fig 104).

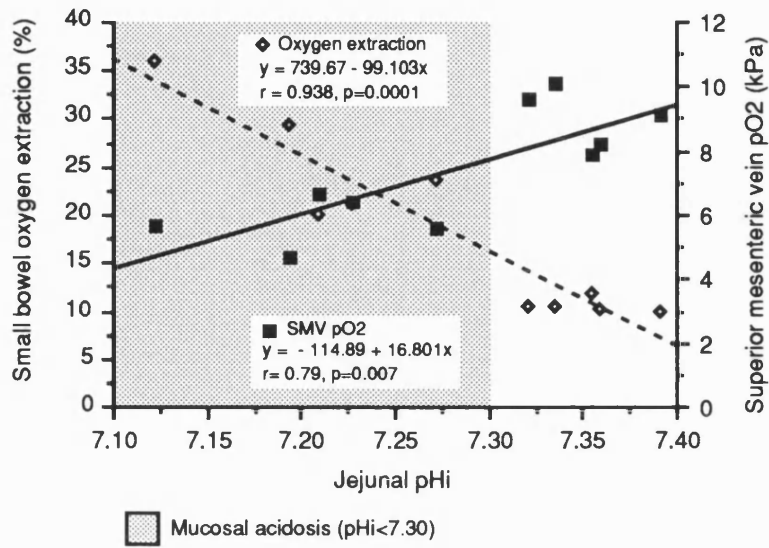
Fig 104: The correlation between small bowel oxygen consumption, delivery and the jejunal pH_i.



(b) Oxygen Extraction, SMV pO₂ & Jejunal pH_i

The increase in $\dot{V}O_2$ was met by an increase in the oxygen extraction fraction. Thus the pH_i declined as the oxygen extraction increased, with a tendency towards intramucosal acidosis (pH_i < 7.3), when the extraction fraction became >20%. Similarly, the SMV pO₂, which was mixed venous pO₂ draining from the small bowel, declined with increases in oxygen extraction, with a tendency towards mucosal acidosis when the SMV pO₂ fell below 7 kPa (Fig 105). Both the oxygen extraction fraction and the SMV pO₂, an indicator of mucosal oxygenation, were significantly correlated with the pH_i ($r=0.94, p=0.0001$ and $r=0.79, p=0.007$ respectively, Fig 105).

Fig 105: The correlation between jejunal pHi, small bowel oxygen extraction and superior mesenteric vein pO₂



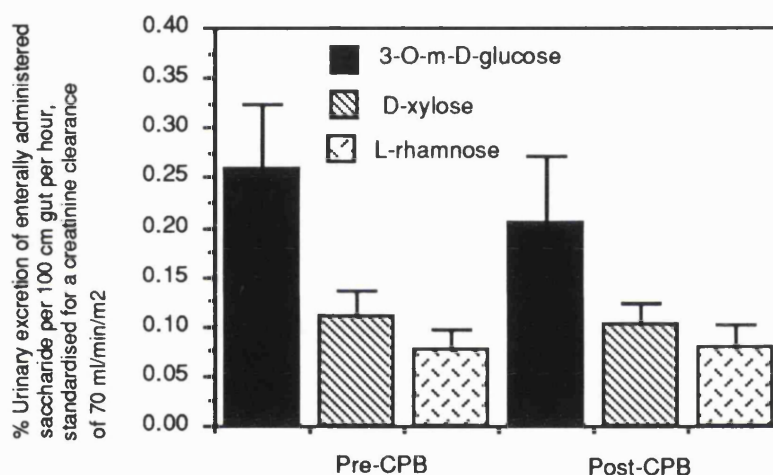
Jejunal Saccharide Transport & Permeability

Values have been standardised to a creatinine clearance of 70 ml/min/m² and are expressed as % urinary excretion per 100 cm of jejunum per hour.

(a) 3-O-m-D-glucose, D-xylose & L-rhamnose transport.

The results are illustrated below in Fig 106. The transport of the monosaccharides was reduced by 29.3%, 17.1% and 4.1% for 3-O-m-D-glucose, D-xylose and L-rhamnose respectively, but this did not reach statistical significance (Fig 106).

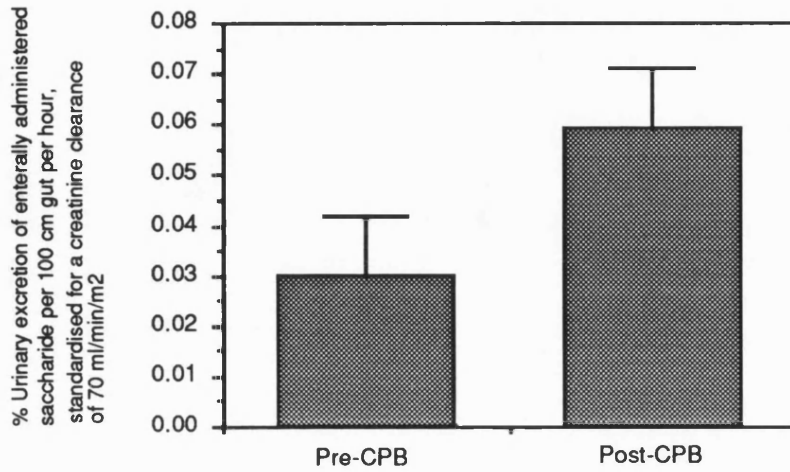
Fig 106: The effect of CPB on the absorption of 3-O-m-D-glucose, D-xylose & L-rhamnose using isolated dog jejunal loop.



(b) The Permeation of Lactulose.

The urinary excretion of this disaccharide increased in dogs following CPB (n=5) compared to dogs who underwent the study immediately prior to CPB (n=5). The permeation of lactulose increased by 38.8%, but this did not reach statistical significance (Fig 107).

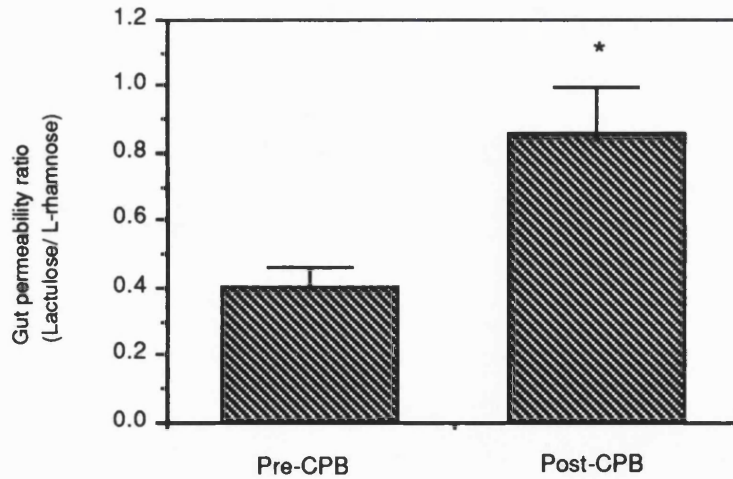
Fig 107: The effect of CPB on the permeation of lactulose using isolated dog jejunal loop



(c) Gut permeability

The lactulose/ L-rhamnose ratio increased by 80.6% from 0.402 pre-CPB to 0.726 post-CPB ($p=0.014$, Fig 108).

Fig 108: The effect of CPB on gut permeability using isolated dog jejunal loop



Compared to pre-CPB value, * $p=0.014$

Neurohistochemical Changes in Dog Jejunum

Haematoxylin and eosin staining of the tissues found swelling of the villi with an increase in the thickness of all layers of the gut wall particularly the submucosa, which was due to oedema (Fig 109 a & b, 110 a & b) and was most pronounced for tissue which had sustained both CPB and ischaemia-reperfusion injury (Fig 109c & 110c). In addition to oedema, dilatation of submucous vessels was a consistent feature in gut tissue exposed to CPB and ischaemia-reperfusion injury (Fig 110c). Villus tip damage was not a consistent histological feature following CPB, but was evident in the gut tissue which had undergone both CPB and ischaemia-reperfusion injury (Fig 109 b & c).

Fig 109a: Haematoxylin and eosin stained section of dog jejunal villi (x90) pre-CPB.

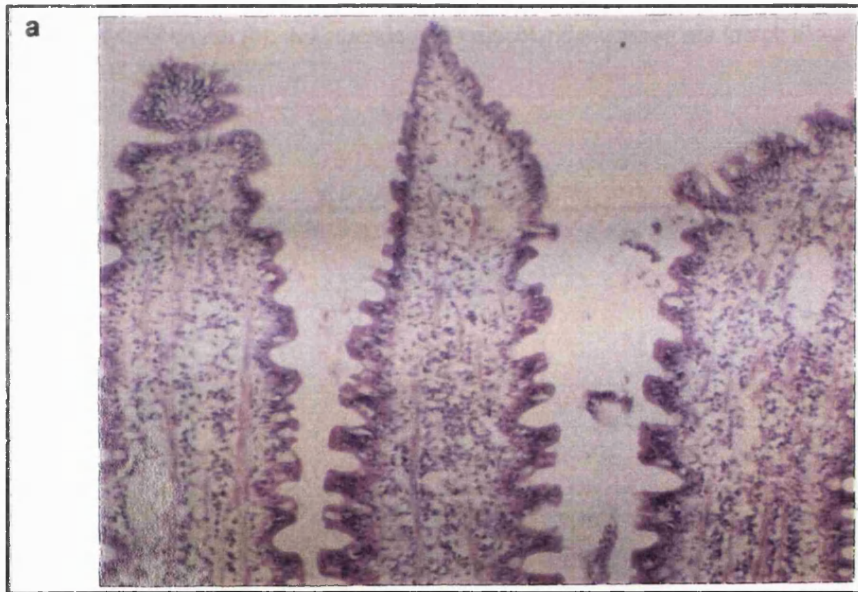
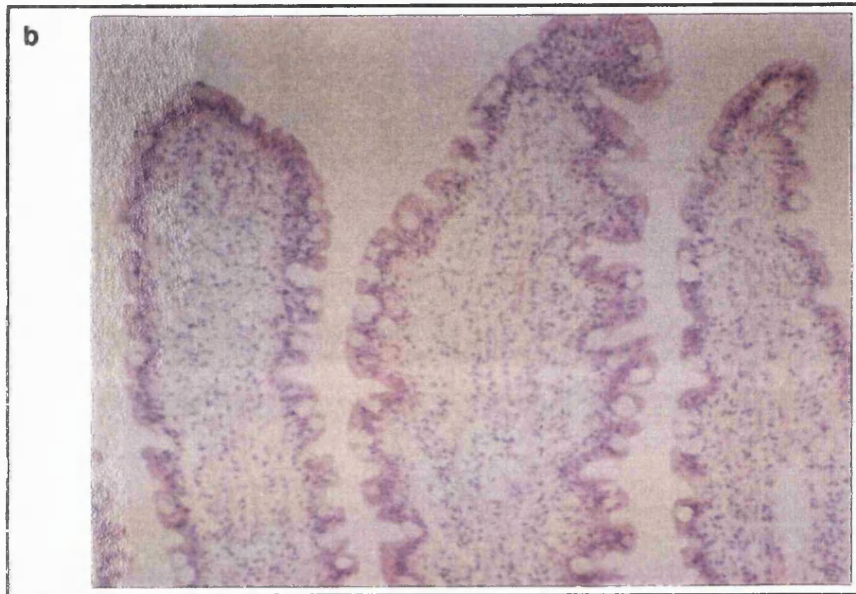
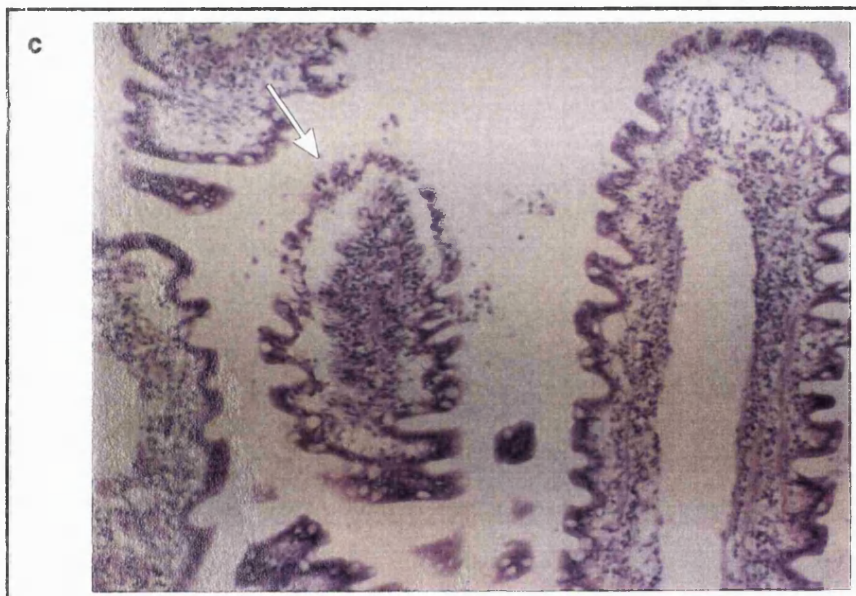


Fig 109b & c: Haematoxylin and eosin sections of jejunal villi (x90), (b) post-CPB & (c) post-CPB following 20 min ischaemia & 20 min reperfusion injury.

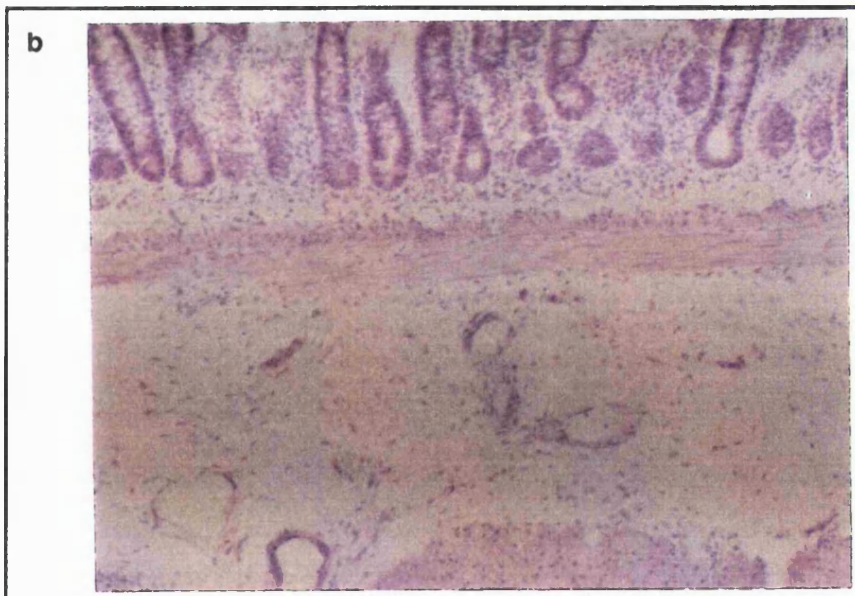
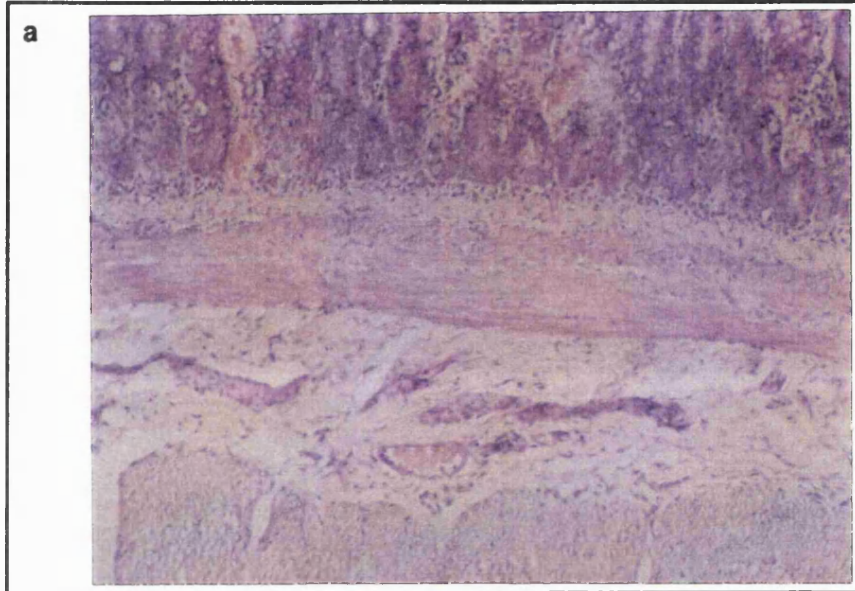


The post-CPB tissue (b), the mucosa and muscularis mucosae are intact; there is oedema of villus tissue.



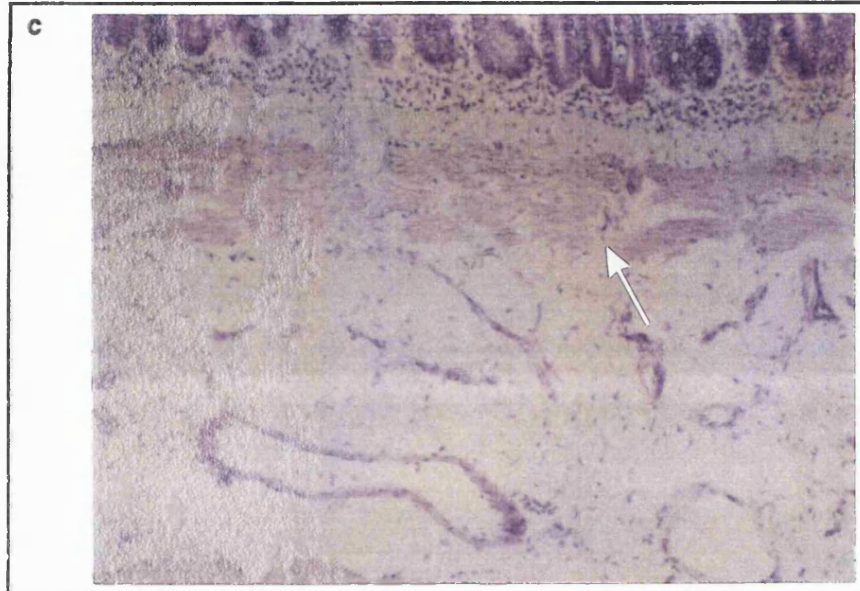
Post-CPB/ ischaemia-reperfusion jejunum (c): there is tissue oedema with disruption of the muscularis mucosae and separation of the mucosa from the underlying lamina propria. The villus tip in the middle of the section shown (marked with arrow) has necrosis of the mucosa with early features of villus tip loss.

Fig 110a & b: Haematoxylin and eosin sections of jejunal submucosa (x90),
(a) pre-CPB & (b) post-CPB



Post-CPB (b): there is tissue oedema with an increase in the thickness of the submucosal layer; the muscularis mucosae is intact.

Fig 110c: Haematoxylin and eosin stained section of dog jejunal submucosa (x90) post-CPB and following 20 min ischaemia and 20 min reperfusion injury.



Tissue oedema with an increase in the thickness of the submucosa is a more marked feature than post-CPB tissue (Fig 110b). There is disruption of the muscularis mucosae (marked with arrow) and the vessels of the submucosa are dilated.

The most consistent change in immunohistochemical staining was found for VIP, which showed an increased intensity of staining in the nerves of the submucous plexus and within the villi (Fig 111a,b & c, Fig 112a, b &c).

Fig 111a: Dog jejunal villi, stained for VIP (x120) pre-CPB.

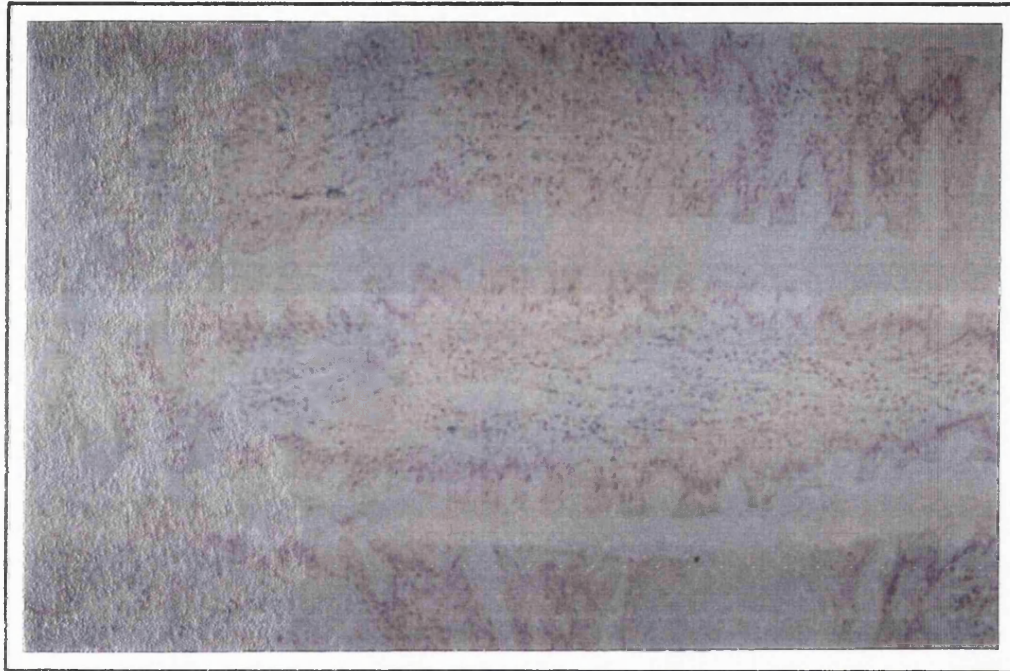
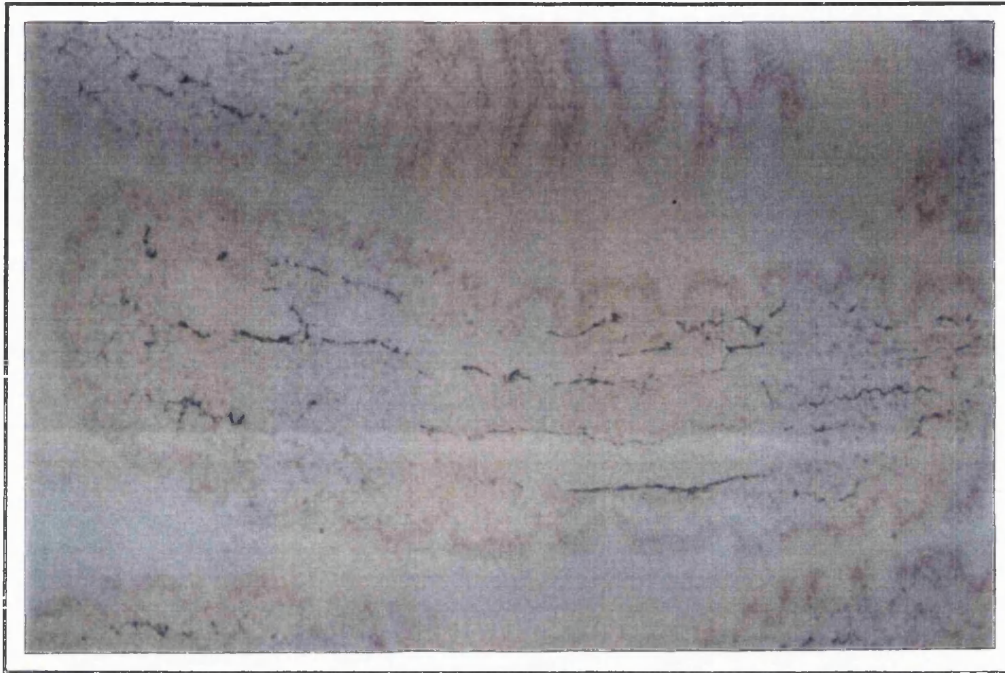


Fig 111b & c: Dog jejunal villi, stained for neural VIP (x120).

(b) Post-CPB



(c) Post-CPB/ ischaemia -reperfusion injury



Fig 112a & b: Dog jejunal submucosa, stained for neural VIP (x120).

(a) Pre-CPB



(b) Post-CPB

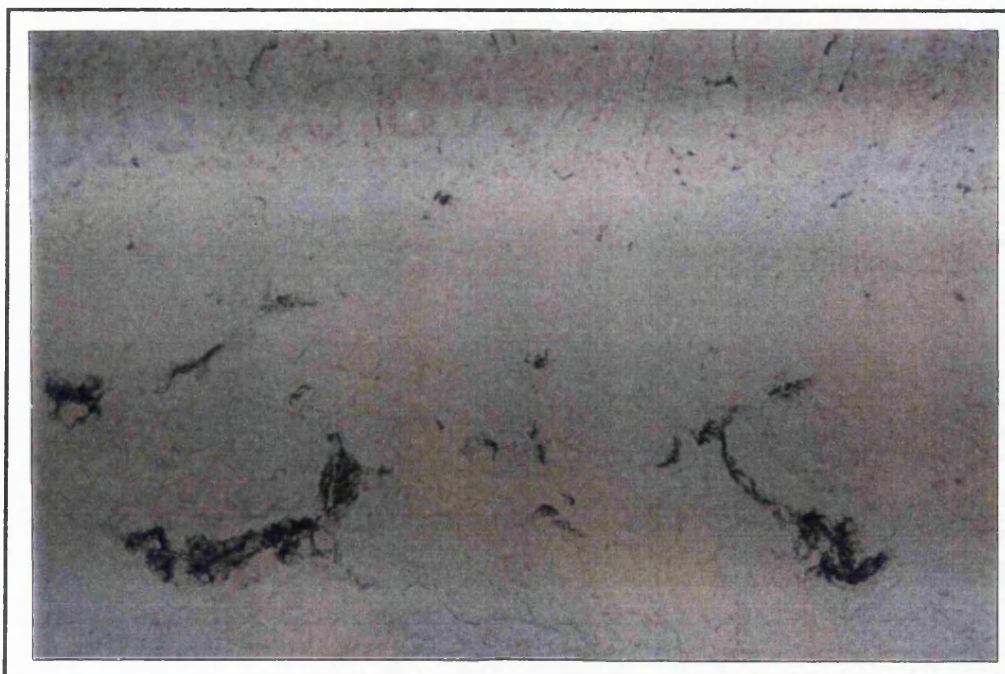
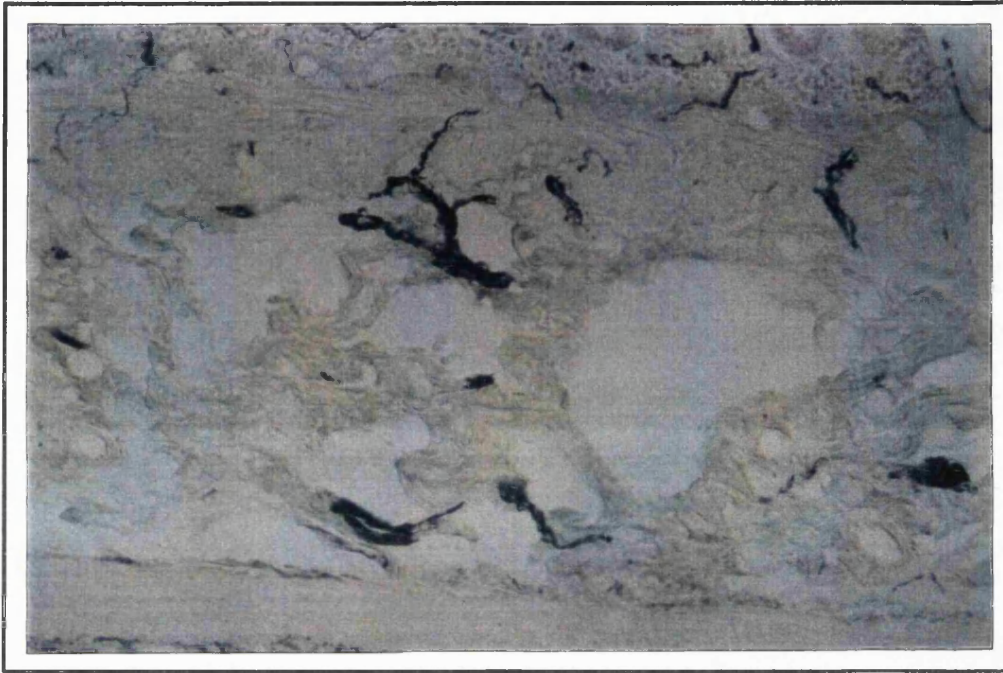


Fig 112c: Dog jejunal submucosa, stained for neural VIP (x120), post-CPB/ ischaemia-reperfusion injury.



Histological features are similar to those observed in haematoxylin eosin stained sections, with dilated submucosal vessels and an increase in the thickness of the submucosal layer. Neural tissue in the submucosa and the lamina propria is stained heavily for VIP.

Statistical analysis of VIP stained Gut tissue

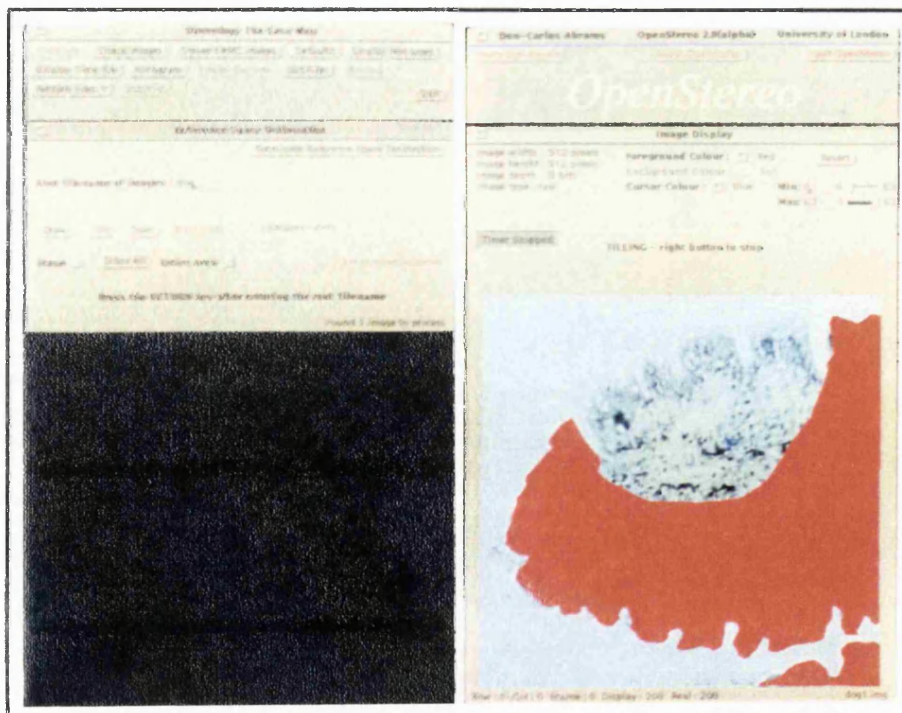
Separate analyses were performed for each compartment due to differences in the distribution of results in the three compartments. The object count (area of VIP stained tissue, Fig 113) was positively skewed, as was the ratio (object count/reference space; reference space delineation illustrated in Fig 114).

Fig 113: Thresholding of captured images using OpenStereo



These images have been photographed directly from the computer screen. The image on the left is the reference image which has been captured from the VIP stained section. The image on the right has been delineated and demonstrates how VIP stained tissue can be shaded red, leaving behind the unstained background tissue. The computer then captures this stained area and records the area shaded in pixels.

Fig 114: Delineation of captured images using OpenStereo



The total area under study can be marked using the mouse and then shaded by the computer. The defined area then becomes the reference space (recorded in pixels). The thresholded area over the reference area is expressed as a ratio. Areas captured from the original section eg the space around the tissue or spaces within the tissue can be excluded by delineation.

A log₁₀ transformation was employed to make the distribution more symmetrical in the mucosa and submucosa, but could not be used for the muscularis compartment. This was because the majority of values for the muscle compartment both before and post-CPB were zero. The number of zero values for each compartment is illustrated below in Tab 29, out of a total number of 75 fields.

Tab 29: Statistical analysis: the number of zero values in each tissue compartment.

COMPARTMENT	TIME	No. of Zero values	(%)	No. of Positive values
Submucosa	Pre-CPB	7	9	68
	Post-CPB	1	1	74
Mucosa	Pre-CPB	19	25	56
	Post-CPB	4	5	71
Muscularis	Pre-CPB	73	97	2
	Post-CPB	61	81	14

Because of the paucity of VIP staining in the muscularis, there was a large number of zero counts (97% pre-CPB & 81% post-CPB) and it was not possible to undertake a suitable transformation for this compartment. For each dog, the number of positive fields out 75 fields examined was determined (Tab 30).

Tab 30: Statistical analysis: the proportion of positive values for VIP staining in the muscularis compartment.

Dog	Pre-CPB	Post-CPB
1	0	0
2	0	5
3	0	1
4	1	4
5	1	4
Total	2/75 (3%)	14/75 (19%)

The proportions were analysed using the GLIM (generalised linear modelling) computer program with binomial errors (Healy 1988 p.80). The change in deviance, when time is included in the model has a chi-squared distribution ($\chi^2=11.83$,

$p < 0.001$). This result indicates a significant increase in VIP staining between pre and post-CPB for muscularis fields containing stained nerve tissue.

For the remaining two compartments, a \log_{10} transformation was undertaken with analysis of the data using REML (restricted maximum likelihood) procedure, using Genstat computer software (Genstat 5 1990 p.89). The results are provided in Tab 31.

Tab 31: Statistical analysis of \log_{10} -transformed and untransformed data for submucosal and mucosal compartments.

Compartment log ₁₀ ratio	Pre-CPB	Post-CPB	Post-Pre difference	standard error	t	p value
Submucosa	-2.950	-2.295	0.6548	0.1381	4.74	0.009
Mucosa	-3.64	-3.039	0.6010	0.3071	1.957	0.12
Untransformed (%)			Post/Pre%	95% confidence interval		
Submucosa	0.11	0.51	452	187-1092		
Mucosa	0.023	0.091	399	56-2841		

Discussion

This study has confirmed the findings of the clinical studies (Chapter 4, 5, & 6), but in addition, has shown the importance of simultaneously measuring small and large vessel blood flow in conjunction with a technique determining the overall adequacy of tissue oxygenation. Previous studies examining the effect of CPB upon intestinal blood flow have produced a conflicting picture. Recently Lazenby et al (Lazenby et al 1992), found a reduction in SMA flow with hypothermia (25°C) and a flow index during CPB of $3.0 \text{ l min}^{-1} \text{ m}^{-2}$. Previous reports have documented either an increase (Kawashima et al 1976, Utley et al 1981) or no change in blood flow (Mavroudis et al 1984). However, a significant alteration in intestinal blood flow was not found during hypothermic (28°C) non-pulsatile flow ($2.4 \text{ l min}^{-1} \text{ m}^{-2}$). This may in part be explained by the intrinsic autoregulatory ability of the intestine to maintain blood flow and tissue oxygenation in the face of haemodynamic stress. With normothermic ischaemia, $\dot{V}O_2$ is not governed by $\dot{D}O_2$, until $\dot{D}O_2$ becomes reduced by more than 60% (Grum et al 1984). Granger and Norris (Granger and Norris 1980) showed that a 64% fall in MAP resulted in only a 40% fall in intestinal flow due to reduced intestinal vascular resistance. Furthermore, a super-regulatory mechanism also exists which can increase intestinal blood flow despite reductions in MAP when food is present in the gut lumen (Granger and Norris 1980).

Changes in large intestinal vessel haemodynamics do not reflect the more dynamic alterations which may occur in the microvasculature. The gut mucosa is the most metabolically active part of the gut wall and therefore normally receives a greater proportion of the total gut blood flow. In dogs the mucosa and submucosa receives 65-92% of the blood flow and the muscularis and serosa 8-35% (Bond et al 1979, Chou and Grassmick 1978, Fan et al 1979). During CPB, the greatest reduction in LDF was found in the serosal aspect of the gut wall, whilst the mucosal aspect, after an initial reduction in blood flow, returned to pre-CPB levels. The gut controls blood flow through modulations in arteriolar smooth muscle tone; this governs total flow to

the gut wall (Shepherd 1982). However, adjustments in blood flow at a capillary level are made by pre-capillary sphincters. The vasomotor tone of the capillary sphincters may be explained by the metabolic demand theory of local vasoregulation (Granger and Nyhof 1982). This suggests that capillary sphincter tone is independent of nervous or humoral influences, which may be active at an arteriolar level, but is governed by the local pO_2 of the tissues (Granger and Nyhof 1982). Although a correlation was found between reductions in mucosal laser Doppler blood flow and hyperoxia, it must be acknowledged that in this study protocol, the pO_2 and pCO_2 were not controlled independently. To dissociate the effects pO_2 and pCO_2 on intestinal blood flow, a further study is required in which either parameter is held constant whilst the other is changed.

Findings from this study suggest that during the hypothermic phase of CPB blood may be shunted away from the bowel wall, since mucosal blood flow which appears to receive prioritised flow, returned to pre-CPB values, but serosal blood flow remained depressed. Shunting of blood flow away from the GI tract in a dog model with diversion of blood to the liver was commented on by a discussant in a paper on blood flow distribution during CPB in monkeys (Rudy et al 1972). Although in this study alterations in portal venous blood flow were not determined, Desai et al found increased portal vein flow in a canine model of during hypothermic non-pulsatile CPB (Desai et al 1993).

During the hypothermic phase of CPB, $\dot{D}O_2$ is reduced by haemodilution. Although cellular protection may occur by metabolic deceleration associated with hypothermia, hypothermia also decreases the dissociation of O_2 from haemoglobin. During the re-warming phase of CPB, tissue delivery is unaltered (flow and PCV kept constant in this study), whilst metabolism returns to higher rates with normothermia. Increased tissue requirements are met by pre-capillary sphincter vasodilatation, which in our study would account for the 140% surge in mucosal LDF from baseline values, with

an associated increase in O_2 extraction. Increased O_2 extraction was shown by Shepherd to correspond to an increase in density of perfused capillary bed as indicated by ^{86}Rb extraction technique (Shepherd 1978). Oxygen extraction is the main method by which the gut maintains $\dot{V}O_2$. Granger and Norris found that in a canine model, only at very high $\dot{V}O_2$ did functional hyperaemia occur (Granger and Norris 1980). Furthermore, there would appear to be the capacity for vigorous O_2 extraction; combined hypoxia and hypoxaemic stress increased O_2 extraction to 54% (Granger and Norris 1980).

The situation in hypothermic CPB is complicated by cooling and re-warming, but data from this study would indicate that increases in $\dot{V}O_2$ cannot be met by $\dot{D}O_2$. This may in part be due to the anatomy of the villus with the arrangement of capillaries and venules in a system promoting counter-current exchange of O_2 (Jacobson and Noer 1952). The reduced MAP during CPB will increase plasma transit time and further reduce villus tip pO_2 (Chapter 6, Fig 78) (Lundgren and Svanvik 1973). Therefore, the decrease in SMV pO_2 during the re-warming phase of CPB does not accurately reflect villus tip pO_2 which may be expected to be considerably lower than mixed SMV blood. The tonometer more closely reflects tissue hypoxia due to its juxtaposition to the mucosa. Poole et al in a canine model found a linear relationship between decreasing pHi and decreasing intestinal blood flow (Poole et al 1987), and pHi as determined by tonometry has shown very good correlation with microprobe determined intramucosal pH (Antonsson et al 1990).

The rise in small bowel $\dot{V}O_2$ during the re-warming and post-CPB periods may be secondary to metabolic derangements which occur following anaesthesia, CPB and surgery. Although endotoxaemia was not investigated in this study, sepsis increases splanchnic and total body $\dot{V}O_2$ (Dahn et al 1987). An oxygen debt was incurred during the hypothermic phase of CPB as a consequence of the sudden drop in $\dot{D}O_2$ with haemodilution, whilst cooling and reductions in metabolism may occur more

slowly. These differences in $\dot{V}O_2$ were reflected in the Q_{10} before and after CPB. The small bowel Q_{10} for the pre-CPB and hypothermic phase of CPB was 2.44. This was lower than previously reported for whole body Q_{10} for dogs (Hegnauer and D'Amato 1954, Kent and Pierce 1974), but pentobarbitone used for the induction of anaesthesia would reduce the initial $\dot{V}O_2$ (Kent and Pierce 1974). Secondly, during the hypothermic phase, gut tissue temperature and therefore $\dot{V}O_2$ may have been greater than expected for any given core temperature as determined by an oesophageal probe, as a result of tissue temperature gradients (Kent and Pierce 1974). Both these factors would tend to reduce the slope of the graph and the determined Q_{10} . Nevertheless, the small bowel Q_{10} was significantly elevated during the re-warming/ post-CPB period (3.39), confirming an elevated gut $\dot{V}O_2$ at normothermia post-CPB.

In previous clinical studies of CPB, in which impairment of gut function and increases in gut permeability were found (Chapter 6), the absorption of 3-O-m-D-glucose was impaired approximately 73% immediately following CPB with recovery 5 days after surgery, except for patients who had received non-pulsatile perfusion and membrane oxygenation. This monosaccharide is normally transported by a Na⁺-glucose co-transporter from the lumen of the small bowel into the cell and subsequently into the blood stream via an ATP-dependent process (Hediger et al 1987). The mucosal hypoxia found in this animal model may in part explain these clinical findings, since gut mucosal hypoxia would limit cellular ATP availability for all active cellular processes. Although using the isolated jejunal loop, statistically significant reductions were not found in the transport of 3-O-m-D-glucose, its absorption was still reduced by approximately 29% post-CPB compared to animals who had the study immediately prior to CPB. The main advantage of this experimental design was that pre-gastric factors such as delayed gastric emptying and delivery into the small bowel could be excluded as variables limiting absorption. However, the severity of canine and human gut damage may not be directly

comparable, since mucosal blood flow was reduced to the jejunal mucosa by 38% initially with recovery to 11% after 20 minutes of hypothermic CPB. In contrast, clinical studies found that gastric LDF was reduced by over 45% during the hypothermic phase of CPB and did not recover until the re-warming period of CPB. These difference may be due to reductions in cardiac index down to 1.7 l/min/m² for patients undergoing cooling to 28°C, whilst in this animal model, flow was maintained at 2.4 l/min/m² throughout CPB, or they may represent intrinsic differences in responses to CPB by the two species.

The gut permeability ratio which is independent of pre & post-mucosal factors increased approximately 700% in clinical studies (Chapter 5 & 6), whilst in this canine model the permeability ratio increased by approximately 81%, which may also indicate that the magnitude of the insult to the gut in man and dog is quite different. Furthermore, the baseline gut permeability using saccharide test substances was approximately 0.4, which is much higher than the human permeability ratio of 0.06-0.08. Similar findings have been previously reported using ⁵¹Cr EDTA as the test substance, with a urinary excretion value of 13% for dogs, compared to a reference range of 1.0-2.6% for human gut (Hall et al 1989). This difference in baseline gut permeability may indicate a higher rate of enterocyte turnover, since sites of cell extrusion are areas of reduced intercellular binding or it may represent a major difference in the total surface area available for absorption (Hall et al 1989). The latter finding would also enable the canine jejunum to be more resistant to reductions in monosaccharide transport following episodes of mucosal hypoperfusion.

One of the mechanisms involved in the reduction in monosaccharide transport following hypoperfusion/ischaemia has recently been ascertained. In vitro cultures of renal tubular cells, which share the glucose co-transporter found in the small bowel, when exposed to episodes of ischaemia did not alter the amount of carrier present within the cells, but the carrier was mis-directed to the basolateral aspects of

the cell (G Wild 1993, personal communication). This significantly reduced the normal uptake of glucose, even after brief periods of hypoxia. Although this mechanism may predominate and needs validation in a gut model, the role of extra-intestinal inflammation in the impairment of normal intestinal absorption also needs to be addressed. This latter phenomenon may be more important in the context of CPB where a 'whole body inflammatory response' occurs. Inflammation affects membrane integrity and anti-inflammatory drugs such as ibuprofen have a role in preserving lysosomal and cytoplasmic membrane integrity. Formalin-induced peripheral inflammation has been found to decrease the absorption of ^{14}C -leucine and ^{14}C -glucose in the jejunum of rats, with restoration of absorption after anti-inflammatory drug treatment (Somasundaram et al 1983, Somasundaram et al 1983). Prostaglandins may have a role in this malabsorption, since they can increase membrane bound adenylyl cyclase enhancing cAMP production, which inhibits $\text{Na}^+\text{-K}^+$ ATPase necessary for normal glucose and amino acid absorption. Extra-intestinal inflammation with impaired ^{14}C -leucine absorption has been associated with a decrease in ouabain sensitive $\text{Na}^+\text{-K}^+$ ATPase activity, but preserved in the presence of anti-inflammatory drugs such as oxyphenbutazone (Somasundaram and Sadique 1985).

Vasoactive intestinal peptide (VIP) was originally isolated from porcine intestine and was thought to be primarily a gut hormone, but it is now recognised to be mainly found in neurones (Bryant et al 1976). Although VIP has actions upon the gut, stimulating small bowel secretions, it also has significant cardiovascular effects. VIP causes profound vasodilation and hypotension and has been shown to increase cardiac contractility (Said and Mutt 1970). In addition, VIP stimulates hepatic glycogenolysis (Kerins and Said 1973) and lipolysis (Frandsen and Moody 1973), mimicking the cardiovascular and metabolic changes found in sepsis. VIP has been implicated in the development of hypotension in sepsis with increased levels of VIP found in animal models of endotoxic shock and hyperdynamic sepsis (Freund et al 1981, Fuortes et al

1988). Increased levels of VIP was isolated in the portal circulation following sepsis, suggesting production/release of stored VIP from the gut. Intraperitoneal injection of TNF α or IL-1 did not increase portal plasma levels of VIP, but TNF α did increase peptide YY (PYY) levels, a hormone localised to the mucosa of the distal ileum and colon (Zamir et al 1992). However, increased VIP staining in the nerves of the submucous plexus may be due to systemic increases in cytokines other than TNF α or IL-1. VIP has also been found to be released following episodes of gut ischaemia in a porcine model (Mitchell et al 1977). Therefore, it is difficult to determine if increased VIP staining in nerves of the submucous plexus in this canine model is a consequence of extra-intestinal inflammation with cytokine release or secondary to gut hypoperfusion during CPB. VIP may act as a local vasoregulator, dilating the submucous arterioles and maintaining mucosal blood flow at times of gut hypoperfusion. As regards vasodilatory action on gut large blood vessels, maximal vasodilation of the left gastric artery has been found with a 550% increase in conductance following intravenous injection of VIP in a canine model, whilst SMA conductance increased only 20% (Blitz and Charbon 1983). However, the effect of VIP on these large truncal vessels cannot be extrapolated to the arterioles and the microvasculature. If VIP is an important intramural regulator of blood flow in the small bowel, it may explain the preservation of mucosal blood flow during the hypothermic phase, with profound increases in blood flow in the re-warming phase of CPB. In contrast, there was minimal VIP staining evident within the muscularis/serosal aspect of the gut wall, even though the few VIP neurones present increased their staining post-CPB; this aspect of the gut wall sustained the greatest reductions in LDF during CPB.

In summary, this study has found that the gut wall may undergo considerable alterations in blood flow at a microcirculatory level despite minimal changes in large vessel blood flow. Shunting of blood towards the metabolically active mucosa occurs during CPB, particularly during the re-warming phase of hypothermic CPB. There is

a disparity in $\dot{V}O_2$ and $\dot{D}O_2$ during this period with resultant mucosal acidosis and villus tip ischaemia. These metabolic derangements following hypothermic CPB have also been reported clinically. The alterations in gut oxygen utilisation found in this study may help to explain the pathophysiology of endotoxaemia during and following CPB (Chapter 7).

Chapter 9

Discussion

Overall Discussion of Thesis Findings

The retrospective study (Chapter 2) found a relationship between CPB time and the development of gut-related complications. Indirect evidence for organ hypoperfusion was present, with the finding that patients who required inotropic and intra-aortic balloon support had a higher incidence of intra-abdominal complications. Age (>65 years) was also identified as a risk factor. This may indicate either the presence of more advanced mesenteric atherosclerosis, or more limited myocardial function and reduced cardiac output and thus splanchnic perfusion in the post-CPB period. Patients who develop GI complications post-CPB are also more predisposed to the development of multi-system organ failure. It was not possible to discern from a retrospective study, whether GI injury was the origin or the 'motor' of multi-system organ failure, or if GI complications simply reflect generalised organ hypoperfusion.

The importance of tissue perfusion during CPB and the patency of the microcirculation has been highlighted over recent years, with the advocacy of arterial line filtration during CPB to limit organ micro-embolism, the use of pulsatile flow and the trend towards warmer CPB. In relation to gut injury, the finding that antacid/ H₂-antagonist therapy does not reduce the risk of developing post-CPB gastrointestinal haemorrhage, has underlined the value of maintaining mucosal blood flow for the preservation of the gut barrier.

However, the measurement of macrovascular gut blood flow alone provides little insight into the adequacy of tissue oxygenation. Tissue oxygenation is the solution of a complex dynamic equation which includes blood flow, tissue oxygen extraction, metabolic demands, haemoglobin oxygen carrying ability and the patency of the microcirculation. To this end, the determination of intramucosal pH by tonometry has provided a convenient and clinically applicable method for directly determining gastric or colonic pH_i during CPB. Unfortunately, although several studies purport to

describe the changes in gastric mucosal pHi during CPB, including the original work of Fiddian-Green & Baker, none has addressed the problem of measuring the pHi in hypothermic patients (Fiddian-Green and Baker 1987, Kuttala et al 1990, Landow et al 1991). The validation experiments (Chapter 3) found that temperature correcting the pHi was important to reduce the erroneously high and unphysiological values obtained during the hypothermic phase of CPB. Without these corrections, the gastric pHi values implied that tissue $[H^+]$ was lower than arterial $[H^+]$ i.e. a reverse hydrogen ion gradient between tissues and blood. Furthermore, the accuracy of the correction factors provided by the manufacturing company (Tonometrics, Worcester, Mas, USA) is challenged; the discrepancy is greater for lower equilibration times. Ideally it would be more accurate to use an equilibration time in excess of 40 minutes to improve the accuracy of measured pHi values. However, this would limit the value of this technique in monitoring changes in tissue oxygenation in the very dynamic cardiovascular environment of CPB. Even after temperature corrections and the administration of pre-operative H_2 -antagonist therapy to minimise the influence of H^+ back-diffusion on the determined pHi, the limitation of this technique to determine accurately the pHi must be acknowledged. This may be particularly so in circumstances of mucosal hypoperfusion when the assumption that tissue and arterial $[HCO_3^-]$ may not be valid. Nevertheless, gastric tonometry provides very good qualitative data and is currently the only tool available for the non-invasive measurement of tissue oxygenation. Further, its value in the management of critically ill patients and providing early signals for impending systemic dysfunction has been widely reported and put to commercial advantage by the manufacturing company (Gutierrez et al 1992, Maynard et al 1992).

Although the precise value of the gastric pHi during the hypothermic phase of CPB may be difficult to determine, given the limitations of the methodology, both studies presented in this thesis, and those published in the literature, report the same trends in

the pHi. There is a progressive fall in the gastric pHi with re-warming and release of the aortic X-clamp, with further reductions in the post-CPB period. The development of mucosal hypoxia may indicate a reduction in blood supply, but during this period of mucosal acidosis, mucosal blood flow is increasing to, or above, pre-CPB levels in both clinical and animal studies. This illustrates the concept of relative ischaemia, when mucosal hypoxia occurs in the face of normal or supranormal blood flow. If it is appropriate to extrapolate from an animal model to the clinical situation, intramucosal acidosis is due to a disparity between $\dot{V}O_2$ and $\dot{D}O_2$ during the re-warming and post-CPB period. The increase in gut $\dot{V}O_2$ may be due to increased demand by membrane pumps trying to maintain the integrity of cells injured during episodes of hypoperfusion and/ or damage sustained from the effectors of the inflammatory response of CPB. The activated cells (neutrophils, lymphocytes, monocytes and platelets) would also increase oxygen consumption post-CPB. The role of endotoxaemia during CPB in $\dot{V}O_2$ is not defined, but endotoxaemia occur predominantly during this phase of increased gut $\dot{V}O_2$.

Laser Doppler flowmetry, like tonometry, provides only qualitative data, but again has great clinical applicability. It may be argued that the reduction in laser Doppler blood flow during the hypothermic phase of CPB is due to haemodilution, and certainly the validating experiments in Chapter 4 would lend some support to this argument. However, the increase in laser Doppler gastric and canine jejunal blood flow found in the re-warming phase of hypothermic CPB cannot be attributed to changes in the haematocrit. A recent report of a porcine model of CPB found reductions in mucosal laser Doppler blood flow, even when the haematocrit was kept constant by transfusion to eliminate the effect of haemodilution (Cox et al 1992). Not surprisingly, positive correlations were found between mucosal blood flow and cardiac index, independent of blood pressure, both clinically and in the animal model; demonstrating the autoregulatory ability of the gut to maintain blood flow despite

reductions in perfusion pressure. The high pO_2 used during CPB may contribute to mucosal hypoperfusion; hyperoxia has been previously reported to be a mesenteric vasoconstrictor and an inverse relationship was found between mucosal laser Doppler blood flow and arterial pO_2 . The contribution of hypothermia to mucosal hypoperfusion may become more apparent in studies of normothermic CPB, although from both clinical and animal work, reductions in core temperature were associated with reductions in mucosal blood flow.

The median penetration of a laser Doppler probe and therefore its sampling volume is governed by a number of factors. These include probe geometry (diameter of the optical fibre and separation of the transmitting and receiving fibres for probes with more than one fibre), the wavelength of the laser light interrogating the tissue as well as the intrinsic optical properties of the tissue under investigation (Flock et al 1989, Flock et al 1989, Johansson et al 1991). For a single fibre probe operating at 633 nm, the median sampling depth varies from approximately 90 to 360 μm for brain and liver respectively (Jakobsson 1992). The laser Doppler monitor employed for the reported studies operates between 800-820 nm and will therefore have a greater depth of penetration for any given tissue. From the above it may be estimated that the probe depth approaches 700 μm for gut tissue, which approximates to the mucosal layer of the gut wall. Although in the animal model both mucosal and serosal probes were simultaneously applied, the thickness of the dog jejunum approximates to 3-4 mm. Thus a large part of the intestinal wall i.e. the muscularis was not monitored by laser Doppler flowmetry. However, no significant alteration was observed in superior mesenteric arterial blood flow in the animal model during the re-warming phase of CPB, despite a 140% increase in mucosal/ submucosal blood flow from base-line values. It is therefore plausible that blood flow was shunted or prioritised away from the muscularis/serosa. The autoregulatory mechanisms involved are probably complex. A low tissue pO_2 , as indicated by the development of intramucosal acidosis,

would act as a potent vasodilator of pre-capillary sphincters, but the immunohistochemical analysis of the tissues suggests a possible role of VIP.

Little to no VIP stained neural tissue was found in the muscularis/serosal aspects of the gut wall, whilst the mucosal/ submucosal aspects stained avidly for this peptide hormone. Although a recent study suggested that cytokines may play a role in the release of gut hormones such as VIP (Zamir et al 1992), hypoperfusion/ ischaemia is probably a more potent stimulus for production and release of VIP. This is supported by finding that those segments of gut that had been exposed to both CPB and ischaemia-reperfusion injury had the most pronounced staining for VIP. At present it is not possible to say conclusively if increased production of VIP is due to increased transcription of the VIP genome, because the monoclonal for dog VIP mRNA is not currently available. As regards the clinical importance, VIP may have more important systemic consequences beyond its potential role as a local vasoregulator. VIP is a powerful stimulator of lipolysis, mimicking the metabolic derangement in endotoxaemia and sepsis (Frandsen and Moody 1973). VIP also has profound vasodilatory ability, which may become significant if released in large amounts post-CPB.

A reduction in mucosal perfusion during hypothermia may be offset by reduced rates of metabolism, since the pHi is maintained or slightly reduced during this phase even after temperature corrections. Nevertheless, microcirculatory occlusive phenomena may also occur during this period. Ashford has found (P Ashmore 1993, personal communication) microemboli in the mesenteric circulation during CPB in dogs, which has been confirmed by videomicroscopy of the mesenteric circulation in a canine model of CPB at the Hammersmith Hospital (S Ohri, unpublished data). These emboli are probably platelet/fibrin aggregates, which have been previously reported in a study examining the effects of CPB on the retinal microcirculation, but may also

contain activated neutrophils. If these emboli are not completely cleared following reperfusion in the re-warming phase of CPB, they may impair gut $\dot{D}O_2$, but may also cause cellular damage by the release of oxidative enzymes and oxygen free radicals. Preliminary data from a canine model of bypass has also found evidence of CPB-induced lipid peroxidation (S Ohri, unpublished data), similar to lipid peroxidation previously reported for pulmonary tissue post-CPB (Braude et al 1986).

The functional implications of these alterations in blood flow and gut oxygenation were examined by the saccharide absorption and permeation studies. Clinically, profound increases in gut permeability were found following hypothermic CPB. As already discussed in Chapters 5 & 6, increases in gut permeability cannot be explained by pre or post mucosal factors since a combined saccharide analysis (lactulose/L-rhamnose) ratio was employed. Therefore, increased gut permeability was due to the effect of CPB on the gut barrier. Similar to the retrospective study of GI complications, gut permeability was found to have temporal relationship with the CPB time, even allowing for other patient factors such as age or the consumption of aspirin pre-operatively. In Chapter 6, an inverse correlation was found between the pHi and increases in the gut permeability ratio i.e. patients who developed gastric intramucosal acidosis tended to have a higher gut permeability ratio post-CPB. Although it may be hypothesised that this may result in greater endotoxaemia, a positive correlation between gut permeability or pHi was not found. This may be due in part to the lipid solubility of the endotoxin molecule which enables it to permeate through, as well between enterocytes, so that endotoxin permeation is not specifically dependent on permeability changes assessed by hydrophilic molecules. The permeability ratio is a measure of the integrity of intercellular tight junctions. This may explain the relationship between a low pHi and gut permeability ratio, since the zona occludens between enterocytes are controlled by an intracellular cytoskeleton dependent upon a supply of ATP. Animal studies have demonstrated an association

between bacterial translocation/ endotoxaemia and increases in gut permeability; however, this probably represents one end of the gut injury spectrum. In patients who sustain severe gut damage, a similar correlation between gut permeability and endotoxaemia may become evident. In the majority of patients, although endotoxaemia is present, septicaemia does not ensue, suggesting the injury and increase in gut permeation is self-limiting.

Perhaps more perplexing is the problem of endotoxaemia and its clinical significance. At what level does endotoxaemia become significant ? This problem cannot be addressed by measuring endotoxin levels alone, since naturally occurring antibodies to endotoxin may provide protection from the endotoxin insult during CPB. At present, it is difficult to make comparisons between endotoxin studies of CPB due to the heterogeneity of assay techniques. The validation experiments on the endotoxin assay found that both haemodilution and heparinisation may significantly alter the sensitivity of the LAL chromogenic assay (Chapter 7). Until these limitations are acknowledged, or a more reliable assay becomes available, endotoxin cannot be reliably assayed from CPB samples. Using the modified LAL assay, endotoxaemia was demonstrable in the majority of patients undergoing CPB. There was a progressive increase in the endotoxin concentration in the post X-clamp period, supporting previous findings, and the notion that it may be due to a washout of endotoxin, absorbed during hypoperfusion (Jansen et al 1992). There was no specific correlation between the degree of endotoxaemia and post-operative outcome. When other patient and perfusion factors were assessed in combination with endotoxaemia, endotoxaemia was a good predictor of the length of ICU stay and the duration of intubation (Chapter 7). The significance of the endotoxaemia may have been diluted by the presence of naturally occurring antibodies, which may afford a degree of protection from gut endotoxins. Further 'dilution' may be due to so called environmental endotoxins, which are endotoxins found trapped in the plastic tubing

of the extra-corporeal bypass circuit and in fluids administered during CPB, such as the pump-oxygenator priming fluid and iced slushed saline used for topical cooling of the heart. Although these non-gut derived endotoxins are measured by the LAL assay, they are less potent than gut derived endotoxin. It has been estimated that the environmental endotoxins may amount to approximately 20 -30 ng of 'exogenous' endotoxin. For a 70 kg individual this would only amount to 6 ng/l endotoxin, which is considerably below (mean 47.1 ± 7.8 ng/l, n=28) the levels measured in the clinical studies (Chapter 7). Furthermore, this exogenous endotoxin would not explain the reproducible increase in systemic endotoxin levels following release of the aortic X-clamp. On this basis it is difficult to explain the lack of increase in TNF α with endotoxaemia. TNF α has been reported to become elevated 1-2 hours following the development of endotoxaemia (Jansen et al 1992), especially since the sensitivity of the TNF α assay was doubled to maximise the detection of this cytokine. However, these results would be in keeping with other reports, which have also failed to detect significant increases in TNF α during CPB (Butler et al 1993, Finn et al 1993, Haeffner-Cavallion et al 1989). This may indicate that either the endotoxin measured has a low potency, because of binding to lipoproteins in inflammatory serum, or that other mechanisms exist in vivo during CPB inhibiting TNF generation by monocytes.

The isolated jejunal loop experiments in the canine model supported the clinical findings. Although significant reductions in monosaccharide transport were not observed, the increase in gut permeability was only 80%, despite handling of the bowel, compared to the 700% increase found in clinical studies. This may be attributable to differences in the CPB protocol, since in the canine model the cardiac index was maintained at 2.4 l/min/m², but was reduced clinically to 1.7 l/min/m² with core cooling to 28°C. This may indicate the relevance of CPB protocol in the attenuation of gut injury, especially since a positive correlation was found for mucosal laser Doppler blood flow and the cardiac index. The clinical studies reported

in Chapter 6 have examined the influence of pulsatile flow and the type of oxygenator used for CPB. There was a tendency for pulsatile patients, regardless of the oxygenator used, to maintain a higher gastric pHi following aortic X-clamp release, with less reduction in the arterial $[\text{HCO}_3^-]$. This would be consistent with early reports in the literature, which have postulated the benefits of pulsatile perfusion in the maintenance of tissue oxygenation by promoting microvessel patency (Shepard and Kirklin 1969). Significant differences were not found for the absorption of monosaccharides or for the gut permeability ratio immediately post-CPB, but both bubble and membrane oxygenator patients who had received non-pulsatile perfusion had a higher gut permeability ratio at 5 days post-CPB. Pulsatile flow was also the best predictor for a normal post-CPB permeability ratio. The sensitivity of the test, probably limited its ability to delineate differences in perfusion protocols immediately post-CPB, which became more apparent at 5 days, when most patients were preparing for discharge home. Both patients who developed duodenal ulcers, without a pre-operative history of dyspepsia, belonged to the non-pulsatile group. This would be in keeping with clinical evidence, albeit from retrospective studies, which have found the benefits of pulsatile perfusion in reducing the incidence of post-CPB gastrointestinal complications (Huddy et al 1991).

The presented studies have not resolved the mechanisms behind the reductions in monosaccharide transport. When the problem is viewed with a mechanistic approach, it is difficult to appreciate how such a profound reduction in glucose transport can occur, when the small intestine is known to have such an efficient mechanism for the transport of this monosaccharide. Normally, glucose malabsorption does not occur until 80% or more of the small intestine is resected. However, mucosal hypoxia as determined by tonometry, in clinical and animal studies, should affect the entire length of the small intestine, thus impairing carrier mediated transport. This may explain both the reduction in active and carrier mediated transport, since the

production of carrier proteins may be diminished - or as has been found for renal tubule cells made ischaemic, the carrier protein for glucose becomes mis-directed to the basolateral rather than the apical portion of the cell (G Wild 1993, personal communication). This hypothesis is probably one piece of a much larger jigsaw puzzle. Monosaccharides may still be transported from the gut lumen to the basolateral aspect of the enterocytes, but then become 'trapped'. The transport of nutrients from the basolateral aspect of the cells to the bloodstream is dependent upon passive diffusion and the gradient is maintained by clearance by normal blood flow. As previously mentioned, laser Doppler blood flow appears to become supranormal in the immediate post-CPB period, so this would not appear to be a limiting factor. However, since nutrients move down their concentration gradients by passive diffusion, an increase in the unstirred fluid layer may significantly retard their export from the gut wall. Breckenridge et al found increases in extracellular water of up to 30% in patients following CPB and significant increases in gut interstitial pressure indicating oedema have also been found (Breckenridge et al 1970, Utley 1990). The histological sections of dog jejunum would also lend support to these findings, with swelling of the villi and increases in the thickness of the submucosal layer post-CPB, with larger increases in gut segments sustaining CPB and ischaemia-reperfusion injury. However, a reduction in the rate of gastric emptying post-CPB would decrease delivery of the monosaccharides into the small intestine and thus impair the total dose of enterally administered monosaccharide subsequently excreted in the urine over the 5 hour collection period. This may explain the difference in results between the clinical studies (chapter 5 & 6) when the test solution was administered into the stomach compared to the isolated jejunal loop studies when the monosaccharides were delivered directly into the small bowel (chapter 8). Furthermore, the increase in the extracellular compartment post-CPB may be expected to also increase the volume of distribution of the test monosaccharides, which would result in reduced clearance of the monosaccharides in the face of unchanged renal function.

The Future

From the presented study and numerous reported in the literature, the risk factors for the development of GI complications have only begun to be addressed. A larger prospective study, with pre-CPB risk stratification is required in order to delineate pre-operative and CPB-related factors. The high mortality, which has been historically associated with post-CPB GI complications, will undoubtedly become reduced as clinical awareness of these problems increases, which will reduce delays in diagnosis and management.

CPB haemodynamic parameters have traditionally been based upon limits which provide a safe level of cerebral and renal protection. Although these parameters still remain important for cerebral protection, they may need modification and extension if other organs, particularly those of the splanchnic bed, are to receive adequate perfusion during CPB. This thesis is only a primer for further more detailed enquiries of haemodynamic alterations of the splanchnic bed during CPB. Other organs of the splanchnic bed such as the liver, have largely been studied in experimental animal models. Currently, only one study with a heterogeneous mix of CPB protocols exists in the literature describing the effect of CPB on liver perfusion (Hampton et al 1989). Only after the influence of a standardised CPB protocol on perfusion of various aspects of the splanchnic bed has become established, can studies then be undertaken manipulating CPB parameters with the goal of optimising splanchnic perfusion.

The influence of anaesthetic agents and drugs administered to patients during the course of general anaesthesia and CPB has not been examined by the presented studies. Clearly some agents which are splanchnic vasodilators may enhance mesenteric perfusion, such as dopexamine or dopamine, whilst the influence of anaesthetic agents on mesenteric blood flow is poorly understood. However, a

preliminary report has recently suggested that gastric intramucosal acidosis may be attenuated by avoiding enflurane and using isoflurane for patients undergoing cardiac surgery (Mythen et al 1993).

Although these studies have found that intramucosal acidosis develops in the re-warming period and the immediate post-CPB period, the point when the pHi reaches its lowest limits and the influence of CPB protocol needs defining. This may be particularly important with current clinical trends to 'fast track' patients out of intensive care and into high dependency at the earliest possible date. Peripheral assessments of the adequacy of tissue oxygenation, such as oxygen transport and metabolic measurements, have already been shown to be of limited value (Maynard et al 1992). In this context gastric pHi measurements may be a useful adjunct in the clinical timing of extubation. Recently, there has also been a trend towards normothermic CPB, with many surgeons using an intermediate temperature of 32-34°C rather than 28°C. The basis of this clinical practise is anecdotal and the advantages/ disadvantages both for cerebral and splanchnic perfusion have not been determined. In the light of the findings of this thesis, it may be postulated that normothermic CPB may be detrimental to the gut, if current levels of perfusion are employed (2.4 l/min/m²), since mucosal hypoperfusion may occur without reductions in the metabolic rate of the tissue, although splanchnic hypoperfusion due to splanchnic vasoconstriction may be attenuated by not core-cooling patients.

At a molecular level, the effect of CPB on the carrier systems of the gut wall requires further study. This may be achieved by taking biopsy samples from patients if ethically permissible or culturing enterocytes and performing in vitro hypoxia experiments. Furthermore, no current information is available as regards the influence of CPB on the enzymes lining the gut wall or the transport of fats and proteins. If a malabsorptive state does exist following CPB, it may then be relevant to examine the

change in the absorption of enterally administered drugs such as digoxin and atenolol in the immediate post-CPB period; this may help to reduce the rate of post-CPB arrhythmias if these drugs need initial parenteral administration. The nutritional aspects may also provide a therapeutic modality, since the gut is highly dependent upon glutamine as its main source of energy (Windmueller and Spaeth 1974). Glutamine supplementation has been found to reduce translocation in a rodent model of abdominal irradiation- induced gut injury (Souba et al 1990), but also to enhance the functions of the immune system (Parry-Billings et al 1990). Glutamine levels have been found to become profoundly depressed following CPB (Castell et al 1992); early enteral supplementation with glutamine-enriched feed may prove beneficial in limiting increases in gut permeability and/ or enhancing the rate of recovery, but also in aiding the reversal of the immunosuppression of CPB.

Clearly, there are many paths which may be followed in understanding the mechanisms of splanchnic injury post-CPB and thus their potential solutions. However, the approach once advocated by Professor DG Melrose, a pioneer of CPB, may be most appropriate:

'Individually each is important, but it is when ideas combine in synergy that real momentum is established'

Prof DG Melrose

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Glossary of Abbreviations

ADP	adenosine diphosphate
ARDS	adult respiratory distress syndrome
ATP	adenosine triphosphate
BNP	bubble oxygenator with non-pulsatile flow
BP	bubble oxygenator with pulsatile flow
CABG	coronary artery bypass grafting
CGRP	calcitonin gene-related peptide
CI	cardiac index
CMI	cell mediated immunity
CO	cardiac output
CPB	cardiopulmonary bypass
CR	complement receptor
CV	coefficient of variation
CVP	central venous pressure
DC	direct current
DJ	duodeno-jejunal
$\dot{D}O_2$	oxygen delivery
DU	duodenal ulcer
EDTA	ethylenediaminetetraacetate
eep	energy equivalent pressure
ELAM	endothelial leukocyte adhesion molecule
ELISA	enzyme linked immunosorbant assay
EU	endotoxin unit
GI	gastrointestinal
GLIM	generalised linear modelling
HDL	high density lipoprotein
ICAM	intercellular adhesion molecule

ICU	intensive care unit
IL	interleukin
IR	ischaemia-reperfusion
IU	international unit
JVP	jugular venous pressure
LAL	Limulus amoebocyte lysate
LBP	lipopolysaccharide binding protein
LDF	laser Doppler flow
LFA	leukocyte function associated antigen
LPS	lipopolysaccharide
LT	leukotriene
MAP	mean arterial pressure
MHC	major histocompatibility complex
MNP	membrane oxygenator with non-pulsatile flow
MP	membrane oxygenator with pulsatile flow
NO	nitric oxide
NOS	nitric oxide synthase
NP	non-pulsatile
NSAID	non-steroidal anti-inflammatory drug
NYHA	New York Heart Association
OFR	oxygen free radical
P	pulsatile
p	pressure
PAF	platelet activating factor
PBS	phosphate buffered saline
pCO ₂ (ss)	steady state pCO ₂
PCV	packed cell volume
pHa	arterial pH
pHat	temperature corrected arterial pH

pHi	intramucosal pH
pHit	temperature corrected intramucosal pH
PPS	post-perfusion syndrome
R	gas constant
RBC	red blood cell
RBCVF	red blood cell volume fraction
RE	reticuloendothelial
REML	restricted maximum likelihood
RPMS	Royal Postgraduate Medical School
SMA	superior mesenteric artery
SMAI	superior mesenteric flow index
SMV	superior mesenteric vein
SVRI	systemic vascular resistance index
SVRU	systemic vascular resistance units
T	temperature
TAL	Tachypleus tridentatus lysate
TBF	total blood flow
TNF	tumour necrosis factor
Tx	thromboxane
V	volume
VIP	vasoactive intestinal peptide
$\dot{V}O_2$	oxygen consumption
vWF	von Willebrand's factor
x	body temperature
X-clamp	aortic cross-clamp
y	solubility coefficient of CO ₂ and
3-O-m-D-glucose	3-O-methyl-D-glucose
[Ca ²⁺] _f	intracellular calcium concentration
[HCO ₃ ⁻]	bicarbonate concentration