# THE ROLE OF GASTROINTESTINAL MUCOSAL HYPOPERFUSION IN THE PATHOGENESIS OF POST-OPERATIVE ORGAN FAILURE

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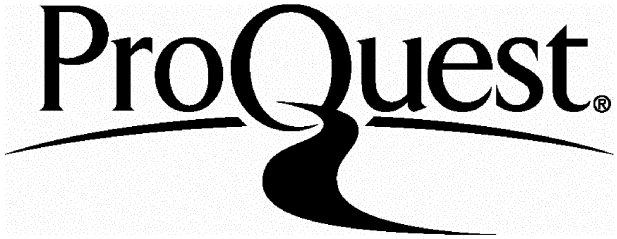

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# ABSTRACT


## THE ROLE OF GASTROINTESTINAL MUCOSAL HYPOPERFUSION IN THE PATHOGENESIS OF POST-OPERATIVE ORGAN FAILURE.

Multiple organ failure is the commonest cause of death in the intensive therapy unit. From a wide variety of stimuli, such as trauma or infection, an excessive and uncontrolled inflammatory response is thought to be the final common pathway in its pathogenesis. Major surgery is a significant pro-inflammatory stimulus that may be compounded by a failure to maintain an adequate blood supply to all organs. The purpose of this thesis was to explore the hypothesis that splanchnic hypoperfusion with subsequent activation of inflammatory pathways, possibly via translocation of endotoxin, occurs during major surgery and is associated with the development of post-operative organ failure.

The gastrointestinal tonometer allows the assessment of splanchnic perfusion by calculation of the gastrointestinal intramucosal pH (pHi). In agreement with previous studies it was demonstrated that the development of a gastric intramucosal acidosis is common during major surgery and is a sensitive predictor of a poor outcome. By measuring descending aortic blood flow, using an oesophageal Doppler, the hypothesis was developed that occult hypovolaemia is a common cause of peri-operative splanchnic hypoperfusion.

In another group of patients undergoing major surgery plasma components of the contact system and neutrophil elastase:$\alpha_1$-antitrypsin complexes were measured. An association was demonstrated between peri-operative gut mucosal hypoperfusion, excessive activation of the contact system, increased neutrophil degranulation and the development of organ failure. The measurement of changes in endotoxin core antibodies were also used to examine the relationship between peri-operative gut mucosal hypoperfusion and

exposure of patients to endotoxin. The results suggested that translocation from the gut lumen is not the sole source of endotoxin during the peri-operative period. The same results showed an unexpected association between exceptionally high levels of endotoxin antibodies and the maintenance of gut mucosal perfusion.

Finally, in a prospective randomised study of patients undergoing elective cardiac surgery, it was demonstrated that per-operative plasma volume expansion reduces the incidence of gut mucosal hypoperfusion. This was asociated with a significant reduction in post-operative morbidity.

# CHAPTER 1.0. THE PROPOSED ROLE OF GASTROINTESTINAL MUCOSAL HYPOPERFUSION IN THE PATHOGENESIS OF POST-OPERATIVE ORGAN FAILURE.

## 1.1. THE LINK BETWEEN GASTROINTESTINAL MUCOSAL HYPOPERFUSION AND ORGAN DYSFUNCTION.

### 1.1.1. Surgery and organ dysfunction.

Despite advances in anaesthetic and intensive care management techniques, multiple organ failure remains the commonest cause of death on surgical intensive care units (Machiedo, Loverme, McGovern et al. 1981). The fact that the resultant organ damage is apparently unrelated to the presenting pathology or the site of surgery can leave clinicians bemused. The surgeon has often performed a macroscopically perfect operation during an ostensibly uneventful anaesthetic, with prophylactic antibiotics given before the first skin incision. Up to the moment of deterioration, often days after surgery, the heart rate, blood pressure, urine output, etc. that have been recorded laboriously on anaesthetic, recovery, intensive therapy unit and ward charts often remain *normal*; yet the patient may still die. This would suggest that either the cardiovascular status of the patient is unrelated to the pathogenesis of organ dysfunction or commonly measured cardiovascular variables are insensitive to the developing pathology. To explore these relationships it was first necessary to examine the hypotheses of the pathogenesis of multiple organ failure.

### 1.1.2. Historical perspective - 'Irreversible shock'.

Blalock (1930) suggested that it was the loss of blood rather than the *"release of evil humors"* that led to death after major trauma and recommended treatment by the administration of intravenous fluids . It was not until the 1940s and the second World War that blood and plasma were widely used for the treatment of blood loss (Blalock

1943). This major development coupled with the enormous number of trauma victims treated at the time resulted in the widespread recognition of a new phenomenon - 'irreversible shock' (Blalock 1943). It became apparent that numerous hypovolaemic casualties could have their vital signs restored with the administration of intravenous blood and plasma only to develop 'secondary shock', often days after the original insult, which rapidly became resistant to any form of treatment. Fifty years later the main thing that seems to have changed is the terminology used to identify the same condition. Even from the time of starting the work for this thesis the terms multiple organ failure syndrome (MOFS), multiple systems organ failure (MSOF) and, most recently, multiple organ dysfunction syndrome (MODS) have all been used to describe 'irreversible shock' and organ failure. Rather like the elephant it seems we have difficulty describing it but we all know one when we see one.

In 1943 Blalock summarised the causes of irreversible shock seen in the Armed Forces as:

> *"...(1) haemorrhage uncomplicated by gross trauma; (2) burns; (3) trauma to large masses of muscle and (4) the re-establishment of circulation in a damaged ischaemic area."*

In the same dissertation he suggests that:

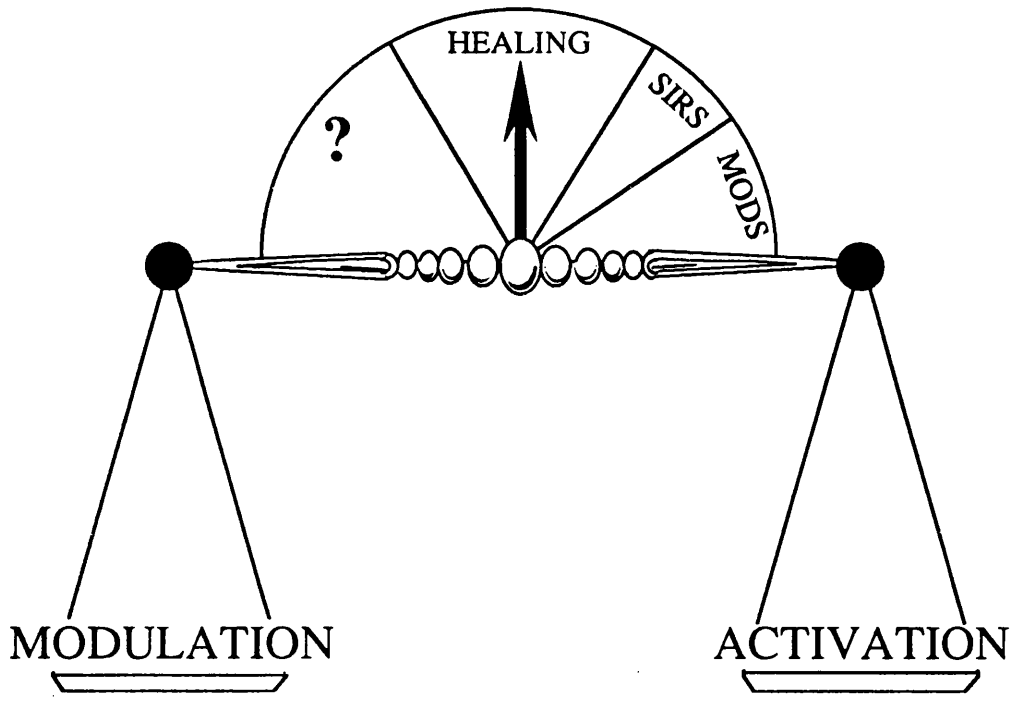
> *"...the initial phases (of shock) are certainly associated with and probably dependent upon a reduction in the volume of effective circulating blood... Investigations have indicated that at some time in the development of irreversible shock there appear effects that may be ascribed either to toxic substances elaborated in areas of tissue damage or to a derangement of metabolism produced by a circulation which, either locally or generally, has been compromised over a long time."*

The currently proposed theories of the pathogenesis of organ failure differ mainly in the fine detail; in particular many of the 'toxic substances' or mediators have been identified. Currently, we recognise and have tried to define more clearly the clinical conditions of the Systemic Inflammatory Response Syndrome (SIRS) and Multiple Organ Dysfunction Syndrome.

### 1.1.3. The Systemic Inflammatory Response Syndrome and Multiple Organ Dysfunction Syndrome.

It is postulated that, as a result of an insult, uncontrolled activation of inflammatory pathways may result in tissue destruction and subsequent organ failure. Inflammation is an essential component of the healing process (Williams and Maier 1992). From a wide variety of stimuli (e.g. trauma, burns, infection) the final common pathway results in vasodilatation, increased vascular endothelial cell permeability, thrombosis and leukocyte migration and activation (Glauser, Zanetti, Baumgartner et al. 1991; Goris 1991; Knaus, Draper, Wagner et al. 1985). Successful localisation to the injury site should result in resolution and healing. The systemic inflammatory response syndrome is the latest term proposed to describe a failure of localisation. The multiple organ dysfunction syndrome, due to tissue damage in organs distant to the site of the original injury, is the clinical manifestation of the systemic inflammatory response syndrome (Glauser, Zanetti, Baumgartner et al. 1991; Goris 1991; Knaus, Draper, Wagner et al. 1985). Irrespective of the initiating stimulus (e.g. surgery, bacterial infection, pancreatitis) the morphology of necropsy specimens in both animal models and patients is remarkably constant. There is microvascular occlusion, vascular endothelial cell destruction, interstitial oedema, leukostasis and thrombosis (Coalson 1975; Tighe, Moss, Boghossian et al. 1989). Therefore, there would seem to be a dichotomy. Inflammation is essential for successful recovery from infection or injury, yet an excessive and uncontrolled inflammatory response can result in organ dysfunction or failure. One hypothesis is that there is a level of stimulation that, once exceeded, leads to uncontrolled activation of inflammatory pathways (Redl and Schlag 1991). There would appear to be a critical balance between activation and modulation (**Figure 1.1**).

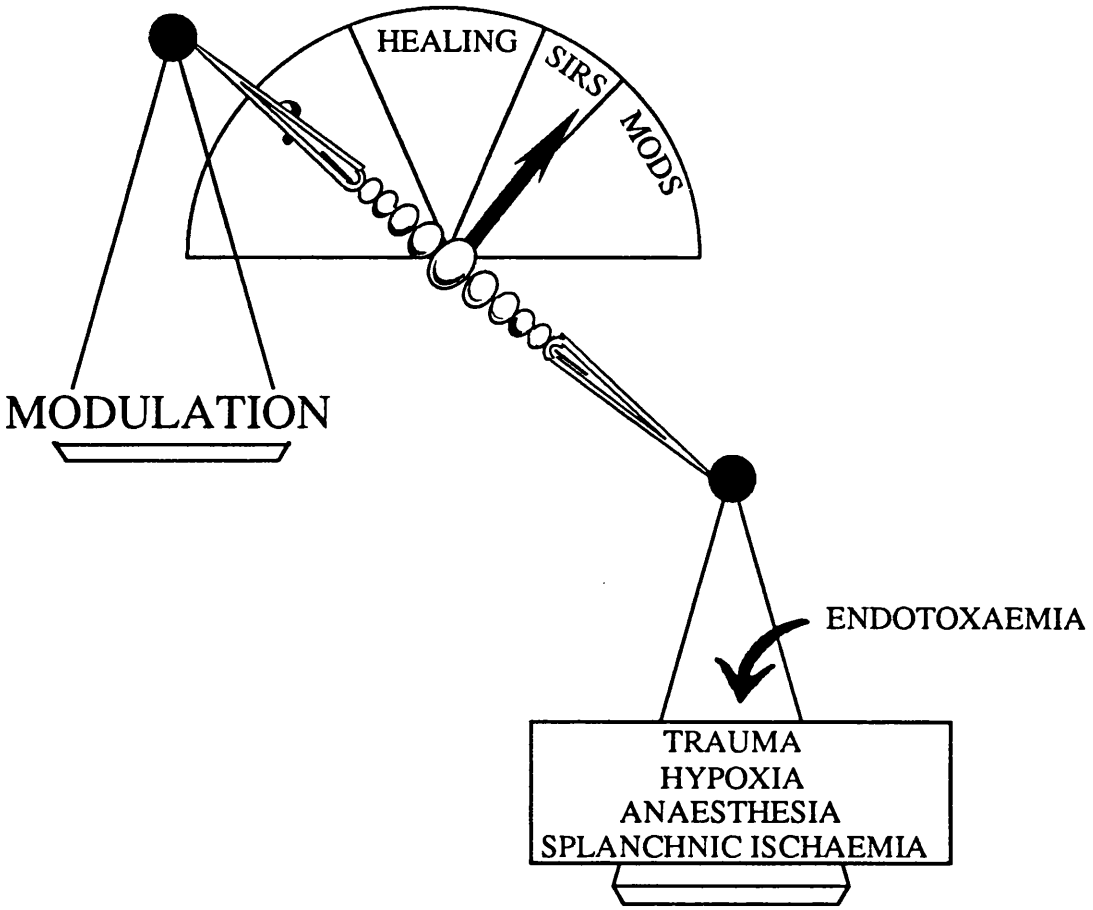
*Figure 1.1. The fine balance between activation and modulation of inflammatory pathways in the pathogenesis of post-operative organ dysfunction.*




### 1.1.4. The link between surgery and organ failure.

Many patients who undergo elective major surgery have an uneventful recovery. However, a considerable number suffer some form of end organ dysfunction. Things like breathlessness, reduced urine output and confusion are so common place that they are regarded as almost normal. A small percentage develop organ failure requiring admission to an intensive care unit for organ support. Irrespective of outcome the presumed initial insult (a particular type of surgery) is usually of a similar magnitude with no obvious variation as judged by type, duration and difficulty in those who have a poor outcome. Therefore, it is proposed that either host responses may vary or additional activation of inflammatory pathways may have compounded the insult of surgery. One of the proposed compounding insults is translocation of bacteria or endotoxin from the lumen of the gut into the blood stream as a result of an occult reduction in gut mucosal blood flow during the peri-operative period (**Figure 1.2**).

*Figure 1.2. Endotoxaemia as a potential compounding factor tipping the balance in favour of uncontrolled inflammatory activation and post-operative organ dysfunction.*




### 1.1.5. Bacterial translocation.

Fine et al. (1965) originally proposed that bacteria and/or endotoxin escaping from the damaged gut were a potential source of systemic infection. The primary function of the gut mucosa is absorption of nutrients. However, it also serves as a mechanical barrier to prevent the invasion of bacteria that are usually contained within the lumen of the gut. In both experimental animals and humans the barrier function of the gut is compromised by various pathological insults, including haemorrhage (Baker, Deitch, Li et al. 1988; Deitch, Morrison, Berg et al. 1990), trauma (Deitch and Bridges. 1987), burns (Carter, Tompkins, Schiffrin et al. 1990; Deitch 1990a), bacterial infection (Ziegler, Smith, O'Dwyer et al. 1988), endotoxinaemia (Fink, Antonsson, Wang et al. 1991a; Fink, Antonsson, Wang et al. 1991b; O'Dwyer, Michie, Ziegler et al. 1988) and sterile inflammation (Deitch, Wen-Jing, Li et al. 1990). Translocation is defined as the

movement of viable bacteria, endotoxin or yeast across grossly intact epithelium from the lumen of the gut into mesenteric lymph nodes, liver, spleen, lung or blood. Translocation is thought to be pathological whereas the appearance of very small amounts of gut derived endotoxin in the mesenteric lymph nodes (but not beyond) is thought to be physiological (Fink 1991). Koziol et al. (1988) demonstrated in a rat haemorrhagic shock model that death was inevitable by 72 hours following a prolonged period of hypotension (a mean of 249 minutes at 30 mmHg) despite successful initial resuscitation. Bacteraemia was demonstrable in some animals as early as two hours into the period of haemorrhagic shock and in all animals by three hours. Bacteraemia resolved in the early post-shock period but then reappeared, as early as two hours later, primarily in portal-venous rather than systemic cultures. The organisms isolated were all common rodent enteric flora and were cultured from the GI tracts of the same rats. Altered barrier function (i.e. increased permeability to non-microbial markers or bacterial translocation) has been demonstrated in human volunteers injected with endotoxin (O'Dwyer, Michie, Ziegler et al. 1988), burn patients with sepsis (Ziegler, Smith, O'Dwyer et al. 1988), non-septic burn patients soon after their injury (Deitch 1990a) and patients with uncomplicated small bowel obstruction (Deitch 1989). Further circumstantial evidence for the gut as a potential source of acquired infection is provided by Marshall et al.(1988) who showed that the bacteriology of nosocomial infections on the intensive therapy unit was the same as that in the proximal gastrointestinal tract of the same group of patients. This could, however, equally well be explained by simple overflow from the stomach and oro-pharynx into the respiratory tract. Against this proposed route of infection and in favour of the translocation theory are the findings of Rush et al. (1988) who demonstrated a significantly greater incidence of positive peripheral blood cultures taken from trauma patients admitted with a systolic blood pressure of 80 mmHg or less (8/10) when compared to patients with a blood pressure of 80-110 mmHg (2/7) or greater than 110 mmHg (1/25) ($p$ <0.01). However, more recently, Moore et al. (1990) found portal venous bacteraemia to be a rare event for the first five days following severe torso trauma. This suggests that dissemination of gut derived organisms beyond the mesenteric

lymph nodes via the portal vein is uncommon in this group of patients, although it does not rule out the possibility of dissemination via the lymphatics.

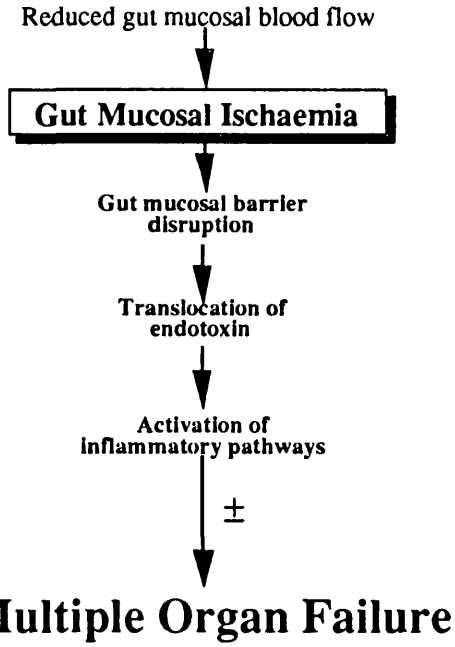
There is much animal, indirect human and circumstantial evidence to support the hypothesis that the gut is a potential motor for organ failure. Although not necessarily an initiating insult, gut mucosal ischaemia and translocation of gut luminal contents may certainly fuel the fire. There is, however, very little direct human evidence to support the hypothesis that bacterial translocation is a constant, causally related event in the pathogenesis of all forms of multiple organ failure.

### 1.1.6. The link between bacterial translocation, endotoxaemia and organ dysfunction.

Bacteria that may translocate from the gut lumen are normally prevented from entering the systemic circulation by the reticulo-endothelial system. The subsequent lysis of bacteria, releasing cell wall products (endotoxin), is associated with stimulation of the cytotoxic pathways whose main purpose is to prevent the invasion of micro-organisms. These pathways, stimulated via the activation of gut and hepatic macrophages are not selective and, as described above, can eventually lead to the characteristic tissue destruction of MOF (Goris 1991). Such mediators and other embolic material are normally cleared by the lungs, but this fails with the onslaught of these tissue destructive agents. Furthermore, activation of pulmonary macrophages is thought to lead to the release of more tissue destructive agents (Glauser, Zanetti, Baumgartner et al. 1991). Endotoxin is a recognised potent activator of various cellular and humoral pathways involved in the generalised inflammatory response (Williams and Maier 1992). In controlled animal, volunteer and laboratory studies administration of endotoxin has been shown to not only stimulate cytokine production from macrophage-monocytes (Michie, Manogue, Spriggs et al. 1988) but also to activate directly complement and the contact and coagulation systems (Mason, Kleeburg, Doland et al. 1970;Morrison and Cochrane 1974; Robinson, Klondnycky, Loeb et al. 1975). Endotoxin is commonly present in the plasma taken from patients with septic shock, including those with no detectable bacteraemia or those

with isolated Gram positive bacteraemia (Danner, Elin, Hosseini et al. 1991). In hospitalised patients with fever, the presence of an endotoxaemia predicts the development of the sepsis syndrome with a sensitivity of 79% and specificity of 96% (van Deventer, Buller, ten Cate et al. 1988). These findings strongly support the notion that endotoxin is an important, though not essential component in the pathogenesis of multiple organ failure.

***Figure 1.3.*** *The proposed link between gut mucosal hypoperfusion and post-operative organ dysfunction*




Therefore, if we accept the above hypothesis, it would seem logical to try and detect a reduced gut mucosal blood flow during surgery as this may be associated with gut mucosal ischaemia. As was suggested earlier the commonly measured cardiovascular variables are poor predictors of outcome. Two explanations were offered for this: i) that the cardiovascular status of the patient is unrelated to the pathogenesis of organ failure or ii) that they are too insensitive to detect the underlying deficits in tissue and, in particular, gut mucosal perfusion. The hypothesis outlined above would support the later

explanation and if this is so then monitoring gut mucosal perfusion should improve sensitivity at predicting post-operative outcome.

## 1.2. CARDIOVASCULAR MONITORING AND PREDICTION OF OUTCOME.

### 1.2.1. The purpose of monitors.

The word 'monitor' is from the Latin monere - to warn. The purpose of a monitoring device is to measure a physiological variable and to indicate trends of change, thus allowing the physician to make appropriate therapeutic interventions and improve outcome.

### 1.2.2. Commonly measured cardiovascular variables and outcome

That most of the commonly measured cardiovascular variables are unreliable predictors of patient outcome is repeatedly acknowledged in the literature (Bland, Shoemaker, Abraham et al. 1985; Eichhorn, Cooper, Cullen et al. 1986; Fiddian-Green and Baker 1987; Shoemaker 1991; Sykes 1992; Tuman, McCarthy, Spiess et al. 1989). Sykes (1992), in a review of the last 60 years of clinical measurement in anaesthetic practice, examines the value of measurements of blood pressure, central venous pressure, pulmonary artery pressure and end tidal carbon dioxide. When considering the measurement of blood pressure, which is the most commonly measured cardiovascular variable in the UK, he asks in desperation:

> *"Surely, we have here a measurement which is easy to make, has acceptable accuracy and which has been shown to be crucial to outcome?"*

He goes on to point out that the only thing in its favour is ease of measurement and concludes that although the use of more advanced monitoring techniques may have enriched the lives of anaesthetists there is little or no evidence to say they have done the same for patients.

Following the introduction of the Harvard Standards of Minimal Monitoring in 1985 there was a temporally related reduction in local Insurance costs (Eichhorn, Cooper, Cullen et al. 1986). This has been interpreted as a direct effect of the increased use of patient monitors on subsequent outcome. However, the first clause of the document stipulated the presence of an anaesthetist (or nurse anaesthetist) throughout the procedure. Many sceptics have proposed that this single mandate had the most significant impact on patient outcome and go on to cite that no one has actually proved that any piece of monitoring equipment has had a similar effect. In their description of the development of the Harvard Standards Eichorn et al. (1986) admit that:

> *"No experimental data exist from which a set of standards for minimal monitoring during anaesthesia can be objectively derived."*

Everybody recognises that if a patient is anuric and has no recordable blood pressure following an elective surgical procedure they will probably not do well. Unfortunately, it is less readily accepted that the converse is simply not true and it remains a continued source of frustration for staff working in Intensive Therapy Units that the majority of cardiovascular variables are normal in patients who subsequently die (Bland, Shoemaker, Abraham et al. 1985;Shoemaker 1991). Is this simply because we are measuring the wrong things?

### 1.2.3. Oxygen flow variables.

Shoemaker et al. (1991) looked at heart rate, blood pressure, central venous pressure, cardiac output and other commonly monitored variables in high risk patients having major, non-cardiac surgery. On analysis of approximately 70,000 measurements they found that 76% had been normalised in the patients who eventually died. They concluded that either the wrong variables were being measured or that normal values (taken from a cohort of healthy people) may be inappropriate following major surgery. In a follow-up study they looked at 220 consecutive patients pre-operatively, intra-operatively, and up to four days post-operatively (Bland, Shoemaker, Abraham et al. 1985). They found a marked and significant increase in cardiac output, oxygen transport and oxygen

consumption in survivors within four hours of the start of surgery. All other measured variables showed little difference between survivors and non-survivors. They hypothesised that an appropriate metabolic response to the trauma of major surgery produces an increased tissue oxygen demand and that the survivors were able to meet this demand. Similarly, in cardiothoracic surgical patients, Krauss et al. (1975) found that a persistently low mixed venous oxygen saturation was strongly associated with morbidity and mortality, in other words there was a failure to meet the body's increased oxygen demands.

The function of the cardio-respiratory system is to maintain an adequate supply of oxygen to all tissues. Failure to achieve this results in tissue hypoxia and cellular dysfunction. This suggested that using oxygen flow variables as monitors would improve outcome.

In a prospective randomised study Shoemaker et al. (1988) prospectively used the median cardiac index, oxygen transport and oxygen consumption levels of survivors of major surgery as therapeutic goals. High risk surgical patients (expected mortality of 30%) admitted to the trial were randomised to one of three groups: a CVP control group (n=30); a pulmonary artery catheter control group (n=30) and a pulmonary artery catheter protocol group (n=28). The control group patients were treated according to a strict protocol with the aim of maintaining normal values for all of the available cardiovascular variables. In the protocol group additional fluids and drugs were given as required to try and achieve cardiac index of >4.5 l/min/$m^2$, oxygen delivery of >600 ml/min/$m^2$ and oxygen consumption of > 170 ml/min/$m^2$. Mortality was reduced significantly in the protocol group to 4% (1/28, $p<0.05$) compared to 23% in the CVP (7/30) control group and 30% (10/30) in the pulmonary artery control group. The phrase 'goal directed therapy' was coined to describe this approach. More recently Boyd et al. (1993) have completed a similar study against a background of serious scepticism of the results of Shoemaker et al. Again in a prospective randomised study of high risk non-cardiac surgical patients they found that by trying to increasing cardiac index and oxygen

delivery (but not oxygen consumption) to the same goals as used by Shoemaker et al. they could reduce mortality from 22% (12/54) in the control group to 5.7% (3/53. $p$=0.15) in the protocol group.

So it seems that the measurement of global oxygen flow variables can be used to improve outcome. The success of the technique also supports the hypothesis that occult tissue hypoperfusion and perhaps gut mucosal hypoperfusion, not detectable by the measurement of the commonly measured cardiovascular variables, are central to the pathogenesis of post-operative organ failure. However, in 'goal directed therapy' the oxygen flow variables are not strictly used as monitors. 'Goal directed therapy' is really a patient management technique. An analogy can be drawn with the technique of pre-oxygenation prior to tracheal intubation. The lungs are prophylactically filled with 100% oxygen so that if there is a delay in successfully instituting positive pressure ventilation the patient is less likely to become hypoxaemic. Both techniques seem to be very logical and demonstrably successful. In support of this observation pre-oxygenation is used prior to almost every emergency tracheal intubation in the UK, if not the world; it is generally believed that if this technique is not used and the patient suffers harm as a result of hypoxia then the practitioner has been negligent. Can the same be said for 'goal directed therapy'?

### 1.2.4. The use of 'goal directed therapy'.

Despite the impressive results goal directed therapy, which requires intense peri-operative usage of a pulmonary artery catheter, is not common practice in the UK (Singer and Bennett 1988). A recent survey revealed that fewer than 10% of hospitals in the UK used pulmonary artery catheters peri-operatively (Singer and Bennett 1988). In particular, poor benefit to risk ratio, lack of necessary expertise and cost were reported as factors restricting their wider usage. The evidence used to support the poor benefit to risk objection is taken largely out of context. Tuman et al. (1989) examined the effects of the use of pulmonary artery catheters on outcome in 1094 consecutive patients undergoing elective cardiac surgery. Patients were managed either with the aid of central venous

pressure monitoring alone (n=557) or with the addition of a pulmonary artery catheter (n=537) according to the practice of the individual anaesthetists. The two groups were well matched and yet they could find no difference in outcome between the groups. The use of pulmonary artery catheters in patients with acute myocardial infarction has actually been associated with an increased morbidity and mortality (Gore, Golberg, Spodick et al. 1987). However, as emphasised in the studies of Shoemaker et al. and Boyd et al. cited above, the use of a pulmonary artery catheter alone, without a treatment strategy, adds only the risk associated with the placement of the catheter.

### 1.2.5. Problems with the 'goal directed therapy' technique.

Even clinicians who find the Shoemaker philosophy attractive may feel uncomfortable starting inotrope infusions just because a patient is not achieving the *magic numbers*. There is increasing concern that the residual mortality in *goal directed therapy* patients may be as a result of over treatment, particularly with inotropes. This is reinforced by the findings of Hayes et al. (1994) who, in a prospective randomised study, found an increased mortality in intensive care unit patients treated with high dose dobutamine to try and achieve Shoemaker style supranormal oxygen flow variables compared to matched controls. Again this objection, although of great concern, should not be used in direct reference to the Shoemaker and Boyd studies quoted above. The essential difference is that their studies were preventive whereas the Hayes study was reactive. The hypothesis that the maintenance of blood flow and oxygen delivery to all tissues via a patent microcirculation may avoid tissue hypoxia and thus limit the damage resultant from major surgery seems very attractive. The hypothesis that trying to reverse hypoxia in organs that already have a blocked microcirculation (a central and highly reproducible feature of multiple organ failure) by simplying increasing global oxygen delivery seems fundamentally flawed.

It would seem therefore that there is reasonable evidence to support the following: i) that the MODS is associated with excessive activation of inflammatory pathways; ii) that tissue hypoxia, and in particular, gut mucosal hypoperfusion may compound the insult of

surgery and lead to excessive activation of inflammatory pathways; iii) that treatment regimens based on increasing oxygen delivery to tissues prophylactically with the aim of avoiding tissue hypoxia (and presumably gut mucosal hypoperfusion) are successful in reducing post-operative multiple organ failure. However, the blunderbuss approach used in the 'goal directed therapy', with its potential inherent risks of iatrogenic morbidity and mortality, has limited the widespread use of an otherwise very rational approach to avoiding tissue hypoxia. To this end a reliable and easy measure of tissue oxygen utilisation remains the Holy Grail of many anaesthetists and intensive care specialists.

## 1.3. MONITORING TISSUE OXYGENATION.

### 1.3.1. Surrogate monitors of tissue oxygenation.

The surrogate markers of tissue oxygenation, such as oxygen consumption, can only provide very gross indications of cellular bioenergenetics. Animal experiments show a classical non-linear relationship between oxygen consumption and delivery. Oxygen consumption is independent of delivery until a critical level is reached and then they are linearly related (Schumacker and Cain 1987) . This would suggest that the relationship between oxygen delivery and consumption measured with a pulmonary artery catheter would provide a good index of tissue oxygenation. However, oxygen delivery and consumption measurements taken in patients with sepsis commonly show a linear relationship which may be real or artifactual (Smithies, Royston, Makita et al. 1991; Vincent, Roman, De Backer et al. 1990). The lack of a clearly identifiable critical oxygen delivery above which consumption is independent of supply makes it impossible to determine the level at which all cells are undergoing aerobic metabolism. If inotropes are used to increase oxygen delivery to try and reach a critical point, consumption may continue to rise due to a direct effect of the inotrope on metabolic rate. Also if a shunt exists in a particular tissue bed a critical global oxygen delivery may be reached while that bed remains hypoxic. The measurement of blood lactate, arterial pH, mixed venous pH and $CO_2$ levels, although more closely related to cellular energy status, still have the problem of being pooled indicators and not organ specific. If they are abnormal it

suggests that a significant amount of tissue is hypoxic but if they are normal the converse is not necessarily true (Mizock and Falk 1992). Other metabolic markers have been tested such as the metabolites of adenine nucleotides, inosine, hypoxanthine, xanthine and uric acid. Grum et al. (1985) were able to identify high levels in the blood of critically ill patients but they were still insensitive global indicators. What is required is a direct monitor of tissue oxygenation.

### 1.3.2. Direct monitors of tissue oxygenation.

Magnetic resonance spectroscopy is the closest we have to a direct monitor of tissue oxygenation. It is non-invasive and has been used to determine the levels of high energy phosphate in several important tissues such as brain and heart (Gutierrez and Andry 1989). Unfortunately its size and the magnetic properties of the spectrometer restrict it to research use only. Currently the closest we can get to a practical direct monitor of overall tissue oxygenation is to try and obtain an index of perfusion in a readily accessible tissue bed that is known to be affected early in reduced perfusion states. To this end attention has focused on the gastrointestinal tract.

## 1.4. MONITORING THE GASTROINTESTINAL TRACT.

### 1.4.1. Why choose the gastrointestinal tract?

From a practical point of view the gut is large, robust and accessible without having to breach the skin surface. Its inner-most mucosal layer has a counter-current system of arterioles and venules that improves absorptive function but makes it susceptible to reduced oxygen delivery states (Lundgren 1989). Ischaemia occurs when mitochondrial oxygen utilisation in a tissue falls below metabolic requirements at that time. Splanchnic vasoconstriction is an early response to a reduction in global oxygen delivery as blood is diverted to the *vital* organs such as heart and brain (Lundgren 1989). Whether due to myocardial failure and/or hypovolaemia the reduction in splanchnic blood volume is disproportionately greater than that seen in other beds. In animal experiments it has been demonstrated that increasing degrees of cardiogenic shock induced by cardiac tamponade raises inferior mesenteric resistance up to four times more than total vascular resistance (Bailey, Morrris, Hamilton et al. 1986). Price et al. demonstrated in human volunteers that a 15% reduction in circulating blood volume resulted in a 40% reduction in splanchnic blood volume while heart rate, blood pressure and cardiac output remained unchanged (Price, Deutsch, Marshall et al. 1966).

### 1.4.2. Monitoring of gastrointestinal mucosal perfusion.

There are many methods available to aid the clinician in the diagnosis of gut mucosal ischaemia but few are of practical use in the immediate peri-operative period. Endoscopic visualisation of the gut mucosa cannot detect ischaemia unless extensive mucosal infarction has occurred (Marrone and Silen 1984). Angiographic techniques may reveal macrovascular but not microvascular abnormalities (Bynum, Gallavan and Jacobson 1984). Hepatic vein catheterisation allows total splanchnic blood flow, oxygen saturation and lactate to be measured but does not account for subtle regional differences (Dahn, Lange, Lobdell et al. 1987). Laser Doppler flow probes (Kvietys, Shepherd and

Granger 1985) and tissue $PO_2$ meters could, at least hypothetically, be used but oxygen delivery is no guarantee of adequate oxygen utilisation (Fink, Cohn, Lee et al. 1989). To gauge the adequacy of cellular oxygen utilisation a metabolic marker can be used, such as the ADP/ATP ratio, lactate or tissue pH. The only currently available clinical monitor that can provide such information and is practical for use during a routine surgical procedure is the gastrointestinal tonometer (Fiddian-Green, Amelin, Herrmann et al. 1986; Fiddian-Green 1990; Fiddian-Green and Baker 1987). Tonometry refers to the measurement of the partial pressure of a gas. Gastrointestinal tonometry uses a modified nasogastric tube to allow the measurement of the carbon dioxide tension in the gastrointestinal mucosa. This measurement, when used in combination with the arterial bicarbonate, can be used to estimate the intramucosal pH (pHi) of the gastrointestinal tract. The gastric pHi in healthy volunteers is 7.38 with a standard deviation of 0.03 (Fiddian-Green and Baker 1987), i.e. similar to arterial pH. In spite of a number of fundamental flaws in the tonometric technique experimental studies have shown that a mucosal acidosis (measured with the gastrointestinal tonometer) correlates with reduced mucosal perfusion and/or the onset of anaerobic metabolism in response to hypovolaemia, hypoxia and sepsis (Antonsson, Boyle, Kruithoff et al. 1990; Fink, Cohn, Lee et al. 1989; Fink, Kaups, Wang et al. 1991; Grum, Fiddian-Green, Pittenger et al. 1984; Montgomery, Almqvist, Arvidsson et al. 1990).

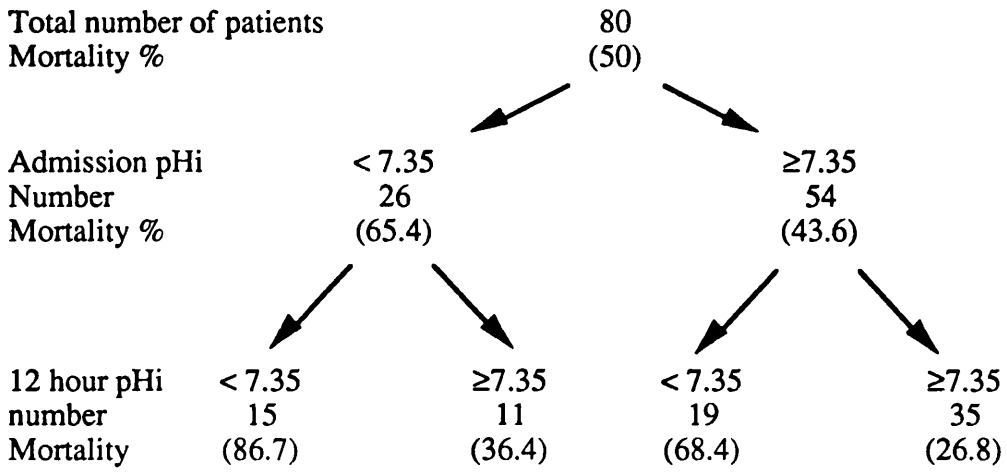
If the gastrointestinal tonometer can be used to estimate gut mucosal perfusion reliably then we would have a monitor of tissue oxygen utilisation in an area that is very sensitive to reduced perfusion states. If this is the case one would expect a low intramucosal pH to be common in intensive care unit patients and associated with a poor outcome.

## 1.5. GASTRIC INTRAMUCOSAL pH MEASUREMENT IN INTENSIVE CARE UNIT PATIENTS.

### 1.5.1. The incidence of a low gastric intramucosal pH in intensive care unit patients and its relationship to outcome.

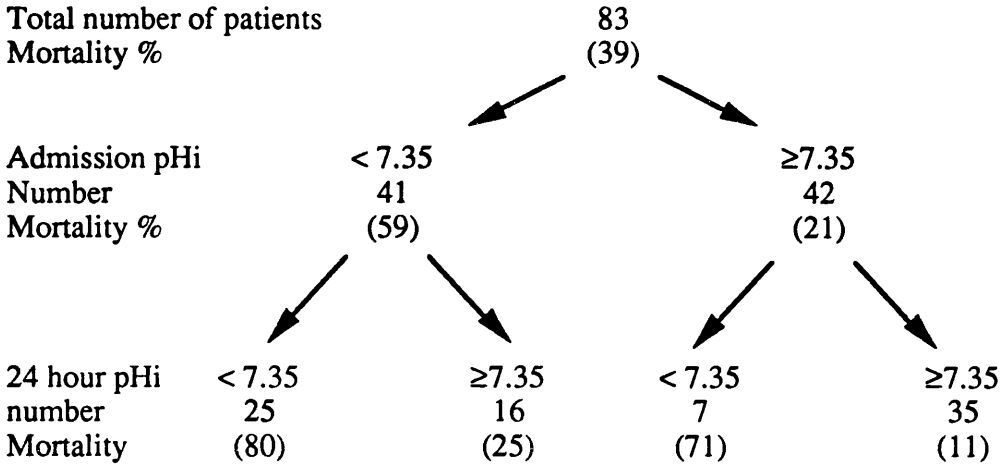
Initially the measurement of pHi in intensive care unit patients was used to investigate stress ulceration. In a study of 103 intensive care unit patients Fiddian-Green et al. (1983) compared risk factors and prophylactic therapies (antacids and cimetidine) with the measurement of gastric intraluminal pH, intramucosal pH and arterial pH. They found that the presence of a low pHi (<7.24) was the most powerful independent predictor ($p<0.001$) of bleeding from the upper GI tract whereas the intraluminal pH had no predictive power at all. Seven patients had a massive bleed from a gastric ulcer confirmed by gastroscopy and six of these died. All of these patients had a low pHi (<7.24), yet the lowest recorded gastric juice pH was 3.45. The lack of association between gastric juice pH and pHi has been confirmed by Knight-George and Bihari (1990) who also noted that non-survivors had a lower mean pHi than survivors. Gys et al. (1988) studied fifty nine patients admitted to intensive care and found a significantly higher early short term mortality in patients with an admission gastric pHi of <7.32 (14/38; $p$ <0.005) compared to those with a pHi of > 7.32 (0/21). Doglio et al.(1991), in a study of 80 patients admitted to two general intensive care units found that patients with a gastric pHi of <7.35 had a significantly higher mortality and that the pattern of change in pHi over the first 12 hours following admission was a major determinant of outcome (**Figure 1.4**). They also reported that the measurement of pHi was the only measured cardiovascular variable (blood pressure, heart rate, arterial pH etc.) that was significantly different in non-survivors when compared to survivors and yet found no significant difference in mean acute physiology and chronic health evaluation (APACHE) score between the patients when stratified according to their pHi. However, there were no pulmonary artery catheter measurements of oxygen flow variables reported in this study.

***Figure 1.4.*** *Patient distribution and mortality according to admission and 12 hour gastric intramucosal pH values as reported in the study by Doglio et al.(1991) of patients admitted to two adult intensive care units.*




Maynard et al.(1993) performed a similar study on 83 patients admitted to a London Teaching Hospital ITU who required pulmonary artery catheterisation. They measured gastric pHi and all the other commonly measured cardiovascular variables on admission and at 12 and 24 hours. Again they found that only gastric pHi independently predicted outcome and could find no difference in cardiac output, oxygen delivery and oxygen consumption between survivors and non-survivors at 24 hours. **Figure 1.5** shows a summary of the results of Maynard et al. in the same fashion as those of Doglio et al. (**Figure 1.4**); they are strikingly similar.

***Figure 1.5.*** *Patient distribution and mortality according to admission and 24 hour gastric intramucosal pH values as reported in the study by Maynard et al.(1993) of patients admitted to an adult intensive care unit.*




Marik (1993) studied 30 critically ill patients with pulmonary artery catheters and gastric tonometers in place and again found the gastric intramucosal pH to be the only predictor of multiple organ dysfunction syndrome and death when compared to all the other commonly measured cardiovascular variables (including arterial lactate and mixed venous pH and $PCO_2$).

It seems therefore that a low gastric intramucosal pH is common among patients admitted to intensive care and, along with other markers of impaired tissue hypoperfusion such as the presence of a metabolic acidosis, is a sensitive predictor of the development of organ failure. If this were so it would seem logical that using a normal gastric pHi as a resuscitation target would be a more rational end point and improve outcome on the intensive care unit.

### 1.5.2. Using gastric intramucosal pH as a resuscitation goal in the intensive care unit.

Gutierrez et al. (1992) studied the impact of pHi guided resuscitation in a prospective randomised trial of 260 patients admitted to general intensive therapy units in Argentina. All patients had a nasogastric tonometer inserted following admission and their pHi measured. They were then stratified into a low (<7.35) or normal (>7.35) pHi group and

randomised into a treatment or protocol groups. Only the physicians attending the protocol group patients were aware of the pHi results and if the pHi was <7.35, or had fallen by 0.1 pH units from the previous reading, then a therapeutic protocol was initiated. Of the 46% (119/260) of patients admitted with a low pHi survival was the same in both control (37%, 23/63) and protocol groups (36%, 20/56), whereas for those admitted with a normal pHi, survival was significantly higher in the protocol group (58%, 46/79) than the control group (42%, 26/62; $p<0.01$). Like the Doglio et al. study cited above criticism can be levelled at this study on two main points. Firstly the survival in the control group (42%) was much lower than would be expected for their average APACHE II scores (60%). Secondly although attempts were made to augment patients' cardiac outputs and oxygen flow variables by the administration of inotropes this was not guided by pulmonary artery catheter measurements. Many would suggest that this is not a standard of practice that is comparable to North America and Europe. However, Gutierrez et al. (1992), Maynard et al. (1993) and Marik et al. (1993) have all failed to demonstrate any correlation between pulmonary artery derived oxygen flow variables and gastric pHi in intensive care unit patients. In his article Marik proposes that:

> *".....once gastrointestinal ischaemia is established, the cascade of events that leads to multiple organ dysfunction syndrome has already been set in motion and measures that improve oxygen delivery will not alter prognosis."*

This hypothesis is in agreement with the findings of Shoemaker (1988) and Boyd (1993) cited above, i.e. the prophylactic increase of oxygen delivery with the aim of avoiding tissue hypoxia is associated with an improved outcome following major surgery. It is also in agreement with the findings of Hayes et al.(1994) which suggest that the reactive driving of cardiac output and oxygen delivery to try and restore tissue oxygenation is futile and may even be associated with iatrogenic morbidity and mortality. It seems, therefore, that if organ failure is to be avoided following major surgery then the abnormality (in this case a low pHi indicative of tissue and in particular gut mucosal hypoperfusion) must be detected as early as possible allowing effective treatment or even better avoided altogether. If this is feasible a low pHi should be detectable during major

surgery and again associated with a poor outcome and in particular with the development of organ failure.

## 1.6. INCIDENCE OF GASTROINTESTINAL MUCOSAL HYPOPERFUSION DURING MAJOR SURGERY AND ITS ASSOCIATION WITH OUTCOME.

### 1.6.1. Incidence of gastrointestinal mucosal hypoperfusion during major vascular surgery.

Fiddian-Green et al. (1986) measured the intramucosal pH of the sigmoid colon in 25 high-risk patients undergoing elective aortic aneurysm repair. They found the incidence of a low sigmoid pHi (taken here as <6.86, which was two standard deviations below the group baseline mean) on the day of surgery was 24% (6/25). All six subsequently had evidence of blood in their stools, four developed major complications (one acute myocardial failure and three multiple organ failure) and subsequently died. Of the 19 patients who had a sigmoid pHi of ≥ 6.86 none had evidence of blood in their stools or major complications and all survived. Stepwise logistic regression showed that the duration of presence of a low sigmoid pHi was the best predictor of a poor outcome. In the patients who had a poor outcome the pHi was abnormal by the end of surgery or shortly after admission to the intensive therapy unit. Soong et al. (1992a) found the incidence of a low sigmoid pHi (< 7.00) during, or shortly after aortic aneurysm repair to be 48% (10/21). Of the low pHi group four developed diarrhoea, two major complications (one myocardial failure and one multiple organ failure) and both subsequently died. In the same study the post-operative levels of endotoxin and tumour necrosis factor were found to be significantly higher in the patients who developed a low pHi. Unfortunately the commonly measured cardiovascular variables were not reported in either study so it is not possible to judge whether there were any obvious global defects (hypotension, arterial acidosis) in the patients who did badly. Also gastric intramucosal pH was not being measured so it is not possible to say with confidence whether the sigmoid ischaemia observed was as a result of the surgical interference with

local blood supply or an overall reduction in splanchnic blood supply, for example due to hypovolaemia or cardiac failure. Maynard et al. (1992) studied a group of 22 patients admitted to the intensive therapy unit following emergency aortic aneurysm repair who had an overall mortality of 45.5% (10/22). They found that the mean gastric pHi measured 24 hours after admission was significantly lower in non-survivors compared to survivors (7.25 vs 7.42; $p$ <0.001) and was the most sensitive predictor of death when compared to the other routinely measured cardiovascular variables (including cardiac output and arterial lactate).

### 1.6.2 Incidence of gastrointestinal mucosal hypoperfusion during cardiac surgery.

Fiddian-Green et al.(1987) examined the predictive value of measurement of gastric pHi, blood pressure, cardiac output, urine output and arterial pH taken within eight hours following induction of anaesthesia in 85 patients undergoing elective cardiac surgery. They found a low pHi in 49% (42/85) of patients, eight of whom developed life threatening complications (six acute heart failure, one pulmonary embolus and one pancreatitis) and six of these subsequently died. The other 43 patients had an uneventful recovery. A low gastric pHi following surgery was found to be the most sensitive (100%), though not so specific (56%), predictor of a poor outcome. If the magnitude and duration of the intramucosal acidosis was taken into account its specificity increased greatly (>90% for a pHi <7.1 for >120 minutes). In the post-cardiopulmonary by-pass period blood pressure and urine output were both highly specific (>98%) but insensitive (<25%). Cardiac output measurement was neither sensitive nor specific. Again the data was not presented in such a way that a relationship between global cardiovascular variables and intramucosal pH can be examined. Kuttila et al. (1991) examined the relationship between gastric pHi and peripheral tissue perfusion in ten patients following admission to the intensive therapy unit after elective coronary artery by-pass graft surgery. They found that although the mean pHi was within the normal range on admission (7.46) it dropped steadily to a mean of 7.33 by four hours as the patients core

temperature increased to normal. From four hours to eight hours the pHi increased again to 7.37. Over the same eight hour period they recorded a steady improvement in peripheral perfusion as judged by subcutaneous and transcutaneous tissue oxygen tension, laser-Doppler skin red cell flux and finger tip temperature all measured on the same arm. They found no change in the central haemodynamics (including cardiac output).

Landow et al. (1991) studied eight patients undergoing elective cardiac surgery to determine the relationship between gastric pHi and several other more invasively derived indices of splanchnic perfusion. They found that the mean pHi increased during the period of hypothermic cardiopulmonary by-pass and then fell to <7.32 15 minutes after by-pass and was still <7.32 one hour after the end of surgery. They also demonstrated a correlation between gastric pHi and hepatic venous pH, lactate and oxygen saturation but a dissociation between pHi and systemic oxygen delivery and between mixed venous oxygen saturation and hepatic vein oxygen saturation. They propose that following hypothermic cardiopulmonary by-pass there is a regional oxygen consumption/delivery mismatch. However, there is no adequate explanation as to how the pHi was measured at core temperatures below 36°C (e.g. during cardiopulmonary by-pass (see **Chapter 2.1.**). This could account for some of the observed changes in pHi which may in fact have been artifactual. Anderson et al. (1993) studied 10 patients undergoing elective coronary artery bypass graft surgery to examine the association between gastric intramucosal pH and splanchnic endotoxin, antibody to endotoxin and tumour necrosis factor-$\alpha$. They found the same changes in pHi as Landow et al. with the mean pHi becoming abnormal (<7.35) by 15 minutes after by-pass and remaining low at the end of surgery (7.30 $\pm$ 0.02). They also report an increase in hepatic venous lactate over the same time course that pHi fell and no association between these and systemic oxygen delivery or mixed venous oxygen saturation. However, they were unable to demonstrate a relationship between pHi and endotoxin or tumour necrosis factor-$\alpha$ concentrations in plasma.

### 1.6.3. Summary of the association between gastrointestinal mucosal acidosis and outcome following major surgery.

Gastrointestinal hypoperfusion occurs commonly during major cardiovascular surgery and may be associated with a poor outcome. It also seems that the commonly measured cardiovascular variables are less able to predict outcome or the level of intramucosal pH. Although 'goal directed therapy' may be an effective way of avoiding tissue hypoxia there does not seem to be a direct relationship between the global oxygen flow variables and intramucosal pH once organ perfusion is compromised. If gut mucosal hypoperfusion is associated with a poor outcome then it is reasonable to assume that avoiding it would be associated with a better outcome. In order to try and avoid gut mucosal hypoperfusion we must first explore its pathogenesis.

## 1.7. PATHOGENESIS OF GASTROINTESTINAL MUCOSAL ACIDOSIS.

Gut mucosal ischaemia may occur as a result of anything that decreases oxygen supply or increases oxygen demand.

### 1.7.1. Specific types of surgery and gastrointestinal mucosal acidosis.

Certain types of surgery are thought to result in an inevitable reduction in splanchnic oxygen delivery. During non-pulsatile cardiopulmonary by-pass large quantities of angiotensin II are released (Taylor 1986). In an animal model angiotensin II was found to be a potent selective splanchnic vasoconstrictor (Porter, Sussman and Bulkley 1989). Cardiopulmonary by-pass also causes activation of platelets, neutrophils and inflammatory pathways (Royston, Fleming, Desai et al. 1986). Resultant microaggregate formation may cause microvascular occlusion and a local reduction in oxygen delivery (Blauth, Kohner, Arnold et al. 1986). Supra-renal cross clamping of the aorta, which is also associated with increased angiotensin II release, abdominal distension and portal venous outflow obstruction should all result in decreased splanchnic oxygen delivery. Although there is indirect human evidence (Fiddian - Green,

Amelin, Herrmann et al. 1986; Porter, Sussman and Bulkley 1989) and animal work (Diebel, Dulchavsky and Wilson 1992) to support these hypotheses no systematic human studies have been reported. It is also interesting to note that only around 50% of patients develop gut mucosal hypoperfusion during cardiopulmonary by-pass and aortic surgery (Fiddian - Green, Amelin, Herrmann et al. 1986; Fiddian-Green 1990; Fiddian-Green and Baker 1987) suggesting an epi-phenomenon rather than a cause and effect relationship.

### 1.7.2. Anaemia and gastrointestinal mucosal acidosis.

A decrease in total haemoglobin results in a proportionate decrease in oxygen delivery if the cardiac output remains unchanged. However, Grum et al. (1984) found that reducing oxygen delivery by isovolaemic haemodilution in a canine model caused the heart to stop before a gut mucosal acidosis developed. If flow and in particular blood volume are maintained it seems that the gut mucosa can extract oxygen very efficiently. Silverman and Tuma (1992) found that increasing oxygen delivery by dobutamine infusion but not by the transfusion of packed red blood cells improved pHi in a group of septic patients. Disturbingly, in the same study, the transfusion of packed red blood cells to a subset of septic patients with a normal pHi increased oxygen delivery but worsened the mucosal acidosis. Marik et al. (1993) have demonstrated that transfusion of donor red cells that are more than 15 days old makes the pHi fall but the transfusion of younger cells improves pHi. They suggested that the impaired deformability of older stored cells results in microvascular occlusion and therefore improves global oxygen delivery but not necessarily tissue oxygenation. Similar work has not been reported in surgical patients.

### 1.7.3. Oxygen supply and gastrointestinal mucosal acidosis.

Any increase in metabolic demand for oxygen and/or an impaired ability to extract or utilise oxygen may result in splanchnic ischaemia. The trauma of surgery and subsequent activation of inflammatory pathways results in a de-novo metabolic demand for oxygen. As discussed above there has been no association demonstrated between global oxygen flow variables and pHi once organ perfusion is compromised (Gutierrez, Bismar, Dantzker et al. 1992; Marik 1993; Maynard, Bihari, Beale et al. 1993). However, the association between pHi and global oxygen flow variables in the immediate peri-operative period (i.e. starting before microcirculatory flow has become compromised) has not been reported.

### 1.7.4. Sepsis and gastrointestinal mucosal acidosis.

Sepsis and in particular endotoxin exposure are potent causes of tissue hypoxia (Dahn, Lange, Lobdell et al. 1987; Ledingham and Ramsay 1989). In animals the administration of endotoxin results in a marked and sustained intramucosal acidosis despite maintained or even increased splanchnic oxygen delivery and oxygen consumption (Fink 1991; Fink, Antonsson, Wang et al. 1991b; Fink, Kaups, Wang et al. 1991). Several authors have found a relationship between a low pHi and systemic endotoxaemia (Merino, Martinez, Alvarez et al. 1992; Soong, Halliday, Hood et al. 1992b; Welch, Durrans, Carr et al. 1992). It was assumed that the endotoxaemia was as a result of translocation through an ischaemic gut mucosa. However, Rouman et al. (1992) found that endotoxaemia preceded an increase in bowel permeability in severely traumatised patients. Similarly Anderson et al. (1993) in a study of patients undergoing cardiopulmonary by-pass found a significant rise in mixed venous endotoxin levels (and not in hepatic vein levels) before gastric pHi became abnormal. Similarly Deitch et al. (1990) found that the extent of tissue damage and mortality in a rat model of haemorrhagic shock was unaffected by using a germ free strain. As stated in **1.1.5**

(above) although gut mucosal ischaemia is associated with endotoxaemia the cause and effect relationship has not been settled.

### 1.7.5. Anaesthesia and gastrointestinal mucosal acidosis.

Different anaesthetic techniques and agents may have significant effects on the splanchnic perfusion. Halothane, for example, is notoriously associated with the idiosyncratic development of a post-operative hepatitis. Although hepatocyte antibodies have been identified in some susceptible individuals (Hubbard, Roth, Gandolfi et al. 1988) the high level of hepatic metabolism and consequent effects on splanchnic oxygen requirements distinguishes halothane from agents such as enflurane and isoflurane (Stoelting 1991). All general anaesthetic agents cause a degree of myocardial depression and therefore would be expected to reduce oxygen delivery and the effects of the different agents on the splanchnic circulation is well documented in animals (Debaene, Goldfarb, Braillon et al. 1990; Stoelting 1991). Of the agents commonly used for the maintenance of anaesthesia isoflurane has the most favourable cardiovascular profile for the maintenance of tissue perfusion. Isoflurane is said to cause less myocardial depression than halothane, enflurane or propofol and maintain flow in gut, kidney, heart, brain, skin, and muscle (Eger 1981; Gelman, Fowler and Smith 1983). Nitrous oxide is highly soluble and can thus accumulate in and distend gas containing cavities such as the bowel (Eger and Saidman 1965). The subsequent reduction in gut perfusion is thought to be a significant factor in the breakdown of bowel anastomoses. Epidural or sub-arachnoid blocks with local anaesthetics to the mid-thoracic level should ablate any neurohumoral splanchnic vasoconstriction and thus maintain splanchnic blood flow. However, most of the effects of the different anaesthetic techniques and agents on gut perfusion have not been demonstrated in human studies.

## 1.8. SUMMARY AND AIM OF THIS THESIS.

Gastrointestinal mucosal hypoperfusion is common during major surgery and is associated with the development of post-operative organ failure. There are human and animal studies to support the hypothesis that gastrointestinal mucosal hypoperfusion may result in failure of gut barrier function, translocation of bacteria or endotoxin, and excessive activation of inflammatory pathways resulting in organ failure. However, there are no studies that have examined the relationship between the development of gut mucosal hypoperfusion and changes in global haemodynamic variables possibly accounting for the regional perfusion defect. Nor are there any studies that have examined treatment regimens aimed at reducing the incidence of gut mucosal hypoperfusion and improving patient outcome.

The purpose of this thesis was to explore the hypothesis that per-operative splanchnic hypoperfusion is associated with post-operative organ failure and is as a result of a perturbation of cardio-respiratory function.

The gastrointestinal tonometer and an oesophageal Doppler were used to determine changes in gastrointestinal mucosal perfusion and cardiac output during major (mainly cardiovascular) surgery. A relationship was sought between these variables and the development of post-operative complications. An association between peri-operative gut mucosal hypoperfusion, markers of inflammatory pathway activation and exposure of patients to endotoxin was explored. Based on the results of these studies, a treatment regimen aimed at reducing the incidence of post-operative organ dysfunction was tested in a prospective randomised trial of patients undergoing major surgery.

# CHAPTER 2.0. GENERAL METHODS, ASSESSMENT OF TISSUE PERFUSION, HAEMODYNAMICS AND ROUTINE LABORATORY DATA.

## 2.1. THE GASTROINTESTINAL TONOMETER

### 2.1.1. The gastrointestinal tonometer.

For the experiments described in this thesis a sigmoid tonometer (Tonometrics, Inc., Worcester, MA) was used for the determination of gastric intra-mucosal pH. It was decided to use this rather than the combined gastric tonometer and sump tube available from the same company as it has a much finer bore and is therefore potentially less traumatic to the patient. To facilitate passage of the sigmoid tonometer into the patients stomach via the nose and allow confirmation of correct placement (by injection of air while auscultating over the stomach) it was sutured to a fine bore naso-gastric feeding tube using cat gut (**figure 2.1.**).

The tonometer consists of a gas impermeable sampling tube with a gas permeable silicone balloon on one end and a three-way tap on the other end (**Figure 2.1**). If it is inserted into the lumen of the stomach it allows the relatively non-invasive assessment of the carbon dioxide tension in the lumen of the stomach (**Figure 2.2**). This is done by using a routine blood gas analyser to measure the $PCO_2$ of a sample of saline aspirated from the tonometer balloon after time has been allowed for equilibration with the $PCO_2$ in the lumen of the gut.

***Figure 2.1.*** *The sigmoid gastrointestinal tonometer (with 5ml syringe connected to its 3-way tap) sutured to a fine bore nasogastric feeding tube to facilitate easy passage of both tubes into the stomach via the patients nose.*

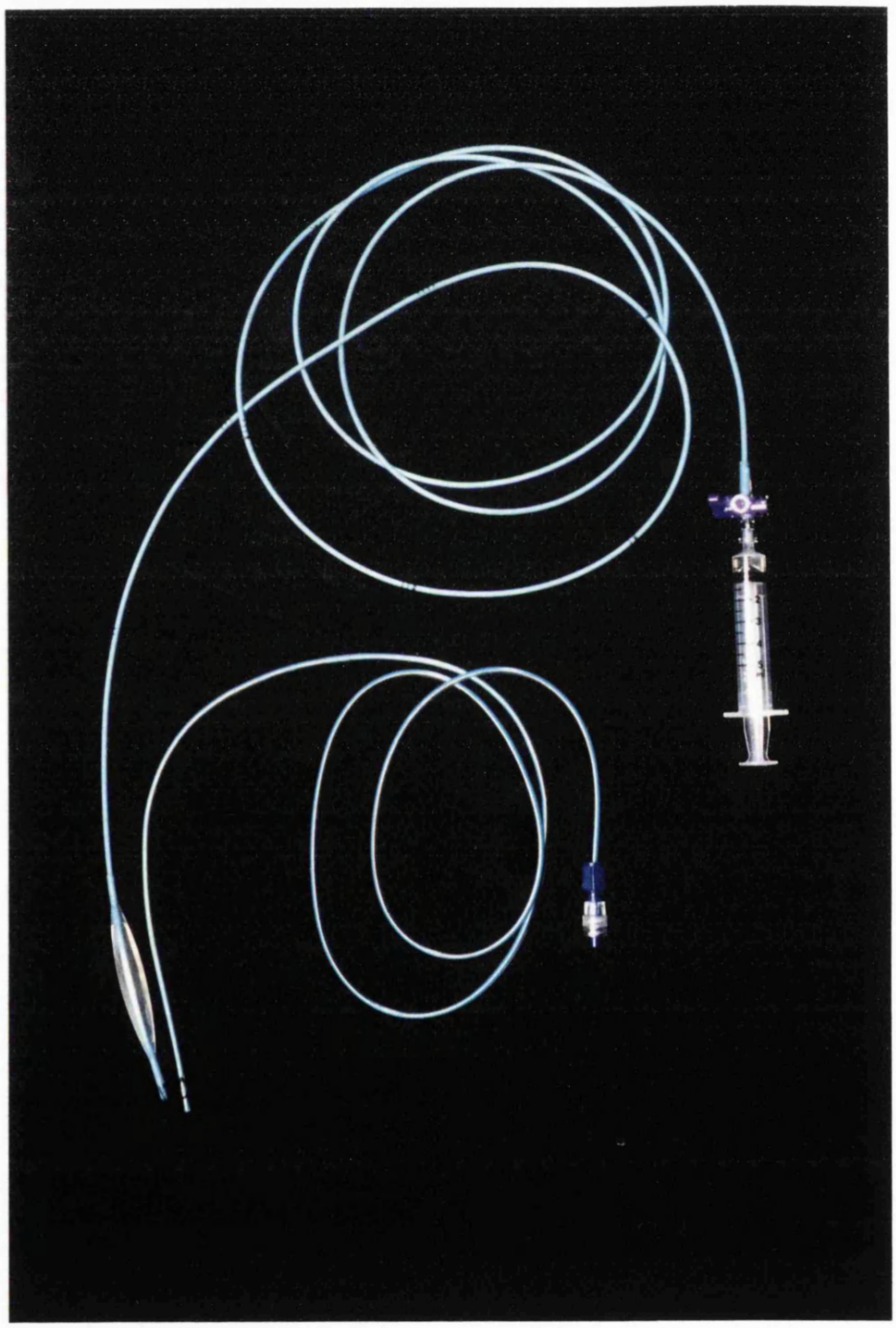
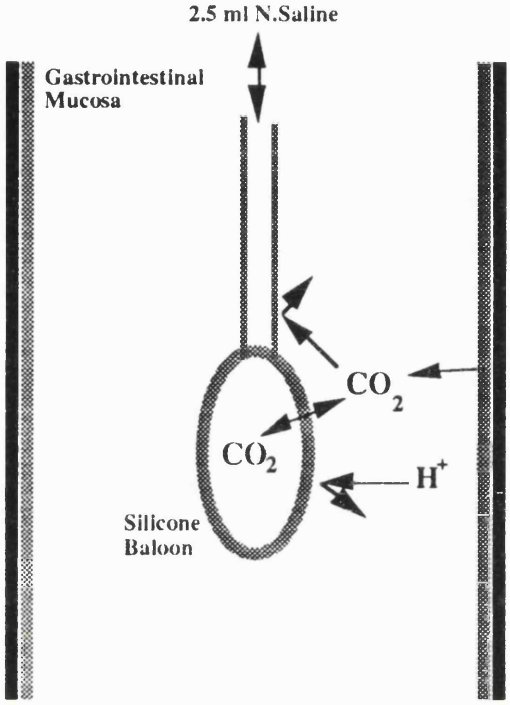


***Figure 2.2.*** *Diagrammatic representation of the gastrointestinal tonometer*




The pHi is calculated from a modified Henderson - Hasselbach equation:

$$pHi = 6.1 + \text{Log}_{10}\left(\frac{\text{arterial } [HCO_3^-]}{PCO_2(\text{tonometer}) \times K}\right)$$

where K is a time dependant equilibration constant provided by the manufacturer that corrects for the concentration gradient that exists across the tonometer membrane. It has been developed by the manufacturers *in-vitro* using multiple readings over a wide range of equilibration periods and $CO_2$ tensions at 37°C (Boyle and Kent, Tonometrics Inc. - personal communication).

Full equilibration of luminal $CO_2$ with that in the balloon requires 90 minutes. Measurements can be made after as little as 10 minutes but the manufacturers recommend a minimum period of 30 minutes to reduce the errors associated with incomplete

equilibration. With readings taken at 10 minutes after initial insertion of the tonometer there may be up to a 20% underestimation of the $PCO_2$ in the gastrointestinal lumen. This is due to the first 1ml of dead space saline in the tonometer tubing being non-carbonated whereas all subsequent dead space saline will contain $CO_2$ at the level of the immediate preceding sample. For first readings taken with an equilibration period of 15 minutes the error is less than 5% and for readings at 30 minutes less than 2% (Boyle and Kent, Tonometrics Inc. – personal communication).

### 2.1.2. The tonometric technique for determining gastrointestinal intramucosal pH.

The tonometer is intended to be a monitor of tissue perfusion and relies on the assumption that reduced perfusion will result in anaerobic metabolism and in a fall in local pH. Increased lactic acid production is commonly thought to be the cause of tissue acidosis during hypoxia. However, Gores et al. (1989) have calculated that unreversed hydrolysis of high energy phosphates accounts for half of the observed decrease in intracellular pH in an in vitro model of cellular hypoxia. In ischaemic myocardium Lange et al. (1983) found a good correlation between intracellular pH and tissue levels of adenosine triphosphate but similar work has not been reported for the gastrointestinal mucosa.

If tissue hypoxia results in a local increase in hydrogen ions and these are buffered by bicarbonate then additional $CO_2$ to that produced from aerobic metabolism will be generated. Carbon dioxide produced in the superficial layers of the mucosal surface of a viscus will rapidly equilibrate with the luminal contents. Bergofsky (Bergofsky 1964) and Dawson (1965) showed that the $PCO_2$ of a hollow viscus could be determined by measuring the intraluminal fluid $PCO_2$. Kivisaari and Niinikosky (1973) refined the tonometric technique for the determination of the $PCO_2$ of tissues by using a gas permeable saline filled balloon. Although a correction factor was now required to

compensate for the $PCO_2$ gradient across the balloon the use of clean saline samples overcomes the potential problem of gastric contents soiling gas analysers. Fiddian - Green at al. (1982) adopted the tonometric technique for assessing intramucosal $PCO_2$ and extended its use to the calculation of the intramucosal pH (pHi), initially without the use of a silicone balloon catheter. If it is assumed that the arterial bicarbonate (determined by arterial blood gas analysis) is the same as intramucosal bicarbonate then the pHi can be calculated by inserting the arterial bicarbonate and intraluminal $PCO_2$ values into the Henderson - Hasselbach equation. Using this technique Fiddian-Green et al. demonstrated a correlation between calculated pHi and pHi measured directly with a microprobe placed in the submucosa of the stomach, small bowel and colon of 16 dogs (114 paired readings, r=0.68, $p$ <0.001).

### 2.1.3. Validation of the gastrointestinal tonometer.

In animal models of septic and haemorrhagic shock directly measured microprobe $PCO_2$ and tonometer measured $PCO_2$ in the gastrointestinal tract have been shown to be the same (Desai, Weil, Tang et al. 1993;Nok, Weil, Sun et al. 1993). In anaesthetised pigs in a steady state Antonsson et al. (1990) demonstrated that the indirect tonometric method of pH measurement was the same as that made with a microprobe placed in the interstitial layer of the gut mucosa. They also demonstrated a correlation between the two methods of gut mucosal pH measurement in pigs following the administration of endotoxin (n=4, weighted mean correlation coefficient = 0.94), partial vascular occlusion (n=5, weighted mean correlation coefficient = 0.92) and total vascular occlusion (n=4, weighted mean correlation coefficient = 0.74) (Antonsson, Boyle, Kruithoff et al. 1990). In anaesthetised dogs Grum et al. (1984) showed a linear relationship between splanchnic $VO_2$ and pHi whether $DO_2$ was reduced by limiting the flow of blood or oxygen content. Similarly they showed that pHi was maintained until splanchnic $DO_2$ was equal to $VO_2$ when it fell precipitously (Grum, Fiddian-Green, Pittenger et al. 1984). Again in animal models a persistently low pHi induced by vascular occlusion, endotoxaemia or faecal peritonitis was associated with histological features of ischaemia (Fiddian-Green 1989;

Fink 1991). The direct infusion of oxygenated saline into the lumen of a section of hypoperfused gut corrected a low pHi and prevented histological damage (Fiddian-Green 1989). In a study of patients undergoing cardiac surgery Landow et al. (1991) found that the gastric pHi was correlated with lactate concentration, pH and $O_2$ saturation measured directly from the hepatic vein. It seems therefore that a low pHi is highly suggestive of inadequate gut mucosal and possibly splanchnic perfusion. However, a low pHi is not necessarily an absolute indicator of gut mucosal hypoxia.

### 2.1.4. Flaws in the gastrointestinal tonometric technique for the determination of pHi

Gut mucosal hypoxia is not the only reason for an increase in gut luminal $PCO_2$. The reflux of duodenal bicarbonate into acid in the stomach will liberate $CO_2$ independent of the gut mucosal redox state. This effect has been studied by Heard et al. (1990) in healthy volunteers. Using the group as their own controls they found that the administration of two oral doses of Ranitidine 150mg reduced the range of pHi measurements and increased reproducibility. A primary respiratory acidosis or even consuming a carbonated drink will have a similar effect on gut luminal $PCO_2$. An abnormal pHi in the face of a high $PaCO_2$ cannot be taken as evidence of gut mucosal hypoperfusion. Benjamin et al. (1992) have confirmed this finding in a porcine haemorrhagic shock model. Likewise a respiratory alkalosis will artificially lower the calculated pHi (Desai, Weil, Tang et al. 1993).

During periods of gut mucosal hypoxia the assumption that arterial bicarbonate is the same as intramucosal bicarbonate or even that in local flowing capillary blood is fundamentally flawed. The basic premise for the tonometric measurement depends on changes in gut luminal $PCO_2$ secondary to local bicarbonate buffering of $[H^+]$. If this is the case then intramucosal bicarbonate concentration should be lower than arterial bicarbonate concentration and the pHi calculation should underestimate the magnitude of intramucosal acidosis. This hypothesis is borne out by the validation experiments of Antonsson et al. (1990) mentioned above. When a mucosal acidosis was induced in pigs

by total vascular occlusion the tonometer pHi calculation underestimated the degree of acidosis measured directly with a microprobe. Desai et al. (1993) have made direct measurements of gastrointestinal $PCO_2$ and pH using microeletrodes placed into the ileal mucosa of rats. They calculated that the mucosal bicarbonate was actually higher than the arterial bicarbonate in normal but not hypotensive rats. However, the results are difficult to interpret as no indication is given in the methodology as to the calibration of the four different sets of electrodes (two pH and two $PCO_2$) used to calculate the different bicarbonate concentrations cited. In the same experiments, in spite of the proposed bicarbonate discrepancies, the directly measured pHi compared favourably with the calculated pHi using the Fiddian-Green technique.

Any change in the arterial bicarbonate concentration will modify the calculated pHi. For example a low pHi in a patient with a metabolic acidosis due to renal failure does not necessarily imply gut mucosal hypoxia. Similarly, a normal pHi in a patient who has a metabolic alkalosis does not necessarily imply tissue normoxia. The administration of bicarbonate will raise arterial bicarbonate concentration and therefore can correct the calculated pHi despite the presence of mucosal ischaemia (Benjamin, Polokoff, Oropello et al. 1992). This was elegantly demonstrated in another study by Benjamin et al. (1992). They showed that the administration of bicarbonate to hypovolaemic pigs apparently corrects the calculated gastric pHi. Closer scrutiny of the data shows that the administration of bicarbonate produces a metabolic alkalosis and a sharp increase in tonometer $PCO_2$ compared to arterial $PCO_2$. Therefore, the magnitude of the gap between the arterial pH and pHi remained unchanged until the hypovolaemia was corrected.

In a study of 20 critically ill patients Boyd et al. demonstrated a close correlation between pHi and base deficit . They found that a blood gas analyser calculated base deficit of 4.65 predicted a gastric pHi of <7.32 with a specificity of >95%. As stated above patients with a low bicarbonate must have a low calculated pHi unless the gut lumenal $PCO_2$ is lower than arterial $PCO_2$ which is unlikely. Also base deficit and pHi must correlate as the same bicarbonate concentration is common to both calculations. However, as also

demonstrated in the study of Boyd et al., not all patients with a low pHi have a metabolic acidosis. In order to solve many of these problems the difference between the arterial pH and the pHi or the arterial $PCO_2$ and tonometer $PCO_2$ may be a more useful index of gut mucosal perfusion. However, neither method has yet been properly validated.

### 2.1.5. The measurement of $PCO_2$ in saline using routine blood gas analysers.

There is a systematic error when a routine blood gas analyser, calibrated for the determination of the $PCO_2$ of blood, is used for the determination of $PCO_2$ in saline samples. The bias is small and comparable for the Corning 178 (Corning, Medfield, Massachusetts, USA) and the Radiometer ABL 300 (Radiometer, Copenhagen, Denmark) (Tonometrics Inc, personal communication). For other blood gas analysers such as the Nova Stat Profile 7 (Nova Biomedical, Waltham, Massachusetts, USA) the precision is good but the bias is large (Riddington, Venkatesh, Clutton et al. 1992). In the validation experiments performed by Antonsson et al. (1990) the $PCO_2$ measurements were made using the Corning 178 analyser; for all of the experiments reported in this thesis a Radiometer ABL 300 was used. The same machine was used throughout and calibrated daily by a skilled technician. The accuracy of this instrument for the determination of $PCO_2$ in saline was confirmed at regular intervals by the repeated analysis of carbonated 0.9% saline .

### 2.1.6. Normal range for gastric intramucosal pH.

In a group of 47 volunteers with known cardiac disease Fiddian-Green et al. (1991) found the group mean of triplicate recordings of gastric pHi to be 7.38 with a standard deviation of 0.03. Fiddian-Green's first peri-operative study took a gastric pHi of less than 7.32 as evidence of intramucosal acidosis, i.e. two standard deviations below the mean of the group reported above (Fiddian-Green and Baker 1987). However, most studies on ITU patients have used 7.35 as the lower limit of normality (Doglio, Pusajo, Egurrola et al. 1991; Gutierrez, Bismar, Dantzker et al. 1992; Gutierrez, Palizas, Doglio et al. 1992; Marik 1993; Maynard, Bihari, Beale et al. 1993). No explanation is offered for this inconsistency in reporting. For the purpose of this thesis a level of 7.32 has been taken as the lower limit of normality.

### 2.1.7. The gastrointestinal tonometer for the determination of gastrointestinal pH – Conclusion.

The gastrointestinal tonometric technique is a validated method for measuring the $PCO_2$ of the lumen of the gastrointestinal tract. The tonometer (Tonometrics, Inc., Worcester, MA) can be used to calculate reliably gastrointestinal intramucosal pH. The calculation of pHi is a validated method for determining gastrointestinal perfusion and estimating tissue oxygenation.

## 2.2. THE OESOPHAGEAL DOPPLER.

For the determinations of cardiac output (CO) it was decided not to use a pulmonary artery floatation catheter and the thermodilution method. There were two main reasons for this: firstly, it was not common practice in the United Kingdom (Singer and Bennett 1988) or routine practice in the patients that were studied; secondly, PA catheterisation has been associated with an increased morbidity and mortality if used simply for monitoring (Gore, Golberg, Spodick et al. 1987; Tuman, McCarthy, Spiess et al. 1989) rather than a therapeutic guide (Boyd, Grounds and Bennett 1992). Therefore, it was

decided that if the ultimate aim was to develop a therapeutic regimen that would improve post-operative outcome that may require the measurement of CO a relatively non-invasive method was more appropriate. The oesophageal Doppler was chosen as it was easy to use, reliable, validated and there was already a high level of local expertise and familiarity.

### 2.2.1. The oesophageal Doppler - summary.

For the estimations of cardiac output and stroke volume reported in this thesis the ODM1 Oesophageal system (Doptek, Chichester, UK) was used. The ODM1 uses a 5.1 MHz continuous wave oesophageal Doppler connected to a spectral analyser system. The transducer was inserted via the patient's mouth and positioned approximately 35–40cm from the teeth where well defined aortic blood flow signals could be detected. The ODM1 displays a continuous spectral analysis of velocity waveforms of descending aortic blood flow. Using an internal nomogram an estimation of volumetric cardiac output is calculated and displayed along with heart rate and stroke volume. Using the ODM 1 measurements of cardiac output have been made to approximately 85-90% accuracy when compared with thermodilution (Belot, Valtier, de-la-Coussaye et al. 1992; Lefraynt, Aya, de-LA-Coussaye et al. 1992; Singer, Clarke and Bennett 1989) and the coefficient of variation has been shown to be considerably lower.

### 2.2.2. The Doppler effect.

The Doppler effect was described by Christian Doppler in 1842. He observed that the shift in frequency of sound or light waves emitted by, or reflected off, a moving object is proportional to the relative velocity between the object and observer. The Doppler equation relates velocity to the frequency shift, the frequency and speed of the emitted (in this example) sound and angle between the emitted beam and velocity vector. The speed of sound is constant so if the emitted frequency and angle can also be kept constant then velocity can be calculated from the observed frequency shift as follows:

$$V=\left(\frac{c\, f_d}{2\, f_T \cos\theta}\right)$$

Where v is the flow velocity, c is the speed of sound (in body tissue = 1540 m/sec) and $f_d$ is the frequency shift (Hz). Cos θ is cosine of the angle between the sound beam axis and velocity vector, $f_T$ is the frequency of transmitted ultrasound (Hz) and this is multiplied by 2 as reflected ultrasound off a moving object is being used.

Ultrasound refers to acoustic waves with a frequency that is above the range of the human ear, i.e. greater than 20 kHz. Doppler ultrasound measurement of aortic blood flow usually uses sound waves of 2-5 MHz which allows good penetration of tissues and insonation of the aorta. Different tissues have different sound impedance. When ultrasound reaches an interface between tissues with different sound impedance then part of the wave form is reflected back. If the tissues are stationary then there will be no frequency shift between the transmitted and reflected sound frequencies. Blood flowing in the aorta has components with different acoustic impedance (e.g. blood cells and plasma). So if an acoustic beam is focused on the aorta a Doppler shift can be detected that is proportional to the velocity of the flowing blood. If the blood is flowing away from the transmitter then the reflected ultrasound will have a lower frequency (a negative shift) and vice-versa. The Doppler shift is dependent on the angle between the sound beam and the velocity vector and this is represented in the Doppler equation by cosine θ. Maximal Doppler shifts are detected if the ultrasound beam is transmitted parallel to the flow of blood ($\theta=0^o$) and no shift is detected if the beam is perpendicular ($\theta=90^o$). Because of the non-linear nature of the cosine curve there are greater errors as the insonation angle increases.

### 2.2.3. The ODM1 oesophageal Doppler.

The ODM1 uses a 5.1 MHz continuous wave oesophageal Doppler connected to a spectral analyser system. The probe (**Figure 2.3**) has a transducer at its tip with two piezo-electric crystals, one to transmit a 5.1 MHz pure tone and the other receiving back-

scattered shifts reflected from any flow within the entire beam volume. The transducer beam is angled at 45°; thus the cos θ term in the Doppler equation is 0.71 if the velocity vector is parallel to the probe. The transducer is introduced via the mouth and passed approximately 35-40 cm from the upper incisors into the oesophagus until signals are obtained from flowing blood in the descending aorta (**Figure 2.4**).

***Figure 2.3.*** *Diagrammatic representation of the Doppler probe*

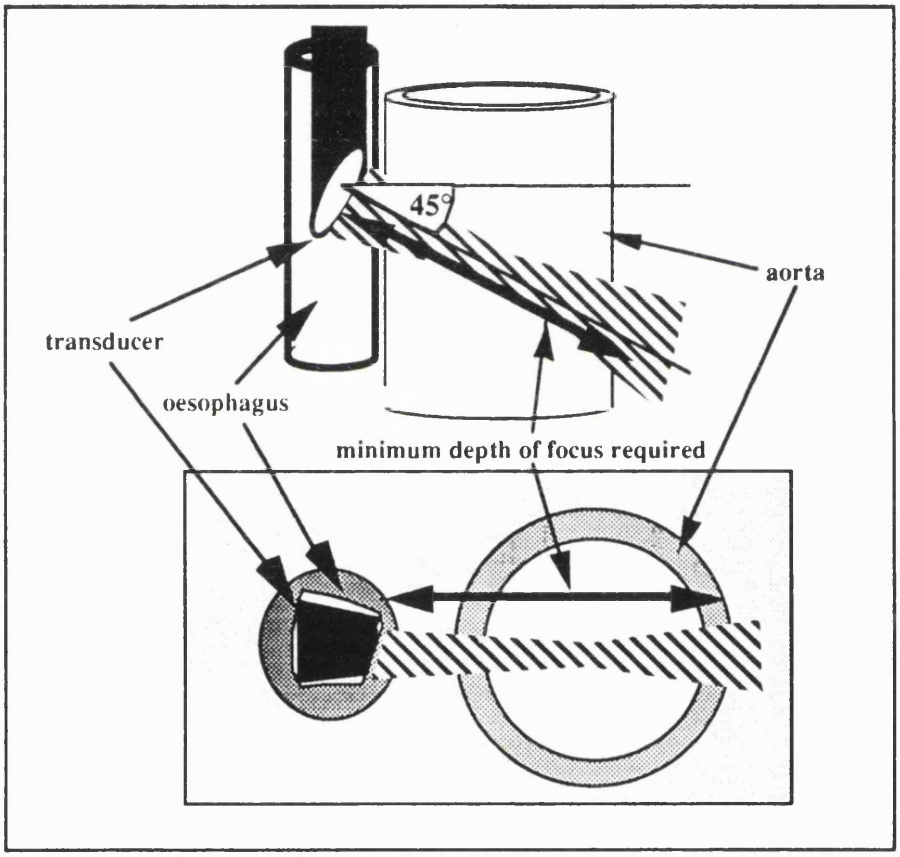
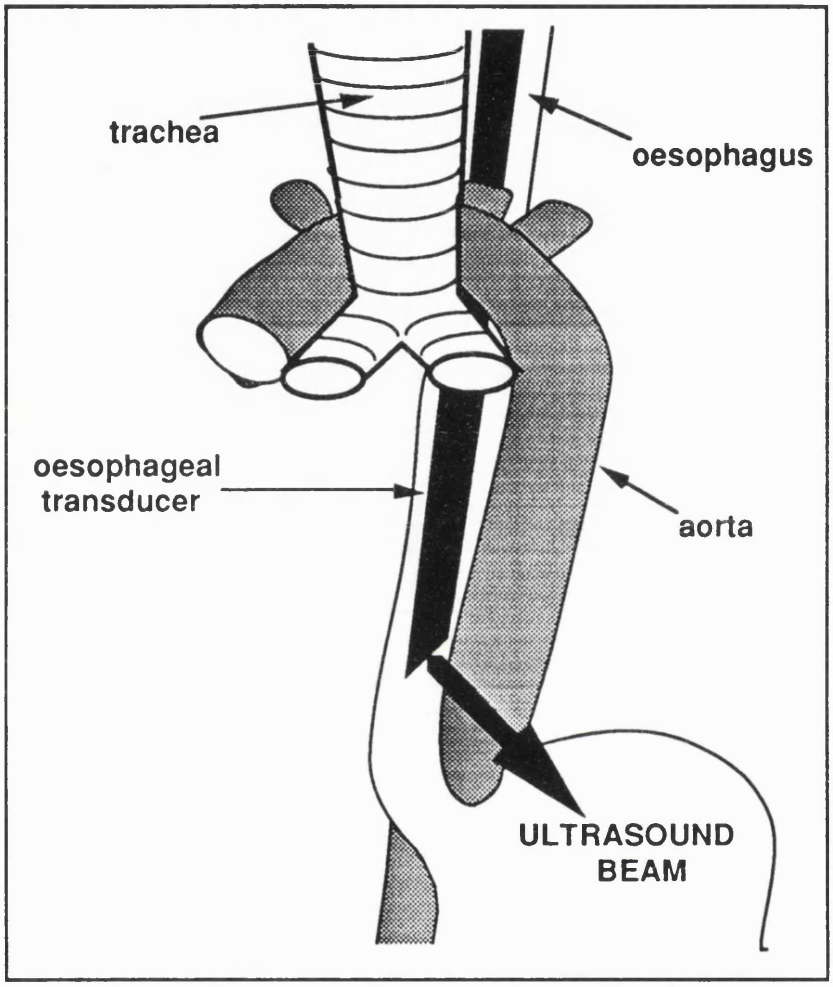
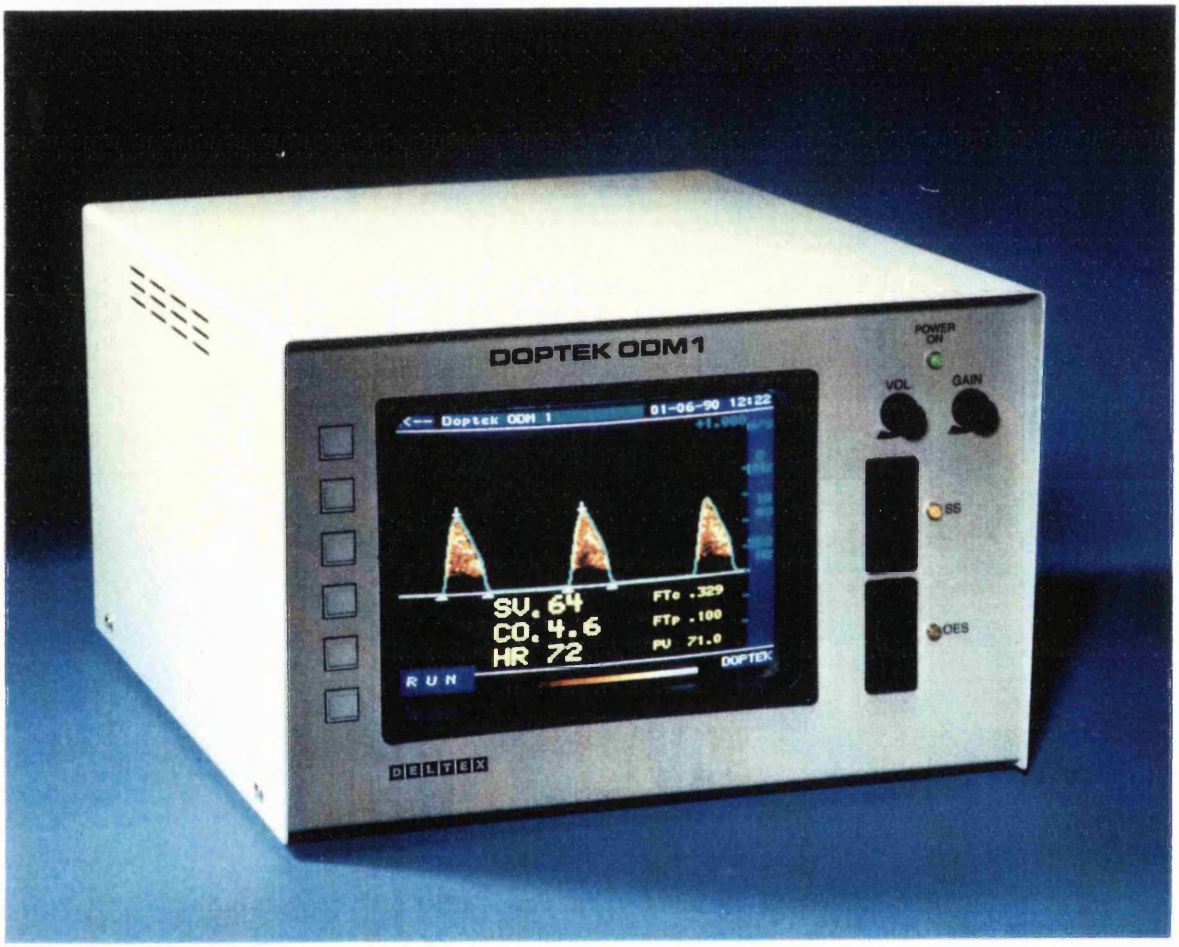


***Figure 2.4.*** *Diagrammatic representation of the position of the Doppler probe in the oesophagus.*




The Doppler frequency shifts detected are processed by spectral analysis and displayed as a velocity waveform envelope. The waveforms contain all the different velocities that correspond to the different Doppler frequency shifts. They are displayed in a power density distribution derived from the thermal colour scale (**Figure 2.5**). The intensity of the signal (e.g. white most intense, red less intense) is proportional to the number of cells moving at that velocity at that time. Different waveforms can be detected with intracardiac, venous, pulmonary vessel and aortic blood flow.

***Figure 2.5.*** *The ODM1 with Doppler waveform display showing the thermal colour scale used to indicate different cell velocities.*



The probe is rotated on its long axis to face posteriorly and detect midstream descending aortic blood flow. Correct focusing is indicated by maximal pitch heard through a sound system that converts ultrasound to audible sound and sharply defined velocity waveforms. As the transducer is at an angle to the probe and descending aortic flow is being recorded two assumptions must be made if the numbers recorded are to be taken as representative of the cardiac output. First is that the oesophagus and aorta are parallel at the level of the probe, such that the angle of the transducer (45°) is θ in the Doppler equation (**Figure 2.4**). Second is that a fixed proportion of left ventricular outflow passes down the descending aorta over a range of cardiac outputs and states of vascular resistance. Fourcade et al. (1980) found that a probe placed at 35-40 cm from the upper incisors lay at the level of the 5th and 6th thoracic vertebrae where the aorta and oesophagus are normally parallel. Wade and Bishop (1962) found that approximately three-quarters of the total cardiac output passes through the descending aorta over a wide range of output states.

The ODM1 computer software traces the outline of the velocity waveform and marks the beginning and end of each waveform and the peak velocity (**Figure 2.5**). The systolic velocity-time integral (stroke distance) is calculated and displayed. This is the distance a column of blood travels through the aorta with each ventricular contraction. Assuming that a fixed proportion of cardiac output passes down the descending aorta then the stroke distance is a linear index of left ventricular stroke volume. If one now assumes that the cross sectional area of the aorta changes little during systole then the product of stroke distance and aortic cross sectional area is directly proportional to stroke volume.

The ODM 1 is pre-programmed with a height, weight and age nomogram (Singer. 1989) that provides an estimate of aortic cross sectional area. The full screen display is of the waveform, heart rate, stroke volume and cardiac output (**Figure 2.5**). Other measured and derived variables are available on a separate menu .

### 2.2.4. Validation of the ODM1 for the estimation of cardiac output.

Singer et al.(1989) compared cardiac output measurements made by the thermodilution technique with the cardiovascular variables measured with a prototype of the ODM 1. The prototype was identical in all aspects (probes, analyser, calculations etc) with two exceptions: i) the orientation of the spectral display showed the waveform displacement below rather than above the baseline and ii) the calculations were only semi-automatic requiring the machine to be stopped and the waveform points marked by the observer with a light-pen. Inter- and intra-observer variability was found to be 1.0 and 0.7%, respectively, for the light-pen system. A total of 238 paired measurements of thermodilution and Doppler variables were reported on 38 ventilated patients (ITU and cardiac surgery). A good signal was obtained for all patients within 2-3 minutes of insertion and cardiac outputs ranged from 1.5 to 10 L/min. In comparing the Doppler minute distance (stroke distance x heart rate) to thermodilution cardiac output the data was presented with the patients arbitrarily divided into 3 age bands - 18 to 39, 40 to 59, and 60 to 78 years to represent young, middle age and old. These are the same age bands as used in the aortic-cross sectional area nomogram. There was a reasonable correlation for all three groups. However, there were only two patients in the young group (18 to 39) years. The coefficient of variation of three measurements made a total of 20 times in ten patients while in a steady haemodynamic state was 6.2% for thermodilution and 3.8% for the Doppler. Taking the first paired measurement in all patients (n=38) as a baseline, a total of 200 changes in cardiac output were obtained ranging from -72.6% to +125.4%. Using a Bland-Altman analysis, close agreement was reported between the two techniques with a mean difference of 0.6%, confidence interval bias of -1.2% to 2.0% and limits of agreement of -13.5% to 14.7%. This means that a change in cardiac output can be detected by the Doppler to within approximately 85% of thermodilution detected change. Similar validation experiments have been performed by two groups in France on ventilated critically ill patients and have provide very similar results (Belot, Valtier, de-la-Coussaye et al. 1992; Lefraynt, Aya, de-LA-Coussaye et al. 1992).

Subsequent studies by Singer et al. (1989a, 1991) have provided further evidence supporting the validation of the ODM 1 oesophageal Doppler when it has been used to

optimise left ventricular performance during positive pressure ventilation and the administration of boluses of fluid. The same group have also demonstrated that there are certain situations where thermodilution measurement of cardiac output is unreliable but the oesophageal Doppler can still be used (eg. patients with tricuspid valve regurgitation) (Singer and Bennett 1989b).

### 2.2.5. Limitations and potential errors

Like any monitoring system there are potential limitations in the use of the oesophageal Doppler. As pointed out above the Doppler, like the pulmonary artery catheter, can only provide an estimate of cardiac output. It actually measures linear velocity and uses a correction factor to provide an estimate of volumetric cardiac output. This is derived from a nomogram using height, weight and age which are a well recognised source of imprecision. However, as stated above, in the validation experiments the coefficients of variation reported were favourable and actually better than for the thermodilution method (Belot, Valtier, de-la-Coussaye et al. 1992; Lefraynt, Aya, de-LA-Coussaye et al. 1992; Singer, Clarke and Bennett 1989). Incorrect focusing may result in detection of non-aortic flow signals and therefore a source of error. However, to the skilled user the detection of the correct descending aortic signal is obvious.

Although under normal circumstances it is reasonable to assume that a fixed proportion of cardiac output passes down the descending aorta this assumption may not be valid during situations where there are acute adjustments in peripheral resistance. To over come this problem all readings for this thesis were taken during periods of presumed cardiovascular stability and stable states of anaesthesia. No recordings were made during periods when the aorta was clamped as this has not been validated.

## 2.3. GENERAL ROUTINE METHODOLOGY.

### 2.3.1. Determination of full blood counts.

Haematocrit, platelet and white blood cell counts were determined from venous blood samples collected into EDTA vacutainers (Becton-Dickinson, Rutherford, New Jersey, USA) by the routine haematology laboratories at University College London Hospitals using a Coulter STKS automated blood counter (Coulter Inc., Miami, USA).

### 2.3.2. Measurement of arterial blood gases.

All blood gas analyses were made from arterial samples collected into pre-heparinised syringes (Radiometer, Copenhagen, Denmark). The same Radiometer ABL 300 (Radiometer, Copenhagen, Denmark) was used for all experiments. This machine undergoes automatic one and two point calibrations on a timed cycle and a skilled technician routinely performed a daily quality control check.

### 2.3.3. Measurement of arterial lactate.

Lactate determinations were made from the same arterial samples used for blood gas analysis (see **2.6.2.**). 0.3 ml of blood was transferred into bottles containing Fluoride Heparin Nitrite (Analox, London, UK) these were then stored at room temperature for batch testing on the day of collection or stored at 4°C for a maximum of 48 hours. Each sample was tested in duplicate and the mean of the two readings calculated. Prior to each batch test the machine was re-calibrated using a 8.0 mmol standard lactate solution (Analox, London, UK). A skilled technician routinely performed a daily two point calibration according to the manufacturers guidelines.

### 2.3.4. Measurement of arterial oxygen saturation and haemoglobin

Arterial oxygen saturation and haemoglobin determinations were made from the same arterial samples used for blood gas analysis (see **2.6.2.**) using a Haemoximeter (OSM2,

Radiometer, Copenhagen, Denmark). A skilled technician routinely performed a twice daily quality control check.

### 2.3.5. Measurement of blood pressure, central venous pressure and end tidal $CO_2$.

Blood pressure and central venous pressure was measured using pre-packed transducer kits and Hewlett-Packard Merlin Monitors (Hewlett-Packard, CCC). End tidal carbon dioxide was measured using the Hewlett-Packard *on line* system and the Merlin monitor with the $CO_2$ cell placed immediately next to the endotracheal tube. The Merlin monitor was calibrated twice weekly by a skilled technician and a separate $CO_2$ check was performed before each case.

## 2.4. ETHICAL CONSIDERATIONS.

All studies reported in this thesis were approved by the Joint Ethics Committee at University College London Hospitals. Informed consent was obtained from all participating patients at a pre-operative visit.

## 2.5. STATISTICS.

### 2.5.1. Determining study population size.

In the planning stages of the studies reported in this thesis and for subsequent presentation and analyses of data advice was sought from epidemiologists and statisticians at the University of London. Numbers required to demonstrate statistical significance with a predetermined power were calculated using standard methods (Kirkwood 1988).

### 2.5.2. Randomisation.

Randomisation was performed using a sealed envelope technique. The envelopes containing randomisation codes were shuffled and placed into a pile in a small box.

Following entry of a patient into the respective randomised studies the next envelope in the pile was opened to reveal the code. There were no sub-set randomisations.

### 2.5.3. Determining frequency distribution.

Frequency distribution was estimated by reference to previous related studies.

### 2.5.4. Presentation of data.

If there was any doubt regarding the distribution of data stem and leaf diagrams were plotted (Kirkwood 1988). Normally distributed data are presented as the group means and 95% confidence intervals. Non-normally distributed data were checked for logarithmic correction to normality. If data corrected then they are presented as the geometric mean and 90th centiles. Otherwise non-normally distributed data is presented as the group medians and 90th centiles or (for demographic details) means, medians and ranges. All raw data are presented in the appendices.

Graphical presentation of data show the individual patient data whenever practical. Graphs were plotted using a commercial computer programme (see **2.8.5**) using the data generated by the statistics packages or raw data.

### 2.5.5. Computer hard and soft ware.

All calculations were performed using the computer programme Stat View™ SE + Graphics (Abacus Concepts Inc., Berkley, USA) and an Apple Macintosh Plus computer with a 40 MB external hard disk drive (Apple Computer Inc., Cupertino, USA). Random checks were made by two individual parties with the aid of a calculator and standard tables.

### 2.5.6 Statistical analyses

The appropriate parametric or non-parametric tests were used to determine statistical significance as outlined in the individual chapters and a $p$ value of less than 0.05 was taken as significant. However, the actual $p$ values are cited. The Z value for the Mann-Whitney U-tests are corrected for ties (Kirkwood 1988).

# CHAPTER 3.0. GUT MUCOSAL HYPOPERFUSION AND POST-OPERATIVE ORGAN DYSFUNCTION

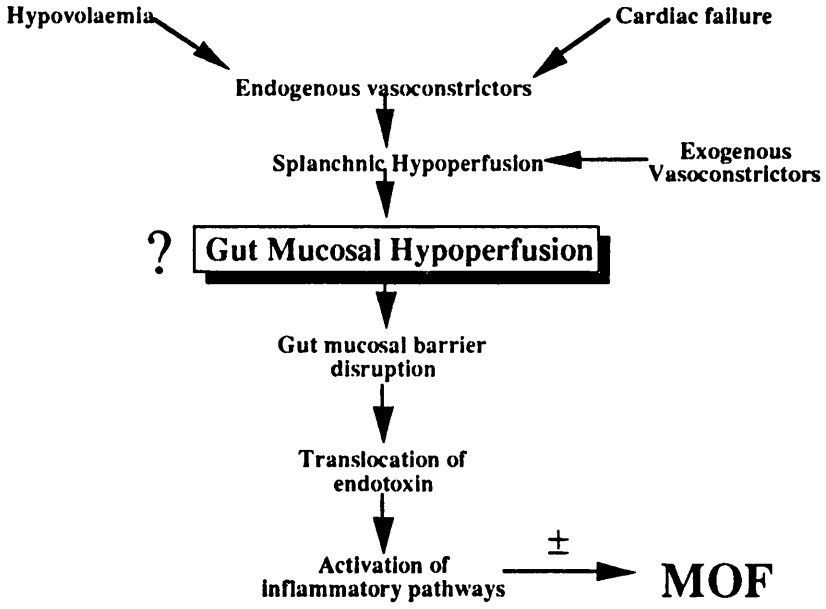
## 3.1. INTRODUCTION.

Irrespective of the initiating stimulus (surgery, cardiopulmonary bypass, bacterial infection) the morphology of necropsy specimens in both animal models and patients with multiple organ failure is remarkably constant suggesting a final common pathway. Post-operative organ dysfunction involves the whole organism with tissue destruction distant from the site of surgery secondary to uncontrolled activation of inflammatory pathways. Established multiple organ failure remains an essentially incurable disease (Knaus, Draper, Wagner et al. 1985). Therefore, attention needs to be focused on prevention. Tissue trauma at the site of major surgery is a necessary evil. Thus, attention has been focused on the patient's haemodynamic state in the peri-operative period in the hope that a compounding and potentially avoidable insult may be identified.

As discussed at length in the introduction to this thesis gut mucosal hypoperfusion, as determined by the presence of a low pHi, is associated with increased morbidity and mortality in patients undergoing cardiac and major vascular surgery and in patients admitted to the intensive care unit (Doglio, Pusajo, Egurrola et al. 1991; Downing, Beard, Cottam et al. 1992; Fiddian - Green, Amelin, Herrmann et al. 1986; Fiddian - Green, McGough, Pittenger et al. 1983; Fiddian-Green 1988; Fiddian-Green and Baker 1987; Gutierrez, Bismar, Dantzker et al. 1992; Gutierrez, Palizas, Doglio et al. 1992; Gys, Hubens, Neels et al. 1988; Maynard, Bihari, Beale et al. 1993; Maynard, Taylor, Bihari et al. 1992; Soong, Halliday, Hood et al. 1992a; Soong, Halliday, Hood et al. 1992b). Also the maintenance of a normal pHi is associated with reduced mortality in the intensive care unit (Doglio, Pusajo, Egurrola et al. 1991; Gutierrez, Palizas, Doglio et al. 1992).

Gut mucosal hypoperfusion is an early consequence of hypovolaemia and inadequate cardiac output and is demonstrable long before systemic blood pressure falls (Gilmour, Aitkenhead, Hothersall et al. 1980; Price, Deutsch, Marshall et al. 1966). Undetected hypovolaemia and other low cardiac output states may thus lead to inadequate tissue oxygenation and subsequent complications which may not become apparent until several days post-operatively. It is postulated that the integrity of the gut mucosa is compromised by ischaemia and this leads to translocation of bacteria and endotoxin from the gut lumen (Koziol, Rush, Smith et al. 1988; Rush, Sori, Murphy et al. 1988). The subsequent stimulation of inflammatory pathways may eventually lead to the characteristic tissue destruction seen in multiple organ failure (**figure 3.1**).

***Figure 3.1** flow diagram summarising the hypothesised link between gut mucosal ischaemia and multiple organ failure*




Previous studies in intensive care unit patients have failed to demonstrate any correlation between global cardiovascular variables and pHi (Gutierrez, Bismar, Dantzker et al. 1992; Marik 1993; Maynard, Bihari, Beale et al. 1993). One hypothesis is that once the gut mucosal microcirculation is occluded then manipulations of the global circulation will

not in themselves restore mucosal perfusion (Marik 1993). However, the manipulation of cardiovascular variables in high risk surgical patients with the aim of avoiding tissue hypoxia has been associated with a reduced morbidity (Shoemaker, Appel, Kram et al. 1988; Boyd, Grounds and Bennett 1993). These patients presumably start with a patent microcirculation and attempts to maintain microvascular perfusion would seem well founded. Although Fiddian-Green et al. (1987) found the measurement of gastric pHi to be a better predictor of outcome in cardiac surgical patients than cardiac output the specific relationships between global cardiovascular variables and gut mucosal perfusion have not been studied in patients undergoing major elective surgery.

## 3.2. AIMS.

The aims of this study were:

i) To determine the incidence of a low gastric pHi in a non-homogeneous group of patients undergoing high-risk elective major surgery, with a case mix that was representative of the Middlesex Hospital ITU's throughput.

ii) To compare changes in gastric pHi with the development of post-operative complications and in particular multiple organ failure.

iii) To examine the relationship, if any, between pHi and changes in the commonly measured cardiovascular variables, in particular cardiac output.

iv) To use these findings as the basis for investigating treatment regimens aimed at avoiding gut mucosal hypoperfusion and improoving outcome following elective major surgery.

## 3.3. PATIENTS AND METHODS.

The study was approved by the local ethics committee and all patients gave written informed consent. Patients were studied if they were i) having elective major surgery of an anticipated duration of greater than 2h which required the routine placement of a urinary catheter, an arterial catheter and central venous catheter and ii) at risk of developing gut mucosal hypoperfusion (i.e. patents having cardiac and vascular surgery) (Fiddian - Green, Amelin, Herrmann et al. 1986; Fiddian-Green and Baker 1987) and/or at risk of developing post-operative organ failure according to the criteria of Shoemaker et al. (1988) (**table 3.1**).

***Table 3.1.*** *Criteria for patients at high risk of developing peri-operative gut mucosal hypoperfusion and/or post-operative organ failure.*

| Criteria | Source |
|---|---|
| Aortic aneurysm repair | (Fiddian - Green et al. 1986) |
| Cardiopulmonary by-pass | (Fiddian-Green and Baker 1987) |
| Late stage vascular disease involving aorta | (Shoemaker et al. 1988) |
| Extensive ablative surgery for carcinoma | (Shoemaker et al. 1988) |
| Age over 70 years with limited physiologic reserve of one or more vital organ | (Shoemaker et al. 1988) |

The following exclusion criteria were observed: age less than 18 years and pregnancy (for ethical reasons); coagulopathies, perforated viscus and oesophageal or gastric pathology (for safety reasons); oesophageal or gastric surgery (due to uncertainty in interpretation of pHi results). All patients were given Ranitidine 150mg p.o. on the night before and on the morning of surgery to increase the precision of the assessment of gastric pHi (Heard, Helmsmoortel, Kent et al. 1990). All patients were anaesthetised using a balanced general anaesthetic technique. Depth of anaesthesia was maintained at a constant level as judged by standard clinical criteria until the last set of study variables

had been recorded. Otherwise, no attempt was made to influence the anaesthetic or surgical management of the patients.

Heart rate and blood pressure were measured directly via a 20g radial artery cannula and central venous pressure directly via a 14g internal jugular catheter 15-20 min after induction of anaesthesia, before the first surgical stimulus, and at the end of surgery following the completion of skin closure. These timings were chosen in an attempt to have the patients in a stable anaesthetised state without the cardiovascular instability that can be associated with surgical stimulation. Hypotension was defined as a mean blood pressure of less than 60 mmHg. Urine flow was measured hourly. Oliguria was defined as a urine output of less than 0.5ml/kg/h.

The following non-routine techniques were used: immediately after induction of anaesthesia a tonometer (Sigmoid Tonomitor, Tonometrics Inc., Worcester, MA.) was introduced into the patient's stomach having been sutured to a fine-bore nasogastric tube with catgut. Correct placement was confirmed by the injection of air down the nasogastric tube while auscultating over the epigastrium. The tonometer was used to measure $PCO_2$ in the stomach following an equilibration period of 60 minutes and again at the end of surgery, following the completion of skin closure. This timing was chosen to allow almost full equilibration of the $CO_2$ in the stomach with the saline in the balloon and thus reduce any errors. The pHi was calculated as described in **chapter 2.1.** using a correction factor of 1.19 as recommended by the manufacturers. Particular note was made of bicarbonate administration as this can affect the interpretation of the calculated pHi (Benjamin, Polokoff, Oropello et al. 1992). An oesophageal Doppler probe (ODM 1, Deltex, Chichester, UK) was inserted after induction of anaesthesia and used to measure cardiac output and stroke volume 15-20 minutes after induction of anaesthesia (before the first surgical stimulus) and again at the same time as the final pHi measurement. Operating theatre personnel were blind to the results of all non - routine techniques.

Post-operative complications were recorded – a major complication being defined as one that resulted in an overall post-operative hospital stay of greater than 14 days or death (Webb, Newman, Taylor et al. 1989). Organ failure was determined according to the criteria proposed by Knaus and Wagner (1985) (**table 3.2**). Multiple organ failure was defined as two or more organ failures present at the same time but more than 24 hours post-operatively.

***Table 3.2.*** *Organ failure criteria as proposed by Knaus and Wagner.*

If the patient had one or more of the following during a 24 hour period (regardless of other values), organ system failure existed on that day.

I. Cardiovascular failure (presence of one or more of the following):

A. Heart rate ≤ 54/minute

B. Mean arterial pressure ≤ 49 mmHg

C. Occurrence of ventricular tachycardia or fibrillation.

D. Serum pH ≤ 7.24 with a $PaCO_2$ of ≤ 6.5 kPa

II. Respiratory failure (presence of one or more of the following):

A. Respiratory rate ≤ 5/minute or ≥ 49/minute

B. $PaCO_2$ ≥ 6.7 kPa

C. $A_a DO_2$ gradient ≥ 47 kPa ($A_a DO_2 = 95 \times FiO_2 - PaCO_2 - PaO_2$)

D. Dependent on the ventilator on the fourth day of organ failure

III. Renal failure (presence of one or more of the following):

A. Urine output ≤479 ml/24 hours or ≤ 159 ml/8 hours

B. Serum urea ≥ 16.5 mmol/l

C. Serum creatinine ≥ 308 µmol/l

IV. Haematological failure (presence of one or more of the following):

A. White cell count ≤ $1 \times 10^9$/l

B. Platelets ≤ $20 \times 10^9$/l

C. Haematocrit ≤ 20%

V. Glasgow coma scale ≤ 6 (in absence of sedation at any one point in the day)

Study group size was estimated with reference to previous studies (Fiddian - Green, Amelin, Herrmann et al. 1986;Fiddian-Green and Baker 1987). As a guideline it was assumed that 20% of patients would develop major complications, 40% would develop a pHi < 7.32 and this would have a sensitivity of >90% for predicting a major complication. Therefore, to have a >80% power of demonstrating the ability of a low pHi to predict major complications at the 5% level at least 44 patients needed to be studied (Kirkwood 1988). Statistical analysis was performed after 6 months data collection. Data are shown as mean (95% confidence interval) or mean (median) [range]. A $p$ value of <0.05 was considered significant. Analysis groups were defined according to the presence or absence of a low pHi (< 7.32) at the end of surgery.

## 3.4. RESULTS.

### 3.4.1. Study population.

A total of 51 patients were studied. There were 32 patients (63%) who had evidence of gastric mucosal hypoperfusion (pHi <7.32) at the end of surgery. Of these 32, forming the low pHi group, 12 (23%) also had a low pHi at 1 hour. None of the other 19 patients, forming the normal pHi group, showed mucosal ischaemia at 1 hour. No patients were given bicarbonate during the study period. Distribution of surgical procedures between low and normal pHi groups are shown in **table 3.3**. There were no significant differences in demographic characteristics between the two groups as shown in **table 3.4**.

***Table 3.3.*** *Distribution of surgical procedures between low (<7.32) and normal (≥7.32) pHi groups.*

| Surgical procedure | pHi at end of surgery | |
|---|---|---|
| | ≥7.32 | <7.32 |
| Coronary artery by-pass grafts | 7 | 14 |
| Re-do coronary artery by-pass grafts | - | 1 |
| Coronary artery by-pass grafts and aortic valve replacement | 1 | 2 |
| Aortic valve replacement | 4 | 1 |
| Mitral and tricuspid valve replacement | - | 2 |
| Aortic, mitral and tricuspid valve replacement | 1 | - |
| Mitral valve replacement | - | 1 |
| Re-do aortic and mitral valve replacement | - | 2 |
| Re-do mitral and tricuspid valve replacement | - | 3 |
| Ventricular aneurysectomy (recent MI) | 1 | - |
| Major vascular surgery - with clamping of the aorta | 1 | 4 |
| Major vascular surgery - no clamping of the aorta | 1 | 1 |
| Cystectomy for invasive carcinoma | 1 | 1 |
| Whipples procedure | 2 | - |

***Table 3.4.*** *Demographic characteristics of study population and duration of surgery. Where appropriate data are shown as mean (median) [range].*

| | pHi ≥7.32 (n=19) | pHi<7.32 (n=32) |
|---|---|---|
| Male | 11 | 24 |
| Female | 8 | 8 |
| Age (years) | 62 (64) [28-82] | 62 (64) [27-79] |
| Weight (kg) | 71.3 (68) [46-99] | 73.3 (75) [46-102] |
| Height (cm) | 167.4 (170) [145-187] | 168.2 (170) [128-183] |
| Duration (minutes) | 203.0 (180) [120-350] | 232.7 (240) [130-330] |

### 3.4.2. Cardiovascular variables.

The cardiovascular changes are shown in **tables 3.5** and **3.6** with individual cardiac outputs indexed to body surface area plotted in **figure 3.2**. There were no significant differences between groups in baseline values of heart rate, blood pressure, central venous pressure, stroke volume cardiac output or cardiac index. None of the 51 patients were oliguric or hypotensive at the end of surgery. The low pHi group showed no significant change in blood pressure or cardiac output from baseline to the end of surgery. However, there was a significant rise in heart rate ($p = 0.0006$) and central venous pressure ($p = 0.009$) and a fall in stroke volume ($p = 0.006$). The normal pHi group showed no significant change in blood pressure or heart rate but a highly significant increase in both cardiac output (48.4%, 95% confidence interval 21.3% – 75.6%, $p = 0.001$) and stroke volume (31.1%, 95% confidence interval 9.6% – 52.5%, $p = 0.007$).

***Table 3.5.*** *Cardiovascular variables for patients who had a pHi ≥ 7.32 at the end of surgery. Data are shown as mean (95% confidence interval).*

| | Baseline | End of surgery | p value |
|---|---|---|---|
| Heart rate (beats/min) | 71 (64 to 79) | 77 (72 to 83) | NS |
| Systolic BP (mmHg) | 117 (109 to 125) | 121 (110 to 132) | NS |
| Mean BP (mmHg) | 79 (72 to 85) | 82 (74 to 89) | NS |
| CVP (mmHg) | 5.3 (2.6 to 8.0) | 5.8 (2.7 to 8.9) | NS |
| Cardiac output (l/min) | 4.3 (3.8 to 4.8) | 6.4 (5.0 to 7.7) | 0.001 |
| Stroke volume (ml) | 63 (55 to 72) | 82 (65 to 99) | 0.007 |
| pHi | 7.39 (7.36 to 7.42) | 7.37 (7.35 to 7.40) | NS |
| Arterial pH | 7.42 (7.39 to 7.43) | 7.41 (7.38 to 7.44) | NS |
| Urine flow (ml/kg/h) | | 1.42 (0.94 to 1.54) | |

***Table 3.6.*** *Cardiovascular variables for patients who had a pHi < 7.32 at the end of surgery. Data are shown as mean (95% confidence interval).*

| | Baseline | End of surgery | p value |
|---|---|---|---|
| Heart rate (beats/min) | 74 (65 to 83) | 89 (83 to 95) | 0.0006 |
| Systolic BP (mmHg) | 114 (107 to 121) | 120 (115 to 126) | 0.09 |
| Mean BP (mmHg) | 78 (73 to 83) | 81 (76 to 86) | NS |
| CVP (mmHg) | 4.3 (2.8 to 5.8) | 8.1 (6.3 to 9.9) | 0.009 |
| Cardiac output (l/min) | 4.7 (4.0 to 5.3) | 4.5 (4.0 to 4.9) | NS |
| Stroke volume (ml) | 67 (57 to 78) | 53 (46 to 60) | 0.006 |
| pHi | 7.34 (7.31 to 7.37) | 7.20 (7.16 to 7.25) | 0.0001 |
| Arterial pH | 7.38 (7.35 to 7.40) | 7.33 (7.30 to 7.35) | 0.01 |
| Urine flow (ml/kg/h) | | 1.13 (0.85 to 1.41) | |

### 3.4.3. Patient outcome.

The patients in the low pHi group spent an average of 4.7 (median 1.0) [range 0–33] days in the intensive care unit and 15.1 (median 9.5) [range 1 – 97] days in hospital. Of these 32 patients, 14 developed major post-operative complications and 6 subsequently died (**table 3.7**). Two patients having cardiac surgery died in the immediate post-operative period; one from uncontrollable bleeding, the other from heart failure which became resistant to inotropic support. The sensitivity of the pHi measured at the end of surgery for predicting subsequent complications was 93.3% with a specificity of 50%. The sensitivity of pHi at 1 hour for predicting complications was 53.3% with a specificity of 88.9%. Only 2 patients developed overt gastrointestinal complications. Seven patients developed multiple organ failure of whom 4 (57%) subsequently died. Only one patient had a pulmonary artery catheter placed pre-operatively compared to 7 post-operatively (6 with multiple organ failure and 1 with cardiac failure). Of the 51 patients 12 had a low baseline pHi at 1 hour after induction of anaesthesia. Of these 12 patients 8 developed post-operative complications of whom 4 subsequently died. The 19 patients who did not develop evidence of gastric mucosal hypoperfusion (pHi >7.32) by the end of surgery spent an average of 1.0 (median 1.0) [range 0 – 4] day in the

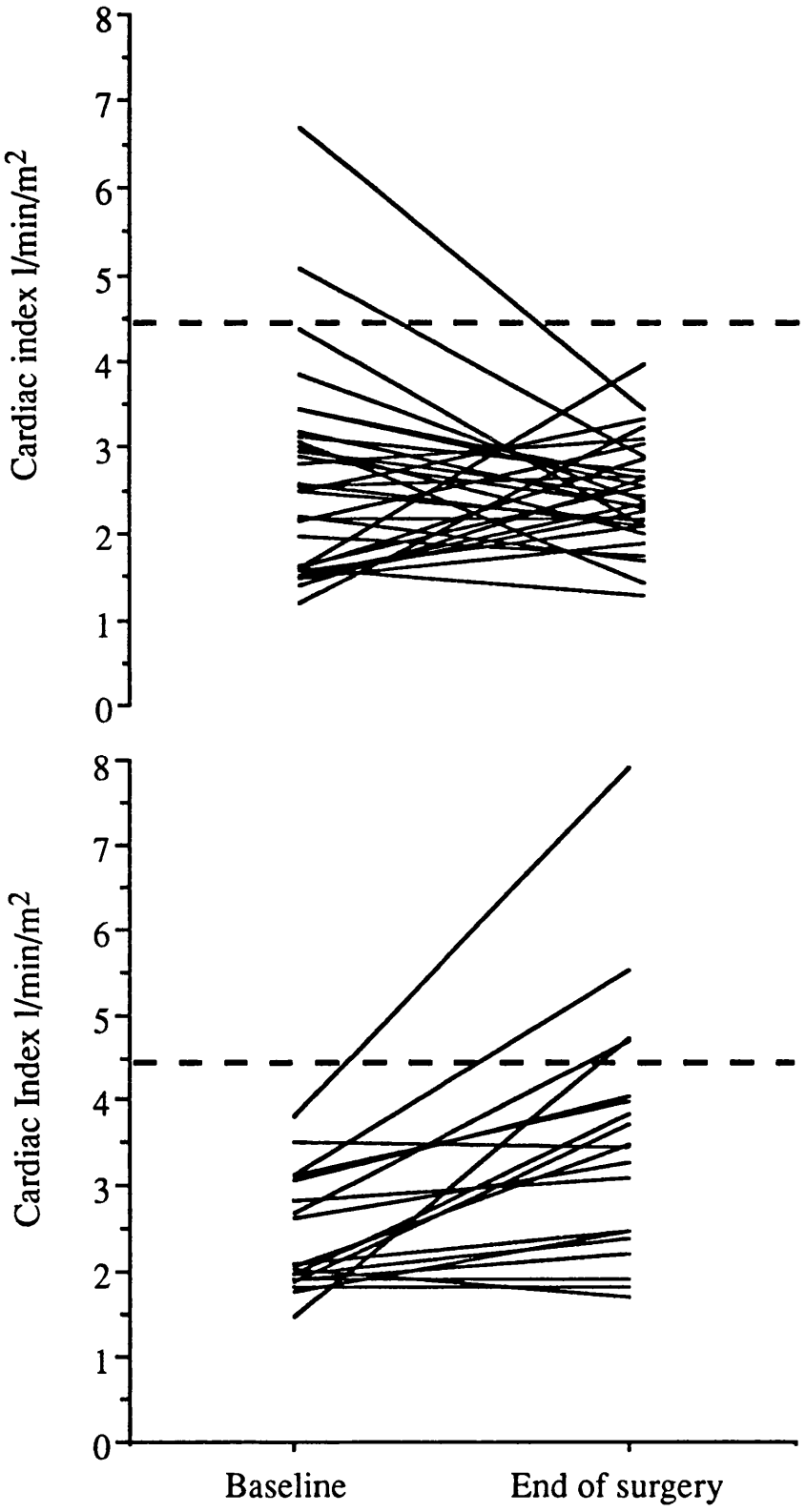
intensive care unit and 8.9 (median 7.0) [range 6 – 24] days in hospital. Of these patients only one developed a major post-operative complication and none died.

***Table 3.7.*** *Individual post-operative complications.*

| Age (years) | Surgical procedure | Post-operative stay | | Complications | Outcome |
|---|---|---|---|---|---|
| | | ITU (days) | Hospital (days) | | |
| **pHi <7.32 at end of surgery** | | | | | |
| 71 | AAA repair | 2 | 22 | Respiratory failure | Survived |
| 79 | MVR | 9 | 9 | MOF | Died |
| 50 | Mesenteric re-vascularisation | 6 | 97 | MOF | Survived |
| 77 | AAA repair | 1 | 21 | Pseudo-obstruction | Survived |
| 67 | CABG (re-do) | 33 | 33 | MOF | Died |
| 69 | AVR | 1 | 14 | Wound infection* | Survived |
| 70 | Femoro-popliteal by-pass (re-do) | 0 | 21 | Wound-infection* | Survived |
| 27 | AVR/MVR (re-do) | 1 | 21 | CVA | Survived |
| 73 | MVR/TVR (re-do) | 1 | 1 | Surgical bleeding | Died |
| 71 | MVR/CABG | 20 | 20 | MOF | Died |
| 68 | MVR/TVR | 21 | 30 | MOF | Survived |
| 76 | Cystectomy | 19 | 19 | MOF | Died |
| 58 | CABG | 1 | 1 | Cardiac failure | Died |
| 63 | CABG | 13 | 24 | MOF | Survived |
| **pHi ≥7.32 at end of surgery** | | | | | |
| 68 | femoro-femoral arterial bypass | 0 | 24 | Wound dehiscence | Survived |

** Wound infection was determined according to the criteria of Peel and Taylor (1991). AAA - abdominal aortic aneurysm. MVR - mitral valve replacement. AVR - aortic valve replacement. CABG - coronary artery by-pass grafts. TVR - tricuspid valve replacement. MOF multiple organ failure.*

***Figure 3.2.*** *Change in cardiac index from baseline to end of surgery. The upper graph shows the individual changes recorded in patients who had a pHi <7.32 at the end of surgery (n=32). The lower graph shows the individual changes in the patients who had a pHi ≥ 7.32 at the end of surgery (n=19). The broken horizontal line indicates Shoemaker's goal for CI.*



## 3.5. DISCUSSION.

### 3.5.1. Study population.

The aim of this study was to measure cardiac output and gastric pHi during elective major surgery and to compare changes in these variables to the development of post-operative complications, in particular multiple organ failure. The patients studied were undergoing mainly cardiac and vascular surgery, the final distribution being representative of the case mix of elective major surgery seen at the Middlesex Hospital. The level of morbidity (30%) and mortality (12%) was appropriate for the case mix studied which included many complex major surgical procedures (**Table 3.7**).

### 3.5.2. Gastrointestinal mucosal hypoperfusion and outcome.

An abnormally low gastric mucosal pH (< 7.32) was found at the end of surgery in 63% of patients. In agreement with previous studies of patients undergoing major surgery a low pHi measurement was predictive of post-operative major complications and mortality (Fiddian - Green, Amelin, Herrmann et al. 1986;Fiddian-Green and Baker 1987) and more sensitive than other forms of monitoring such as blood pressure or urine flow measurement (Fiddian-Green and Baker 1987). The pHi was also measured 1 hour after induction of anaesthesia. The original intention was that this would form a "baseline" measurement, allowing 1 hour for equilibration of gastric $CO_2$ with the tonometer saline. While a normal "baseline" pHi was not seen in all patients it is of interest that this measurement was able to predict post-operative complications with greater specificity though with less sensitivity than the pHi taken at the end of surgery. What is not clear from these data is whether the pHi was abnormally low at the beginning of surgery or became abnormal over the one hour after induction of anaesthesia. Certainly there was no difference between the global cardiovascular variables recorded at baseline but this was some 30 to 45 minutes earlier than the first pHi reading so there may have been some deterioration in that time. One possible

confounding factor may have been a difference in the anaesthetic agents used as these were not dictated by protocol. At least hypothetically, some agents should maintain splanchnic perfusion better than others over the course of time. However, no obvious relationship between the use of particular agents and changes in pHi were noted.

Again in agreement with previous studies the presence of a low pHi was associated with the development of post-operative complications that were only rarely directly related to the gastro-intestinal tract (Fiddian - Green, Amelin, Herrmann et al. 1986; Fiddian-Green and Baker 1987). Of the 15 major complications only one was a pseudo-obstruction and with the exception of the one surgical bleed the others were a mixture of isolated organ dysfunction (lung, brain, heart and skin) and multiple organ failure. This is consistent with the hypothesis that gut mucosal hypoperfusion is central to the pathogenesis of organ dysfunction as proposed in **figure 3.1.** but also with the suggestion that the presence of a low pHi may just be a marker of tissue hypoperfusion, albeit in a very sensitive area. This study does not help in answering that particular question.

### 3.5.3. Using a pHi of 7.32 as the lower limit of normality.

Due to the inherent bias in blood gas analysers for the measurement of $PCO_2$ in Saline caution must be observed in drawing direct comparisons with other studies when comparing absolute values of pHi measurements. In spite of this reservation, and in agreement with Fiddian-Green et al.(1987), 7.32 proved to be a very sensitive cut off point. Of greater import is the changes in the group means over time and in particular that all of the patients who had a poor outcome showed a marked deterioration in pHi over the duration of surgery.

### 3.5.4. Gastrointestinal mucosal perfusion and cardiac output

In agreement with the findings of Bland et al. (1985) and Shoemaker et al.(1988) the group of patients who showed evidence of tissue hypoxia, manifest here as gut mucosal hypoperfusion, and had a poor outcome, demonstrated no change in the group mean

cardiac output. Whereas, the group that maintained gut mucosal perfusion demonstrated a significant and spontaneous increase in mean cardiac output (48.4±13%) with 37% of patients achieving greater than 50% increase in cardiac output. This is consistent with the hypothesis that an appropriate metabolic response to the trauma of major surgery produces an increased tissue oxygen demand and that the survivors are able to meet this demand. It would seem likely that increasing cardio-respiratory variables to greater than normal levels may be a pre-requisite to the perfusion of areas such as the gut mucosa during states of increased demand. However, these patients maintained gut mucosal perfusion without intervention so it is not yet clear which therapeutic interventions, if any, would be most useful for increasing global cardio-respiratory variables and, indeed, whether such manoeuvres would necessarily result in better gut mucosal perfusion and a reduction in complications.

At deference to the findings of Shoemaker et al. (1988) and Boyd et al. (1993) that the prospective use of global oxygen flow variables as therapeutic goals resulted in an improved outcome, there was no correlation found between individual cardiac output recorded at the end of surgery and pHi. Although there are some limitations to direct comparison (eg. the patients were anaesthetised in this study and a different technique was used to determine cardiac output) many of the patients who developed gut mucosal hypoperfusion in this study did so inspite of an increase in cardiac output. Similarly many patients who maintained gut mucosal perfusion demonstrated minimal change in cardiac output and, although 37% increased their cardiac output by greater than 50%, only 4 achieved Shoemaker's goal of 4.5 l/min/$m^2$. Data for total body oxygen transport or consumption were not collected but we believe these data support the findings of other studies in animals and ITU patients that adequacy of the global circulation judged by determination of cardiac output does not guarantee adequacy of perfusion in vulnerable tissues such as the gut mucosa (Fiddian-Green and Baker 1987; Fink, Cohn, Lee et al. 1989; Grum, Fiddian-Green, Pittenger et al. 1984; Gutierrez, Bismar, Dantzker et al. 1992; Hartmann, Montgomery, Jonsson et al. 1991).

### 3.5.5. pHi and acid-base balance.

In agreement with the findings of Boyd et al. (1993) there was a positive correlation between pHi and base deficit. However, pHi was a far more sensitive predictor of a poor outcome. Of the 15 patients who developed a major complication 14 had a low pHi whereas only five had a metabolic acidosis (base excess < 4.65). In the minority of patients who had both a metabolic acidosis and a low pHi at the end of surgery and subsequently had a poor outcome (5 of 14) the degree of gastric intramucosal acidosis was greatest. Due to the infrequent peri-operative measurements made during the peri-operative period it is not possible to say which occurred first.

### 3.5.6. Duration of gastrointestinal hypoperfusion.

Fiddian-Green et al. reported previously that the magnitude and duration of a low pHi were major determinants in outcome (Fiddian-Green and Baker 1987). As there were no recordings made in the post-operative period it is not possible to comment directly on this. However, the very high sensitivity of the presence of a low pHi at the end of surgery in these patients which lacked specificity is in agreement with the findings of Doglio et al. (1991), Gutierrez et al. (1992) and Maynard et al. (1993) who all reported that the presence of a low pHi on admission to the intensive care unit that could not be corrected in the first 12 to 24 hours inspite of resuscitative efforts was associated with the highest mortality. The restoration of splanchnic perfusion in the immediate post-operative period may have occurred in the patients who subsequently did well inspite of an initially low pHi.

### 3.5.7. Using the observed global cardiovascular changes to form the basis of a therapeutic regimen.

From this study there is only indirect evidence from the global cardiovascular changes to form the basis of a therapeutic regimen that may improve peri-operative splanchnic perfusion. Although the patients who maintained gut mucosal perfusion demonstrated increases in group mean cardiac output and stroke volume this was not the case for individual patients. Perhaps this should come as no surprise. Although a reduction in splanchnic blood volume is an immediate response to hypovolaemia as increased sympathetic tone diverts blood to more *vital* organs such as the brain, heart and kidneys this is not readily detected in the measurement of global cardiovascular variables. Price et al. (1966) demonstrated that a 15 – 20% reduction in total blood volume was associated with no significant change in blood pressure, cardiac output or central blood volume in healthy volunteers although there was a 40% reduction in splanchnic blood volume. Similarly, the patients who developed gut mucosal hypoperfusion in this study maintained their blood pressure, cardiac output and urine flow. However, they did show a reduction in stroke volume and increase in heart rate consistent with hypovolaemia and a compensatory increase in sympathetic tone. Patients having major surgery are at particular risk of hypovolaemia. Pre-operative preparation may include prolonged starvation and the administration of enemas. During surgery there is bleeding and oedema formation due to tissue trauma, increased evaporation from exposed surfaces and from the lungs due to the administration of dry anaesthetic gases. It is well known that hypovolaemia can be marked before blood pressure falls (Gilmour, Aitkenhead, Hothersall et al. 1980; Price, Deutsch, Marshall et al. 1966). Yet blood pressure measurement remains the most commonly used method for the peri-operative assessment of cardiac function in the UK (Association of Anaesthetists 1988). The patients that developed gut mucosal hypoperfusion also demonstrated a significant increase in CVP from baseline to the end of surgery. The measurement of a normal central venous or even pulmonary artery wedge pressure will not exclude hypovolaemia without assessing

the dynamic effect of a fluid challenge since the venous system constricts in response to increased sympathetic tone (Weil, Shubin and Rosoff 1965) Baek et al. (1975) demonstrated this point in high risk post-operative patients. Of the patients with high CVP measurements, more than half showed a decrease in CVP and pulmonary artery wedge pressure in response to volume loading. Pulmonary and total vascular resistance's fell and cardiac output increased. Since hypovolaemia is such a powerful factor in the reduction of gut mucosal perfusion it is likely that volume substitution will be most useful. Indeed, in the study of 'goal directed therapy' reported by Shoemaker et al. (1988) and the study of pHi guided therapy reported by Gutierrez et al. (1992) therapeutic goals were achieved with volume replacement alone in the majority of cases.

### 3.5.8. Additional questions that may help in developing a therapeutic regimen.

The following questions need to be addressed before any further progress can be made in developing a therapeutic regimen aimed at reducing the incidence of peri-operative gastrointestinal hypoperfusion:

i) Is the development of a low pHi and the subsequent development of organ dysfunction associated with an endotoxaemia and the subsequent excessive activation of inflammatory pathways as proposed in **figure 3.1**?

ii) Does a variable host response to the proposed insults of endotoxaemia and excessive activation of inflammatory pathways account for the lack of specificity of a low pHi for the prediction of organ dysfunction?

iii) Does the choice of anaesthetic agents used to maintain anaesthesia have a direct bearing on the development of a low pHi?

## 3.6. CONCLUSION.

A low gastric pHi measured during the intra-operative period was found to be common and a sensitive, though non-specific, predictor of post-operative complications including organ failure. There was no causal relationship found between pHi and changes in any of the commonly measured cardiovascular variables such as blood pressure or cardiac output. Therefore, if a therapeutic regimen is to be developed that may result in improved peri-operative gastrointestinal mucosal perfusion it will first be necessary to investigate other potential areas for intervention. As there is a non-specific relationship between gastrointestinal mucosal perfusion and the development of post-operative organ dysfunction the degree of activation of inflammatory pathways maybe a key issue.

# CHAPTER 4.0. CONTACT ACTIVATION, NEUTROPHIL DEGRANULATION AND GUT MUCOSAL HYPOPERFUSION.

## 4.1. INTRODUCTION.

### 4.1.1. Overview.

As discussed in **chapter 1.0** it is postulated that the multiple organ dysfunction syndrome occurs as a result of uncontrolled activation of inflammatory pathways (**chapter 1.1.3**). During major surgery a critical balance is thought to exist between modulation and activation of inflammatory pathways (**figure 1.1**). A degree of inflammation is an essential component of the normal healing process yet excessive activation is said to result in a systemic inflammatory response syndrome and eventually organ dysfunction. It is further postulated that gut mucosal hypoperfusion and subsequent activation of inflammatory pathways could tip the balance in favour of a deleterious systemic response and thus organ dysfunction (**chapter 1.1.4-6, figure 1.2**).

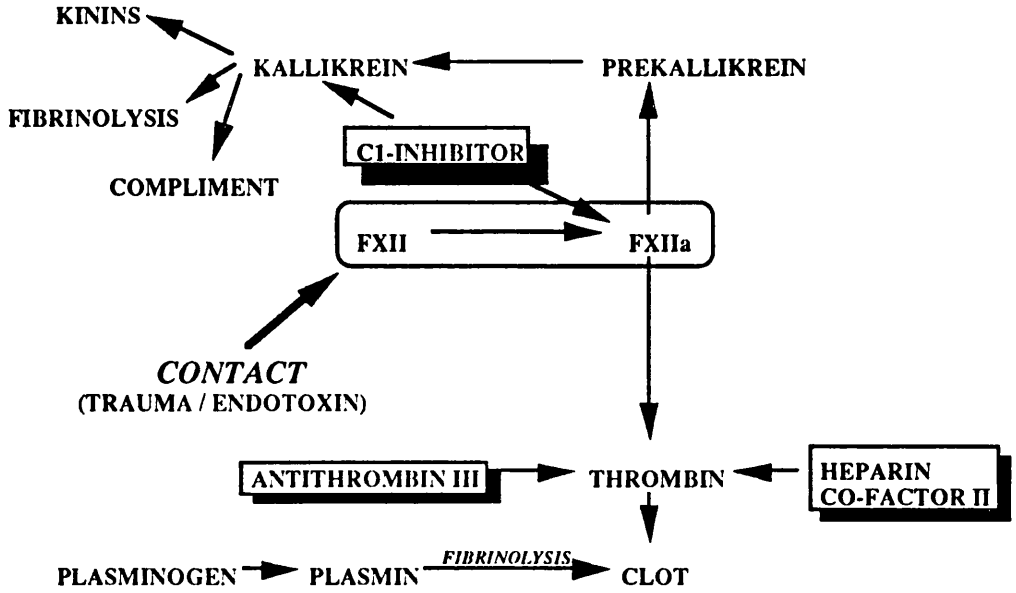
In **chapter 3.0** an association was reported between peri-operative gastric mucosal hypoperfusion and a poor outcome following major (mainly cardiovascular surgery). However, this was not a causal relationship and as in previous studies a low gastric pHi was a sensitive predictor of a poor outcome, although it was by no means specific (**chapter 1.6-1.7, 3.5**). One possible explanation for this is a variable degree of insult or host response. In order to investigate this further it was decided to examine the degree of activation of certain inflammatory pathways in relationship to changes in peri-operative gastric mucosal perfusion and subsequent outcome following high risk major surgery. It would have been impossible to measure all of the factors implicated in the pathogenesis of post-operative organ dysfunction so two specific areas were chosen: contact activation and neutrophil degranulation. The justifications for choosing these two areas are given

below. However, a high level of established local expertise was a not inconsiderable factor in the decision.

### 4.1.2. Contact activation

Contact activation refers to the activation of coagulation Factor XII and subsequent activation of the intrinsic pathway classical coagulation cascade (Griffin andBouma 1987). As well as sub-endothelial collagen and smooth muscle, certain proteases and endotoxin can directly activate Factor XII. Factor XII sits at the hub of a web of enzyme cascades including intrinsic and extrinsic coagulation, fibrinolysis, kinin generation and complement activation (**figure 4.1**).

*Figure 4.1. Key components of the contact system. Inhibitors are shown in boxes*




Prekallikrein is a precursor for the formation of kallikrein which acts on kininogens to liberate bradykinin, a potent vasoactive agent on vascular smooth muscle. The resultant amplification in signal generation through these systems with clot formation and the release of vaso-active peptides would result in mayhem if it were not for a series of feed-back loops via natural inhibitors of all the enzyme systems. The main inhibitors of the contact system are C1-esterase inhibitor, $\alpha_2$-macroglobulin, antithrombin III and protein C inhibitor. C1-esterase inhibitor accounts for 52% of the capacity of normal plasma to inhibit kallikrein and 98% of its abilty to inhibit activated Factor XII (de Agostini, Lijnen, Pixley et al. 1984; Schapira, Scott and Colman 1982; Van der Graaf, Koedam and Bouma 1983). Heparin co-factor II specifically inhibits thrombin, cathepsin G and other chymotrypsin like enzymes, but not kallikrein or activated Factor XII (Mackie andBull 1989).

### 4.1.3 Potential role of contact activation in determining outcome from major surgery.

The measurement of various plasma components of the contact system such as Factor XII, prekallikrein and C1-esterase inhibitor, as well as plasminogen and antithrombin III have all been suggested as diagnostic tools and predictors of outcome in adult respiratory distress syndrome and multiple organ dysfunction syndrome (Aasen, Smith-Erichsent, Gallimore et al. 1980; Colman 1989; Glauser, Zanetti, Baumgartner et al. 1991; Hellgren, Egberg and Eklund 1984; Kalter, Daha, ten Cate et al. 1985; Martinez-Brotons, Oncins, Mestres et al. 1987; Velasco, Torres, Guerrero et al. 1986). The depletion of precursors is interpreted as evidence of activation. The combined depletion of precursors and natural inhibitors is interpreted as uncontrolled activation and has been associated with a significantly higher mortality in patients with established organ failure (Martinez-Brotons, Oncins, Mestres et al. 1987; Nuijens, Abbink, Wachtfogel et al. 1992). In support of this hypothesis there are preliminary results suggesting that the administration of clinical concentrates of inhibitors of the contact system such as antithrombin III and C1-esterase inhibitor may modify the outcome in established sepsis (Hack, Voerman, Eisele et al. 1992; Hellgren, Javelin, Hagnevik et al. 1984).

Therefore, it would seem that the magnitude of contact activation and/or the level of naturally occurring inhibitors may be an important determinant of the outcome following major surgery. Peri-operative gastric mucosal hypoperfusion and subsequent endotoxaemia may be a potential cause of contact activation compounding the inevitable response to the trauma of surgery (**figure 4.2**). Furthermore, many of the natural inhibitors of contact activation are acute phase proteins produced and stored in the liver so splanchnic hypoperfusion can, in isolation, rapidly lead to an imbalance between pro-enzymes and inhibitors of the contact system. These acute changes have been demonstrated in animal models (Borgstrom and Haglund 1989; Omland and Mathisen 1991).

### 4.1.4. Potential role of neutrophil elastase in outcome following major surgery.

Neutrophils are also thought to have a major role in the development of multiple organ dysfunction syndrome (Goris 1991; Williams and Maier 1992). Once again a dichotomy exists, the neutropenic patient has impaired healing and is prone to overwhelming infection, yet degranulating neutrophils, as part of a systemic inflammatory response, are said to cause microvascular injury and promote organ dysfunction (Goris 1991; Williams and Maier 1992). In support of the later, depleting animals of granulocytes makes them resistant to endotoxin induced pulmonary damage (Heflin and Brigham 1981). However, the central role of the neutrophil is confused by two sets of conflicting observations, firstly, neutropenic patients die from MODS (Ognibene, Martin, M. et al. 1986) and secondly, in on going animal studies the promotion of a neutrophilia by the administration of a specific growth factor, GCSF (Granulocyte Colony Stimulating Factor), has been associated with a decreased mortality from the injection of an otherwise fatal dose of endotoxin (M. P. Fink. Boston, MA. personal communication). It seems, therefore, that although the degranulating neutrophil may play a central role in the pathogenesis of MODS it is not an essential component. In studies of patients undergoing major surgery it has been demonstrated that the trauma of surgery is associated with alterations in peripheral neutrophil counts but in a rather non-specific fashion (Davies, Sheppard and Fletcher 1983). Of potentially greater interest is the persistence of increased plasma levels of neutrophil derived proteases such as elastase and lactoferrin in patients who have had a poor outcome following major surgery (Davies, Sheppard andFletcher 1983; Duswald, Jochum, Schramm et al. 1985; Rocker, Wiseman, Pearson et al. 1988).

### 4.1.5 The neutrophil elastase: $\alpha_1$-antitrypsin complex as a marker of neutrophil degranulation.

Following neutrophil degranulation elastase is released from the azurophillic granules along with cathepsin G and collagenase (Dewald, Rindler-Ludwig, Bretz et al. 1975). If

elastase is released into the blood it is rapidly bound and inactivated by $\alpha_1$-antitrypsin and $\alpha_2$-macroglobulin (Ohlsson and Olsson 1978). The former accounts for 90% of the inhibitory capacity in normal blood. High levels of elastase:$\alpha_1$-antitrypsin complexes are a marker of increased neutrophil degranulation and have been associated with the development of the multiple organ dysfunction syndrome (Davies, Sheppard and Fletcher 1983; Duswald, Jochum, Schramm et al. 1985; Nuijens, Abbink, Wachtfogel et al. 1992; Nuytinck, Goris, Redl et al. 1986; Rocker, Wiseman, Pearson et al. 1988).

## 4.2. HYPOTHESIS.

The development of gut mucosal hypoperfusion in the immediate peri-operative period is a sensitive predictor of the subsequent development of organ dysfunction (**chapter 3.0**). Excessive uncontrolled activation of the contact pathway and elevated plasma levels of neutrophil derived proteases have also been associated with the development of organ dysfunction. Therefore, it was hypothesised that gut mucosal hypoperfusion occurring during major surgery would probably be associated with a greater degree of contact activation and degranulation of neutrophils.

## 4.3. AIMS.

To examine the changes in components of the contact system and neutrophil elastase: $\alpha_1$-antitrypsin following major surgery and to relate these findings to per-operative gut mucosal perfusion and subsequent outcome.

## 4.4. PATIENTS AND METHODS.

### 4.4.1. Patient selection.

Patients were studied if they were: i) having elective major surgery of an anticipated duration of greater than 2 hours which required the routine placement of an arterial catheter and central venous catheter and ii) at risk of developing gut mucosal hypoperfusion (i.e. patents having cardiac and vascular surgery) (Fiddian-Green, Amelin, Herrmann et al. 1986; Fiddian-Green and Baker 1987) and/or at risk of developing post-operative organ failure according to the criteria of Shoemaker et al. (1988) (**table 3.1**). The following exclusion criteria were observed: age less than 18 years and pregnancy (for ethical reasons); coagulopathies, perforated viscus and oesophageal or gastric pathology (for safety reasons); the administration of aprotinin (as this interferes with many of the laboratory assays and may modify the degree of contact activation); regional anaesthesia (as this may have a variable effect on splanchnic perfusion).

### 4.4.2. Anaesthetic management.

All patients were given an oral dose of Ranitidine 150mg the night before and on the morning of surgery to permit accurate assessment of gastric pHi (Heard, Helmsmoortel, Kent et al. 1990). All patients were anaesthetised according to a standardised protocol to try and reduce variability in splanchnic perfusion, contact and neutrophil degranulation that might have been as a result of idiosyncratic drug effects. The protocol was as follows: Patients were premedicated with papaveretum 15-20mg and hyoscine 0.3-0.4mg. Prior to induction patients were given alfentanil as a bolus of 25μg/kg, followed by an infusion of 25 μg/kg/hour. Anaesthesia was induced with a dose of thiopentone sufficient to obtund the eyelash reflex and the trachea intubated after administration of atracurium 0.5mg/kg. This was followed by an infusion of atracurium 0.5mg/kg/hour. The lungs were ventilated with 50% nitrous oxide in oxygen. Ventilation was adjusted to

maintain $PaCO_2$ at 4.5-5.5 kPa throughout. Anaesthesia was maintained with Isoflurane or Enflurane. Depth of anaesthesia was gauged by clinical signs according to the anaesthetists standard practice. For those patients undergoing cardiopulmonary bypass the alfentanil and atracurium infusions were continued but the nitrous oxide and volatile anaesthetic agent was stopped and patients were given a single bolus of lorazepam 2mg i.v. Otherwise, no attempt was made to influence the anaesthetic or surgical management of the patients.

### 4.4.3. Tonometric measurements and blood samples

Immediately after induction of anaesthesia a tonometer (Tonometrics Inc., Worcester, MA.) was inserted into the patients stomach and its position confirmed by the injection of air down the nasogastric tube whilst auscultating over the epigastrium. The tonometer was used to measure pHi in the stomach at the end of surgery (see **chapter 2.1**). Theatre staff were blind to the results of all non-routine measurements. Immediately after induction of anaesthesia, and again 24 hours after induction of anaesthesia blood samples were collected for routine blood counts, assays of components of the contact system and neutrophil elastase:$\alpha_1$-antitrypsin (see below).

### 4.4.4. Definitions of post-operative complications.

Post-operative complications were recorded – a major complication defined as one that resulted in an overall post-operative hospital stay of greater than 14 days or death (Webb, Newman, Taylor et al. 1989). Organ failure was determined according to the criteria proposed by Knaus and Wagner (1985) (**table 3.2**).

## 4.5. LABORATORY ASSAYS.

### 4.5.1. Blood collection, plasma preparation and general principles.

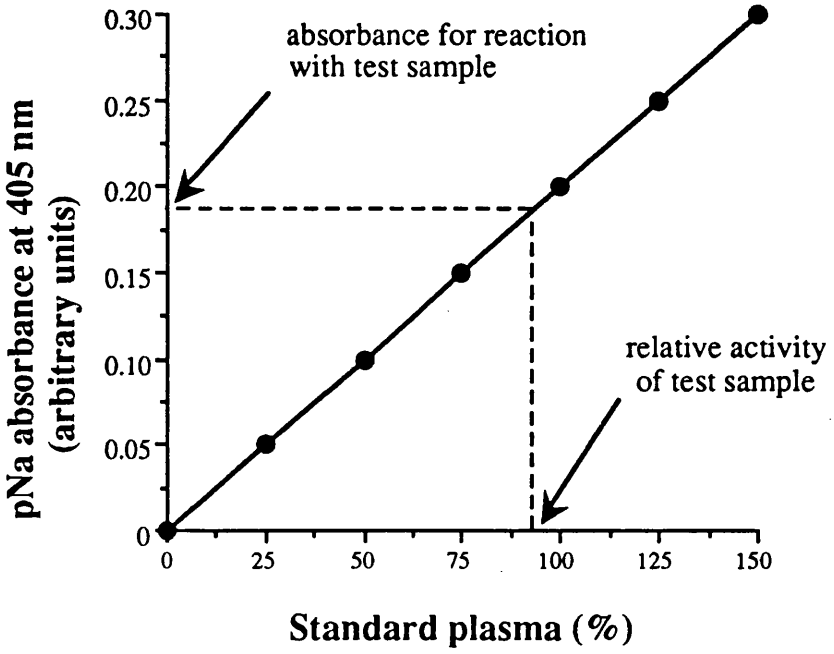
#### 4.5.1.1. Collection of blood and preparation of plasma.

Blood was aspirated from either a fresh puncture of the internal jugular vein with a 14G needle (at the time of the routine placement of central venous pressure catheters) or a 16G non-heparinised lumen of a central venous catheter having first removed a 10 ml dead space sample. For assays of components of the contact system, neutrophil elastase complexes and endotoxin antibodies 9 ml of blood was mixed with 1.0 ml of 0.106M Tri-sodium citrate. Plasma was separated within 20 minutes of collection by centrifugation at 2,000g for 15 minutes at room temperature, divided into aliquots and stored immediately at -70$^{o}$C for later analysis. For routine full blood counts blood was collected into the routinely used EDTA vacutainers.

#### 4.5.1.2. Chromogenic substrate assays – basic principle

The chromogenic substrate assays work on a common principle where the final pathway is the cleavage of p-nitroaniline (pNa) from a specific substrate. The amount of pNa generated can be determined photometrically using a 405 nm light source (hence *chromogenic*) and is proportional to the concentration of test substance. If a standard chromogenic curve is constructed using known concentrations of the test substance then the relative plasma concentration of test samples can be determined from the amount of colour generated in the same reaction (**figure 4.2**).

*Figure 4.2. Example of a standard curve for chromogenic assays.*




Depending on the intermediate steps involved in the final cleavage of pNa from the chromogenic substrate the correlation between the test substance plasma concentration and the change in absorbency may be direct (above) or inverse and linear (above) or logarithmic.

### 4.5.1.3. The ACL 300R analyser.

Many of the assays were performed using the ACL 300R (Instrumentation Laboratories, Warrington, UK.) analyser. The basic principle was as described above but the final mixing of reagents (which had been prepared by hand), standard plasma curve samples and test samples were performed automatically. The dynamic colour generation from the final reaction was then analysed by dedicated computer software and a standard curve drawn.

#### 4.5.1.4. Standardisation.

Unless stated otherwise reference plasma 100% (Immuno Ltd., batch 1AOA) was used for standardisation, assuming a potency of 100 U/dl for each parameter as there is no international standard.

#### 4.5.1.5. Calculation of results

From the standard curve data an x/y graph of absorbency against concentration was plotted. If the graph characteristics were acceptable ($r^2 > 0.95$) the test absorbencies were used to determine the relative concentrations in the test samples.

#### 4.5.1.6. Quality control

Prior to performing any test sample assays multiple assays (>20) were performed on aliquots of plasma taken from a single unit collection of donor plasma (my own) which had been stored at -70°C. These demonstrated the inter- and intra-assay co-efficients of variation shown in **table 4.1**. A quality control check was performed with each run and results only accepted if they lay within the normal range as shown in **table 4.2.**

***Table 4.1.*** *Inter- and intra-assay co-efficients of variations (cv)*

| | Inter-assay cv | Intra-assay cv |
|---|---|---|
| Antithrombin III | 3.8% | 5.1% |
| C1-inhibitor | 3.1% | 5.9% |
| Plasminogen | 3.2% | 5.5% |
| Prekallikrein | 2.1% | 5.9% |
| Heparin co-factor II | 4.8% | 5.6% |
| $\alpha_2$ -macroglobulin | 3.3% | 6.9% |
| $\alpha_1$- antitrypsin | 3.7% | 5.5% |
| Factor XII | 4.1% | 4.1% |

#### 4.5.1.7. Interpretation of data.

A normal range was established by the examination of plasma from at least 20 healthy human adult volunteers (Department of Haematology UCL - on file) and these are shown in **table 4.2.**

*Table 4.2. Normal ranges (2SD about the mean) for plasma components of the contact system determined by chromogenic assays (Department of Haematology UCL - on file).*

| | Normal range (U/dl) |
|---|---|
| Antithrombin III | 70 - 130 |
| C1-inhibitor | 70 - 130 |
| Plasminogen | 70 - 140 |
| Prekallikrein | 70 - 130 |
| Heparin co-factor II | 65 - 148 |
| $\alpha_2$ -macroglobulin | 70 - 150 |
| $\alpha_1$- antitrypsin | 70 - 130 |
| Factor XII | 70 - 140 |

More specific details of the individual assays are given below.

### 4.5.2. Determining antithrombin III in plasma by chromogenic substrate assay.

#### 4.5.2.1. Principle.

Heparin was used as a catalyst for the reaction between a known concentration of bovine thrombin and plasma antithrombin III (AT III). Residual thrombin activity was measured using a chromogenic substrate for thrombin (Odegaard, Lie andAbildgaard 1975). The rate at which pNa was generated was measured photometrically at 405 nm. The inverse correlation between the change in absorbency and the [AT III] was linear up to 125% of the normal plasma value.

#### 4.5.2.2. Reagents

- Lyophilised thrombin substrate (2AcOH.H-D-CHG-Gly-Arg-pNA) with added mannitol as a bulking agent reconstituted in sterile water to a final concentration of 0.1 µmol/ml.

- Lyophilised bovine thrombin reconstituted in 1% bovine albumin to a concentration of 3.6 NIH U/ml.

- 0.05M Tris-HCL buffer containing 0.175M NaCl, 7.5mM EDTA, and Heparin 3.0 U/ml, pH8.4.

#### 4.5.2.3. Preparation of a standard curve

50 µl standard plasma was mixed with 3ml buffer. From this the standard curve was prepared from 25-125% with dilutions as shown in **appendix 4.1.** The zero blank was diluted buffer only. 50 µl of each test plasma was also mixed with 3000 µl of buffer and then 200 µl of this mixture was diluted with 1000 µl of buffer to give the same final dilution as the 100% standard.

#### 4.5.2.4. Assay methods.

The standard curve and test samples were loaded into the ACL 300R autosampler tray and a sample cup containing assay buffer placed in the position marked 'DIL'. Using the chromogenics research mode on the ACL 300R the assay conditions were programmed as shown in **appendix 4.2** :

#### 4.5.2.5. Calculation of results.

From the standard curve data a linear x/y graph of absorbency against concentration was plotted and the test absorbencies used to determine the relative concentration of AT III in the test samples.

#### 4.5.2.6. Interpretation of data.

AT III may be reduced in; congenital deficiency, nephrotic syndrome, patients receiving 1-Asparaginase therapy, patients taking the oral contraceptive pill and after long periods (>24h) of i.v. heparin therapy (Machin and Mackie 1989).

### 4.5.3. Determining C1-esterase inhibitor in plasma by chromogenic substrate assay.

#### 4.5.3.1. Principle.

A known excess concentration of human C1-esterase reacts with C1-inhibitor. Residual C1-esterase activity can then be measured using a chromogenic substrate for C1-esterase (Immuno Ltd., Dutton Green, UK). The rate at which pNa is generated is measured photometrically at 405 nm. The correlation between the change in absorbency and the C1-inhibitor is linear.

#### 4.5.3.2. Reagents

- C1-esterase substrate (AcOH. $C_2H_5CO$-lys-(e-Cbo)-Gly-Arg-pNA) reconstituted in buffer B to a final concentration of 6 μmol/ml.
- Human C1-esterase reconstituted in sterile water.
- Buffer A – Tris (0.61%) NaCl (1.4%) pH 7.4.
- Buffer B – Tris (0.61%) NaCl (1.4%) pH 8.4.

#### 4.5.3.3. Preparation of a standard curve.

The standard curve samples were prepared as shown in **appendix 4.1.** The zero blank was diluted buffer only. 25 µl of each test plasma were also mixed with 250 µl of buffer to give the same final dilution as the 100% sample in the standard curve .

#### 4.5.3.4. Assay methods.

The standard curve and test samples were loaded into the autosampler tray and a sample cup containing assay buffer placed in the position marked 'DIL'. Using the chromogenics research mode on the ACL 300R the assay conditions were programmed as shown in **appendix 4.2.**

#### 4.5.3.5. Calculation of results

From the standard curve data a linear x/y graph of absorbency against concentration was plotted and used to determine the relative concentration of C1–inhibitor in the test samples.

### 4.5.4. Determining plasminogen in plasma by chromogenic substrate assay.

#### 4.5.4.1. Principle.

A known concentration of streptokinase reacts with plasminogen. The plasminogen-streptokinase complex can then be measured using a chromogenic substrate for the complex (Unicorn diagnostics Ltd., London, UK). The rate at which pNa is generated is measured photometrically at 405 nm. The correlation between the change in absorbency and plasminogen is linear up to 150% of the normal plasma value.

#### 4.5.4.2. Reagents.

- Plasminogen substrate (2AcOH.HD-Ala-CHT-Lys-pNA), with added mannitol as a bulking agent, reconstituted in sterile distilled water to a concentration of 2μmol/ml. This is then mixed with an equal volume of assay buffer to obtain a final concentration of 1 mmol/ml.

- Streptokinase reconstituted in sterile water to a final concentration of 1600 U/ml.

- Buffer: Tris HCl 0.05M containing 0.1M NaCl, pH 7.4.

#### 4.5.4.3. Preparation of a standard curve.

The standard curve samples were prepared as shown in **appendix 4.1.** The zero blank was diluted buffer only. 50 μl of each test plasma was also mixed with 2000 μl of buffer to give the same final dilution as the 100% standard curve sample.

#### 4.5.4.4. Assay methods.

The standard curve and test samples were loaded into the autosampler tray and a sample cup containing assay buffer placed in the position marked 'DIL'. Using the chromogenics research mode on the ACL 300R the assay conditions were programmed as shown in **appendix 4.2.**

#### 4.5.4.5. Calculation of results.

From the standard curve data a linear x/y graph of absorbency against concentration was plotted and used to determine the relative concentration of plasminogen in the test samples.

### 4.5.5. Determining prekallikrein in plasma by chromogenic substrate assay.

#### 4.5.5.1. Principle.

Prekallikrein is converted to kallikrein by an activator mixture containing ellagic acid, factor XII and high molecular weight kininogen. The kallikrein liberated is measured using a chromogenic substrate. The pNa released is measured photometrically at 405 nm. The correlation between the change in absorbency and the prekallikrein is linear.

#### 4.5.5.2. Reagents

- Prekallikrein activator (Channel Diagnostics Ltd., Deal, Kent, UK) reconstituted sterile water.
- Kallikrein substrate (MBz-Pro-Phe-Arg-pNA, Channel Diagnostics Ltd., Deal, Kent) reconstituted in sterile water.
- Buffer – 0.05M Tris HCl, pH 7.9.
- Acetone.
- 1M citric acid was used as a stopping agent.

#### 4.5.5.3. Preparation of a standard curve.

Standard plasma was mixed 3:1 with acetone and left for 15 minutes at 4°C. This inactivated the natural kallikrein inhibitors (C1-INH, AT III, etc.) and prevented their possible interference in the assay. The standard curve samples were then prepared as shown in **appendix 4.1.** The zero blank was diluted buffer only. Each test plasma was mixed 3:1 with acetone and left for 15 mins at 4°C. 50 µl was then diluted with 2950 µl of buffer to give the same final dilution as the 100% standard .

#### 4.5.5.4. Assay method.

The substrate and activator were kept in a water bath at 37°C and the acetone treated plasma samples at ambient temperature. 50 µl of plasma dilution (or buffer) was pipetted into the wells of a microtitre plate. Following 2 minutes incubation at 37°C 50 µl of the prekallikrein activator was added to each well. This was then mixed and incubated for 5 minutes at 37°C. 50 µl of kallikrein substrate was then added, mixed and re-incubated at 37°C. Following the addition of 50 µl of stopping agent (citric acid) the absorbency of the individual cell was determined using a plate reader set at 405 nm.

#### 4.5.5.5. Calculation of results.

From the standard curve data a linear x/y graph of absorbency against concentration was plotted and was used to determine the relative concentration of prekallikrein in the test samples.

### 4.5.6. Determining Heparin cofactor II in plasma by chromogenic substrate assay.

#### 4.5.6.1. Principle.

Dermatan sulphate acts as a catalyst for the binding of heparin cofactor II to thrombin. A known excess concentration of human thrombin is added to the test plasma with dermatan sulphate. Residual thrombin is then measured with a chromogenic substrate. The rate at which pNa is generated is measured photometrically at 405 nm. The change in absorbency has an inverse linear relation to the heparin cofactor II concentration.

#### 4.5.6.2. Reagents.

- Thrombin substrate (2AcOH.H-D-CHG-Gly-Arg-pNA, Channel Diagnostics Ltd., Deal, Kent, UK), reconstituted in sterile distilled water to a final concentration of 1µmol/ml.

- Human thrombin (Channel Diagnostics Ltd., Deal, Kent, UK), reconstituted in 1% bovine serum albumin.

- 1% bovine serum albumin (Sigma Ltd, Basle, Switzerland)

- Dermatan sulphate (Channel diagnostics Ltd., Deal, Kent, UK), reconstituted in sterile water.

- Buffer. Tris (0.66%), NaCl (0.88%), disodium EDTA (0.28%), polybrene (0.0002%), pH 8.2.

#### 4.5.6.3. Preparation of a standard curve

The standard curve samples were prepared as shown in **appendix 4.1.** The zero blank was diluted buffer only. 25 µl of each test plasma was also mixed with 2000 µl of buffer to give the same final dilution as the 100% standard .

#### 4.5.6.4. Assay methods

The standard curve and test samples were mixed with an equal volume of Dermatan Sulphate and loaded into the autosampler tray and a sample cup containing assay buffer placed in the position marked 'DIL'. Using the chromogenics research mode on the ACL 300R the assay conditions were programmed as shown in **appendix 4.2.**

#### 4.5.6.5. Calculation of results.

From the standard curve data a linear x/y graph of absorbency against concentration was plotted and used to determine the relative concentration of heparin cofactor II in the test samples.

### 4.5.7. Determining $\alpha_2$-macroglobulin in plasma by chromogenic substrate assay.

#### 4.5.7.1. Principle

Excess trypsin was added to test plasma and formed complexes with alpha-2-macroglobulin. Residual trypsin was then blocked with soya bean trypsin inhibitor. The $\alpha_2$-macroglobulin:trypsin complex was measured using a chromogenic substrate. The pNa released was measured photometrically at 405 nm. The correlation between the change in absorbency and the $\alpha_2$-macroglobulin was linear.

#### 4.5.7.2. Reagents.

- Porcine trypsin (Channel Diagnostics Ltd., Deal, Kent, UK) reconstituted in 1 mM HCl to a final concentration of 0.002%.
- Soya bean trypsin inhibitor (SBTI) 20 mg reconstituted in sterile water to a final concentration of 0.2%.
- $\alpha_2$-macroglobulin:trypsin substrate (Channel Diagnostics Ltd., Deal, Kent) reconstituted in sterile water.
- Buffer – 0.05M Tris HCl, pH 8.0.
- 1M citric acid was used as a stopping agent.

#### 4.5.7.3. Preparation of a standard curve.

The standard curve samples were prepared as shown in **appendix 4.0.** The zero blank was diluted buffer only. 25 µl of each test plasma was diluted with 3975 µl of buffer to give a final dilution the same as the 100% standard .

#### 4.5.7.4. Assay method.

The substrate was kept in a water bath at 37°C. 50 µl of plasma dilution (or buffer) was pipetted into the wells of a microtitre plate in duplicate. Following 2 minutes incubation at 37°C 50 µl of trypsin was added to each well. This was then mixed and incubated for 2 minutes at 37°C. 50 µl of soya bean trypsin inhibitor was then added, mixed and re-incubated at 37°C for 2 minutes. 50µl of substrate was added, mixed and incubated at 37°C for 2 minutes. Following the addition of 50 µl of stopping agent (citric acid) the absorbency of the individual cell was determined using a microtitre plate reader set at 405 nm.

#### 4.5.7.5. Calculation of results.

From the standard curve data a linear x/y graph of absorbency against concentration was and used to determine the relative concentration of $\alpha_2$-macroglobulin in the test samples.

### 4.5.8. Determining $\alpha_1$-antitrypsin in plasma by chromogenic substrate assay.

#### 4.5.8.1. Principle.

Methylamine is added to test plasma and inactivates $\alpha_2$-macroglobulin. Excess trypsin is then added and a proportion binds to $\alpha_1$-antitrypsin. Residual trypsin is then measured using a chromogenic substrate. The pNa released is measured photometrically at 405 nm. The correlation between the change in absorbency and the $\alpha_1$-antitrypsin is linear.

#### 4.5.8.2. Reagents

- Porcine trypsin (Channel Diagnostics Ltd., Deal, Kent, UK) reconstituted in 1 mM HCl to a final concentration of 0.00005%.

- Trypsin chromogenic substrate (Channel Diagnostics Ltd., Deal, Kent, UK) reconstituted in sterile water.

- Buffer A – 0.05M Tris HCl, pH 8.0.

- Buffer B - 0.05M Tris HCl, 0.15M methylamine, pH 8.9.

- 1M citric acid was used as a stopping agent.

#### 4.5.8.3. Preparation of a standard curve.

The standard curve samples were prepared as shown in **appendix 4.1**. The zero blank was diluted buffer A only. 25µl of each test plasma was diluted with 975µl of buffer A, and then 50µl of this with 950µl of buffer B to give the same final concentration as the 100% standard.

#### 4.5.8.4. Assay method.

The substrate was kept in a water bath at 37°C. 50 µl of plasma dilution (or buffer) was pipetted into the wells of a microtitre plate in duplicate. Following 3 minutes incubation at 37°C 50 µl of trypsin was added to each well. This was then mixed and incubated for 5 minutes at 37°C. 50µl of substrate was added, mixed and incubated at 37°C for 1 minute. Following the addition of 50 µl of stopping agent the absorbency of the individual cell was determined using a microtitre plate reader set at 405 nm.

#### 4.5.8.5. Calculation of results.

From the standard curve data a linear x/y graph of absorbency against concentration was plotted and used to determine the relative concentration of $\alpha_1$-antitrypsin in the test samples.

### 4.5.9. Determining factor XII (Hagemann factor) in plasma by chromogenic substrate.

#### 4.5.9.1. Principle

Factor XII is converted to activated factor XII by an activator mixture containing elagic acid. The kallikrein liberated is inactivated by the addition of kallikrein inhibitor. Activated factor XII is measured with a chromogenic substrate. The pNa released is measured photometrically at 405 nm. The correlation between the change in absorbency and the factor XII is linear.

#### 4.5.9.2. Reagents

- Factor XII activator (Channel Diagnostics Ltd., Deal, Kent, UK) reconstituted sterile water.
- Activated factor XII substrate (Channel Diagnostics Ltd., Deal, Kent, UK) reconstituted in sterile water.
- Kallikrein inhibitor (Channel Diagnostics Ltd., Deal, Kent, UK).
- Buffer – 0.05M Tris HCl, pH 7.9 with 0.12M methylamine and 33.6% EDTA.
- Acetone.
- 1M citric acid was used as a stopping agent.

#### 4.5.9.3. Preparation of a standard curve

Standard plasma was mixed 3:1 with acetone and left for 15 mins at 4°C. This inactivates the natural kallikrein inhibitors (C1-INH, AT III, etc.) and prevents their possible interference in the assay. The standard curve samples were then prepared as shown in **Appendix 4.1.** The zero blank was diluted buffer only. Each test plasma was mixed 3:1 with acetone and left for 15 mins at 4°C. 100 µl was then diluted with 300 µl of buffer to give the same final dilution as the 100% standard .

#### 4.5.9.4.. Assay method.

The substrate and activator were kept in a water bath at 37°C and the acetone treated plasma samples at ambient temperature. 25 µl of plasma dilution (or buffer) was pipetted into the wells of a microtitre plate. Following 2 minutes incubation at 37°C 25 µl of the factor XII activator was added to each well. This was then mixed and incubated for 4 minutes at 37°C. 75 µl of kallikrein inhibitor was then added, mixed and re-incubated at 37°C for 1 minute. Finally 50 µl of activated factor XII substrate was added, mixed and re-incubated at 37°C for 10 minutes. Following the addition of 50 µl of stopping agent (citric acid) the absorbency of the individual cell was determined using a plate reader set at 405 nm.

#### 4.5.9.5. Calculation of results.

From the standard curve data a linear x/y graph of absorbency against concentration was plotted and used to determine the relative concentration of factor XII in the test samples.

### 4.5.10. Determining neutrophil elastase:$\alpha_1$-antitrypsin complex in plasma by an enzyme linked immunosorbant assay.

#### 4.5.10.1. Principle.

Neutrophil elastase is released from the azurophilic granules of activated neutrophils (Dewald, Rindler-Ludwig, Bretz et al. 1975). In blood it is rapidly inactivated by $\alpha_1$-antitrypsin, which accounts for 90% of the plasma inactivating capacity, and $\alpha_2$-macroglobulin (Ohlsson and Olsson 1978). Microtitre plates are coated with antibodies directed against neutrophil elastase, and test and standard plasma samples are added.. After washing off unbound protein, peroxidase conjugated antibodies directed against alpha-1-antitrypsin are added. The bound antibody can then be detected with a chromogenic peroxidase substrate. The colour generated can be determined photometrically at 405 nm and is linearly related to the plasma neutrophil elastase:$\alpha_1$-antitrypsin activity. As with the chromogenic assays described above the relative concentration of plasma neutrophil elastase:$\alpha_1$-antitrypsin can be determined by comparing the test sample colour generation with a standard curve of known concentrations.

#### 4.5.10.2. Reagents.

- Coating antibody (Serotec Ltd., Kidlington, Oxfordshire): Sheep-anti human neutrophil elastase diluted in buffer A to a final concentration of 1ml/ml.

- Conjugated antibody (Serotec Ltd., Kidlington, Oxfordshire): Sheep anti-human $\alpha_1$-antitrypsin peroxidase conjugate diluted in Buffer B to a final concentration of 1µl/ml.

- Peroxidase substrate (3,3',5,5' - Tetramethyl - Benzidine Dihydrochloride, Sigma Ltd., Basle, Switzerland) reconstituted in substrate buffer to a final concentration of 0.01%).

- Buffer A (Sigma P-4417 Phosphate buffer saline , Sigma Ltd, Basle, Switzerland).

- Buffer B - 0.0025M $Na_2HPO_4$. $2H_20$, 0.0075M $Na_2HPO_4$. $12H_20$, 0.145M NaCl, pH 7.2.

- Substrate buffer (Sigma P-9305 Phosphate citrate buffer with urea hydrogen peroxide, Sigma Ltd., Basle, Switzerland)

- Blocking agent - 1% bovine serum albumin in buffer A.

- Stopping agent - 2M sulphuric acid.

#### 4.5.10.3. Standardisation.

Neutrophil elastase:$\alpha_1$-antitrypsin complexes were purified in house as previously described by Bough and Travis (Galloway, Mackie and McVerry 1985). These complexes were then diluted in plasma and incubated for 1 hour at 37°C (Galloway, Mackie and McVerry 1985). This *in-house* standard was then callibrated using a commercial standard (Merck, Lutterworth, Leicestershire).

#### 4.5.10.4. Preparation of standard curve

50 µl of standard plasma was diluted with 1,200 µl of buffer B and then dilutions of 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600 made from this using buffer B. For each test plasma 50 µl was diluted in 1,200 µl of buffer B and then dilutions of 1:50, 1:100, 1:200 made from this using buffer B.

#### 4.5.10.5. Assay method

180 µl of coating antibody was placed into each well of a microtitre plate. The plate was covered, mixed and incubated for 2 hours at ambient temperature. The wells were then washed three times with 200µl of buffer B. 180 µl of blocking agent was added to each well. The plate was covered, mixed and incubated for 1 hour at ambient temperature. The

wells were then washed again three times with 200 µl of buffer B. 180 µl of buffer B was placed in wells A1 and B1. 180 µl of each standard curve dilution was placed in duplicate in wells A2 to B8. 180 µl of the dilutions of each test plasma were placed in duplicate wells through the rest of the plate. The plate was then covered, mixed and incubated at room temperature for 2 hours. Having washed the wells three times with 200 µl of buffer B, 200 µl of substrate was added to each well. This was left at room temperature until there was colour visible in all standard curve wells. Following the addition of 50 µl of stopping agent (citric acid) the absorbency of the individual cells was determined using a plate reader set at 450 nm using A1 as the blank.

#### 4.5.10.6 Calculation of results.

From the standard curve data a log x/y graph of absorbency against concentration was plotted and used to determine the relative concentration of neutrophil elastase:alpha-1-antitrypsin complexes in the test samples. The test dilutions were then corrected for the dilution and the mean result calculated.

#### 4.5.10.7. Quality control.

Prior to performing any test sample assays multiple assays were performed on aliquots of plasma taken from a single unit collection of donor plasma (my own) which had been stored at -70°C. These demonstrated an inter- and intra-assay co-efficient of variation of 3.8% and 8.0% respectively. A quality control check was performed with each run and results only accepted if the result lay within the normal range.

#### 4.5.10.8. Normal range.

A normal range was established in our laboratory by the examination of plasma from healthy volunteers and was found to be 30 - 172 µg/l (mean 101.0 µg/l; SD 35.6µg/l) (Department of Haematology UCL - on file).

## 4.6. RESULTS.

A total of 26 patients having elective major surgery were studied. Type of surgery is shown in **table.4.3.** and demographic details, duration of surgery and outcome in **table 4.4.** Of the 26 patients 16 (61%) developed gut mucosal hypoperfusion (pHi <7.32). Of this group 8 developed major complications (1 cerebrovascular accident, 1 persistent dyspnoea, 1 ileus with persistent nausea and vomiting, 1 haematemesis requiring blood transfusion and 4 multiple organ dysfunction syndrome). Of the 4 patients who developed multiple organ dysfunction syndrome 3 subsequently died. One patient required inotropic support at the end of surgery (Isoprenaline and Dopamine). This patient had an abnormal pHi and subsequently developed multiple organ dysfunction syndrome and died. Ten patients maintained gut mucosal perfusion (pHi ≥7.32); none of these developed major complications.

***Table 4.3.*** *Type of surgery. Patients are grouped according to their pHi at the end of surgery.*

| | pHi ≥ 7.32 | pHi < 7.32 |
|---|---|---|
| Coronary artery by-pass graft | 6 | 9 |
| Heart valve replacement | 3 | 3 |
| Vascular surgery with Aortic cross-clamp | 1 | 4 |

***Table 4.4.*** *Demographic details, duration of surgery and complications. Patients are grouped according to their pHi at the end of surgery.(* Multiple organ dysfunction syndrome).*

| | pHi<7.32 (n=16) | pHi ≥7.32 (n=10) |
|---|---|---|
| Male | 12 | 7 |
| Age (years) | 65 (67) [43-82] | 64 (67) [40-75] |
| Weight (kg) | 72 (75) [46-91] | 73(71) [48-99] |
| Height (cm) | 168 (173) [128-183] | 167 (173) [145-187] |
| Duration (mins) | 231.7 (229) [130-310] | 218.0 (195) [131-362] |
| Major complications | 8 | 0 |
| MODS* | 4 | 0 |
| Deaths | 3 | 0 |

For both groups there was a significant reduction in haematocrit and platelet count but increase in white cell count (**table 4.5**). However, there were no differences in baseline values or magnitude of change between the two groups.

***Table 4.5.*** *Results of blood counts. Patients are grouped according to their pHi at the end of surgery. Data are shown as mean (95% confidence interval).* p< 0.005 from baseline to 24 hours within groups.*

| | pHi < 7.32 (n=16) | | pHi >7.32 (n=10) | |
|---|---|---|---|---|
| | Baseline | 24 hours | Baseline | 24 hours |
| Haematocrit (%) | 42 | 33 | 43 | 31 |
| | (40 - 45) | (30 - 35)* | (39 - 47) | (27 - 34)* |
| Platelets ($10^{12}$ /l) | 227 | 141 | 218 | 137 |
| | (204 - 251) | (114 - 168)* | (192 - 244) | (103 - 170)* |
| White cells ($10^9$ /l) | 7.2 | 9.8 | 7.1 | 9.9 |
| | (6.2 - 8.1) | (8.7 - 10.9)* | (6.22 - 8.1) | (8.8 - 11.1)* |

For both groups there were significant reductions in Factor XII, plasminogen, prekallikrein, antithrombin III, α-2-macroglobulin and heparin co-factor II (**table 4.6**). For both groups there was no significant reduction in α-1 antitrypsin. In the low pHi group there was a significant increase in neutrophil elastase:$\alpha_1$-antitrypsin ($p$<0.005) (**figure 4.6**) and a significant reduction in C1-esterase inhibitor ($p$<0.005) (**Ttble 4.6 and figure. 4.3**). However, In the normal pHi group neutrophil elastase:α-1-antitrypsin was not increased (**figure 4.2**) and C1-esterase inhibitor levels were maintained (**table 4.6 and figure 4.3**). There were no significant differences between groups for any of the baseline measurements. In the low pHi group one patient had an abnormally high baseline level of neutrophil elastase:$\alpha_1$-antitrypsin (**figure 4.2**). There was no obvious clinical reason for this. By 24 hours it had decreased and the patient's post-operative course was uneventful. There was no difference between the groups in the number of patients with a low baseline level of C1-esterase inhibitor (2 in each group). However, the lowest level was recorded in a patient who developed multiple organ dysfunction syndrome and died (46 IU/dl). Of the 16 patients who developed a low pHi

15 had high levels of neutrophil elastase:$\alpha_1$-antitrypsin (> 170 μg/l) at 24 hours compared to 3 in the normal pHi group. All 3 of the patients who developed multiple organ dysfunction syndrome and subsequently died had neutrophil elastase:α-1-antitrypsin levels > 500 μg/l by 24 hours.

***Table 4.6.*** *Changes in components of the contact system and neutrophil elastase:α-1-antitrypsin complexes in patients plasma from Induction of anaesthesia (baseline) to 24 hours later. Patients are grouped according to their pHi at the end of surgery. Data are shown as geometric mean (10th to 90th centiles).* p< 0.05, **p < 0.005, from baseline to 24 hours within groups. † p< 0.005 change from baseline to 24 hours between groups*

| | pHi <7.32 (n=16) | |
|---|---|---|
| | Baseline | 24 hours |
| NE:$\alpha_1$-AT (µg/l) | 134.9 (66.1 - 571.8) | 372.4 (212.9 to 809.4) ** |
| Factor XII (IU/dl) | 73.0 (57.4 - 109.0) | 52.6 (34.1 to 74.8) ** |
| Plasminogen (IU/dl) | 79.0 (47.8 - 110.8) | 51.5 (31.1 to 91.3) ** |
| Prekallikrein (IU/dl) | 94.9 (52.6 - 139.7) | 55.2 (35.0 – 85.1) ** |
| Antithrombin III (IU/dl) | 96.5 (53.1 – 157.9) | 56.6 (16.9 – 103.9) ** |
| Heparin co-factor II (IU/dl) | 80.8 (57.8 – 98.8) | 49.5 (36.4 – 71.3) ** |
| $\alpha_2$-macroglobulin (IU/dl) | 104.4 (67.9 – 147.5) | 60.7 (42.4 – 85.4) ** |
| $\alpha_1$-antitrypsin (IU/dl) | 109.8 (80.8 – 147.8) | 97.5 (68.6 – 161.8) |
| C1-inhibitor (IU/dl) | 102.4 (68.4 – 166.1) | 74.2 (43.0 – 118.0) †** |

| | pHi > 7.32 (n=10) | |
|---|---|---|
| | Baseline | 24 hours |
| NE:$\alpha_1$-AT (µg/l) | 118.1 (75.0 – 223.5) | 170.3 (87.5 – 311.5) |
| Factor XII (IU/dl) | 81.3 (46.0 – 115.0) | 51.6 (32.0 – 70.5)** |
| Plasminogen (IU/dl) | 87.8 (76.0 – 99.0) | 49.8 (22.5 – 70.0)** |
| Prekallikrein (IU/dl) | 104.8 (87.0 – 124.5) | 54.2 (36.0 – 103.0)* |
| Antithrombin III (IU/dl) | 97.3 (80.5 – 117.0) | 68.3 (28.5 – 96.9)* |
| Heparin co-factor II (IU/dl) | 83.5 (61.5 – 103.0) | 58.6 (43.5 – 100.0)* |
| $\alpha_2$-macroglobulin (IU/dl) | 95.6 (70.4 – 132.0) | 59.7 (40.5 – 97.7)** |
| $\alpha_1$-antitrypsin (IU/dl) | 106.8 (88.5 – 136.5) | 112.2 (82.5 – 143.0) |
| C1-inhibitor (IU/dl) | 87.4 (63.5 – 130.0) | 87.1 (69.0 – 109.5)† |

***Figure 4.3.*** *Changes in Neutrophil elastase; $\alpha_1$-antitrypsin from baseline to 24 hours for individual patients grouped according to their pHi at the end of surgery. The solid lines indicate the change in the geometric mean. ** $p< 0.005$.*

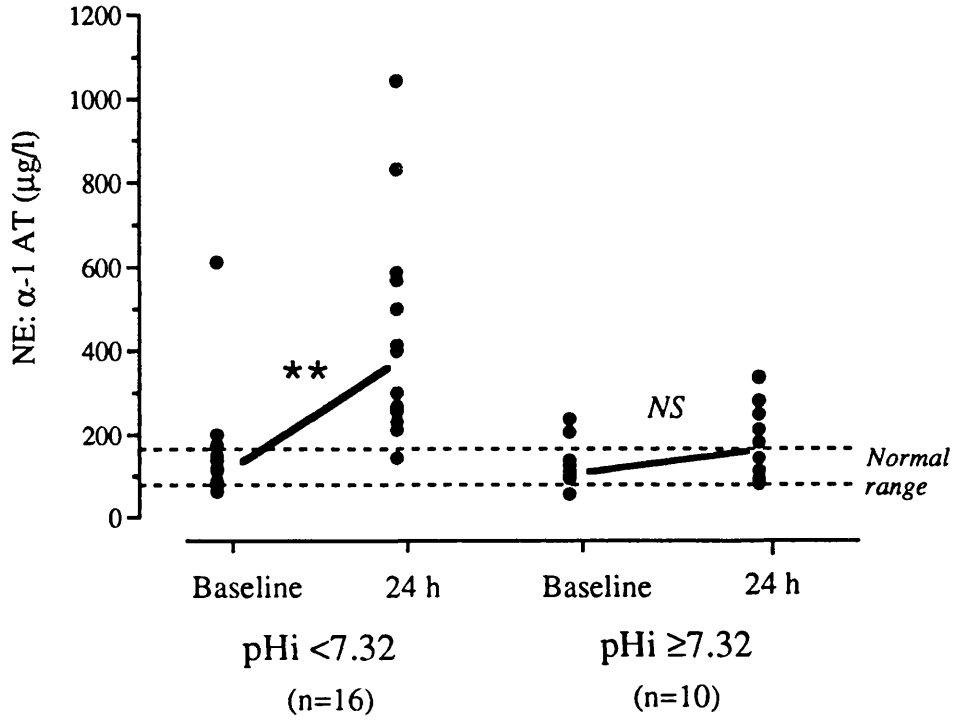
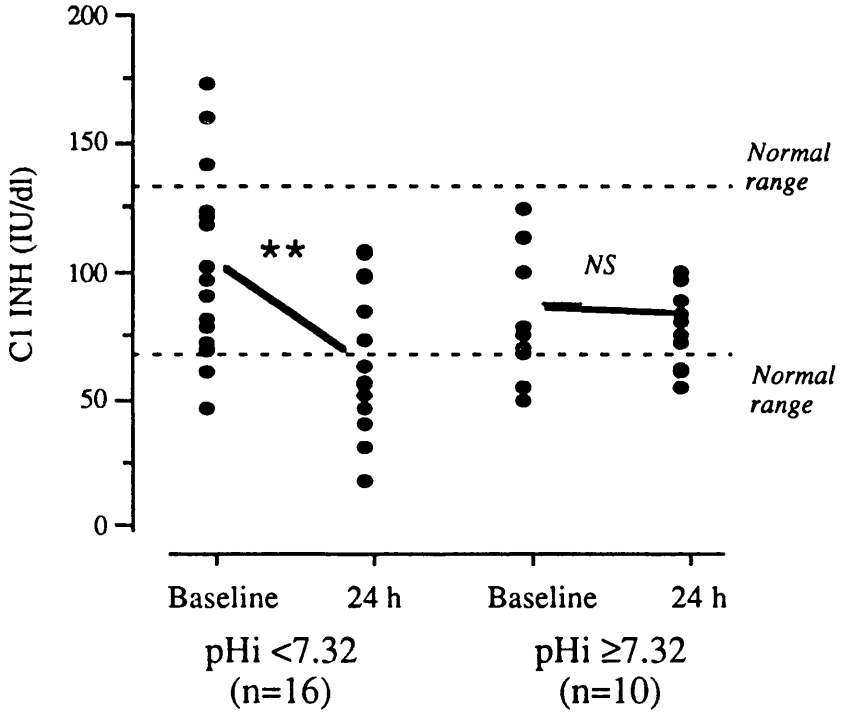


***Figure 4.4.*** *Changes in C1-esterase inhibitor from baseline to 24 hours for individual patients grouped according to their pHi at the end of surgery. The solid lines indicate the change in the geometric mean. ** p< 0.005.*



## 4.7. DISCUSSION.

### 4.7.1. Gut mucosal hypoperfusion, neutrophil degranulation and contact activation.

These data suggest that gut mucosal hypoperfusion, neutrophil degranulation and contact activation are associated with a poor outcome following major surgery if natural inhibitors of inflammatory pathways become depleted. These data also support the hypothesis that the degree of activation of inflammatory pathways is a major determinant in the development of multiple organ dysfunction syndrome (**chapter 1.1.3**). The patients in the low pHi group had significantly higher levels of neutrophil elastase at 24 hours and also depletion of the protease inhibitor (by complexing with a wide variety of possible target proteases) C1-esterase inhibitor.

### 4.7.2. Effects of dilution on plasma concentrations.

The reduction of many of the components of the contact system may have been due to dilution with the administration of crystalloid and colloid solutions or extravisation through leaky vascular endothelium. However, haematocrit correction of plasma concentrations of the various components was unable to support this hypothesis as was the fact that some plasma components, most notably C1-esterase inhibitor and Neutrophil elastase, increased in many patients (**appendix 4.0**).

### 4.7.3. Sampling times.

It was decided that sampling at 24 hours would be the most efficient single point for detecting changes in the plasma components measured. Major surgery and cardiopulmonary by-pass are associated with transient perturbations of the contact system that seem unrelated to subsequent outcome (Mythen, Purdy, Cardigan et al. 1993). However, in healthy volunteers treated with a bolus of endotoxin changes in plasma neutrophil elastase and protease inhibitors persist for at least 24 hours (Suffredini, Harpel

and Parrillo 1989). It seems most likely that the compounding insults of the trauma of surgery plus gut mucosal hypoperfusion plus the translocation of gut luminal contents could lead to excessive activation of inflammatory pathways as demonstrated by the high levels of Neutrophil elastase and depletion of C1-esterase inhibitor in those patients who developed gut mucosal hypoperfusion and had a poor outcome. However, it may equally be that the early depletion of natural inhibitors leads to an imbalance between coagulant and fibrinolytic pathways and results in microvascular thromboembolism manifest as gut mucosal ischaemia.

### 4.7.4. Comparisons with previous studies.

In agreement with previous studies of patients undergoing major surgery the patients who had a poor outcome had the highest levels of Neutrophil elastase at 24hours suggesting increased neutrophil degranulation (Davies, Sheppard and Fletcher 1983; Duswald, Jochum, Schramm et al. 1985; Rocker, Wiseman, Pearson et al. 1988). In support of the hypothesis that peri-operative gut mucosal hypoperfusion may be a compounding factor in the pathogenesis of post-operative organ dysfunction the patients who developed a low pHi had significantly higher levels of neutrophil elastase at 24 hours and also depletion C1-esterase inhibitor. There is no directly comparable work in major surgical patients comparing pHi and the inflammatory markers measured here. Yet some parallels can be drawn with three other studies. Firstly Soong et al. (1992b) found significantly increased levels of tumour necrosis factor-$\alpha$ suggesting increased cytokine release in patients who developed a low sigmoid pHi following elective major vascular surgery. Secondly, Nuijens et al. (1992) found significantly higher levels of Neutrophil elastase in sepsis patients who died compared to those who survived and that Neutrophil elastase was inversely related to C1-esterase inhibitor. Thirdly, Martinez-Brotons et al. (1987) found that the combination of endotoxaemia and contact activation was common among patients with established sepsis and if C1-esterase became depleted that this was associated with the highest mortality.

At deference to previous studies in patients with sepsis or established organ failure (Aasen, Smith-Erichsent, Gallimore et al. 1980; Colman 1989; Glauser, Zanetti, Baumgartner et al. 1991; Hellgren, Egberg and Eklund 1984; Kalter, Daha, ten Cate et al. 1985; Martinez-Brotons, Oncins, Mestres et al. 1987; Velasco, Torres, Guerrero et al. 1986) there was no difference found between the low and normal pHi group in the degree of coagulation or contact system activation, as judged by peri-operative falls in Factor XII and prekallikrein. Similarly there was no difference in the degree of depletion of antithrombin III, $\alpha_2$-macroglobulin, heparin co-factor II and $\alpha_1$-antitrypsin which are natural inhibitors of the coagulation and contact systems.

### 4.7.5. Discrepancies in inhibitor levels.

The discrepancy between the two groups in the fall of C1-esterase inhibitor despite parallel falls in prekallikrein and Factor XII suggests either that additional proteases (perhaps cellular, or from complement activation) are generated in the low pHi group, or that other plasma protease inhibitors have been compromised leading to secondary depletion of C1-esterase inhibitor. The lack of difference between the two groups in the fall of $\alpha$-1 antitrypsin, despite a significant difference in Neutrophil elastase, may be due to the enormous excess of plasma $\alpha_1$-antitrypsin compared to released neutrophil elastase.

### 4.7.6. Limitations in interpreting significance of study data.

The major limitations in the interpretation of the above data are the relatively small numbers of patients used with highly variable pathologies and types of surgery. There is no doubt that the pathogenesis of post-operative MODS is complex and multifactorial. Therefore, considering the complexity of the pathogenesis of post-operative organ failure and the enormous number of interwoven inflammatory pathways involved it is remarkable in itself that any differences were demonstrated between the two groups. Even though there is a large degree of overlap for many of the factors measured in the two groups the three patients who developed post-operative MODS and subsequently

died all had a low pHi that developed over the course of surgery, they had the highest levels of neutrophil elastase α-1-antitrypsin by 24 hours and the greatest falls in C1-esterase inhibitor.

## 4.8. CONCLUSION.

The development of gut mucosal hypoperfusion during elective major surgery is associated with increased activation of the contact system, increased neutrophil degranulation and the development of post-operative organ dysfunction. These do not appear to be causal relationships in that a patient can develop a low pHi over the course of surgery and not demonstrate evidence of either increased contact activation or increased degranulation of neutrophils. Similarly patients may develop a low pHi, depletion of C1-esterase inhibitor (the main inhibitor of the contact pathway) and elevation of neutrophil elastase complexes and have an uneventful recovery. The apparent lack of a causal relationship between these factors may in part be due to a variable host response to gut mucosal hypoperfusion such as different levels of endotoxin exposure or varying degrees of natural immunity to endotoxin.

# CHAPTER 5.0. GUT MUCOSAL HYPOPERFUSION AND ENDOTOXIN IMMUNITY.

## 5.1. INTRODUCTION.

### 5.1.1. The proposed link between gut mucosal hypoperfusion and post-operative organ dysfunction.

Reduced gut mucosal perfusion is associated with the development of post-operative organ dysfunction (**chapter 3.0**), increased degranulation of neutrophils and depletion of natural inhibitors of the contact system (**chapter 4.0**) but these are not causal relationships and the pathophysiological mechanisms involved remain poorly understood. Post operative organ dysfunction is considered to be the end result of a generalised, exaggerated systemic inflammatory response to an initiating insult, which is commonly infection (**chapter 1.1.3 - 1.1.6**). It is postulated that gut mucosal hypoperfusion may result in loss of gut barrier function and translocation of bacteria and/or endotoxin from the gut lumen into the blood stream (Deitch 1990b) (**chapter 1.1.5, 1.1.6**).

### 5.1.2. The evidence for endotoxin's role in the pathogenesis of MODS.

Bacteria have been demonstrated in portal venous and systemic arterial blood within 2 hours of shock and this bacteraemia has been shown to be more severe with increasing levels of hypotension (Rush, Sori, Murphy et al. 1988). Radiolabeled endotoxin placed in the colon of dogs could be recovered in the bloodstream within 30 minutes of inducing local ischaemia (Papa, Halperin, Rubenstein et al. 1983). However, the cause and effect relationship between gut mucosal hypoperfusion, increased permeability, endotoxaemia and multiple organ failure remains unproved in humans. Endotoxin is a recognised potent activator of various cellular and humoral

pathways involved in the generalised inflammatory response (Williams and Maier 1992). In controlled animal, volunteer and laboratory studies administration of endotoxin has been shown to not only stimulate cytokine production from macrophage-monocytes (Michie, Manogue, Spriggs et al. 1988) but also to activate directly complement, the contact and coagulation systems (Mason, Kleeburg, Doland et al. 1970; Morrison and Cochrane 1974; Robinson, Klondnycky, Loeb et al. 1975). Endotoxin is commonly present in the plasma taken from patients with septic shock, including those with no detectable bacteraemia or isolated Gram positive bacteraemia (Danner, Elin, Hosseini et al. 1991). In hospitalised patients with fever, the presence of an endotoxaemia predicts the development of the sepsis syndrome with a sensitivity of 79% and specificity of 96% (van Deventer,Buller,ten Cate et al. 1988). These findings strongly support the notion that endotoxin is important, though not an essential component in the pathogenesis of multiple organ dysfunction syndrome.

### 5.1.3. The measurement of endotoxin.

Endotoxin is difficult to assay reliably. Aside from the problems of environmental contamination the commonly used limulus amoebocyte lysate bioassay has not been standardised as there is no direct method for measuring endotoxin. Also, systemic endotoxaemia may be pulsatile and gut mucosal ischaemia has been shown to be associated with portal endotoxaemia without systemic endotoxaemia (van Deventer, Knepper, Landsman et al. 1988).

### 5.1.4. Anti-endotoxin core anti-bodies.

Endotoxins mostly comprise of lipopolysaccharide. Lipopolysaccharide is composed of three distinct regions; the lipid A moiety, the core lipopolysaccharide region and the oligosaccharide (O)-side chains. Gram negative bacterial endotoxins exhibit an enormous diversity of O-specific serotypes but the core region is nearly identical for most strains. Anti-endotoxin-core antibodies are cross reactive antibodies to Gram-negative bacterial endotoxin which appear in the first year of life (GR Barclay et al personal

communication) and are found comprehensively in healthy adults (Barclay 1990; Barclay and Scott 1987). Endotoxin perturbs endotoxin core antibody homeostasis, and depressed or falling endotoxin core antibody levels can be interpreted as indicating current or recent exposure to endotoxin. Therefore the measurement of endotoxin core antibodies should provide a more robust indicator of recent endotoxin exposure.

## 5.2. HYPOTHESIS.

It was hypothesised that patients who developed gut mucosal hypoperfusion in the peri-operative period would demonstrate significant falls in endotoxin core antibodies as a result of exposure to translocated endotoxin and that these patients would have a higher incidence of post-operative complications. It was also hoped that a variation in endotoxin immunity may help in understanding the lack of specificity for the detection of a low pHi and a poor outcome.

## 5.3. AIM.

To examine the changes in plasma IgG and IgM anti-endotoxin core antibodies occurring during major surgery and relate these findings to the incidence of gut mucosal hypoperfusion and patient outcome.

## 5.4. PATIENTS AND GENERAL METHODS.

### 5.4.1. Patients.

Patients were studied if they were: i) having elective major surgery of an anticipated duration of greater than 2 hours which required the routine placement of an arterial and central venous catheter and ii) at risk of developing gut mucosal hypoperfusion and post-operative organ failure according to published criteria (see **chapter 3.1** and **table 3.1**) The following exclusion criteria were observed: age less than 18 years or pregnancy (for ethical reasons); coagulopathies and perforated viscus (for safety reasons); oesophageal or gastric pathology (due to uncertainty in interpretation of the tonometer results); non-pulsatile cardiopulmonary by-pass (as it has been reported as

a cause of reduced splanchnic perfusion); the administration of aprotinin (as it may modify both splanchnic perfusion and patient outcome); regional anaesthesia (as the effects of blocking the autonomic blood supply to the splanchnic region are unknown). All patients were given an oral dose of Ranitidine 150mg the night before and on the morning of surgery to permit accurate assessment of gastric pHi ( see **chapter 2.0**).

### 5.4.2. Anaesthetic management.

All patients were anaesthetised according to the following protocol: Patients were premedicated with papaveretum 15-20mg and hyoscine 0.3-0.4mg. Prior to induction patients were given alfentanil as a bolus of 25μg/kg, followed by an infusion of 25 μg/kg/hour. Anaesthesia was induced with a dose of thiopentone sufficient to obtund the eyelash reflex and the trachea intubated after administration of atracurium 0.5mg/kg. This was followed by an infusion of atracurium 0.5mg/kg/hour. The lungs were ventilated with 50% nitrous oxide in oxygen. Ventilation was adjusted to maintain $PaCO_2$ at 4.5-5.5 kPa throughout. Anaesthesia was maintained with Isoflurane or Enflurane. Depth of anaesthesia was gauged by clinical signs according to the anaesthetists standard practice. For those patients undergoing cardiopulmonary bypass the alfentanil and atracurium infusions were continued but the nitrous oxide and volatile anaesthetic agent was stopped and patients were given a single bolus of lorazepam 2mg i.v. Otherwise, no attempt was made to influence the anaesthetic or surgical management of the patients and.

### 5.4.3. Study instrumentation.

Immediately after induction of anaesthesia a tonometer (Tonometrics Inc., Worcester, MA.) was inserted into the patients stomach. The tonometer was used to measure $PCO_2$ in the stomach at the end of surgery. The arterial bicarbonate was measured at the same time and the pHi was calculated (see **chapter 2.0**). The anaesthetist in charge of the case was not aware of the results of any non-routine measurements.

### 5.4.4. Blood sampling.

Immediately after induction of anaesthesia, at the time of placement of a multi-lumen central venous catheter, 14 ml of blood was aspirated from an internal jugular vein via a 14G needle; 9ml was anticoagulated with sodium citrate and 5ml with EDTA. A second sample was collected from a 16G non-heparinised lumen of the central venous catheter 24 hours after induction of anaesthesia having first removed a 10 ml dead space sample. Plasma was separated from the citrated sample within 20 minutes of collection by centrifugation at 2,000g for 15 minutes at room temperature, divided into aliquots and stored immediately at -70$^{o}$C for later analysis. From the EDTA samples haematocrit, white blood cell and platelet counts were measured by standard methods (see **chapter 2.3**). From plasma samples IgG and IgM anti-endotoxin-core antibody levels were measured (see **5.5** below).

### 5.4.5. Definitions of post-operative complications.

Post-operative complications were recorded – a major complication defined as one that resulted in an overall post-operative hospital stay of greater than 14 days or death (Webb, Newman, Taylor et al. 1989). Organ failure was determined according to the criteria proposed by Knaus and Wagner (1985) (see **Table 3.2**).

## 5.5. DETERMINATION OF ENDOTOXIN CORE ANTIBODIES IN PLASMA BY AN ENZYME LINKED IMMUNOSORBANT ASSAY.

IgG and IgM anti-endotoxin-core antibody (EndoCAb) levels were measured by an ELISA developed by Barclay et al. (1990; 1987).

### 5.5.1. Principle.

The ELISA used is based on a rough mutant lipopolysaccharide (R-LPS) with no O-serotypic antigens. A R-LPS is used from each of *Escherichia coli, Pseudomonas aeruginosa, Klebsiella aerogenes* and *Salmonella typhimurium,* in equimolar proportions. Each R-LPS contains a complete inner-core structure (lipid A, KDO and heptose) but no complete outer core and therefore no core-type specific antibodies. Each R-LPS is complexed with polymixin B for good coupling to plates and optimum epitope exposure.

### 5.5.2. Materials.

- Human standard reference serum (Chromogenix AB, Molndal, Sweden) reconstituted with 1ml of dilution buffer for the IgG EndoCAb assay and 2ml for the IgM EndoCAb assay.

- Dilution buffer (Chromogenix AB, Molndal, Sweden).

- Plate wash buffer: NaCl (0.138M), Phosphate (0.01M), Tween 20 (0.1%), Sodium Azide (0.15%), pH 7.4.

- Substrate buffer: Sodium Carbonate (0.05M), Sodium Bicarbonate (0.05M), Magnesium Chloride (0.002M), pH 9.8.

- Conjugated antibodies (Sigma Ltd, Basle, Switzerland.): Rabbit anti-human IgG and IgM alkaline phosphatase conjugate diluted with dilution buffer to a final concentration of 0.02%.

- Alkaline phosphatase substrate tablets (Sigma Ltd, Basle, Switzerland.) reconstituted with substrate buffer.

- Microplate coated with endotoxin core antigen-polymixin B cocktail (Chromogenix AB, Molndal, Sweden).

### 5.5.3. Standard curve and test samples.

Standard curves for the IgG and IgM assays were prepared from the reconstituted standard serum. The reconstituted standards contained 784 IgG 'median units' (MU) and 330 IgM MU respectively. The median units are based on the medians of the normal healthy adult range of EndoCAb IgG and IgM antibodies . The units for the different Ig classes are not equivalent.

The reconstituted standard was used for the top point of the respective curves (e.g. 784 IgG MU for the IgG EndoCAb assay). A double dilution series of eight points was used for the remainder of each curve (eg. 784 to 12.2 IgG MU for the IgG EndoCAb assay).

Test samples were diluted 1:200 with dilution buffer.

### 5.5.4. Assay method.

The assay methodology is the same for both IgG and IgM assays. 100 µl of each standard curve dilution was placed in triplicate in wells A1 to A8. 100µl of the diluted test samples were placed in triplicate through the rest of the plate. The plate was then covered, mixed and incubated at 37°C for 1 hour. Having washed the cells three times with wash buffer, 100 µl of diluted conjugated antibody was added to each well. This was then incubated at 37°C for 1 hour. The cells were then washed three times with wash buffer and a further five times with distilled water before adding 100µl of substrate to each well. This was then incubated at room temperature for approximately one hour. The absorbency of the individual cells was then determined using a plate reader set at 450nm using a single row of substrate only in another plate for blanking. If the top of the

standard curve was low (<1.500 OD) then the plate was re-incubated until more colour developed.

### 5.5.5. Calculation of results.

From the standard curve a linear x/y graph of absorbency against concentration was plotted. If the graph characteristics were acceptable ($r^2$ >0.95) the test absorbencies were used to determine the relative concentration of IgG or IgM EndoCAb in the test samples. The test dilutions were then corrected for the dilution and the mean result calculated.

## 5.6. RESULTS.

### 5.6.1. Study population.

We studied 26 patients having elective major surgery. Of the 26 patients 16 had evidence of gut mucosal hypoperfusion at the end of surgery (pHi <7.32). Type of surgery is shown in **table 5.1** and demographic details and duration of surgery in **table 5.2**.

***Table 5.1.*** *Type of surgery. Patients are grouped according to their pHi at the end of surgery.*

| | pHi ≥ 7.32 | pHi < 7.32 |
|---|---|---|
| Coronary artery by-pass graft | 6 | 9 |
| Heart valve replacement | 3 | 3 |
| Vascular surgery with Aortic cross-clamp | 1 | 4 |

***Table 5.2.*** *Demographic details, duration of surgery and complications. Patients are grouped according to their pHi at the end of surgery.(* Multiple organ failure).*

| | pHi<7.32 (n=16) | pHi ≥7.32 (n=10) |
|---|---|---|
| Male | 12 | 7 |
| Age (years) | 65 (67) [43-82] | 64 (67) [40-75] |
| Weight (kg) | 72 (75) [46-91] | 73(71) [48-99] |
| Height (cm) | 168 (173) [128-183] | 167 (173) [145-187] |
| Duration (mins) | 231.7 (229) [130-310] | 218.0 (195) [131-362] |
| Major complications | 8 | 0 |
| MOF* | 4 | 0 |
| Deaths | 3 | 0 |

### 5.6.2. Patient outcome.

No patient was either hypotensive or oliguric at the end of surgery. Only one of the 16 patients who developed gut mucosal ischaemia also had a metabolic acidosis.(arterial blood gas base deficit >4) Of the 16 patients who developed gut mucosal ischaemia 8 developed life threatening complications (4 multiple organ failure). Of the 4 patients who developed multiple organ failure 3 subsequently died. Ten patients maintained gut mucosal perfusion (pHi ≥7.32); none of these developed life threatening complications (**table 5.2**).

### 5.6.3 Results from blood sample analyses

For both groups there was a significant reduction in haematocrit and platelets but increase in white blood cells (**table 5.3**). However, there were no differences in base line values or magnitude of change between the two groups. Results of the IgG and IgM endotoxin core antibody assays are shown in **table 5.4** and **figures 5.1** and **5.2**. Both groups demonstrated a significant reduction in IgG and IgM endotoxin core antibodies. The group that maintained gut mucosal perfusion had significantly higher IgG endotoxin core antibody levels at base line and 24 hours when compared to the group that developed gut mucosal ischaemia and when compared to healthy

volunteers ($p$ <0.001 Fisher's exact test). Of the patients who maintained gut mucosal perfusion 80% had baseline levels of IgG endotoxin core antibody above the 90th centile of the median of 1,000 healthy volunteers. None of the patients who developed gut mucosal ischaemia had supranormal baseline levels of IgG endotoxin core antibody (**figure 5.3**).

***Table 5.3*** *Results of blood counts. Patients are grouped according to their pHi at the end of surgery. Data are shown as mean (95% confidence interval).* p< 0.005 from baseline to 24 hours within groups.*

| | pHi < 7.32 (n=16) | | pHi >7.32 (n=10) | |
|---|---|---|---|---|
| | Baseline | 24 hours | Baseline | 24 hours |
| Hct (%) | 42 | 33 | 43 | 31 |
| | (40 – 45) | (30 – 35)* | (39 – 47) | (27 – 34)* |
| Plt ($10^{12}$ /l) | 227 | 141 | 218 | 137 |
| | (204 – 251) | (114 – 168)* | (192 – 244) | (103 – 170)* |
| WBC ($10^9$ /l) | 7.2 | 9.8 | 7.1 | 9.9 |
| | (6.2 – 8.1) | (8.7 – 10.9)* | (6.22 – 8.1) | (8.8 – 11.1)* |

***Table 5.4.*** *Results of IgG and IgM endotoxin core antibodies (EndoCAb) ELISAs. Results are expressed as a percentage of the median of normal ranges for each class of antibody determined in 1,000 healthy adults. Results are shown as median(10th to 90th centile). *p ≤ 0.05 change within groups from baseline to 24h Wilcoxon signed-rank. †p < 0.05, ††p <0.005 difference between groups at baseline or 24h Mann Whitney.*

| EndoCAb | pHi < 7.32 (n=16) | | pHi >7.32 (n=10) | |
|---|---|---|---|---|
| | Baseline | 24 hours | Baseline | 24 hours |
| IgG | 150.9 | 79.7 | 447.4 | 130.5 |
| | (71.8–233.4)†† | (61.2–197.3)†* | (145.2–1223.3)†† | (76.7 – 519.9)†* |
| IgM | 89.6 | 52.1 | 103.4 | 67.2 |
| | (16.9–186.2) | (15.7–134.6)* | (42.6–254.6) | (25.1–188.3)* |

*Normal ranges of 1,000 healthy volunteers expressed as median (10th to 90th centile): IgG EndoCAb, 100 (32.7 to 240.7); IgM EndoCAb, 100 (39.7 to 263.1).*

***Figure 5.1.*** *Changes in IgG EndoCAb from base line to 24 hours. Patients are grouped according to pHi at the end of surgery. *p ≤ 0.05 change within groups from baseline to 24h Wilcoxon signed-rank. †p < 0.05, ††p <0.005 difference between groups at baseline or 24h Mann Whitney. N.B. logarithmic scale.*

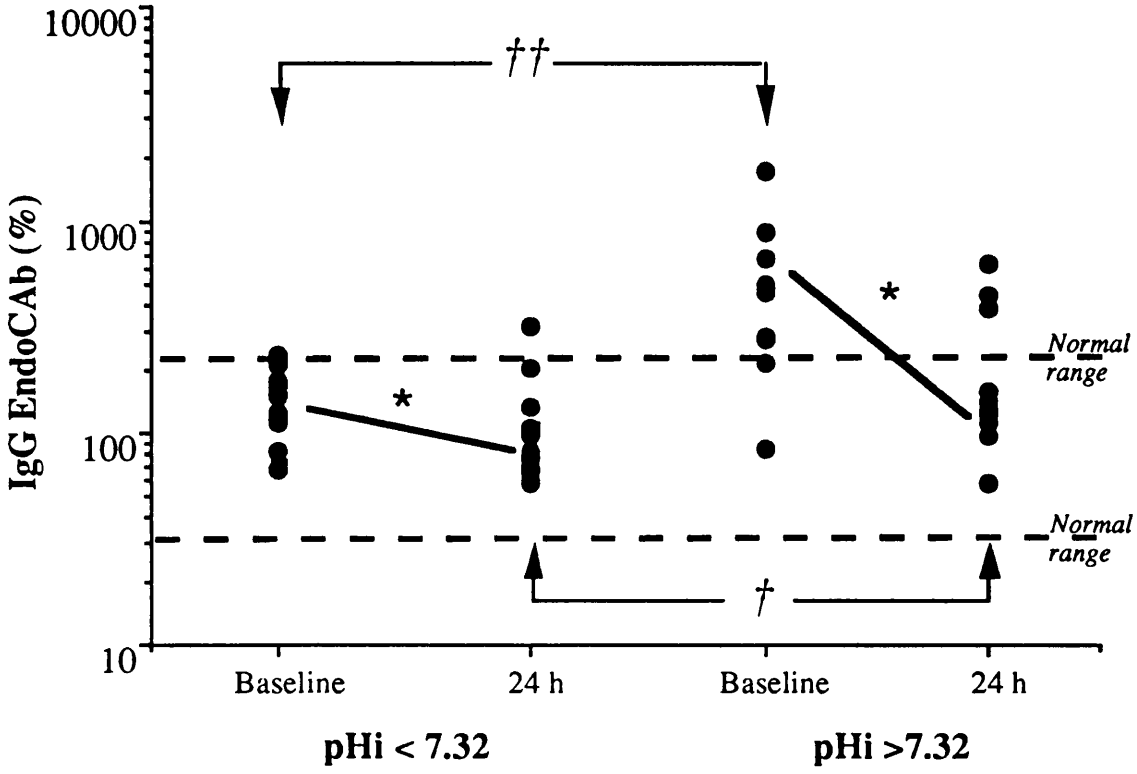
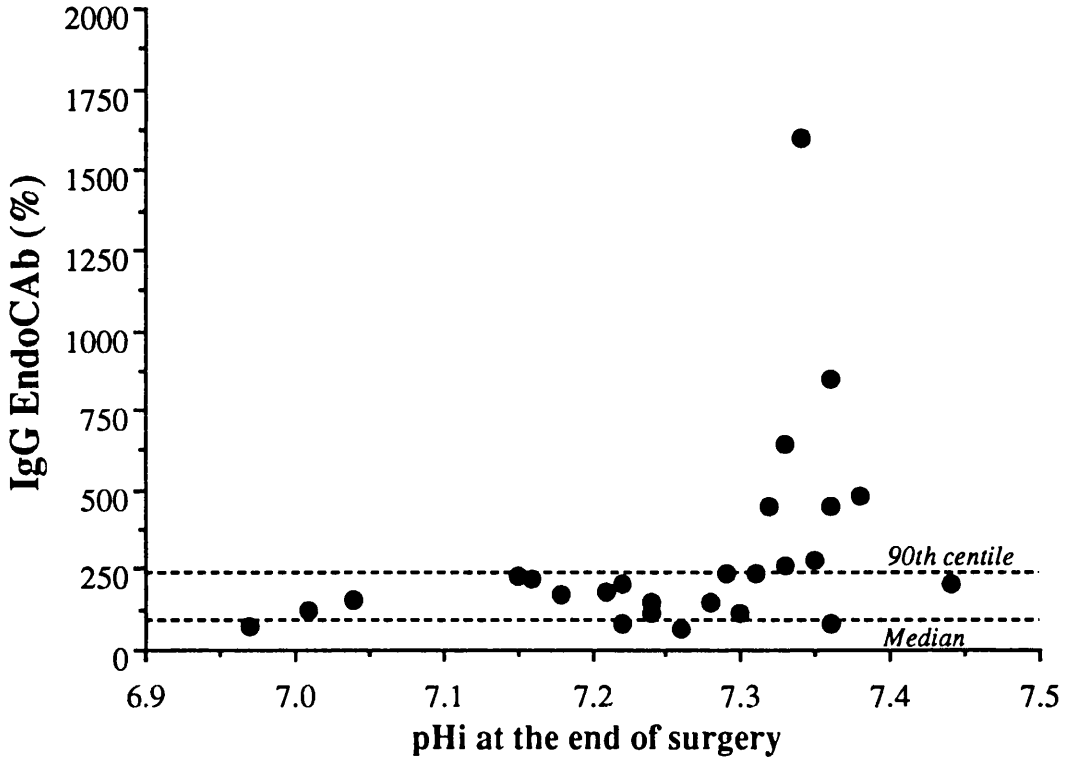


*Figure 5.2 Patients individual data for baseline plasma IgG endotoxin core antibodies (EndoCAb) and intramucosal pH at the end of surgery. A pHi of < 7.32 is an indication of gut mucosal hypoperfusion. The horizontal lines show the median and 90th centile for plasma EndoCAb in a group of 1,000 healthy volunteers.*




## 5.7. Discussion.

Exposure to "environmental" endotoxins is inevitable in the peri-operative period. Patients undergoing cardiopulmonary by-pass routinely receive endotoxin in the solutions used to prime the by-pass machine and the cardioplegic solution (Andersen, Baek, Degn et al. 1987; Rocke, Gaffin, Wells et al. 1987). Many intravenous fluids and indeed heparin, that is routinely used in major cardiovascular surgery, contain endotoxin.

Plasma levels of endotoxin are lowered by the formation of antigen-antibody complexes (Barclay 1990; Gathiram, Gaffin andWells 1986). Effective removal from the circulation is then largely dependent on phagocytosis by the reticulo-endothelial system. This function is compromised by reduced hepatic perfusion and endotoxin exposure (Ledingham and Ramsay 1989).

These data do not support the popular hypothesis that peri-operative endotoxaemia is solely associated with gut mucosal hypoperfusion, loss of gut barrier function and translocation of endotoxin from the gut lumen into the systemic circulation. Although all of the major complications and deaths occurred in the group of patients that developed gut mucosal hypoperfusion both groups of patients demonstrated significant decreases in both IgG and IgM endotoxin core antibodies. Profound or protracted endotoxaemia is necessary to deplete IgG endotoxin core antibody (GR Barclay, personal communication). This suggests that both groups of patients were exposed to significant amounts of endotoxin in the immediate peri-operative period.

The group that maintained gut mucosal perfusion had supranormal base line levels of IgG endotoxin core antibodies. These data suggest that high naturally occurring levels of endotoxin core antibodies are protective against the deleterious effects of increased endotoxin exposure in the immediate peri-operative period. Animal studies have shown that the administration of endotoxin triggers mesenteric vasoconstriction and can itself result in gut mucosal hypoperfusion (Deitch, Berg andSpecian 1987; Navaratnam, Morris, Traber et al. 1990). Human studies have shown that a single dose of 4ng/kg to healthy volunteers resulted in reduced gut barrier function (O'Dwyer, Michie, Ziegler et al. 1988). Similarly high levels of antibody may prevent the increased neutrophil degranulation and contact activation seen in this group of patients (**chapter 4.0**).

These observations are consistent with previous studies that have found that naturally occurring antiendotoxin antibodies are associated with a decrease in post-operative gram negative infection (Freeman and Gould 1985). They are also consistent with the numerous studies that have found that the prophylactic administration of both polyclonal

and monoclonal core antibodies are protective against the deleterious effects of both heterologous lipopolysaccharide and heterologous Gram negative infections in animal models (Fink 1993). This would suggest that passive immunisation with endotoxin core antibody would benefit a sub-set of patients undergoing major surgery who do not have naturally increased endotoxin core antibody levels. However, human trials of antibodies directed against endotoxin have been inconclusive.

Baumgartener et al. (1985) prophylactically administered high risk surgical patients hyperimmune polyclonal antibody serum in a double blind placebo controlled trial. Patients who received the hyperimmune plasma did not have lower rates of Gram negative infection. However, they did show a trend toward reductions in gram negative septic-shock and death rates from gram negative septic shock. More recently a large collaborative study of 352 patients has been completed that compared the effects of prophylactic administration of standard intravenous immune globulin, core polysaccharide hyperimmune globulin and human serum albumin (controls) to surgical patients at risk of infection. Compared with the control group, the standard immune globulin group had fewer infections, less pneumonia due to Gram negative bacteria and shorter stays in ITU and hospital. However, the core antibody group showed no significant difference in these or any other reported variable (The Intravenous Immunoglobulin Collaborative Study Group 1992).

Endotoxins mostly comprise of lipopolysaccharide although they also contain proteins and loosely bound lipids. Most of their biological activity resides in the lipopolysaccharide section. Lipopolysaccharide is composed of three distinct regions; the lipid A moiety, the core lipopolysaccharide region and the oligosaccharide (O)-side chains. Although the polysaccharide components are biologically active (Fink 1993), lipid A is responsible for most of the biological effects seen. Polyclonal or monoclonal antibodies directed against the O-antigenic region provide serotype specific protection against bacterial or endotoxin challenge in experimental animals (Fink 1993). However, there is such enormous diversity of O-specific serotypes that it is difficult to imagine that

specific O-antibodies could prove clinically useful. To that end attention has been focused on the core region which is nearly identical for many strains of Gram negative bacterial endotoxins. In two large multicentre trials of patients with presumed gram-negative infection, antibodies against the core region of endotoxin have been inconclusive (Greenman, Schein, Martin et al. 1991; Ziegler, Fisher, Sprung et al. 1991). More recently, the possibility of increased mortality in patients with Gram-positive infection receiving the HA-1A monoclonal antibody Centoxin has led to withdrawal of its product licence in Europe.

The subset of patients who had high naturally occurring levels of IgG endotoxin core antibody and maintained gut perfusion were a self selecting group. They had supranormal levels of IgG endotoxin core antibody (mean 538.5%) when compared to healthy volunteers (mean 100% for 1,000 volunteers). Of the ten in this group eight had baseline levels of IgG endotoxin core antibody above the 90th centile of the median of 1,000 healthy volunteers. None of the patients who developed gut mucosal ischaemia had supranormal baseline levels of IgG endotoxin core antibody. Raised or rising endotoxin core antibody levels indicate immune response activity reflecting prodromal endotoxin exposure. Endotoxin core antibody levels appear to be in homeostasis in normal healthy adults and can remain at stable levels for years if unperturbed by overt endotoxaemia (Barclay 1990). Therefore this sub set of patients had in some way been actively immunised. Certainly the patient who had the highest recorded baseline IgG endotoxin core antibody level (1,600%) had a documented Gram negative urological infection 18 months prior to his heart surgery. The degree of immunity conferred by active immunisation may have more profound effects than can be offered by passive immunisation with highly specific monoclonal anti-core antibodies in the same way that the prophylactic use of standard immune globulin was more successful than core specific hyperimmune globulin in the study cited above (The Intravenous Immunoglobulin Collaborative Study Group 1992).

## 5.8. Conclusions.

The patients who developed gut mucosal hypoperfusion did not exclusively demonstrate falls in endotoxin core anti-bodies. All patients demonstarted some reduction suggesting exposure to endotoxin was ubiquitous. Unexpectedly the patients who maintained gut mucosal perfusion had supranormal levels of endotoxin antibodies. Therefore, naturally occurring high levels of anti-endotoxin antibodies may contribute to the prevention of endotoxin induced gut mucosal ischaemia occurring during major surgery and thus reduce the incidence of multiple organ dysfunction syndrome. This is of particular interest as pre-operative immunisation may be a practical therapeutic option for patients undergoing planned major surgery.

# CHAPTER 6.0. ANAESTHESIA AND GUT MUCOSAL PERFUSION.

## 6.1. INTRODUCTION.

In the observational study (**chapter 3.0**) it was demonstrated that gastric mucosal hypoperfusion present at the end of surgery was associated with a poor outcome (Fiddian - Green, Amelin, Herrmann et al. 1986; Fiddian-Green and Baker 1987). It was also noted that the group that maintained gut mucosal perfusion had >50% increase in cardiac output over the course of surgery and that a significant number of patients (24%) had an abnormally low pHi within 1 hour of induction of anaesthesia. One suggestion was that the various anaesthetic agents, that had not been stipulated in a protocol, may have had a direct effect on splanchnic blood flow. Even if this were not the case the well documented diverse effects of the commonly used anaesthetic agents on the global circulation may have accounted for many of the changes seen (Stoelting 1991).

All of the patients reported in **chapter 3.0** had a general anaesthetic without supplementary regional blockade. The techniques used conformed to the gross classification of *balanced anaesthesia*. The patients were all premedicated with an intramuscular injection of an opioid and hyoscine. Anaesthesia was induced with a barbiturate or propofol and a bolus of an opioid. The patients were then paralysed with a non-depolarising neuromuscular blocking drug (e.g. atracurium, pancuronium, or vecuronium) and the trachea intubated and lungs ventilated with nitrous oxide in oxygen supplemented either with isoflurane, enflurane or intravenous propofol. Further opioids and neuromuscular blocking drugs were given throughout surgery, commonly by infusion. The depth of anaesthesia was always gauged by clinical signs and the amount of maintenance agent adjusted empirically without additional vapour concentration monitoring. No association was noted between type of agent used and peri-operative changes in pHi. However, this may have been because the pathogenesis of reduced splanchnic perfusion in such a non-homogeneous group of patients is complex and multifactorial. Therefore, it seemed essential at this stage, prior to attempting any

interventions aimed at modifying peri-operative pHi, to identify any causal link or association between the choice of anaesthetic agent used and changes in peri-operative pHi. The greatest degree of variation in the cocktails of anaesthetic agents that had been used lay in the choice of maintenance agents (either enflurane, isoflurane or propofol) and previous animal and human investigations implied that this was the most likely source of variation in effects on both global and regional perfusion.

Enflurane is reported to be a potent myocardial depressant and may reduce splanchnic blood flow (Vollmar and Habazettl 1993). Propofol's effects on the global circulation have been an area of much debate but on balance it would seem to cause a reduction in blood pressure by a combination of mild myocardial depression and reduced peripheral vascular resistance; its effects on splanchnic perfusion are less well understood (Coates et al. 1985; Stoelting 1991). Isoflurane has the most extensively investigated and favourable cardiovascular profile (Vollmar and Habazettl 1993). In both humans and animal models it has been shown to cause minimal myocardial depression, a reduction in systemic vascular resistance and an increase in splanchnic blood flow. There have been no reported investigations of the specific effects of these agents on gastric mucosal perfusion in humans. It was therefore decided to investigate the effects of these three agents on peri-operative pHi measurements in a prospective randomised trial.

## 6.2. HYPOTHESIS.

It was hypothesised that gut mucosal perfusion would be preserved if isoflurane rather than enflurane or propofol was used as part of a balanced anaesthetic technique during elective coronary artery by-pass surgery.

## 6.3 AIM

To study the effects of propofol, isoflurane and enflurane on the global and splanchnic circulation during elective coronary artery by-pass graft surgery utilising oesophageal Doppler measurement of descending aortic blood flow and gastric tonometry.

## 6.4. PATIENTS AND METHODS.

### 6.4.1. Patients.

Study group size was estimated with reference to the study described in **chapter 3.0** and previous studies (Fiddian - Green, Amelin, Herrmann et al. 1986; Fiddian-Green and Baker 1987). As a guideline we assumed that 60% of patients would develop a pHi < 7.32. Therefore, to have a >80% power of demonstrating a causal link between the agent used for maintenance of anaesthesia and the development of a low pHi at the 5% level we needed to study 30 patients (10 in each group) (Kirkwood 1988). Patients were studied if they were: i) having elective coronary artery by-pass graft surgery (>2 vein grafts ± internal mammary artery graft) performed by a consultant surgeon ii) being anaesthetised under the supervision of a consultant anaesthetist and iii) reported to have good left ventricular function (left ventricular ejection fraction >50% and left ventricular end-diastolic pressure <7mmHg) at routine pre-operative cardiac catheterisation. The following exclusion criteria were observed: age less than 18 years and pregnancy (for ethical reasons); coagulopathies and perforated viscus (for safety reasons); oesophageal or gastric pathology (due to uncertainty in interpretation of the tonometer results); non-pulsatile cardiopulmonary by-pass (as it has been reported as a cause of reduced splanchnic perfusion); the administration of aprotinin (as it may modify patient outcome). Following admission to the trial patients were randomised into three groups by a closed envelope system to receive either Isoflurane (group I), Enflurane (group E) or Propofol (group P) as part of a standardised balanced anaesthetic technique. All patients were given Ranitidine 150mg p.o. on the night before and on the morning of surgery to

increase the precision of the assessment of gastric pHi (Heard,Helmsmoortel,Kent et al. 1990).

### 6.4.2. Anaesthetic protocol.

Anaesthetic management was according to the following protocol: group P patients were premedicated with papaveretum 15-20mg and hyoscine 0.3-0.4mg. Prior to induction patients were given midazolam 2.5 mg as a bolus and alfentanil as a bolus of 25μg/kg, followed by an infusion of 25 μg/kg/hour. Anaesthesia was induced with a dose of propofol sufficient to obtund the eyelash reflex and the trachea intubated after administration of atracurium 0.5mg/kg. This was followed by an infusion of atracurium 0.5mg/kg/hour. The lungs were ventilated with 50% nitrous oxide in oxygen. Ventilation was adjusted to maintain $PaCO_2$ at 4.5-5.5 kPa throughout. Anaesthesia was maintained with an infusion of propofol 5-10mg /kg/hour. Depth of anaesthesia was gauged by clinical signs according to the anaesthetists standard practice. During cardiopulmonary bypass the alfentanil and atracurium infusions were continued but the propofol infusion was stopped and patients were given a single bolus of lorazepam 2mg i.v. The anaesthetist in charge of the case was not aware of the results of any non-routine measurements. If the anaesthetist in charge of the case decided that the management of the patient should differ from protocol the patient was excluded from the study and data kept for separate analysis. Group E and I patients were managed as group P patients with the following exceptions: i) for both group E and I induction of anaesthesia was with a dose of thiopentone sufficient to obtund the eyelash reflex; ii) for group E anaesthesia was maintained with enflurane 1-3%; iii) for group I anaesthesia was maintained with isoflurane 0.5-2.5%.

### 6.4.3. Cardiopulmonary by-pass.

Cardiopulmonary by-pass was standardised. After insertion of vena caval and aortic canulae, pulsatile cardiopulmonary by-pass was conducted in a standard fashion using a membrane oxygenator. The essential features of the by-pass technique used were: i) the

pump was primed with Hartmann's solution; ii) unfractionated heparin was used for anticoagulation with protamine reversal after by-pass; iii) myocardial protection was augmented with 1litre of St. Thomas' cardioplegia solution via the aortic root at the start of cardiopulmonary by pass after application of an aortic cross clamp; iv) all patients were cooled to 28°C; v) pump flow rates of approximately 2.5 l/min/$m^2$ and a mean arterial pressure of 45 to 60 mmHg were maintained. Patients were weaned from cardiopulmonary by-pass once the heart began to beat spontaneously, the core temperature was above 37°C and haemostasis was satisfactory. Duration of cardiopulmonary by-pass, duration of aortic cross-clamp time, use of vasoactive drugs and administration of i.v. fluids were recorded.

### 6.4.4. Measurement of cardiovascular variables.

Heart rate and blood pressure were measured directly via a 20g radial artery cannula 15-20 min after induction of anaesthesia, before the first surgical stimulus, and at the end of surgery following the completion of skin closure. Hypotension was defined as a mean blood pressure of less than 60 mmHg while not on by-pass. Urine flow was measured hourly. Oliguria was defined as a urine output of less than 0.5ml/kg/hour. The following non-routine techniques were used: immediately after induction of anaesthesia a tonometer (Sigmoid Tonomitor, Tonometrics Inc., Worcester, MA.) was introduced into the patient's stomach. The tonometer was used to measure pHi (see **chapter 2.0**) 15 minutes after induction of anaesthesia and again at the end of surgery, following skin closure A pHi of 7.32 was taken as the lower limit of normality (see **chapter 2.0**). Particular note was made of bicarbonate administration as this can effect the interpretation of the calculated pHi (Benjamin, Polokoff, Oropello et al. 1992). An oesophageal Doppler probe (ODM 1, Deltex, Chichester, UK) was inserted via the patient's mouth and positioned approximately 35–40 cm from the teeth where well defined aortic blood flow signals were detected(see **chapter 2.0**). Doppler estimated cardiac output and stroke volume were recorded before the first surgical stimulus at 15-

20 minutes after induction of anaesthesia and again at the same time as the final pHi measurement. Theatre personnel were blind to the results of all non-routine techniques.

### 6.4.5 Post-operative morbidity

Post-operative complications were recorded - a major complication being defined as one that resulted in an overall post-operative hospital stay of greater than 10 days or death, a minor complication was defined as an unexpected complication that did not result in a post-operative stay of greater than 10 days or death.

## 6.5. RESULTS.

### 6.5.1. Study population.

A total of 30 patients were admitted to the study, 10 in each of groups E, I and P. There were no exclusions following randomisation. No patients were given bicarbonate during the study period. There were no significant differences in demographic characteristics or duration of surgery between the three groups as shown in **table 6.1**.

***Table 6.1**. Demographic details and duration of surgery for study population. Patients are grouped according to the agent randomly selected for the maintenance of anaesthesia as part of a balanced anaesthetic protocol.*

| | Isoflurane (n=10) | Enflurane (n=10) | Propofol (n=10) |
|---|---|---|---|
| | Mean, [median] (range) | Mean, [median] (range) | Mean, [median] (range) |
| Age (years) | 63.6, [61.0] (51-74) | 63.9, [65.5] (52-79) | 62.4, [62.5] (42-82) |
| Height (cm) | 172.4, [174] (161-180) | 171.9, [172.5] (156-187) | 169.4, [172.0] (145-181) |
| Weight (kg) | 75.6, [77.0] (48-97) | 79.3, [78.5] (52-99) | 77.1, [77.0] (45.9-100) |
| Duration of surgery (minutes) | 199, [185] (130-300) | 203.0, [195.0] (180-270) | 207.6, [205.0] (160-266) |

### 6.5.2 Cardiovascular variables

The cardiovascular changes are shown in **tables 6.2, 6.3** and **6.4**. Mean heart rate, blood pressure, cardiac output, stroke volume, central venous pressure and pHi are plotted in **figure 6.2**.. Volumes of intravenous fluids given are shown in **table 6.5** None of the 30 patients were oliguric or hypotensive at the end of surgery. However, 15 of the 30 patients (50%) had evidence of gastric mucosal hypoperfusion (pHi <7.32) at the end of surgery. There were no significant differences in baseline values for mean heart rate, blood pressure, cardiac output, stroke volume, central venous pressure or pHi between the three study groups. Group I patients maintained mean pHi from baseline to end of surgery and demonstrated a significant rise in heart rate ($p$ <0.05), cardiac output ($p$ <0.05) and stroke volume ($p$ <0.05) while blood pressure and central venous pressure were unchanged. Group E and P patients had significant reductions in mean pHi from baseline to end of surgery. In group E patients this was despite a significant increase in cardiac output ($p$ <0.05) although, stroke volume and blood pressure were unchanged and heart rate and central venous pressure significantly increased ($p$ < 0.001 and 0.05 respectively). In group P patients there was a similar increase in heart rate ($p$ <0.01) but no change in cardiac output, stroke volume, blood pressure or central venous pressure.

***Table 6.2.*** *Changes in cardiovascular variables from baseline to end of surgery for patients in group I (Isoflurane; n=10). There were no statistical differences between the baseline cardiovascular variables for group I and groups E and P. *p<0.05 paired Student's t-test .*

| | Baseline<br>mean (95% conf. int.) | End of surgery<br>mean (95% conf. int.) |
|---|---|---|
| Heart rate | 63.0 (56.1 to 68.9) | 72.2 (64.1 to 80.3)* |
| Systolic BP (mmHg) | 109.6 (99.2 to 120.0) | 117.2 (104.7 to 129.7) |
| Diastolic BP (mmHg) | 61.0 (54.8 to 67.2) | 63.6 (55.1 to 72.1) |
| Mean BP (mmHg) | 75.7 (68.4 to 83.0) | 76 (65.8 to 86.2) |
| Cardiac output (l/minute) | 4.5 (3.4 to 5.5) | 6.6 (4.7 to 8.6)* |
| Cardiac index (l/minute/$m^2$) | 2.4 (1.7 to 2.9) | 3.6 (2.8 to 4.6)* |
| Stroke volume (ml) | 70.9 (55.8 to 86.0) | 93 (68.8 to 116.4)* |
| Stroke volume index (ml/$m^2$) | 38.0 (30.6 to 46.4) | 50.1 (37.6 to 62.0)* |
| CVP (mmHg) | 6.3 (4.1 to 8.5) | 6.9 (3.9 to 9.9) |
| pHi | 7.38 (7.34 to 7.43) | 7.36 (7.32 to 7.40) |
| $PCO_2$ tonometer (kPa) | 4.98 (4.2 to 5.7) | 5.50 (4.8 to 6.3) |
| pHa | 7.41 (7.39 to 7.44) | 7.41 (7.36 to 7.46) |
| $PaCO_2$ (kPa) | 4.90 (4.5 to 5.3) | 4.64 (4.04 to 5.24) |
| $PaO_2$ (kPa) | 22.6 (18.1 to 27.2) | 23.5 (14.6 to 32.4) |
| Bicarbonate (mmol) | 23.4 (21.7 to 25.0) | 21.9 (20.5 to 23.3) |
| Base deficit (mmol) | 0.6 (2.2 to -1.0) | 2.4 (4.4 to 0.4)* |
| Lactate (mmol/l) | 1.31 (0.95 to 1.67) | 1.86 (1.35 to 2.37) |

***Table 6.3.*** *Changes in cardiovascular variables from baseline to end of surgery for patients in group E (Enflurane; n=10). There were no statistical differences between the baseline cardiovascular variables for group E and groups I and P. *p<0.05, **p<0.01, ***p<0.001 paired Student's t-test.*

| | Baseline<br>mean (95% conf. int.) | End of surgery<br>mean (95% conf. int.) |
|---|---|---|
| Heart rate | 60.9 (52.2 to 69.6) | 87.4 (79.9 to 95.0)*** |
| Systolic BP (mmHg) | 119.0 (106.0 to 132.0) | 124.2 (111.6 to 136.8) |
| Diastolic BP (mmHg) | 58.4 (50.2 to 66.6) | 62.5 (53.1 to 71.9) |
| Mean BP (mmHg) | 78.3 (67.6 to 89.0) | 79.1 (70.7 to 83.4) |
| Cardiac output (l/minute) | 4.5 (3.4 to 5.5) | 6.6 (4.7 to 8.5)* |
| Cardiac index (l/minute/$m^2$) | 2.6 (2.0 to 3.2) | 3.5 (2.6 to 4.4)* |
| Stroke volume (ml) | 77.6 (59.4 to 95.8) | 76.0 (54.9 to 97.1) |
| Stroke volume index (ml/$m^2$) | 41.4 (33.8 to 50.5) | 40.1 (29.3 to 51.2) |
| CVP (mmHg) | 4.0 (2.2 to 5.8) | 7.8 (5.2 to 10.4) |
| pHi | 7.39 (7.34 to 7.44) | 7.29 (7.24 to 7.34)** |
| $PCO_2$ tonometer (kPa) | 5.0 (4.2 to 5.7) | 6.0 (5.5 to 6.7)** |
| pHa | 7.40 (7.38 to 7.42) | 7.34 (7.30 to 7.38)* |
| $PaCO_2$ (kPa) | 5.1 (4.7 to 5.5) | 5.1 (4.6 to 5.6) |
| $PaO_2$ (kPa) | 26.3 (17.8 to 34.8) | 19.9 (10.7 to 29.1) |
| Bicarbonate (mmol) | 23.3 (19.9 to 21.6) | 20.7 (19.9 to 21.6)** |
| Base deficit | 0.8 (1.9 to -0.3) | 4.3 (3.5 t0 2.4)** |
| Lactate (mmol/l) | 1.08 (0.9 to 1.3) | 1.98 (1.21 to 2.75)* |

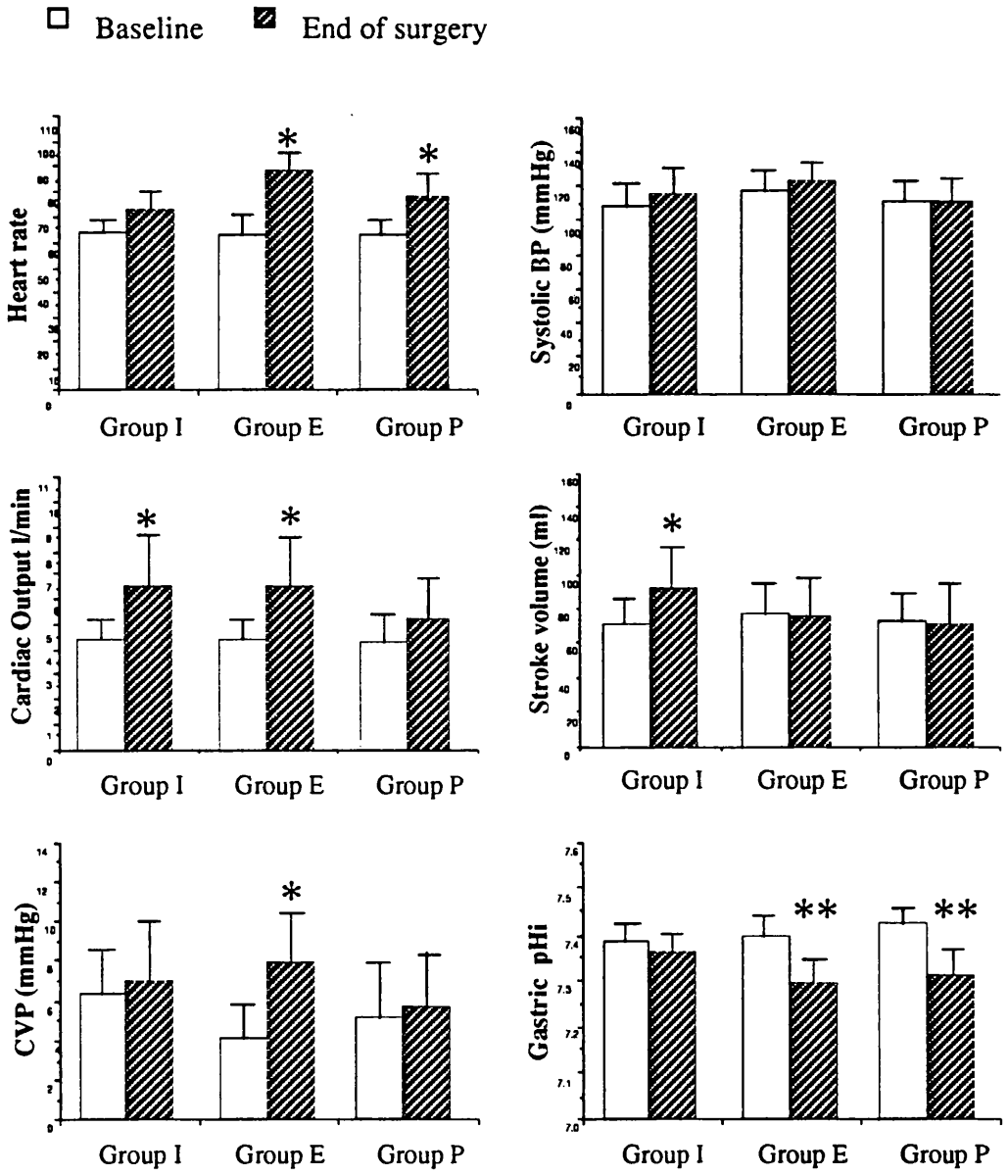
***Table 6.4.*** *Changes in cardiovascular variables from baseline to end of surgery for patients in group P (Propofol; n=10). There were no statistical differences between the baseline cardiovascular variables for group P and groups I and E. *p<0.05, **p<0.01, ***p<0.001 paired Student's t-test.*

| | Baseline mean (95% conf. int.) | End of surgery mean (95% conf. int.) |
|---|---|---|
| Heart rate | 60.5 (54.1 to 66.9) | 76.5 (66.0 to 87.0)** |
| Systolic BP (mmHg) | 111.8 (99.4 to 124.2) | 112.3 (98.9 to 125.7) |
| Diastolic BP (mmHg) | 58.4 (49.8 to 66.9) | 59.9 (52.4 to 67.3 ) |
| Mean BP (mmHg) | 75.4 (64.0 to 86.8) | 70.5 (62.9 to 78.1) |
| Cardiac output (l/minute) | 4.4 (3.3 to 5.4) | 5.3 (3.7 to 6.8) |
| Cardiac index (l/minute/$m^2$) | 2.3 (1.7 to 2.8) | 2.8 (2.0 to 3.5) |
| Stroke volume (ml) | 73.0 (55.4 to 90.6) | 72.0 (47.8 to 96.2) |
| Stroke volume index (ml/$m^2$) | 38.6 (29.9 to 47.4) | 38.3 (26.4 to 40.3) |
| CVP (mmHg) | 5.1 (2.4 to 7.8) | 5.5 (2.4 to 8.6) |
| pHi | 7.42 (7.39 to 7.46) | 7.30 (7.24 to 7.38)** |
| $PCO_2$ tonometer (kPa) | 4.3 (3.8 to 4.8) | 6.2 (5.5 to 6.9)*** |
| pHa | 7.41 (7.38 to 7.43) | 7.36 (7.32 to 7.40)* |
| $PaCO_2$ (kPa) | 5.0 (4.5 to 5.5) | 4.8 (4.4 to 5.2) |
| $PaO_2$ (kPa) | 21.9 (18.7 to 25.0) | 31.9 (17.5 to 46.3) |
| Bicarbonate (mmol) | 24.1 (22.4 to 25.9) | 22.0 (20.4 to 23.6)** |
| Base deficit | 0.0 (1.4 to -1.4) | 2.0 (4.1 to 0.2)* |
| Lactate (mmol/l) | 1.3 (0.8 to 1.7) | 1.9 (1.22 to 2.51)** |

***Table 6.5.*** *Volumes of intravenous fluids given during surgery. Patients are grouped according to the agent randomly selected for the maintenance of anaesthesia as part of a protocolised balanced anaesthetic technique. Group I (Isoflurane), Group E (Enflurane), Group P (Propofol). † p < 0.05 between groups for colloid.*

| | Crystalloid (ml) mean (95% confidence interval) | Colloid (ml) mean (95% confidence interval) |
|---|---|---|
| Group I | 1,210 (947.8 to 1472.2) | 1,370 (1055.3 to 1684.7)† |
| Group E | 1,100 (873.8 to 1,326.2) | 1,020 (866.2 to 1,173.8) |
| Group P | 970 (788.2 to 1,151.8) | 1,360 (845.0 to 1,875 .0)† |

***Figure 6.1.*** *Changes in mean heart rate, systolic blood pressure, cardiac output, stroke volume, CVP and gastric intramucosal pH for patients undergoing coronary artery by-pass graft surgery. Patients are grouped according to the agent used for the maintenance of anaesthesia as part of a standardised balanced anaesthetic technique. Group I (isoflurane; n=10), group E (enflurane; n=10), group P (propofol; n=10). *p<0.05, **p,0.001.*



### 6.5.3. Post operative morbidity

Of the 15 patients who had a low pHi at the end of surgery 8 developed minor and 3 developed major complications. The patients who maintained gut mucosal perfusion (pHi >7.32) had 4 minor and no major complications as shown in **Table 6.6**. Of the 15 patients who had a low pHi at the end of surgery 3 were from group I (30%) compared to 6 from both groups E and P (60%).

***Table 6.6.*** *Post-operative morbidity, Patients grouped according to their gastric intramucosal pH (pHi) at the end of surgery. * p<0.05.*

| | pHi <7.32 (n=15) | pHi >7.32 (n=15) |
|---|---|---|
| Minor complications | 8 | 4 |
| Major complications | 3 | 0 |
| Deaths | 0 | 0 |
| Duration of Hospital stay (days) | 8 (6-12) | 6 (5-8) * |
| Group I | 3 | 7 |
| Group E | 6 | 4 |
| Group P | 6 | 4 |

## 6.6. DISCUSSION.

In agreement with previous studies of patients undergoing coronary artery by-pass graft surgery and cardiopulmonary by-pass an abnormally low pHi (<7.32) was present in 50% of the patients studied by the end of surgery (Fiddian - Green, Amelin, Herrmann et al. 1986; Fiddian-Green and Baker 1987). A causal relationship could not be demonstrated between the choice of anaesthetic agent used for maintenance of anaesthesia and the maintenance of a normal gastric intramucosal pH. However, gastric intramucosal pH was better maintained when isoflurane rather than enflurane or propofol was used as part of a standardised anaesthetic technique.

It was decide to study patients undergoing routine coronary artery by-pass graft surgery with good left ventricular function. This was the largest homogenous group of patients

available that previous work on peri-operative pHi measurements had been reported on (Fiddian-Green 1990; Fiddian-Green and Baker 1987; Kuttila, Niinikoski andHaglund 1991; Landow, Phillips, Heard et al. 1991). This group of patients had numerous advantages from a standardisation point of view. They constituted a fairly uniform demographic group who were otherwise completely fit and well. Cardiac function was estimated at the time of cardiac catheterisation as a part of their routine preparation for surgery. There were only two surgeons and three anaesthetists involved with their peri-operative care and the techniques specified in the protocol were familiar to their routine practices. All patients were managed post-operatively on the same intensive care unit and hospital ward where management protocols were already in operation and there was a stable and experienced group of nurses and resident doctors. The main disadvantage in studying this group of patients was that cardiopulmonary by-pass was used to facilitate surgery and this is thought to result in an inevitable reduction in splanchnic blood flow (Taylor 1986). However, this has mainly been reported with non-pulsatile pump flow and here pulsatile flow was already in routine use. Otherwise the by-pass technique and equipment were standardised so any effects of cardiopulmonary by-pass on pHi should have been common to all study patients.

The mean global cardiovascular changes were similar to those reported previously for enflurane, isoflurane and propofol (Coates et al. 1985; Vollmar and Habazettl 1993; Stoelting 1991). There is little or no human data to draw comparisons with regarding the changes observed in pHi as an index of splanchnic perfusion or indeed other indices of tissue perfusion However, certain parallels can be drawn with previous human and animal studies. Enflurane is the most commonly used volatile anaesthetic in the United Kingdom (Association of Anaesthetists 1988) yet, of the available agents, it causes the greatest dose related decrease in cardiac output (Calvery, Smith and Prys-Roberts 1989). Systemic arterial pressure is better maintained with enflurane as it has little effect on systemic vascular resistance and this may mask hypovolaemia. In keeping with this group E patients demonstrated a small increase in cardiac output yet pHi, arterial pH and base excess fell and plasma lactate rose. The cardiac output increase seen was secondary

to a significant increase in heart rate with no change in stroke volume or blood pressure but a rise in central venous pressure. These are all cardinal signs of hypovolaemia (Baek, Makabali, Byron-Brown et al. 1975; Weil, Shubin andRosoff 1965) and it is of particular note that this group received significantly less colloid than the other two groups **(table 6.5)**.

Coates et al. (1985) found that when propofol was used to induce anaesthesia in patients undergoing elective surgery systemic vascular resistance (SVR) decreased initially by 18-22%. However, when propofol was given as an infusion to the same patients for the maintenance of anaesthesia SVR increased significantly and cardiac output fell by 17.5-31% depending on the rate of infusion Here the Group P patients received significantly more colloid than group E patients, yet pHi fell despite cardiac output, stroke volume, blood pressure and central venous pressure all being unchanged. This may have been as a result of a combination of decreased systemic vascular resistance and a degree of myocardial depression or incomplete correction of hypovolaemia.

Isoflurane causes a dose related reduction in systemic arterial pressure by a reduction in systemic vascular resistance whilst cardiac output is unaffected (Eger 1981; Stoelting 1991). Isoflurane decreases resistance and maintains flow in gut, kidney, heart, brain, skin, and muscle (Eger 1981; Gelman, Fowler and Smith 1983; Stoelting 1991; Vollmar and Habazettl 1993). In studies on cirrhotic rats isoflurane has been shown to preserve splanchnic circulation more efficiently than ketamine, halothane or enflurane (Debaene, Goldfarb, Braillon et al. 1990). In support of the findings in animal models the patients who received Isoflurane had the lowest incidence of gut hypoperfusion (30%) compared to those who received either Enflurane (60%) or Propofol (60%). The mean cardiovascular data for the group I patients demonstrates a significant increase in cardiac output and stroke volume over the course of surgery and maintenance of pHi. They also received significantly more colloid than group E patients. In agreement with the observations reported in **chapter 3.0** the group that maintained their pHi during surgery demonstrated an increase in cardiac output and stroke volume which further supports the

hypothesis that greater than normal oxygen delivery is required to satisfy total oxygen requirements with the additional insult of surgery (Krauss, Verdauw, Hugenholtz et al. 1975; Shoemaker, Appel, Waxman et al. 1982; Bland, Shoemaker, Abraham et al. 1985; Shoemaker 1986; Schumacker and Cain 1987; Shoemaker 1987; Shoemaker, Appel, Kram et al. 1988; Shoemaker 1991; Boyd, Grounds and Bennett 1993). However, the differences in colloid volumes given to the three groups adds further circumstantial support to the hypothesis that an increase in cardiac output without maintenance of circulating blood volume is not in itself enough to maintain gastric mucosal perfusion.

As suspected a causal relationship was not demonstrated between the choice of anaesthetic agent used and the maintenance of gastric mucosal perfusion, or indeed any of the variables studied. This is again probably because, despite rigorous attempts to standardise things, there are too many uncontrollable features that bear influence on tissue perfusion. One of the obvious limitations in the interpretation of these results was the use of more than one anaesthetist and not fixing the doses of study drugs. The study was designed to try and mimic the every day usage of such agents where clinical signs are used to assess depth of anaesthesia i.e. the drugs were not given at a fixed dose. Therefore, as heart rate and blood pressure are the main indices of both awareness and, in the absence of the routine measurement of cardiac output, cardiovascular function, the anaesthetists perception of the drugs cardiovascular effects are of paramount importance. It became apparent over the course of the study that the anaesthetists involved tended to react as follows: with Isoflurane, as it is believed to be minimally myocardially depressant but a potent vasodilator (Eger 1981; Gelman, Fowler and Smith 1983; Vollmar and Habazettl 1993), a fall in blood pressure was interpreted as hypovolaemia and more fluid was given; with Enflurane, although it is believed to be a myocardial depressant , reassurance was taken by the maintenance of blood pressure and central venous pressure. When blood pressure and heart rate went up this was interpreted as increased patient awareness and the Enflurane dose was increased. The cardiovascular changes may have been reflex responses to hypovolaemia but SVR is well preserved with Enflurane (Calvery, Smith and Prys-Roberts 1989); with Propofol, which is

thought to decrease vascular resistance and also to be a potent myocardial depressant (Coates, Prys-Roberts, Spelina et al. 1985), some fluid was given when the blood pressure fell but the dose of propofol was also reduced and the blood pressure restored before the circulating volume. Therefore, the failure to use a single anaesthetist and a more rigorous fluid management protocol makes it impossible to interpret whether one drug actually has a more favourable effect on the global and splanchnic circulation than another. It may just be that by using Isoflurane the anaesthetist is more likely to diagnose and treat hypovolaemia.

## 6.7. CONCLUSION.

Gut mucosal hypoperfusion was found in 50% of the patients undergoing coronary artery by-pass graft surgery in this study. In agreement with previous studies the tonometer was found to be a sensitive predictor of post-operative complications. Gut mucosal hypoperfusion was less likely to occur in those patients who received Isoflurane rather than Enflurane or Propofol for maintenance of anaesthesia as part of a balanced anaesthetic technique.

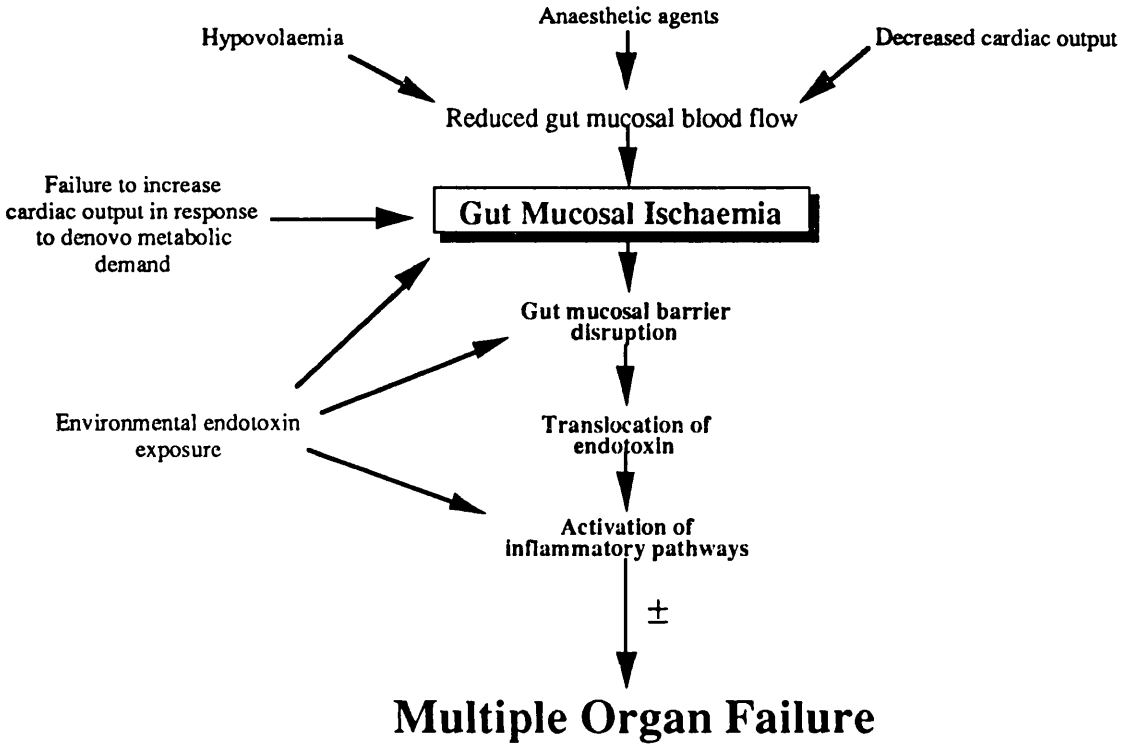
# CHAPTER 7.0. PERI-OPERATIVE GUT MUCOSAL HYPOPERFUSION AND PLASMA VOLUME EXPANSION.

## 7.1. INTRODUCTION.

### 7.1.1. Overview of previous chapters.

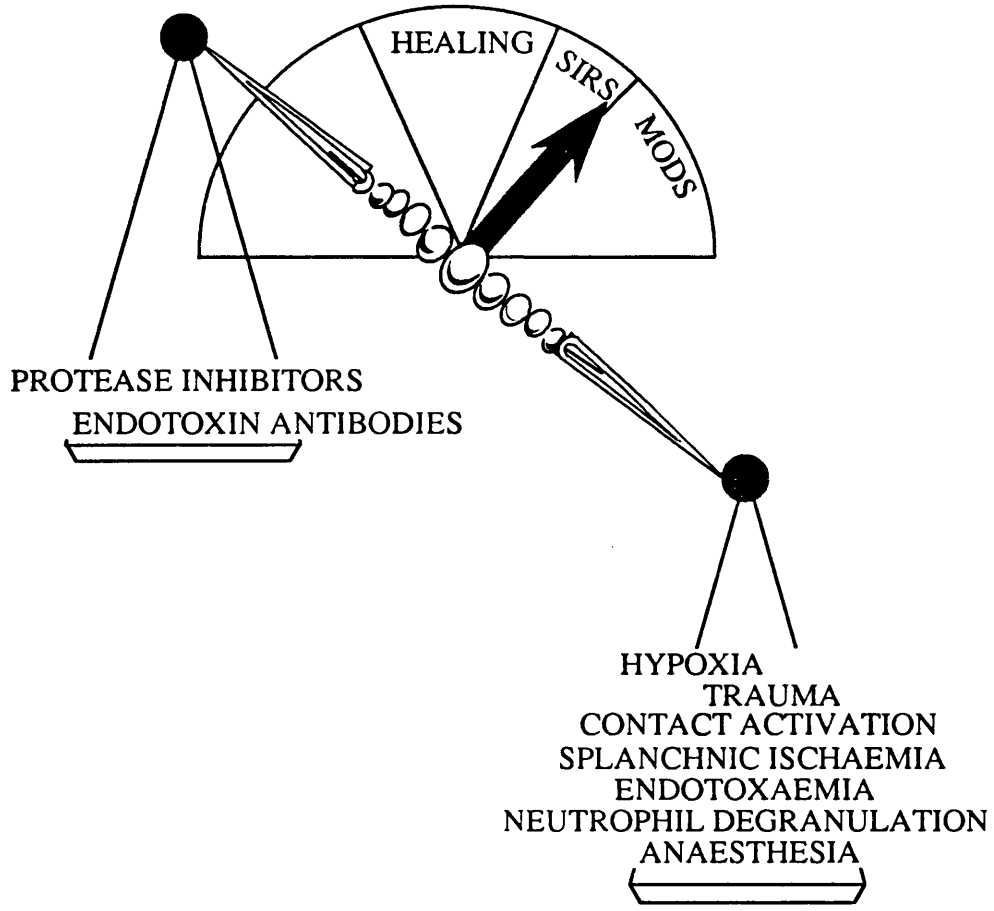
In previous chapters it has been shown that an abnormally low pHi occurs commonly during cardiovascular surgery and is associated with a poor outcome (**chapters 3.0, 4.0, 5.0, 6.0**). In **chapter 3.0** it was also noted that none of the commonly measured cardiovascular variables were predictive either of gut mucosal hypoperfusion or subsequent outcome. In **chapter 4.0** an association was reported between gut mucosal hypoperfusion, increased neutrophil degranulation, contact activation with depletion of C1-esterase inhibitor and a poor outcome. In **chapter 5.0** a high naturally occurring level of endotoxin antibodies was found to be associated with the maintenance of gastric mucosal perfusion and a favourable outcome. Finally, in **chapter 6.0** it was demonstrated that the choice of anaesthetic agents used for the maintenance of anaesthesia may have a bearing on the maintenance of splanchnic perfusion. Although, as one might expect, no causal link has been demonstrated between any individual factor and the development of post operative organ dysfunction a version of the pathogenesis flow diagram shown in **chapter 1.0** (**figure 1.3**) might be re-drawn as below (**figure 7.1**).

***Figure 7.1.*** *Revised flow diagram of the role of gut mucosal hypoperfusion in the pathogenesis of multiple organ failure.*




Similarly the diagram of the balance between modulation and activation of inflammatory pathways (**figure 1.2**) may be modified as below (**figure 7.2**).

*Figure 7.2. Revised diagram of the balance between activation and modulation of inflammatory pathways in the pathogenesis of post-operative organ dysfunction.*




### 7.1.2. Gut mucosal hypoperfusion and post-operative outcome.

The pathogenesis of post-operative dysfunction is undoubtedly complex and multi-factorial and there seems no doubt that whatever variable facet of the peri-operative pathophysiology and management were examined it would reveal yet another potentially compounding factor. However, accepting the fact that the presence of a low pHi may just be a marker of tissue hypoperfusion and that the gut may not be the motor of MODS, one recurring feature exists in the studies reported in this thesis and by all other investigators to date. That is, that the presence of a low pHi is an extremely sensitive indicator of a poor outcome following major surgery. If this is the case then one would anticipate that avoiding the development of a low pHi should be associated with a favourable outcome.

There are numerous reasons that this should be so. Not only would the potentially pro-inflammatory insults of splanchnic hypoperfusion and translocation of endotoxin be avoided but as the gut mucosa is such a low priority organ in stressed states then the maintenance of gut mucosal blood flow would be highly suggestive of normoxia in all tissues. Also, as stressed in the previous chapters, it is most likely that host response to the trauma of surgery is highly variable due to enormous population variability in such things as endotoxin immunity. The splanchnic bed and in particular the liver is responsible for many of our natural defences and the generation of acute phase proteins such as C-reactive protein, Lipopolysaccharide binding protein and protease inhibitors such as C1-esterase inhibitor (Wardle 1993). Therefore, it seems appealing that the maintenance of splanchnic perfusion would leave the host far better equipped to handle the insult of surgery even if they are not primed with high levels of specific endogenous antibodies such as those to endotoxin reported in **chapter 6.0.** In that case what can be done to avoid peri-operative gut mucosal hypoperfusion.

### 7.1.3. Effects of increasing global oxygen delivery on gut mucosal perfusion.

As discussed in **chapter 1.0** and **chapter 3.0** in established organ failure there does not seem to be any relationship between global oxygen flow variables and pHi (Gutierrez, Bismar, Dantzker et al. 1992; Marik 1993) and in peri-operative patients a falling pHi has not been associated with a reduction in cardiac output or global oxygen delivery (Anderson, Landow, Baek et al. 1993; Kuttila, Niinikoski and Haglund 1991; Landow, Phillips, Heard et al. 1991). However, in both Shoemaker's (1988) and Boyd's (1993) studies , also mentioned above, a prophylactic increase in patients' oxygen delivery to supranormal levels with the aim of avoiding a tissue oxygen debt produced a reduction in post-operative organ failure and mortality. If an abnormal pHi is as a result of a mismatch between splanchnic oxygen delivery and consumption then prophylactically increasing global oxygen delivery would seem a rational step in avoiding

gut mucosal hypoperfusion. However, as pHi measurements were not made in either study we do not know if there was any effect on this variable.

### 7.1.4. The effects of increased fluid administration.

In Shoemaker's (1988) study the majority of patients achieved their supranormal goals with fluid administration alone. In the Gutierrez (1992) study the commonest therapeutic intervention that restored a falling pHi was the administration of fluid. Hypovolaemia is a far more potent cause of gut mucosal hypoperfusion than other causes of a reduced oxygen delivery such as anaemia or hypoxia (Gilmour, Aitkenhead, Hothersall et al. 1980; Grum, Fiddian-Green, Pittenger et al. 1984). The effects of peroperative plasma volume expansion with the aim of avoiding gut mucosal hypoperfusion has not been tested though.

### 7.1.5. The effects of inotropes.

The effect of inotropes on pHi, at least in ITU patients, seems to be unpredictable. Silverman and Tuma (Silverman and Tuma 1992) found that increasing global oxygen delivery with dobutamine corrected pHi in established ITU patients. Price et al. have reported similar results (Price, Clark and Guttierrez 1992). However, the same group have also reported a normal pHi becoming abnormal when dobutamine was infused into a patient with sepsis who subsequently died (Gutierrez 1991). Maynard et al. found that dopexamine but not dopamine corrected pHi in septic ITU patients (Maynard, Smithies, Mason et al. 1992). Yet, Bennett claims that dopamine up to 2.5 μg/kg/min increases pHi and further increases in dosage reduces it again (personal communication). There are only anecdotal reports of the effects of other inotropic agents on pHi. Dopexamine would seem to have the most favourable effects on splanchnic blood flow in animal models but its effect on pHi in the peri-operative period has not been systematically assessed in human studies. It is, however, interesting to note that in Boyd's study dopexamine was the inotrope used to achieve the supranormal oxygen flow variables in patients undergoing major surgery (Boyd, Grounds and Bennett 1993).

### 7.1.6. Manoeuvres only tested in animals

Angiotensin II is a potent splanchnic vaso-constrictor and released in increased quantities during surgery that is associated with a high incidence of gut mucosal hypoperfusion (Taylor 1986). Therefore, it might be reasonable to expect that an angiotensin converting enzyme (ACE) inhibitor would be effective in treating or avoiding gut mucosal hypoperfusion. Animal studies support this hypothesis (Porter, Sussman and Bulkley 1989) but no human studies have been reported. Again in animal studies, and unsubstantiated in any human studies, non-steroidal anti-inflammatory drugs and free-radical scavengers have modified the incidence of gut mucosal hypoperfusion and/or modified the extent of histological damage (Fink 1991; Fink, Rothschild, Deniz et al. 1989).

### 7.1.7 Protease inhibitors and endotoxin anti-bodies

The results of **chapters 5.0** and **6.0** would suggest that the administration of clinical concentrates of protease inhibitors or the bolstering of endotoxin immunity might reduce the damage resulting from gut mucosal perfusion or even prevent it. However, considering the level of morbidity and mortality associated with most major elective surgical procedures and the cost of such agents many would argue that a sledge hammer was being used to crack a nut. Certainly with regard to the protease inhibitors one would probably be involved in a damage limitation exercise whereas it should be more effective just to avoid the tissue hypoperfusion in the first place.

### 7.1.8. The choice of anaesthetic technique

From what was said in **chapter 6.0** a balanced anaesthetic technique using Isoflurane as the maintenance agent is most likely to result in maintenance of splanchnic blood flow provided that hypovolaemia is avoided.

### 7.1.9. A treatment regimen aimed at reducing the incidence of peri-operative gut mucosal hypoperfusion.

On balance it would seem that gut mucosal hypoperfusion should be avoidable in the peri-operative period simple by avoiding hypovolaemia if the patients myocardial function is sufficient to meet the de-novo metabolic demands of surgery.

In **chapter 3.0** it was demonstrated that of the 51 patients studied the group that developed a low pHi (<7.32, n=32) by the end of surgery demonstrated no significant change in mean blood pressure or cardiac output from baseline to the end of surgery. However, there was a significant rise in heart rate and central venous pressure and a fall in stroke volume. The group that maintained gut mucosal perfusion (pHi >7.32, n=19) demonstrated no significant change in blood pressure or heart rate over the same time course but a highly significant increase in both cardiac output and stroke volume. These results were consistent with the hypothesis that hypovolaemia was a potential cause of the gut mucosal hypoperfusion observed. Hypovolaemia occurs commonly during any type of surgery and is a potent cause of splanchnic hypoperfusion. The routinely measured global cardiovascular variables such as blood pressure and cardiac output are unreliable indicators of hypovolaemia (Price, Deutsch, Marshall et al. 1966). The immediate response to a reduction in circulating volume is redirection of blood away from the splanchnic bed in favour of the more *vital* organs (Price, Deutsch, Marshall et al. 1966). The measurement of a normal central venous pressure or even pulmonary artery wedge pressure will not exclude hypovolemia unless the response to a fluid challenge is considered (Baek, Makabali, Byron-Brown et al. 1975; Weil, Shubin and Rosoff 1965). The venous system constricts in response to increased sympathetic tone in a similar fashion to the arterial system. Of high risk post-operative patients with raised central venous pressure, more than half had a decrease in central venous pressure and pulmonary artery wedge pressure in response to volume loading (Baek, Makabali, Byron-Brown et al. 1975). Pulmonary and total vascular resistance's fell and cardiac output increased.

## 7.2. HYPOTHESIS.

It was hypothesised that per-operative plasma volume expansion with colloid would maintain gastro-intestinal mucosal pH and improve outcome following elective cardiac surgery.

## 7.3. AIM.

To examine the effects of per-operative plasma volume expansion with 6% hydroxyethyl starch (ELO-HES, Oxford Nutrition, Oxford.) on the maintenance of gastro-intestinal mucosal pH during elective cardiac surgery.

## 7.4. PATIENTS AND METHODS.

### 7.4.1. Study population.

With reference to previous studies (**chapters 3.0–6.0**; Fiddian-Green and Baker 1987) the study population size required to give a power of 90% was calculated (Kirkwood 1988). As a guide it was assumed that the incidence of a low pHi could be reduced from 50% in the control group to 10% in the protocol group. Patients were studied if they were scheduled for elective coronary artery by-pass grafts or single heart valve replacement and had a pre-operative left ventricular ejection fraction estimated at the time of cardiac catheterisation of $\geq$ 50% and had been graded by the anaesthetist in charge of the case as American Society of Anaesthesiologists grade III (Owens, Felts and Spitznagel 1978). The following exclusion criteria were observed: age less than 18 years or pregnancy (for ethical reasons); coagulopathies or perforated viscus (for safety reasons); oesophageal or gastric pathology (due to uncertainty in interpretation of the tonometer results); non-pulsatile cardiopulmonary by-pass (as it has been reported as a cause of reduced splanchnic perfusion); the administration of aprotinin (as it may modify both splanchnic perfusion and patient outcome); previous cardiac surgery or pre-existing respiratory, hepatic or renal disease (as these are recognised factors for increasing the risk of post-operative morbidity). Written consent was obtained from patients who met the eligibility criteria. Patients were then randomised according to the contents of a sealed envelope to either a control group or protocol group.

### 7.4.2. Anaesthetic protocol.

All patients were given Ranitidine 150mg p.o. on the night before and on the morning of surgery to increase the precision of the assessment of gastric pHi (Heard, Helmsmoortel, Kent et al. 1990). On the morning of surgery all patients received papaveretum 15-20 mg and hyoscine 0.3-0.4 mg. After cannulation of the radial artery patients were anaesthetised and the trachea intubated using a combination of midazolam 2.5 mg, alfentanil 25μg/kg, thiopentone sufficient to obtund the eyelash reflex and atracurium 0.5mg/kg. This was followed by an infusion of alfentanil 25 μg/kg/h and atracurium 0.5mg/kg/h. The lungs were ventilated with 50% nitrous oxide in oxygen. Ventilation was adjusted to maintain $PaCO_2$ at 4.5-5.5 kPa throughout. Anaesthesia was maintained with Isoflurane. Depth of anaesthesia was gauged by clinical signs according to the anaesthetists' standard practice. During cardiopulmonary by-pass the alfentanil and atracurium infusions were continued but the nitrous oxide and volatile anaesthetic agents were stopped and patients were given a single bolus of lorazepam 2mg i.v. Depth of anaesthesia was maintained at a constant level as judged by standard clinical criteria until the last set of study variables had been recorded.

### 7.4.3. Measurement of cardiovascular variables.

A tonometer (Sigmoid Tonomitor, Tonometrics Inc., Worcester, MA.) was introduced into the patient's stomach having been sutured to a fine-bore nasogastric tube with catgut. Correct placement was confirmed by the injection of air down the nasogastric tube while auscultating over the epigastrium. An oesophageal Doppler probe (ODM 1, Deltex, Chichester, UK) was inserted via the patient's mouth and positioned approximately 35-40cm from the teeth where well defined aortic blood flow signals were detected. Approximately 15 minutes after induction of anaesthesia, before the first surgical stimulus, a set of baseline cardiovascular variables were recorded. Heart rate and blood pressure were measured directly via a 20g radial artery cannula and central venous pressure directly via a 14g internal jugular catheter. Urine flow was measured via a bladder catheter. The pHi was calculated from the values of gastric tonometer $PCO_2$

and the arterial bicarbonate determined by arterial blood gas analysis (see **chapter 2.0**). A pHi of 7.32 was taken as the lower limit of normality. The same arterial blood gas sample was used for measuring haemoglobin concentration and arterial oxygen saturation ($SaO_2$) and lactate concentration. An estimate of Doppler cardiac output (CO) and stroke volume were recorded. An estimate of oxygen delivery ($DO_2$) was calculated (Eq. 1)

$$DO_2 \text{ (ml/min)} = CO \text{ (dl/min)} \times Hb \text{ (g/dl)} \times 1.34 \left(\frac{SaO_2 \text{ (\%)}}{100}\right) \qquad (1)$$

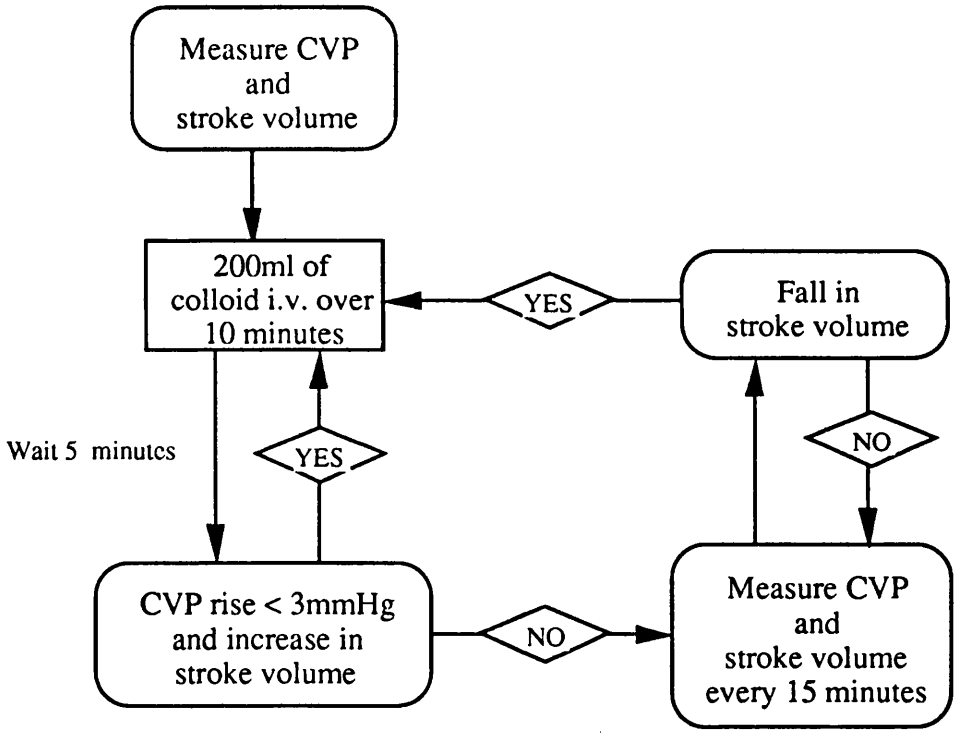
cardiac output, stroke volume and $DO_2$ were indexed to body surface area. Theatre personnel involved with the care of control group patients were blind to the measurements obtained from the tonometer and oesophageal Doppler.

At the end of surgery, after the insertion of the last skin suture and before the level of anaesthesia changed all of the cardiovascular measurements were repeated and urine flow was recorded. The duration of surgery was recorded. Following surgery all patients were transferred intubated sedated and ventilated to the intensive care unit where they were managed according to a routine post-operative protocol.

### 7.4.4 Fluid management protocol

The protocol group received, in addition, 200 ml boluses of 6% hydroxyethyl starch (ELO-HES, Oxford Nutrition, Oxford.) to obtain a maximum stroke volume and a rise in central venous pressure of > 3 mmHg (**figure 7.3**). This procedure was repeated every 15 minutes until the end of surgery except when the patient was on cardiopulmonary by-pass.

***Figure 7.3.*** *Fluid challenge algorithm used for protocol group.*




### 7.4.5. Cardiopulmonary by-pass.

After insertion of vena caval and aortic canulae, pulsatile cardiopulmonary by-pass was conducted in a standard fashion using a membrane oxygenator. The essential features of the by-pass technique used were: i) the pump was primed with Hartmann's solution; ii) unfractionated heparin was used for anticoagulation with protamine reversal post by-pass; iii) cardiac stand-still was either by administration of cardioplegic solution or defibrillation and iv) pump flow rates of approximately 2.5 l/min/$m^2$ and a mean arterial

pressure of 45 to 60 mmHg were maintained. Patients were weaned from cardiopulmonary by-pass once the heart began to beat spontaneously, the core temperature was above 37°C and haemostasis was satisfactory. Duration of cardiopulmonary by-pass, duration of aortic cross-clamp time, use of vasoactive drugs and administration of i.v. fluids were recorded. During cardiopulmonary by-pass anaesthesia was maintained with an opioid-lorazepam-atracurium technique as described above. After patients were successfully weaned from by-pass the same anaesthetic technique as pre-bypass was employed.

### 7.4.6. Post operative morbidity.

Post-operative complications were recorded – a minor complication being defined as anything unexpected that did not result in a hospital stay of greater than 10 days and a major complication being defined as one that resulted in an overall post-operative hospital stay of greater than 10 days or death. Organ failure was determined according to the criteria proposed by Knaus and Wagner (1985; see **table 3.2**). Post-operative nausea and/or vomiting was only regarded as unexpected if it persisted for greater than 3 days after surgery.

## 7.5. RESULTS.

### 7.5.1. Study population.

There were no significant differences between control and protocol groups in demographic characteristics, duration of surgery, cardiopulmonary by-pass or aortic cross clamp time (**table 7.1**). There were no differences between control and protocol groups in distribution of surgical procedures (**table 7.2**).

***Table 7.1*** *Demographic details of study population. Duration of surgery, cardiopulmonary by-pass (CPB) and aortic cross clamp times.*

| | Control (n=30) mean (median) [range] | Protocol (n=30) mean (median) [range] |
|---|---|---|
| Age (years) | 64 (64) [44-86] | 63 (63) [42-89] |
| Height (cm) | 171.0 (175.0) [143-190] | 169.8 (169.5) [148-187] |
| Weight (kg) | 73.8 (75.0) [50-93] | 79.4 (76.0) [58-118] |
| Duration of surgery (min) | 224.3 (225.0) [150-320] | 215.9 (210.0) [150-330] |
| CPB (min) | 76.1 (73.0) [37-155] | 74.0 (70.0) [46-127] |
| Aortic cross clamp (min) | 41.2 (41.0) [29-72] | 42.9 (40.0 [26-76] |

***Table 7.2*** *Distribution of surgical procedures.*

| | Control (n) | Protocol (n) |
|---|---|---|
| Coronary artery bypass grafts | 25 | 23 |
| Aortic valve replacement | 2 | 4 |
| Mitral valve replacement | 3 | 3 |

### 7.5.2. Drugs and fluids given.

Intravenous fluids administered in the operating theatre are shown in **table 7.3** and vasoactive drugs in **table 7.4**. The protocol group received significantly more colloid before cardiopulmonary by-pass ($p<0.001$) and in total ($p<0.001$) than the control group. However, there was no difference between the two groups in the amount of colloid given in theatre after cardiopulmonary by-pass. The control group received significantly more crystalloid in total than the protocol group ($p<0.05$).

***Table 7.3*** *Intravenous fluids given per-operatively. * p<0.05, **p<0.01, ***p<0.001 un-paired Student's t-test.*

| Intravenous fluids given per-operatively | Control (n=30) mean (95% CI) | Protocol (n=30) mean (95% CI) |
|---|---|---|
| Crystalloid pre-CPB (ml) | 800 (682–918) | 810 (693–927) |
| Crystalloid post-CPB (ml) | 293 (119–467) | 47 (10–104)** |
| Crystalloid TOTAL (ml) | 1093 (945–1241) | 857 (685–968)* |
| Colloid pre-CPB (ml) | 250 (137–363) | 898 (744–1,053)*** |
| Colloid post-CPB (ml) | 478 (328–628) | 468 (358–579) |
| Colloid TOTAL (ml) | 728 (547–909) | 1,367 (1,204–1,529)*** |
| Blood TOTAL (ml) | 211 (48–375) | 227 (94–360) |

***Table 7.4.*** *Number of patients given non-anaesthetic drugs per-operatively. † p<0.05 Chi-square.*

| Drug (reason for use) | Control (n=30) | Protocol (n=30) |
|---|---|---|
| Dopamine (oliguria on CPB) | 6 | 1† |
| Frusemide (oliguria on CPB) | 6 | 2† |
| Phenylepherine (hypotension on CPB) | 7 | 1† |
| Isoprenaline (hypotension/bradycardia) | 1 | 1 |
| Adrenaline (hypotension) | 1 | 0 |
| Bicarbonate (metabolic acidosis) | 2 | 0 |

### 7.5.3. Cardiovascular variables.

The cardiovascular variables recorded at baseline and at the end of surgery for the control and protocol groups are shown in **tables 7.5 and 7.6.** There were no differences in any baseline cardiovascular variables between the two groups. At the end of surgery one patient in the protocol group was hypotensive (mean blood pressure 58mmHg) and made an uneventful recovery. No patient was oliguric at the end of surgery and there was no significant difference in urine flow between the two groups.

*Table 7.5 Cardiovascular variables at baseline (after induction of anaesthesia) and end of surgery for control group patients (n=30).* p<0.5, **p< 0.01, ***p<0.001 baseline to end of surgery within control group patients. † p<0.5, ††p< 0.01, †††p<0.001 between control and protocol groups at baseline or end of surgery.*

| | Baseline<br>Mean (95% CI) | End of surgery<br>Mean (95% CI) |
|---|---|---|
| Heart rate | 63.7 (58.6 -68.8) | 83.7 (76.7 - 90.6)*** |
| Systolic BP (mmHg) | 112.1 (105.7 - 118.6) | 112.7 (106.6 -118.8) |
| Diastolic BP (mmHg) | 61.4 (56.4 - 66.3) | 60.0 (56.3 - 63.7) |
| Mean BP (mmHg) | 78.0 (73.2 - 82.8) | 73.9 (69.2 - 78.6) |
| CVP (mmHg) | 6.1 (4.8 - 7.5) | 7.3 (5.94 - 8.6)† |
| Cardiac output (l/min) | 4.7 (4.2 - 5.3) | 5.6 (4.9 - 6.2)*† |
| Stroke volume (ml) | 75.5 (67.2 - 83.9) | 69.6 (61.0 - 78.2)†† |
| Cardiac index (l/min/$m^2$) | 2.5 (2.2 - 2.8) | 3.0 (2.6 - 3.3) * |
| $DO_2$ (ml/min) | 738.5 (646.3 - 830.7) | 681.7 (591.0 - 772.5) |
| $DO_2$ index (ml/min/$m^2$) | 390.1 (340.7 - 439.5) | 358.5 (311.9 - 405.1) |
| Tonometer $CO_2$ (kPa) | 4.99 (4.62 - 5.38) | 5.96 (5.60 - 6.32) ***††† |
| pHi | 7.42 (7.40 - 7.45) | 7.29 (7.26 - 7.32)***††† |
| Arterial pH | 7.40 (7.39 - 7.41) | 7.33 (7.31 - 7.35) ***††† |
| $PaO_2$ (kPa) | 23.7 (19.6 - 27.9) | 26.8 (22.0 - 31.7) |
| $PaCO_2$ (kPa) | 5.1 (4.9 - 5.3) | 5.3 (5.1 - 5.6) |
| $HCO^3$ (mmol) | 23.6 (22.8 - 24.3) | 21.2 (20.4 - 21.9)*** |
| Base deficit | 0.8 (0.1 - 1.5) | 4.0 (3.3 - 4.8)***† |
| Hb (g/dl) | 11.6 (11.1 - 12.2) | 9.0 (8.6 - 9.4)***† |
| Lactate (mmol/l) | 1.24 (1.0 - 1.4) | 2.5 (2.1 - 2.9)***† |
| Urine flow ml/kg/min | | 1.7 (1.0 – 2.4) |

***Table 7.6*** *Cardiovascular variables at baseline (after induction of anaesthesia) and end of surgery for protocol group patients (n=30).* p<0.5, **p< 0.01, ***p<0.001 baseline to end of surgery within control group patients. † p<0.5, ††p< 0.01, †††p<0.001 between control and protocol groups at baseline or end of surgery.*

| Cardiovascular variable | Baseline<br>Mean (95% CI) | End of surgery<br>Mean (95% CI) |
|---|---|---|
| Heart rate | 68.2 (62.1 - 74.2) | 76.7 (72.7 - 80.7)** |
| Systolic BP (mmHg) | 110.1 ( 105.1 - 115.1) | 110.2 (105.7 - 114.7) |
| Diastolic BP (mmHg) | 63.9 (59.6 - 68.2) | 58.9 (55.2 - 62.7) |
| Mean BP (mmHg) | 77.7 (72.8 - 82.6) | 74.4 (70.9 - 77.9) |
| CVP (mmHg) | 5.7 (4.7 - 6.8) | 5.5 (4.5 - 6.5)† |
| Cardiac output (l/min) | 4.5 (4.1 - 5.0) | 6.6 (6.0 - 7.2)***† |
| Stroke volume (ml) | 68.2 (60.5 - 76.0) | 86.8 (79.0 - 94.5)***†† |
| Cardiac index (l/min/$m^2$) | 2.5 (2.2 - 2.7) | 3.6 (3.2 - 3.9)*** |
| DO2 (ml/min) | 690.8 (612.8 - 768.8) | 733.7 (665.4 - 802.1) |
| DO2 index (ml/min/$m^2$) | 372.3 (332.9 - 411.8) | 396.2 (360.0 - 432.4) |
| Tonometer CO2 (kPa) | 5.1 (4.7 - 5.6) | 5.1 (4.7 - 5.5)††† |
| pHi | 7.42 (7.38 - 7.45) | 7.39 (7.36 - 7.42)††† |
| Arterial pH | 7.40 (7.39 - 7.41) | 7.38 (7.36 - 7.41)††† |
| $PaO_2$ (kPa) | 31.8 (24.7 - 39.0) | 35.6 (29.1 - 42.1) |
| $PaCO_2$ (kPa) | 5.2 (5.0 - 5.4) | 5.0 (4.6 - 5.4) |
| $HCO^3$ (mmol) | 24.1 (23.4 - 24.7) | 22.0 (21.4 - 22.5)*** |
| Base deficit | 0.2 (0.3 - 0.9) | 2.4 (1.6 - 3.3)***† |
| Hb (g/dl) | 11.5 (10.9 - 12.1) | 8.3 (8.0 - 8.6)***† |
| Lactate (mmol/l) | 1.2 (1.0 - 1.4) | 2.0 (1.9 - 2.2)***† |
| Urine flow ml/kg/min | | 1.6 (1.3 - 1.9) |

By the end of surgery there was no significant change in blood pressure within the two groups and no difference between the two groups. However, the control group demonstrated a significant increase in mean heart rate ($p<0.01$) and cardiac output ($p<0.001$), no change in stroke volume or central venous pressure but a reduction in pHi ($p<0.001$) and arterial pH ($p<0.001$). The protocol group demonstrated a significant increase in mean heart rate ($p<0.01$), cardiac output ($p<0.001$) and stroke volume

($p<0.001$) with no significant change in pHi or arterial pH. There was no significant difference between or within the two groups for arterial $PaCO_2$ but in the control group there was a significant rise in tonometer $PCO_2$ ($p<0.001$) by the end of surgery. Both groups demonstrated a significant reduction in arterial bicarbonate ($p<0.001$), and increase in base deficit ($p<0.001$) and arterial lactate ($p<0.001$). However, the final mean value of arterial bicarbonate was less and base deficit and lactate greater in the control group than in the protocol group ($p<0.05$). Both groups had a significant fall in Hb but the final mean value was lower in the protocol group ($p<0.05$) than the control group. There was no difference between the two groups in the mean volume of transfused donor packed red cells. There was no significant difference within or between the two groups for oxygen delivery or oxygen delivery indexed to body surface area.

### 7.5.4. Post-operative complications.

**Table 7.7** shows the sensitivity and specificity of the ability to predict complications from the main cardiovascular variables. For the prediction of a major complication or death pHi and arterial pH were the most sensitivity (100% and 83% respectively) and urine flow, blood pressure and cardiac output indexed to body surface area the least (17%, 0% and 0% respectively). Base deficit had a sensitivity of 50%. For the prediction of a major complication or death cardiac output indexed to body surface area and blood pressure were the most specific (94% and 93% respectively) and arterial pH the least (50%). pHi had a specificity of 70%.

***Table 7.7.*** *Comparison of ratios (%) characterising the predictive value of abnormal cardiovascular variables at the end of surgery predicting the development of a major complication.*

| Cardiovascular variable (abnormality) | Sensitivity | Specificity |
|---|---|---|
| pHi (<7.32) | 100 | 70.4 |
| pHa (<7.36) | 83 | 50 |
| Heart rate (>90 beats /min) | 50 | 79.6 |
| Base deficit (>4) | 50 | 72.2 |
| Urine flow (<0.5 ml/kg/min) | 16.6 | 83.3 |
| Blood pressure (mean <60 mmHg) | 0 | 92.5 |
| Cardiac index (<2/l/min/m2) | 0 | 94 |

Incidence of gut mucosal hypoperfusion, numbers of complications and duration of ITU and Hospital stays are shown in **table 7.8**. Types of complications for individual patients are shown in **table 7.9**. The incidence of gut mucosal hypoperfusion (pHi < 7.32) at the end of surgery was significantly reduced in the protocol group (7% *vs* 56%; $p < 0.01$) as were the number of patients developing minor (4 *vs* 11; $p < 0.01$) and major complications (0 *vs* 6; $p < 0.01$), the days spent in hospital (6.4 (6.5) [range 5–9] *vs* 10.1 (7.0) [range 5–48]; $p < 0.01$) and days spent in ITU (1(1) [range 1 – 1] *vs* 1.7 (1) (range 1 – 11); $p < 0.01$). One death occurred in the control group.

***Table 7.8*** *Incidence of gut mucosal hypoperfusion (pHi <7.32), post-operative complications, duration of stay in ITU and hospital. Where appropriate data are shown as mean (median) [range] .* $p<0.05$, * $p<0.01$ Mann-Whitney U corrected for ties. † $p <$ 0.01 Chi-square.*

| | Control (n=30) | Protocol (n=30) |
|---|---|---|
| pHi < 7.32 (n) | 17 (57%) | 2 (6.7%) † |
| Minor complications (n) | 11 | 4 † |
| Major complications (n) | 6 | 0† |
| Deaths (n) | 1 | 0 |
| Days in ITU | 1.7 (1.0) [1 - 11] | 1.0 (1.0) [1.0 - 1.0] ** |
| Days in Hospital | 10.1 (7.0) [5 - 48] | 6.4 (6.5) [5 - 9] ** |

*Table 7.9 Post-operative complications for individual patients.*

| Patient number | Group | Complication | Days in Hospital |
|---|---|---|---|
| 2 | Control | Respiratory failure | 9 |
| 10 | Control | Chest Infection | 8 |
| 16 | Control | Chest infection/pleural effusion/disorientation | 11 |
| 17 | Control | Chest infection/nausea and vomiting | 5 |
| 20 | Control | Nausea and vomiting | 8 |
| 23 | Control | MOF* | 48 |
| 24 | Control | Nausea and vomiting | 7 |
| 27 | Control | Nausea and vomiting | 8 |
| 28 | Control | Wound infection/nausea and vomiting | 7 |
| 29 | Control | Dyspnoea limiting mobilisation/pleural effusion | 8 |
| 32 | Control | TIA†/persistent ulna nerve palsy | 7 |
| 37 | Control | Respiratory failure/nausea and vomiting | 11 |
| 39 | Protocol | Re-sternotomy for bleeding | 7 |
| 41 | Control | Cerebrovascular accident | 12 |
| 43 | Control | Nausea and vomiting | 8 |
| 46 | Control | MOF* | 37 |
| 47 | Protocol | Nausea and vomiting | 9 |
| 49 | Protocol | Dyspnoea limiting mobilisation | 7 |
| 50 | Protocol | Nausea and vomiting | 5 |
| 55 | Control | Respiratory failure | 7 |
| 58 | Control | Paralytic ileus/pericardial effusion/disorientation | 21 |

**Multiple organ failure. † Transient ischaemic attack.*

## 7.6. DISCUSSION.

### 7.6.1. Per-operative plasma volume expansion and pHi.

In agreement with previous studies the development of a low pHi over the course of surgery was the most sensitive predictor of a poor outcome (**chapters 3.0 - 6.0**; Doglio, Pusajo, Egurrola et al. 1991; Fiddian - Green, Amelin, Herrmann et al. 1986; Fiddian-Green and Baker 1987; Gutierrez, Bismar, Dantzker et al. 1992; Gutierrez, Palizas, Doglio et al. 1992; Maynard, Beale, Smithies et al. 1992; Maynard, Taylor, Bihari et al. 1992; Soong, Halliday, Hood et al. 1992a; Soong, Halliday, Hood et al.

1992b). A significant reduction in mortality has been reported in a prospective randomised trial of pHi guided therapy in patients whose pHi was normal on admission to ITU (Gutierrez, Palizas, Doglio et al. 1992). However, this is the first study to report that the per-operative expansion of blood volume using a colloid, with the aim of avoiding hypovolaemia and gut mucosal hypoperfusion, reduces the incidence of a low pHi and improves outcome following elective cardiac surgery.

### 7.6.2. Hypovolaemia as a cause of gut mucosal hypoperfusion.

Hypovolaemia is common among patients who are scheduled for surgery. Aside from the inevitable losses in the per-operative period due to trauma, evaporation and the use of dry anaesthetic gases the majority of patients are still starved for a minimum of 6 hours pre-operatively to try and reduce the risk of acid aspiration syndrome. There is, however, little evidence to support this practice (Phillips, Hutchinson and Davidson 1993). On the contrary it has been shown that drinking small quantities up to 3 hours before surgery is potentially safer (Agarwal, Chari and Singh 1989). Even for relatively minor surgery under general anaesthetic the administration of intravenous fluids in an attempt to replace a presumed pre-operative deficit has resulted in decreased morbidity (Cook, Anderson, Riseborough et al. 1990). General anaesthesia almost always results in some degree of myocardial depression and vasodilatation which will have more profound effects if the patient is hypovolaemic. How much fluid to give during major surgery is extremely difficult to judge. The commonly measured cardiovascular variables and even central venous pressure and pulmonary artery wedge pressure only become abnormal once the degree of hypovolaemia is quite marked (Baek, Makabali, Byron-Brown et al. 1975; Price, Deutsch, Marshall et al. 1966). Similarly it is difficult to detect fluid overload particularly when non-colloidal solutions are used (Baek, Makabali, Byron-Brown et al. 1975; Shoemaker, Schluchter, Hopkins et al. 1981). This is thought to be a particular problem in cardiac surgery (Edmunds and Stephenson 1983). The majority of the patients have impaired myocardial function and there is concern that the administration of iv fluids will produce or exacerbate heart failure. The patients also receive an additional

fluid load from the solution (commonly a crystalloid) used to prime the by-pass machine. These factors and the vascular endothelial leak that is thought to be an inevitable insult following cardiopulmonary by-pass have led to the commonly held belief that fluid is bad for patients having cardiac surgery. However, the same patients having non-cardiac surgery would have their fluid losses replaced in normal fashion, guided by central venous pressure and less commonly cardiac output measurement. Intravenous fluids can be administered safely to any patient if appropriate monitoring is used (Weil, Shubin and Rosoff 1965). The additional use of the oesophageal Doppler with a central venous pressure catheter allows a continuous assessment of the effects of a colloid fluid challenge on cardiac stroke volume (Singer, Allen, Webb et al. 1991). If the fluid bolus (200ml) is given to a patient with good LV filling the stroke volume will not increase and the central venous pressure will rise. A similar process is practised by many cardiac surgeons as they wean the patient from cardiopulmonary by-pass; the only difference is that they have the luxury of seeing the heart directly.

### 7.6.3. Type of intravenous fluid used.

A 6% hydroxyethyl starch ELO-HES® (Oxford Nutrition, Oxford) was used which is the starch solution routinely used in our institution. ELO-HES® has a weight average molecular weight of 200,000 dalton. As cardiopulmonary by-pass may be associated with the development of a leaky vascular endothelium the presence of molecules with a higher molecular weight should allow better maintenance within the circulation than a gelatine or crystalloid solution, allowing the use of smaller volumes to produce the same sustained effects (Webb, Barclay and Bennett 1989). Starches have also been shown to have favourable effects on microcirculatory flow in animal shock models (Webb, Tighe, Moss et al. 1991) and in vitro to reduce the adhesion of blood cells to the vascular endothelium (Collis, Collins, Gutteridge et al. 1994). It remains to be shown in adults whether the same beneficial effects of using fluid challenges can be achieved irrespective of the solution used.

### 7.6.4. Timing and presumed effects of fluid loading.

In this study the use of colloid fluid challenges resulted in significantly more colloid being given to the protocol group. This was not associated with any increased morbidity traditionally associated with fluid overload. In particular no protocol group patient developed clinical evidence of pulmonary oedema. The use of colloid fluid challenges was associated with a significant reduction in end organ hypoperfusion, morbidity and duration of hospital stay. The majority of colloid in the protocol group was given in the pre-bypass period. Before the patient is put onto cardiopulmonary by-pass there is quite extensive surgery. This surgery results in inevitable fluid loss and may involve substantial blood losses. The protocol group presumably received enough additional intravenous fluid in the pre by-pass period to replace any pre-existing deficit, compensate for any anaesthesia induced vasodilatation and replace any new losses. If this were so then the protocol group would have been put onto cardiopulmonary by-pass with a larger total circulating blood volume and thus have a greater likelihood of maintaining perfusion to all tissues. This is supported by the observation that during cardiopulmonary by-pass more patients in the control group required treatment for hypotension (a mean BP < 45 mmHg for > 3 minutes - 7/30 *vs* 1/30; $p<0.05$) and oliguria (< 20 mls urine after 30 minutes of by-pass - 12/30 *vs* 3/30; $p<0.01$). Following cardiopulmonary by-pass there was no significant difference between the amount of colloid given to the two groups. The prototol group presumably still had a larger circulating total blood volume. This is supported by the findings of an increase in cardiac output and stroke volume and improved organ perfusion and oxygenation as judged by pHi, pHa and arterial lactate.

### 7.6.5. Gut mucosal hypoperfusion and outcome.

The major complications reported in the control group all occurred in patients who developed a low pHi. They were all consistent with organ hypoperfusion resulting in organ dysfunction. These data are consistent with the hypothesis that gut mucosal hypoperfusion may result in translocation of endotoxin and additional activation of inflammatory pathways resulting in tissue destruction and dysfunction (Deitch 1990b;

Fiddian-Green 1988). However, they are also consistent with the hypothesis that a low pHi is just another marker of tissue hypoperfusion.

The control group had a significantly longer mean duration of stay in the ITU and hospital, not all of which could be accounted for by the higher incidence of major morbidity. Although not a planned study outcome variable it was also noted that there was a much higher incidence of minor complications in the control group when compared to the protocol group. These minor complications such as disorientation, dyspnoea limiting mobilisation, wound infection and persistent nausea and vomiting beyond the third post-operative day are difficult to study in an objective fashion but contribute significantly to patient suffering and duration of hospital stay. In the control group 13 patients stayed in hospital for more than 7 days compared to 2 in the protocol group. It would be rash at this stage to suggest that all of these complications are related to peri-operative tissue hypoperfusion but it may well be a compounding factor in a complex multi-factorial process.

### 7.6.6. Fluid loading and haematocrit.

One of the objections to fluid pre-loading cardiac patients is that it reduces the measured haemoglobin concentration, although not the red cell mass. The protocol group had a significantly lower haemoglobin at the end of surgery. However, there was no difference between the two groups in the amount of transfused blood given. As a result of the greater increase in cardiac output in the protocol group there was no difference in oxygen delivery at the end of surgery between the two groups. There is no predictable association between haemoglobin concentration, oxygen delivery and pHi as an index of tissue oxygenation (Gutierrez, Bismar, Dantzker et al. 1992; Silverman and Tuma 1992; Mythen and Webb 1993). The myocardium is far more sensitive to anaemic hypoxia than the gut mucosa due to its high oxygen extraction ratio (Grum, Fiddian-Green, Pittenger et al. 1984) but there was no recorded morbidity associated with anaemia in the protocol group.

### 7.6.7. Relationship between global oxygen flow variables and pHi.

Previous studies on high risk major surgical patients have shown a reduction in post-operative morbidity and mortality by using predetermined supranormal levels of cardiac index and oxygen delivery as therapeutic goals (Shoemaker, Appel, Kram et al. 1988; Boyd, Grounds and Bennett 1993). Such a technique relies on the peri-operative use of a flow directed pulmonary artery catheter, which in itself carries a morbidity and mortality, and has not proved to be popular in the UK. However, for an individual patient the goals may be inadequate or unduly excessive. In our study, admittedly on a much lower risk group of cardiac patients, the protocol group did demonstrate a significantly greater increase in cardiac output than the control group but there was no significant increase in $DO_2$ in either group. In agreement with Gutierez et al.(1992), Marik et al.(1993), and Maynard et al. (1993) there does not seem to be a relationship between global oxygen flow variables and gastric pHi. At least in this group of patients the maintenance of gastric pHi and arterial pH and lactate as indices of general tissue perfusion are far less dependent on global $DO_2$ than on the adequacy of circulating blood volume (Price, Deutsch, Marshall et al. 1966; Fink, Cohn, Lee et al. 1989; Gutierrez, Bismar, Dantzker et al. 1992; Silverman and Tuma 1992). This would support the suggestion that a marker of tissue perfusion, such as gastric pHi, would be a far more rational end point for resuscitation (Marik 1993).

## 7.7. CONCLUSION.

Blood volume expansion with colloid, guided by oesophageal Doppler measurement of cardiac stroke volume, can be given safely to patients with moderate left ventricular function having cardiac surgery. The combined use of the oesophageal Doppler and the gastric tonometer allows both quantitative and qualitative assessment of cardiorespiratory function relatively non-invasively. The use of colloid challenges with the aim of optimising the circulating blood volume in elective cardiac surgical patients reduced the incidence of gut mucosal hypoperfusion and improved patient outcome.

# CHAPTER 8.0. THESIS SUMMARY

## 8.1. AIM.

The aim of this thesis was to explore the hypothesis that per-operative splanchnic hypoperfusion is associated with post-operative organ failure and is a result of perturbation of cardio-respiratory function. The gastrointestinal tonometer and an oesophageal Doppler were used to determine changes in gastrointestinal mucosal perfusion and cardiac output during major (mainly cardiovascular) surgery. A relationship was sought between these variables and the development of post-operative complications. It was hoped that by further exploring the pathogenesis of peri-operative gut mucosal hypoperfusion it would be possible to devise a treatment regimen that would reduce the incidence of gastric mucosal hypoperfusion and thus improve patient outcome.

## 8.2. GUT MUCOSAL HYPOPERFUSION AS A PREDICTOR OF A POOR OUTCOME FOLLOWING MAJOR SURGERY.

In an observational study of 51 patients undergoing elective major surgery (**chapter 3.0**) it was demonstrated that gut mucosal hypoperfusion was associated with a poor outcome. The presence of a low pHi at the end of surgery was found to be the most sensitive predictor of post-operative morbidity and mortality when compared to the other commonly measured cardiovascular variables such as blood pressure or urine flow measurement. These findings were in agreement with previous studies of patients undergoing high risk major surgery. However, for the first time, a causal relationship was sought between gastric pHi and changes in the commonly measured cardiovascular variables and cardiac output measurements. No such relationship was identified but two particular changes were noted. Firstly, the group of patients that maintained gut mucosal perfusion had a greater than 50% increase in CO from beginning to end of surgery whereas the group that had a reduction in gut mucosal perfusion only maintained CO at

the baseline level. Secondly, the group that failed to maintain gut mucosal perfusion had a significant reduction in stroke volume and thus only maintained mean CO by an increase in heart rate. These findings suggested that hypovolaemia was a common cause of gut mucosal hypoperfusion.

## 8.3. THE LINK BETWEEN PERI-OPERATIVE GUT MUCOSAL HYPOPERFUSION AND A POOR OUTCOME.

As no causal relationship had been identified between a low pHi and changes in the commonly measured cardiovascular variables other potential areas for therapeutic intervention were examined. The main hypothesis relating gut mucosal hypoperfusion to the development of post-operative organ dysfunction is that excess activation of inflammatory pathways results from endotoxin translocation through a compromised gut mucosal barrier. Therefore, these two areas were studied in more detail.

### 8.3.1. Gut mucosal hypoperfusion, contact activation and neutrophil degranulation.

In an observational study of 26 patients, the development of gut mucosal hypoperfusion during elective major surgery was found to be associated with increased activation of the contact system, increased neutrophil degranulation and the development of post-operative organ dysfunction. However, once again these were not causal relationships. Although the patients who had the worst outcome demonstrated the greatest degree of neutrophil degranulation and depletion of circulating C1-esterase inhibitor levels (the main inhibitor of the contact pathway) some patients developed a low pHi over the course of surgery and did not demonstrate evidence of either increased contact activation or increased degranulation of neutrophils. Similarly other patients developed a low pHi, depletion of C1-esterase inhibitor and elevation of neutrophil elastase complexes yet had an uneventful recovery. These findings led to the conclusion that the lack of a causal relationship may have been due to a variable host response to the results of gut mucosal

hypoperfusion such as different levels of endotoxin exposure or varying degrees of natural immunity to endotoxin.

### 8.3.2. Gut mucosal hypoperfusion and endotoxin immunity.

In the same group of 26 patients the association between gut mucosal hypoperfusion and perturbations in endogenous endotoxin core antibodies were then examined (**chapter 5.0**). It was hoped that the changes in endotoxin antibody levels would not only act as markers of recent exposure to endotoxin but also help to clarify the non-specific relationship between a low pHi and a poor outcome. Irrespective of the state of gut mucosal perfusion as judged by the pHi at the end of surgery all patients demonstrated reductions in IgG and IgM endotoxin antibodies from the beginning of surgery to 24 hours. Rather unexpectedly the group of patients who maintained gut mucosal perfusion and had a favourable outcome not only had higher levels of IgG endotoxin core antibodies when compared to the other study patients but also had supranormal baseline levels when compared to healthy volunteers (80% > the 90th centile). This suggeted that high levels of endotoxin core anti-bodies may be an important determinant in both the maintenance of gastric mucosal perfusion and outcome.

## 8.4. CHOOSING A TREATMENT REGIMEN THAT MIGHT IMPROVE GUT MUCOSAL PERFUSION.

Three main areas had been identified that offered potential for therapeutic regimens aimed at modifying gastric mucosal perfusion and/or the development of post-operative organ dysfunction. These were peri-operative hypovolaemia, the magnitude of activation of the contact pathway and neutrophil degranulation and the level of endotoxin core-antibodies. For this thesis it was decided to concentrate on the hypothesis that peri-operative hypovolaemia was the commonest potential cause of a low pHi . As mild peri-operative hypovolaemia is virtually impossible to diagnose it was decided to explore the effects of plasma volume expansion on gut mucosal perfusion. However, before proceeding it was

felt to be important to exclude any link between the anaesthetic agents that had been used for the maintenance of general anaesthesia in the above studies and changes in pHi.

## 8.5 GUT MUCOSAL PERFUSION AND ANAESTHETIC DRUGS.

Although there was no human data, animal work suggested that pHi would be better preserved with some anaesthetic agents (e.g. Isoflurane). Therefore, 30 patients undergoing elective coronary artery by-pass surgery were studied in a prospective randomised study (**chapter 6.0**) of a balanced anaesthetic technique where either Isoflurane, Enflurane or Propofol was used as part of an otherwise standardised technique. In keeping with the previous animal data it was demonstrated that gut mucosal perfusion was better preserved when Isoflurane was used for the maintenance of anaesthesia. As a result of these findings Isoflurane was used as part of a strict anaesthetic drug protocol for all patients in the final study.

## 8.6. PERI-OPERATIVE PLASMA VOLUME EXPANSION AND GUT MUCOSAL PERFUSION.

In a prospective randomised trial of 60 patients undergoing elective cardiac surgery, it was demonstrated that plasma volume expansion with the aim of avoiding peri-operative hypovolaemia resulted in a highly significant reduction in the incidence of gut mucosal hypoperfusion. The patients in the volume expanded group also had an associated reduction in post-operative morbidity and duration of hospital stay when compared to controls.

## 8.7. OVERALL CONCLUSION.

In the patients studied gut mucosal hypoperfusion was common, associated with a poor outcome and most likely due to peri-operative hypovolaemia. This hypothesis is supported by the demonstration that the incidence of gut mucosal hypoperfusion following elective cardiac surgery could be dramatically reduced by peri-operative plasma volume expansion and that this was associated with an improved outcome.

## 8.8. IMPLICATIONS AND POTENTIAL FUTURE WORK.

The above studies imply that there is potential for using a similar approach in an attempt to reduce morbidity and mortality in the routine clinical management of patients undergoing elective cardiac surgery and following other types of major surgery. However, one cannot assume that this would necessarily be so as there remain a number of unanswered questions.

### 8.8.1. The persistence of a low pHi despite adequate fluid loading.

In the final study 2 patients (7%) developed a low pHi despite apparently adequate volume expansion. Was this because optimising stroke volume by the method employed does not always guarantee euvolaemia or is hypovolaemia not the sole cause of a low pHi in these patients? These low readings may have been artifactual or even normal; the tonometric method is by no means flawless or the normal range for pHi as well established as for other cardiovascular variables.

### 8.8.2. Volume expansion in patients with poor ventricular function.

There is genuine concern that plasma volume expansion may not be entirely safe in patients with poor ventricular function. This needs to be addressed in a separate study as such patients may require a higher level of monitoring, such as the measurement of pulmonary artery occlusion pressure, or inotropic support. If this is so then the choice of inotropic drug, for example, could be a critical component in the maintenance of gut mucosal perfusion and thus outcome.

### 8.8.3. Unavoidable gut mucosal hypoperfusion.

There may be some patient groups where a degree of gut mucosal hypoperfusion is inevitable. For example in major vascular surgery the lower end of the gastrointestinal tract may be rendered ischaemic as a result of infra-renal cross clamping and poor collateral flow through the superior mesenteric vessels. In this situation the maintenance

of a normal gastric pHi may have far less influence on outcome. Similarly during major bowel surgery there is local release of vaso-active substances that may compromise mucosal perfusion in some parts of the bowel irrespective of intravascular volume or the gastric pHi. In the former example it may be that sigmoid tonometry would allow the identification of an *at risk* sub-group and prompt the use of an alternative method of maintaining mucosal perfusion such as a shunt or extracorporeal support. On the other hand the administration of agents to reduce the severity of an inevitable reperfusion injury may prove useful. In the later example it may be that the regonal use of specific vasoactive drugs, for example, could overcome local vasospasm and thus maintain perfusion of the handled bowel.

### 8.8.4. Using peri-operative pHi as a therapeutic index.

The blunderbuss approach of volume expanding all patients to try and avoid presumed hypovolaemia probably results in over treatment of some patients. This was one of the objections raised to Goal Directed Therapy in **chapter 1.0** where the practice of chasing 'magic numbers' with no account taken of the individual patients'needs was criticised. By the same token it would be far more logical to identify and rapidly correct a low pHi than treat all patients in an attempt to avoid it. However, the current tonometric method is too slow and laborious to allow the widespread practical use of such an approach. What is required is an automatic continuous or fast response intermittent system.

### 8.8.5. Is there a need to measure pHi at all?

In the above study the patients were volume expanded with colloid guided by oesophageal Doppler measurement of cardiac output. The tonometer was only used to demonstrate one of the effects of plasma volume expansion, i.e. the maintenance of a normal pHi. This may have been a purely coincidental finding, and to a large extent, the tonometric readings made no contribution to outcome. However, in considering the thesis as a whole the measurement of gastric pHi identified a treatable abnormality and resulted in a change in practice for the better. If this process could be translated into every

day clinical practice it may be that the widespread use of the gastric tonometer would result in an overall change in clinical practice such that the incidence of a low pHi at the end of surgery became so low that the measurement of pHi became redundant. This issue could be addressed in a clinical audit or impact study.

### 8.8.6. Using pHi measurement to identify a sub-group of patients who may benefit from treatments aimed at preventing organ dysfunction?

As alluded to above, it may be that if different groups of patients were studied then cardiovascular manipulations alone would not so dramatically reduce the incidence of a low pHi. Therefore, despite pHi measurement being a non-specific predictor of MODS, one may be able to identify a high risk group of patients that may thus justify the prophylactic administration of therapeutic agents aimed at preventing MODS. Clinical concentrates of C1-esterase inhibitor, the less specific serine protease inhibitor Aprotinin or a cocktail of agents aimed at interfering with the inflammatory process, such as TNF antibodies or Interleukin-1 receptor antagonists, may all have a role.

### 8.8.7. Immunisation against endotoxin.

The finding of such extraordinarily high levels of endotoxin core antibodies in the patients that maintained gut perfusion and had a favourable outcome (**chapter 5.0**) raises the possibility of immunisation for high risk patients where gut mucosal hypoperfusion may not be avoidable by cardiovascular manipulations alone. This warrants investigation in a larger study to see first if the findings are reproducible. As discussed in **chapter 5.0** the high levels of antibodies may just be coincidental markers of *fitter* patients which may explain the apparent failure of other passive immunisation studies. However, if the findings were to bear out then active immunisation may prove to be a realistic option for a high risk subset who are likely to develop gut mucosal hypoperfusion either because of their pre-operative myocardial function or the type of surgery they are scheduled for (e.g Aortic aneurysm repair).

## PEER REVIEWED PUBLICATIONS FROM THIS THESIS.

**Chapter 1.0:** Mythen MG, Webb AR. (1994). The role of gut mucosal hypoperfusion in the pathogenesis of post-operative organ dysfunction. Intensive Care Medicine. 20: 203-209.

**Chapter 3.0:** Mythen MG, Webb AR. (1994) Intra-operative gut mucosal hypoperfusion is associated with increased post-operative complications and cost.Intensive Care Medicine. 20: 99-104.

**Chapter 4.0:** Mythen MG, Purdy GC, McNally T, Mackie IJ, Machin SJ. (1993). Post-operative multiple organ dysfunction syndrome associated with gut mucosal hypoperfusion, increased neutrophil degranulation and C1-esterase inhibitor depletion. British Journal of Anaesthesia. 71: 858-863.

**Chapter 5.0:** Mythen MG, Barclay GR, Hamilton-Davies C, Webb AR, Machin SJ. (1993). The role of endotoxin immunity, neutrophil degranulation and contact activation in the pathogenesis of post-operative organ failure. Blood, Coagulation and Fibrinolysis. 4: 999-1005.

**Chapter 6.0:** Mythen MG, Browne D, Hamilton-Davies C, Webb AR. (1993). Gastric mucosal perfusion is better preserved during cardiac surgery when isoflurane rather than enflurane or propofol is used to maintain anaesthesia. British Journal of Anaesthesia. 70: 15.

**Chapter 7.0:** Mythen MG and Webb AR. (1994). Per-operative plasma volume expansion reduces the incidence of gut mucosal hypoperfusion during cardiac surgery. Archives of Surgery (in press).

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# APPENDICES

## ABBREVIATIONS USED IN THE APPENDICES

| | |
|---|---|
| α-1 antitrypsin | $Alpha_1$ -antitrypsin |
| α-2M | $Alpha_2$ .macroglobulin |
| AAA | Abdominal aortic aneurysm repair |
| ATIII | Antithrombin III |
| AVR | Aortic valve replacement |
| b | Indicates measurements taken at baseline |
| BE | Arterial blood gas calculated base excess |
| BSA | Body surface area |
| C1-INH | C1-esterase inhibitor |
| CABG | Coronary artery by-pass graft surgery |
| CO | Cardiac output |
| CVP | Central venous pressure |
| DBP | Diastolic blood pressure |
| e | Indicates measurements taken at end of surgery |
| FXII | Factor XII |
| Hb | Haemoglobin concentration |
| HCO3 | Arterial bicarbonate concentration |
| Hct | Haematocrit |
| Hep-co II | Heparin co-factor II |
| HR | Heart rate |
| MBP | Mean blood pressure |
| MVR | Mitral valve replacement |
| NE | Neutrophil elastase:$alpha_1$ antitrypsin complexes |
| PO | Plasminogen |
| PaCO2 | Arterial carbondioxide tension |
| PaO2 | Arterial oxygen tension |
| pHi | Intramucosal pH |
| PKK | Prekallikrein |
| Plt | Platelet count |
| $P_TCO2$ | Carbon dioxide tension in tonometer balloon saline |
| SBP | Systolic blood pressure |
| SV | Stroke volume |
| TVR | Tricuspid valve replacement |
| Urine | Urine flow |
| WBC | White blood cell count |

# A3.0. APPENDIX TO CHAPTER 3.0.

*A3.1. Data used in Table 3.4*

| Patient No. | Sex | Age (years) | Height (cm) | Weight (kg) | BSA ($m^2$) |
|---|---|---|---|---|---|
| 1 | M | 59 | 183 | 88 | 2.10 |
| 2 | F | 50 | 150 | 47 | 1.40 |
| 3 | M | 46 | 172 | 77 | 1.90 |
| 4 | F | 77 | 128 | 55 | 1.33 |
| 5 | M | 67 | 180 | 73 | 1.92 |
| 6 | F | 62 | 150 | 56 | 1.50 |
| 7 | M | 67 | 172 | 74 | 1.87 |
| 8 | F | 68 | 170 | 70 | 1.81 |
| 9 | M | 67 | 180 | 75 | 1.94 |
| 10 | M | 69 | 166 | 68 | 1.76 |
| 11 | M | 63 | 181 | 83 | 2.04 |
| 12 | M | 54 | 175 | 64 | 1.78 |
| 13 | F | 28 | 164 | 53 | 1.57 |
| 14 | M | 70 | 180 | 85 | 2.05 |
| 15 | M | 59 | 170 | 68 | 1.79 |
| 16 | F | 73 | 175 | 93 | 2.09 |
| 17 | M | 68 | 168 | 75 | 1.85 |
| 18 | M | 64 | 170 | 84 | 1.96 |
| 19 | M | 27 | 182 | 79 | 2.00 |
| 20 | F | 54 | 163 | 53 | 1.56 |
| 21 | F | 61 | 168 | 62 | 1.70 |
| 22 | F | 73 | 153 | 46 | 1.40 |
| 23 | M | 67 | 175 | 64 | 1.78 |
| 24 | M | 55 | 180 | 79 | 1.99 |
| 25 | M | 71 | 168 | 71 | 1.81 |

*A3.2. Data used in Table 3.4*

| Patient No. | Sex | Age (years) | Height (cm) | Weight (kg) | BSA ($m^2$) |
|---|---|---|---|---|---|
| 26 | F | 68 | 170 | 73 | 1.84 |
| 27 | M | 53 | 177 | 102 | 2.19 |
| 28 | F | 38 | 150 | 57 | 1.51 |
| 29 | F | 40 | 150 | 57 | 1.51 |
| 30 | M | 71 | 168 | 82 | 1.92 |
| 31 | M | 43 | 175 | 80 | 1.96 |
| 32 | M | 60 | 164 | 70 | 1.76 |
| 33 | F | 79 | 153 | 59 | 1.56 |
| 34 | M | 52 | 172 | 78 | 1.91 |
| 35 | F | 73 | 161 | 68 | 1.72 |
| 36 | M | 58 | 180 | 82 | 2.02 |
| 37 | M | 76 | 168 | 58 | 1.66 |
| 38 | F | 69 | 165 | 48 | 1.51 |
| 39 | F | 72 | 160 | 46 | 1.44 |
| 40 | M | 60 | 180 | 81 | 2.01 |
| 41 | M | 64 | 187 | 99 | 2.25 |
| 42 | M | 74 | 173 | 75 | 1.89 |
| 43 | M | 59 | 180 | 79 | 1.99 |
| 44 | M | 63 | 165 | 94 | 2.01 |
| 45 | M | 79 | 167 | 74 | 1.83 |
| 46 | M | 57 | 165 | 96 | 2.03 |
| 47 | M | 58 | 174 | 91 | 2.05 |
| 48 | M | 67 | 174 | 76 | 1.91 |
| 49 | M | 52 | 180 | 94 | 2.14 |
| 50 | M | 75 | 145 | 68 | 1.59 |
| 51 | M | 67 | 179 | 70 | 1.88 |

*A3.3. Data used in Tables 3.3, 3.4 and 3.7.*

| Patient No. | Surgical procedure | Durn. surgery (mins) | Days ITU |
|---|---|---|---|
| 1 | CABG | 210 | 1 |
| 2 | Mesenteric re-vascularisation | 300 | 1 |
| 3 | Aorto-bi-femoral graft | 210 | 0 |
| 4 | AAA | 240 | 1 |
| 5 | CABG | 210 | 1 |
| 6 | Cystectomy | 350 | 1 |
| 7 | AVR | 180 | 1 |
| 8 | Femero-femoral graft | 120 | 0 |
| 9 | CABG (re-do) | 240 | 33 |
| 10 | AVR | 180 | 1 |
| 11 | AAA | 180 | 1 |
| 12 | Whipples procedure | 120 | 0 |
| 13 | Whipples procedure | 270 | 0 |
| 14 | Femero-poplitcal by-pass (re-do) | 150 | 0 |
| 15 | AVR | 120 | 1 |
| 16 | AVR | 180 | 1 |
| 17 | CABG | 240 | 1 |
| 18 | CABG | 240 | 1 |
| 19 | AVR/MVR (re-do) | 255 | 1 |
| 20 | MVR/TVR | 240 | 1 |
| 21 | MVR/TVR (re-do) | 210 | 2 |
| 22 | MVR/TVR redo | 300 | 1 |
| 23 | CABG/AVR | 135 | 1 |
| 24 | Ventricular Aneurysectomy | 180 | 1 |
| 25 | MVR/CABG | 240 | 20 |
| 26 | MVR/TVR | 240 | 21 |
| 27 | CABG | 220 | 1 |
| 28 | AVR/MVR (re-do) | 240 | 2 |
| 29 | AVR/MVR/TVR | 210 | 1 |
| 30 | AAA | 300 | 2 |
| 31 | CABG | 240 | 2 |
| 32 | CABG | 330 | 1 |
| 33 | MVR (re-do) | 210 | 9 |
| 34 | CABG | 210 | 1 |
| 35 | CABG | 180 | 4 |
| 36 | CABG | 300 | 1 |
| 37 | Cystectomy | 210 | 19 |
| 38 | CABG | 180 | 1 |
| 39 | AVR | 160 | 1 |
| 40 | CABG | 130 | 1 |
| 41 | CABG | 180 | 1 |
| 42 | CABG | 240 | 1 |
| 43 | CABG | 210 | 2 |
| 44 | CABG | 240 | 13 |
| 45 | CABG/AVR | 240 | 1 |
| 46 | CABG | 180 | 1 |
| 47 | CABG | 540 | 1 |
| 48 | CABG | 180 | 2 |
| 49 | CABG | 180 | 2 |
| 50 | CABG | 240 | 1 |
| 51 | CABG | 270 | 1 |

*A3.4. Data used in 3.4.3 and Table 3.7.*

| Patient No. | Days in Hospital | Major comp.s (n) | Death |
|---|---|---|---|
| 1 | 6 | 0 | |
| 2 | 97 | 1 | |
| 3 | 10 | 0 | |
| 4 | 21 | 1 | |
| 5 | 8 | 0 | |
| 6 | 12 | 0 | |
| 7 | 6 | 0 | |
| 8 | 24 | 1 | |
| 9 | 33 | 1 | YES |
| 10 | 14 | 0 | |
| 11 | 13 | 0 | |
| 12 | 8 | 0 | |
| 13 | 12 | 0 | |
| 14 | 21 | 1 | |
| 15 | 7 | 0 | |
| 16 | 7 | 0 | |
| 17 | 6 | 0 | |
| 18 | 5 | 0 | |
| 19 | 21 | 1 | |
| 20 | 9 | 0 | |
| 21 | 11 | 0 | |
| 22 | 1 | 1 | YES |
| 23 | 8 | 0 | |
| 24 | 7 | 0 | |
| 25 | 20 | 1 | YES |
| 26 | 30 | 1 | |
| 27 | 11 | 0 | |
| 28 | 9 | 0 | |
| 29 | 9 | 0 | |
| 30 | 22 | 1 | |
| 31 | 11 | 1 | |
| 32 | 7 | 0 | |
| 33 | 9 | 1 | YES |
| 34 | 8 | 0 | |
| 35 | 8 | 0 | |
| 36 | 7 | 0 | |
| 37 | 19 | 1 | YES |
| 38 | 6 | 0 | |
| 39 | 7 | 0 | |
| 40 | 6 | 0 | |
| 41 | 6 | 0 | |
| 42 | 9 | 0 | |
| 43 | 9 | 0 | |
| 44 | 24 | 1 | |
| 45 | 8 | 0 | |
| 46 | 7 | 0 | |
| 47 | 2 | 1 | YES |
| 48 | 8 | 0 | |
| 49 | 7 | 0 | |
| 50 | 7 | 0 | |
| 51 | 12 | 0 | |

*A3.5. Data used in Tables 3.5 and 3.6.*

| Patient No. | HR b | HR e | SBP b (mmHg) | SBP e (mmHg) |
|---|---|---|---|---|
| 1 | 80 | 112 | 140 | 110 |
| 2 | 100 | 116 | 145 | 143 |
| 3 | 80 | 60 | 116 | 168 |
| 4 | 80 | 103 | 159 | 150 |
| 5 | 84 | 85 | 97 | 110 |
| 6 | 66 | 63 | 140 | 140 |
| 7 | 94 | 83 | 110 | 82 |
| 8 | 55 | 60 | 90 | 110 |
| 9 | 57 | 100 | 100 | 120 |
| 10 | 77 | 80 | 114 | 120 |
| 11 | 67 | 80 | 130 | 140 |
| 12 | 87 | 70 | 128 | 130 |
| 13 | 56 | 70 | 95 | 140 |
| 14 | 59 | 70 | 130 | 120 |
| 15 | 87 | 90 | 130 | 115 |
| 16 | 64 | 100 | 90 | 100 |
| 17 | 51 | 70 | 120 | 120 |
| 18 | 64 | 110 | 130 | 140 |
| 19 | 100 | 107 | 106 | 107 |
| 20 | 94 | 80 | 124 | 131 |
| 21 | 158 | 96 | 150 | 106 |
| 22 | 124 | 130 | 134 | 128 |
| 23 | 70 | 70 | 140 | 120 |
| 24 | 70 | 65 | 103 | 110 |
| 25 | 74 | 87 | 101 | 111 |
| 26 | 56 | 97 | 90 | 134 |
| 27 | 69 | 84 | 103 | 118 |
| 28 | 61 | 69 | 110 | 126 |
| 29 | 100 | 70 | 130 | 100 |
| 30 | 50 | 67 | 110 | 130 |
| 31 | 58 | 92 | 104 | 113 |
| 32 | 70 | 89 | 120 | 110 |
| 33 | 100 | 113 | 100 | 100 |
| 34 | 50 | 70 | 115 | 110 |
| 35 | 62 | 79 | 120 | 140 |
| 36 | 46 | 77 | 82 | 120 |
| 37 | 65 | 70 | 125 | 105 |
| 38 | 64 | 65 | 131 | 154 |
| 39 | 67 | 90 | 115 | 160 |
| 40 | 69 | 71 | 100 | 99 |
| 41 | 54 | 78 | 105 | 84 |
| 42 | 54 | 66 | 123 | 121 |
| 43 | 44 | 65 | 119 | 110 |
| 44 | 110 | 94 | 90 | 108 |
| 45 | 58 | 100 | 123 | 120 |
| 46 | 60 | 86 | 123 | 100 |
| 47 | 63 | 100 | 98 | 120 |
| 48 | 40 | 80 | 100 | 110 |
| 49 | 101 | 89 | 92 | 150 |
| 50 | 74 | 90 | 131 | 110 |
| 51 | 72 | 102 | 91 | 135 |

*A3.6. Data used in Tables 3.5 and 3..6 and Figure 3.2.*

| Patient No. | DBP b (mmHg) | DBP e (mmHg) | CO b (l/min) | CO e (l/min) |
|---|---|---|---|---|
| 1 | 94 | 77 | 6.1 | 4.9 |
| 2 | 110 | 111 | 4.3 | 2.0 |
| 3 | 85 | 123 | 4.2 | 3.2 |
| 4 | 104 | 100 | 8.9 | 4.6 |
| 5 | 60 | 67 | 8.4 | 4.0 |
| 6 | 100 | 100 | 4.7 | 6.0 |
| 7 | 81 | 55 | 3.7 | 4.5 |
| 8 | 60 | 83 | 3.7 | 3.1 |
| 9 | 73 | 80 | 3.8 | 3.4 |
| 10 | 72 | 85 | 5.2 | 4.3 |
| 11 | 90 | 93 | 5.7 | 10.0 |
| 12 | 93 | 98 | 3.9 | 4.5 |
| 13 | 65 | 100 | 3.7 | 4.4 |
| 14 | 90 | 88 | 3.1 | 4.8 |
| 15 | 83 | 77 | 6.3 | 6.2 |
| 16 | 63 | 73 | 4.3 | 7.3 |
| 17 | 80 | 87 | 2.6 | 4.9 |
| 18 | 94 | 78 | 4.8 | 5.0 |
| 19 | 82 | 76 | 2.4 | 6.5 |
| 20 | 88 | 92 | 5.4 | 4.1 |
| 21 | 105 | 67 | 5.5 | 6.1 |
| 22 | 85 | 79 | 3.5 | 3.0 |
| 23 | 87 | 60 | 5.0 | 5.5 |
| 24 | 72 | 63 | 3.7 | 7.4 |
| 25 | 62 | 72 | 2.7 | 4.1 |
| 26 | 64 | 88 | 3.0 | 4.7 |
| 27 | 78 | 88 | 7.0 | 5.0 |
| 28 | 72 | 92 | 5.8 | 3.6 |
| 29 | 90 | 80 | 3.0 | 3.0 |
| 30 | 77 | 79 | 6.4 | 5.6 |
| 31 | 80 | 85 | 3.1 | 2.5 |
| 32 | 89 | 79 | 3.0 | 4.0 |
| 33 | 73 | 67 | 5.4 | 4.0 |
| 34 | 70 | 60 | 4.1 | 5.8 |
| 35 | 90 | 100 | 6.5 | 13.6 |
| 36 | 67 | 88 | 2.4 | 4.3 |
| 37 | 79 | 65 | 8.4 | 4.8 |
| 38 | 85 | 104 | 3.1 | 6.0 |
| 39 | 76 | 84 | 4.4 | 5.8 |
| 40 | 85 | 77 | 5.0 | 6.7 |
| 41 | 75 | 60 | 3.3 | 10.6 |
| 42 | 59 | 88 | 3.3 | 4.7 |
| 43 | 62 | 52 | 2.3 | 2.9 |
| 44 | 60 | 77 | 5.2 | 4.2 |
| 45 | 88 | 84 | 4.0 | 4.0 |
| 46 | 65 | 70 | 4.7 | 8.3 |
| 47 | 45 | 90 | 5.1 | 3.4 |
| 48 | 66 | 68 | 2.8 | 7.0 |
| 49 | 63 | 84 | 5.3 | 6.6 |
| 50 | 101 | 78 | 3.3 | 3.3 |
| 51 | 57 | 80 | 5.9 | 5.1 |

*A3.7. Data used in Tables 3.5 and 3.6.*

| Patient No. | SV b (ml) | SV e (ml) | pHi b | pHi e |
|---|---|---|---|---|
| 1 | 80 | 43 | 7.37 | 7.29 |
| 2 | 45 | 20 | 7.39 | 7.04 |
| 3 | 53 | 50 | 7.34 | 7.28 |
| 4 | 107 | 45 | 7.23 | 7.26 |
| 5 | 126 | 51 | 7.42 | 7.15 |
| 6 | 71 | 91 | 7.33 | 7.36 |
| 7 | 40 | 49 | 7.38 | 7.32 |
| 8 | 67 | 56 | 7.39 | 7.34 |
| 9 | 60 | 34 | 7.49 | 7.24 |
| 10 | 68 | 54 | 7.26 | 7.25 |
| 11 | 85 | 135 | 7.39 | 7.40 |
| 12 | 45 | 53 | 7.42 | 7.41 |
| 13 | 67 | 62 | 7.44 | 7.43 |
| 14 | 53 | 63 | 7.41 | 7.29 |
| 15 | 72 | 67 | 7.51 | 7.50 |
| 16 | 67 | 73 | 7.38 | 7.32 |
| 17 | 51 | 70 | 7.43 | 7.28 |
| 18 | 75 | 47 | 7.46 | 7.17 |
| 19 | 24 | 58 | 7.27 | 7.23 |
| 20 | 58 | 53 | 7.47 | 7.26 |
| 21 | 37 | 63 | 7.28 | 7.17 |
| 22 | 28 | 23 | 7.28 | 7.28 |
| 23 | 80 | 80 | 7.40 | 7.33 |
| 24 | 51 | 114 | 7.32 | 7.33 |
| 25 | 37 | 48 | 7.16 | 7.29 |
| 26 | 54 | 49 | 7.38 | 7.23 |
| 27 | 99 | 60 | 7.24 | 7.24 |
| 28 | 95 | 52 | 7.33 | 7.19 |
| 29 | 30 | 40 | 7.40 | 7.36 |
| 30 | 130 | 100 | 7.29 | 7.21 |
| 31 | 52 | 28 | 7.24 | 7.01 |
| 32 | 40 | 42 | 7.31 | 7.22 |
| 33 | 54 | 40 | 7.21 | 6.73 |
| 34 | 82 | 83 | 7.35 | 7.30 |
| 35 | 105 | 172 | 7.35 | 7.39 |
| 36 | 53 | 56 | 7.31 | 7.19 |
| 37 | 131 | 69 | 7.38 | 7.24 |
| 38 | 48 | 96 | 7.43 | 7.44 |
| 39 | 66 | 64 | 7.46 | 7.33 |
| 40 | 73 | 95 | 7.31 | 7.31 |
| 41 | 60 | 135 | 7.32 | 7.32 |
| 42 | 70 | 71 | 7.32 | 7.35 |
| 43 | 44 | 45 | 7.41 | 7.29 |
| 44 | 47 | 44 | 7.34 | 7.09 |
| 45 | 69 | 40 | 7.39 | 7.24 |
| 46 | 78 | 96 | 7.35 | 7.36 |
| 47 | 81 | 34 | 7.32 | 7.18 |
| 48 | 67 | 88 | 7.25 | 7.22 |
| 49 | 53 | 73 | 7.32 | 7.38 |
| 50 | 45 | 36 | 7.46 | 7.35 |
| 51 | 82 | 50 | 7.48 | 7.16 |

*A3.8. Data used in Tables 3.5 and 3.6.*

| Patient No. | pHa b | pHa e | PaCO2 b (kPa) | PaCO2 e (kPa) |
|---|---|---|---|---|
| 1 | 7.37 | 7.30 | 6.2 | 6.4 |
| 2 | 7.44 | 7.33 | 4.6 | 5.0 |
| 3 | 7.33 | 7.40 | 6.0 | 4.6 |
| 4 | 7.36 | 7.32 | 4.6 | 5.4 |
| 5 | 7.46 | 7.24 | 4.8 | 6.4 |
| 6 | 7.47 | 7.36 | 3.8 | 5.2 |
| 7 | 7.36 | 7.39 | 6.1 | 5.1 |
| 8 | 7.38 | 7.35 | 4.9 | 5.2 |
| 9 | 7.54 | 7.24 | 4.3 | 6.7 |
| 10 | 7.30 | 7.32 | 5.8 | 6.0 |
| 11 | 7.41 | 7.42 | 4.7 | 5.0 |
| 12 | 7.40 | 7.50 | 5.3 | 4.2 |
| 13 | 7.42 | 7.41 | 4.7 | 4.9 |
| 14 | 7.39 | 7.42 | 5.4 | 4.5 |
| 15 | 7.35 | 7.41 | 5.5 | 4.9 |
| 16 | 7.44 | 7.29 | 5.2 | 5.5 |
| 17 | 7.40 | 7.38 | 4.5 | 4.7 |
| 18 | 7.51 | 7.29 | 4.0 | 5.9 |
| 19 | 7.38 | 7.29 | 5.5 | 5.9 |
| 20 | 7.46 | 7.32 | 4.4 | 5.4 |
| 21 | 7.18 | 7.32 | 9.3 | 4.9 |
| 22 | 7.38 | 7.41 | 6.6 | 4.8 |
| 23 | 7.48 | 7.46 | 3.9 | 4.1 |
| 24 | 7.44 | 7.38 | 5.5 | 5.8 |
| 25 | 7.32 | 7.50 | 5.3 | 3.5 |
| 26 | 7.41 | 7.28 | 4.6 | 6.2 |
| 27 | 7.32 | 7.41 | 6.6 | 4.8 |
| 28 | 7.39 | 7.35 | 5.0 | 4.7 |
| 29 | 7.40 | 7.42 | 5.3 | 4.2 |
| 30 | 7.30 | 7.30 | 6.3 | 5.4 |
| 31 | 7.32 | 7.18 | 6.0 | 7.8 |
| 32 | 7.34 | 7.31 | 5.7 | 6.1 |
| 33 | 7.33 | 7.31 | 4.7 | 4.7 |
| 34 | 7.39 | 7.41 | 4.8 | 4.8 |
| 35 | 7.41 | 7.41 | 4.2 | 4.5 |
| 36 | 7.37 | 7.26 | 5.4 | 6.5 |
| 37 | 7.39 | 7.30 | 5.8 | 5.8 |
| 38 | 7.46 | 7.50 | 4.3 | 3.7 |
| 39 | 7.41 | 7.39 | 5.7 | 5.3 |
| 40 | 7.41 | 7.46 | 4.8 | 4.8 |
| 41 | 7.41 | 7.31 | 4.5 | 4.6 |
| 42 | 7.45 | 7.53 | 4.7 | 5.1 |
| 43 | 7.37 | 7.32 | 5.7 | 5.8 |
| 44 | 7.36 | 7.26 | 5.5 | 5.8 |
| 45 | 7.42 | 7.36 | 5.3 | 4.9 |
| 46 | 7.38 | 7.36 | 5.2 | 5.2 |
| 47 | 7.39 | 7.25 | 5.5 | 5.8 |
| 48 | 7.33 | 7.39 | 5.6 | 4.6 |
| 49 | 7.31 | 7.44 | 5.7 | 4.2 |
| 50 | 7.50 | 7.49 | 4.1 | 5.9 |
| 51 | 7.42 | 7.25 | 4.8 | 5.9 |

*A3.9. Data used in Tables 3.5 and 3.6.*

| Patient No. | PaO2 b (kPa) | PaO2 e (kPa) | BE b (mmol) | BE e (mmol) |
|---|---|---|---|---|
| 1 | 26.0 | 23.0 | 1.1 | -2.7 |
| 2 | 23.0 | 19.0 | -0.5 | -5.7 |
| 3 | 23.0 | 21.2 | -2.5 | -3.5 |
| 4 | 19.3 | 19.6 | -5.2 | -5.9 |
| 5 | 25.0 | 20.0 | 2.2 | -6.3 |
| 6 | 21.0 | 21.5 | -2.5 | -3.3 |
| 7 | 25.0 | 23.0 | -0.2 | -1.4 |
| 8 | 23.0 | 21.0 | -2.0 | -4.0 |
| 9 | 28.0 | 20.0 | 4.8 | -5.1 |
| 10 | 20.4 | 22.7 | -4.8 | -2.7 |
| 11 | 22.0 | 24.2 | -1.9 | -0.4 |
| 12 | 24.0 | 24.0 | 0.0 | 0.0 |
| 13 | 24.0 | 23.0 | -1.6 | -1.4 |
| 14 | 25.0 | 23.0 | 0.0 | 0.0 |
| 15 | 23.0 | 22.0 | -2.0 | -2.0 |
| 16 | 26.5 | 19.6 | 2.5 | 6.1 |
| 17 | 21.0 | 20.5 | -3.5 | -3.9 |
| 18 | 25.0 | 20.0 | 1.8 | -4.8 |
| 19 | 23.0 | 21.0 | -1.0 | -4.9 |
| 20 | 24.0 | 19.5 | 0.4 | -6.0 |
| 21 | 26.0 | 18.5 | -3.3 | -6.6 |
| 22 | 29.2 | 23.0 | 3.7 | -1.6 |
| 23 | 22.0 | 21.5 | -1.0 | -2.0 |
| 24 | 27.5 | 25.3 | 3.2 | 0.4 |
| 25 | 20.3 | 22.9 | -5.0 | 2.0 |
| 26 | 22.0 | 21.3 | -2.0 | -4.6 |
| 27 | 24.5 | 23.0 | -1.8 | -1.6 |
| 28 | 22.2 | 19.0 | -2.2 | -5.6 |
| 29 | 25.0 | 22.0 | 0.0 | -4.4 |
| 30 | 22.0 | 20.0 | -3.0 | -5.0 |
| 31 | 22.0 | 18.0 | -2.5 | -8.0 |
| 32 | 23.0 | 23.0 | -2.3 | -3.3 |
| 33 | 20.7 | 18.0 | -4.4 | 1.8 |
| 34 | 21.9 | 24.0 | -2.2 | 0.5 |
| 35 | 19.8 | 18.8 | -3.6 | -7.2 |
| 36 | 23.0 | 21.3 | -1.9 | -4.9 |
| 37 | 26.7 | 20.9 | 1.9 | -4.7 |
| 38 | 23.0 | 21.7 | 0.7 | 1.1 |
| 39 | 26.6 | 23.7 | 2.0 | -0.9 |
| 40 | 22.5 | 24.2 | -1.5 | -0.5 |
| 41 | 21.2 | 21.9 | -2.6 | -3.7 |
| 42 | 24.2 | 21.1 | 0.8 | -1.7 |
| 43 | 24.3 | 21.9 | -0.9 | -3.6 |
| 44 | 23.1 | 18.5 | -2.0 | -7.6 |
| 45 | 25.5 | 21.0 | 1.2 | -4.1 |
| 46 | 22.3 | 21.4 | -2.2 | -3.5 |
| 47 | 24.0 | 18.0 | 0.0 | -8.5 |
| 48 | 21.6 | 20.8 | -3.7 | -3.4 |
| 49 | 21.3 | 22.4 | -4.3 | -1.3 |
| 50 | 24.5 | 24.0 | 1.9 | 1.5 |
| 51 | 23.0 | 18.8 | -0.9 | -7.6 |

*A3.10. Data used in Tables 3.5 and 3.6.*

| Patient No. | Urine (ml) | CVP b (mmHg) | CVP e (mmHg) |
|---|---|---|---|
| 1 | 116 | 9 | 9 |
| 2 | 111 | 7 | 9 |
| 3 | 48 | 8 | 9 |
| 4 | 39 | 6 | 9 |
| 5 | 58 | 4 | 4 |
| 6 | 51 | 9 | 6 |
| 7 | 43 | 7 | 4 |
| 8 | 60 | 10 | 6 |
| 9 | 68 | 4 | 3 |
| 10 | 110 | 11 | 8 |
| 11 | 167 | 4 | 4 |
| 12 | 71 | 6 | 5 |
| 13 | 150 | 3 | 7 |
| 14 | 61 | 12 | 4 |
| 15 | 80 | 7 | 8 |
| 16 | 62 | 8 | 5 |
| 17 | 46 | 4 | 6 |
| 18 | 109 | 3 | 6 |
| 19 | 167 | 10 | 12 |
| 20 | 41 | 5 | 7 |
| 21 | 114 | 7 | 8 |
| 22 | 38 | 8 | 7 |
| 23 | 97 | 7 | 6 |
| 24 | 49 | 4 | 7 |
| 25 | 296 | 10 | 12 |
| 26 | 91 | 3 | 5 |
| 27 | 193 | 8 | 6 |
| 28 | 128 | 5 | 5 |
| 29 | 143 | 6 | 4 |
| 30 | 36 | 6 | 9 |
| 31 | 51 | 5 | 6 |
| 32 | 44 | 8 | 6 |
| 33 | 38 | 8 | 10 |
| 34 | 42 | 9 | 11 |
| 35 | 41 | 5 | 5 |
| 36 | 53 | 4 | 8 |
| 37 | 43 | 7 | 3 |
| 38 | 130 | 6 | 6 |
| 39 | 40 | 6 | 4 |
| 40 | 34 | 3 | 6 |
| 41 | 70 | 4 | 3 |
| 42 | 55 | 10 | 7 |
| 43 | 66 | 10 | 7 |
| 44 | 125 | 6 | 11 |
| 45 | 67 | 2 | 4 |
| 46 | 99 | 12 | 8 |
| 47 | 108 | 7 | 6 |
| 48 | 141 | 9 | 7 |
| 49 | 117 | 10 | 9 |
| 50 | 44 | 8 | 5 |
| 51 | 57 | 5 | 5 |

## A4.0. APPENDIX TO CHAPTER 4.0.

### Dilutions for standard curves used in chromogenic assays

*A4.1. Dilutions for preparation of standard curve for determination of AT III by chromogenic substrate assay (4.5.2.3).*

| Standard (%) | Diluted plasma | Buffer |
|---|---|---|
| 125 | 750 µl | 3000 µl |
| 100 | 600 µl | 3000 µl |
| from the 100% the following were prepared: | | |
| 75 | 600 µl | 200 µl |
| 50 | 400 µl | 400 µl |
| 25 | 200 µl | 600 µl |

*A4.2. Dilutions for preparation of standard curve for determination of C1-inhibitor by chromogenic substrate assay (4.5.3.3).*

| Standard (%) | Plasma | Buffer A |
|---|---|---|
| 150 | 75 µl | 500 µl |
| 125 | 62.5 µl | 500 µl |
| 100 | 25 µl | 250 µl |
| 75 | 37.5 µl | 500 µl |
| 50 | 25 µl | 500 µl |
| 25 | 12.5 µl | 500 µl |

*A4.3. Dilutions for preparation of standard curve for determination of plasminogen by chromogenic substrate assay (4.5.4.3).*

| Standard (%) | Diluted plasma | Buffer |
|---|---|---|
| 150 | 75 µl | 2000 µl |
| 100 | 50 µl | 2000 µl |
| from the 100% the following were prepared: | | |
| 75 | 600 µl | 200 µl |
| 50 | 400 µl | 400 µl |
| 25 | 200 µl | 600 µl |

*A4.4. Dilutions for preparation of standard curve for determination of prekallikrein by chromogenic substrate assay (4.5.5.3).*

| Standard (%) | Acetone treated plasma | Buffer |
|---|---|---|
| 150 | 75 µl | 2925 µl |
| 100 | 50 µl | 2950 µl |
| 75 | 200 µl of 100% | 100 µl |
| 50 | 100 µl of 100% | 200 µl |
| 25 | 12.5 µl of 100% | 400 µl |

*A4.5. Dilutions for preparation of standard curve for determination of heparin co-factor II by chromogenic substrate assay (4.5.6.3).*

| Standard (%) | Diluted plasma | Buffer |
|---|---|---|
| 150 | 25 µl | 1500 µl |
| 100 | 25 µl | 2000 µl |
| from the 100% the following were prepared: | | |
| 75 | 600 µl | 200 µl |
| 50 | 400 µl | 400 µl |
| 25 | 200 µl | 600 µl |

*A4.6. Initial dilutions for preparation of standard curve for determination of alpha-2 macroglobulin by chromogenic substrate assay (4.5.7.3)*

| Standard (%) | Plasma | Buffer |
|---|---|---|
| 200 | undiluted | |
| 100 | undiluted | |
| 75 | 150 µl | 50 µl |
| 50 | 100 µl | 100 µl |
| 25 | 50 µl | 150 µl |

The 25-100% samples were then further diluted by adding 25 ml to 3975 ml of buffer.

The 200% standard was prepared by adding 50 ml to 3950 ml of buffer.

*A4.7. Initial dilutions for preparation of standard curve for determination of $\alpha_1$-antitrypsin by chromogenic substrate assay (4.5.8.3).*

| Standard (%) | Plasma | Buffer A |
|---|---|---|
| 195 | undiluted | |
| 100 | undiluted | |
| 75 | 150 µl | 50 µl |
| 50 | 100 µl | 100 µl |
| 25 | 50 µl | 150 µl |

The 25-100% samples were then further diluted by adding 25µl to 975µl of buffer A. The 195% standard was prepared by adding 50µl to 950µl of buffer A. Standard curve samples were then further diluted by mixing 50µl with 1950µl of buffer B.

*A4.8. Dilutions for preparation of standard curve for determination of factor XII by chromogenic substrate assay (4.5.9.3).*

| Standard (%) | Plasma | Buffer |
|---|---|---|
| 200 | 350µl | 350µl |
| 150 | 150 µl (of 200%) | 50 µl |
| 100 | 400 µl (of 200%) | 400 µl |
| 75 | 300 µl (of 100%) | 100 µl |
| 50 | 200 µl (of 100%) | 200 µl |
| 25 | 100 µl (of 100%) | 300 µl |

**ACL 300R assay conditions programmed for chromogenic assays detailed in chapter 4.0.**

*A4.9. ACL 300R conditions programmed for AT III assay (5.5.2.4):*

| | |
|---|---|
| Loading: | |
| Sample volume | 100 ml |
| Bovine thrombin (position 2) | 25 ml |
| Substrate | 50 ml |
| Incubation and aquisition: | |
| Reaction time | 30 s |
| Inter Ramp interval | 5 s |
| Delay time | 0 s |
| Aquisition time | 60 s |
| Speed | 1200 rpm |

*A4.10. ACL 300R conditions programmed for C1-INH assay (5.5.3.4):*

| | |
|---|---|
| Loading: | |
| Sample volume | 25 ml |
| C1-esterase (position 2) | 25 ml |
| C1 substrate (position 3) | 100 ml |
| Incubation and aquisition: | |
| Reaction time | 300 s |
| Inter Ramp interval | 3 s |
| Delay time | 0 s |
| Aquisition time | 180 s |
| Speed | 1200 rpm |

*A4.11. ACL 300R conditions programmed for plasminogen assay (5.5.3.5):*

| | |
|---|---|
| Loading: | |
| Sample volume | 60 µl |
| Streptokinase (position 2) | 30 µl |
| Substrate (position 3) | 60 µl |
| Incubation and aquisition: | |
| Reaction time | 600 sec |
| Inter Ramp interval | 5 sec |
| Delay time | 0 sec |
| Aquisition time | 300 sec |
| Speed | 1200 rpm |

*A4.12. ACL 300R conditions programmed for Hep-co II assay (5.5.6.4):*

| | |
|---|---|
| Loading: | |
| Sample volume | 100 µl |
| Streptokinase (position 2) | 50 µl |
| Substrate (position 3) | 50 µl |
| Incubation and aquisition: | |
| Reaction time | 300 sec |
| Inter Ramp interval | 3 sec |
| Delay time | 0 sec |
| Aquisition time | 200 sec |
| Speed | 1200 rpm |

*A4.13. Data used in Tables 4.3 and 4.4.*

| Patient no. | Sex | Age (years) | Height (cm) | Weight (kg) | Type of surgery | Major complications |
|---|---|---|---|---|---|---|
| 1 | F | 69 | 165 | 48 | CABG | - |
| 2 | M | 52 | 180 | 94 | CABG | - |
| 3 | M | 75 | 145 | 68 | CABG | - |
| 4 | M | 59 | 183 | 88 | CABG | - |
| 5 | M | 67 | 175 | 64 | AVR | - |
| 6 | F | 40 | 150 | 57 | AVR/MVR | - |
| 7 | M | 60 | 164 | 70 | CABG | - |
| 8 | F | 79 | 153 | 59 | MVR | MODS (Died) |
| 9 | M | 60 | 180 | 81 | CABG | Haematemesis |
| 10 | F | 82 | 160 | 46 | AVR | - |
| 11 | F | 75 | 150 | 68 | AAA | MODS |
| 12 | M | 46 | 188 | 75 | Aorto-bi-fem | - |
| 13 | M | 67 | 172 | 77 | CABG | MODS (Died) |
| 14 | M | 74 | 173 | 75 | CABG | - |
| 15 | M | 64 | 187 | 99 | CABG | - |
| 16 | M | 58 | 174 | 91 | CABG | MODS (Died) |
| 17 | M | 67 | 179 | 70 | CABG | - |
| 18 | M | 46 | 174 | 56 | CABG | - |
| 19 | M | 66 | 173 | 92 | CABG | - |
| 20 | F | 62 | 150 | 56 | AAA | - |
| 21 | M | 67 | 172 | 74 | AVR | - |
| 22 | M | 71 | 168 | 82 | AAA | Respiratory failure |
| 23 | M | 43 | 175 | 80 | CABG | CVA |
| 24 | M | 79 | 167 | 74 | MVR | - |
| 25 | M | 67 | 180 | 73 | CABG | - |
| 26 | F | 77 | 158 | 55 | AAA | Paralytic ileus |

*A4.14. Data used in Table 4.6 and Figure 4.3.*

| Patient no. | pHi at end of surgery | AT III (base) (IU/dl) | AT III (24h) (IU/dl) | C1-INH (base) (IU/dl) | C1-INH (24h) (IU/dl) |
|---|---|---|---|---|---|
| 1 | 7.44 | 115.0 | 92.0 | 77.0 | 86.0 |
| 2 | 7.38 | 106.0 | 76.0 | 86.0 | 66.0 |
| 3 | 7.35 | 84.0 | 83.0 | 66.0 | 83.0 |
| 4 | 7.29 | 106.0 | 56.0 | 98.0 | 52.0 |
| 5 | 7.33 | 87.0 | 17.0 | 89.0 | 108.0 |
| 6 | 7.36 | 77.0 | 40.0 | 124.0 | 111.0 |
| 7 | 7.22 | 148.0 | 104.0 | 131.0 | 118.0 |
| 8 | 6.73 | 159.0 | 12.0 | 149.0 | 84.0 |
| 9 | 7.31 | 125.0 | 109.0 | 72.0 | 68.0 |
| 10 | 7.3 | 86.0 | 52.0 | 80.0 | 42.0 |
| 11 | 7.04 | 106.0 | 69.0 | 181.0 | 95.0 |
| 12 | 7.28 | 51.0 | 67.0 | 168.0 | 74.0 |
| 13 | 7.24 | 72.0 | 46.0 | 110.0 | 63.0 |
| 14 | 7.33 | 95.0 | 82.0 | 61.0 | 91.0 |
| 15 | 7.36 | 100.0 | 86.0 | 77.0 | 73.0 |
| 16 | 7.18 | 46.0 | 40.0 | 58.0 | 29.0 |
| 17 | 7.16 | 75.0 | 80.0 | 68.0 | 58.0 |
| 18 | 7.22 | 106.0 | 93.0 | 86.0 | 109.0 |
| 19 | 7.34 | 85.0 | 87.0 | 76.0 | 72.0 |
| 20 | 7.36 | 116.0 | 91.0 | 136.0 | 99.0 |
| 21 | 7.32 | 118.0 | 100.0 | 111.0 | 94.0 |
| 22 | 7.21 | 73.0 | 34.0 | 128.0 | 110.0 |
| 23 | 7.01 | 105.0 | 15.0 | 125.0 | 118.0 |
| 24 | 7.24 | 113.0 | 103.0 | 78.0 | 68.0 |
| 25 | 7.15 | 102.0 | 83.0 | 89.0 | 67.0 |
| 26 | 7.26 | 169.0 | 102.0 | 105.0 | 119.0 |

*A4.15. Data used in Table 4.6.*

| Patient no. | PKK (base) (IU/dl) | PKK (24h) (IU/dl) | FXII (base) (IU/dl) | FXII (24 h) (IU/dl) | Pϑ (base) (IU/dl) | Pϑ (24h) (IU/dl) |
|---|---|---|---|---|---|---|
| 1 | 99.0 | 41.0 | 107.0 | 63.0 | 79.0 | 52.0 |
| 2 | 102.0 | 50.0 | 123.0 | 61.0 | 82.0 | 54.0 |
| 3 | 118.0 | 65.0 | 87.0 | 56.0 | 98.0 | 60.0 |
| 4 | 111.0 | 77.0 | 110.0 | 110.0 | 92.0 | 60.0 |
| 5 | 89.0 | 40.0 | 97.0 | 51.0 | 73.0 | 24.0 |
| 6 | 131.0 | 87.0 | 37.0 | 38.0 | 95.0 | 21.0 |
| 7 | 100.0 | 64.0 | 88.0 | 52.0 | 64.0 | 51.0 |
| 8 | 86.0 | 65.0 | 65.0 | 51.0 | 65.0 | 32.0 |
| 9 | 123.0 | 50.0 | 75.0 | 64.0 | 121.0 | 97.0 |
| 10 | 67.0 | 26.0 | 68.0 | 35.0 | 46.0 | 33.0 |
| 11 | 93.0 | 44.0 | 69.0 | 45.0 | 76.0 | 51.0 |
| 12 | 147.0 | 60.0 | 90.0 | 64.0 | 109.0 | 59.0 |
| 13 | 50.0 | 40.0 | 55.0 | 34.0 | 19.0 | 31.0 |
| 14 | 105.0 | 42.0 | 82.0 | 33.0 | 100.0 | 59.0 |
| 15 | 103.0 | 45.0 | 98.0 | 64.0 | 90.0 | 66.0 |
| 16 | 67.0 | 35.0 | 61.0 | 50.0 | 75.0 | 47.0 |
| 17 | 51.0 | 35.0 | 57.0 | 54.0 | 67.0 | 23.0 |
| 18 | 99.0 | 55.0 | 119.0 | 76.0 | 96.0 | 76.0 |
| 19 | 85.0 | 32.0 | 55.0 | 31.0 | 82.0 | 69.0 |
| 20 | 116.0 | 66.0 | 94.0 | 76.0 | 88.0 | 58.0 |
| 21 | 108.0 | 119.0 | 74.0 | 65.0 | 95.0 | 71.0 |
| 22 | 103.0 | 69.0 | 100.0 | 52.0 | 104.0 | 58.0 |
| 23 | 141.0 | 60.0 | 65.0 | 27.0 | 106.0 | 43.0 |
| 24 | 105.0 | 62.0 | 67.0 | 50.0 | 106.0 | 58.0 |
| 25 | 121.0 | 86.0 | 72.0 | 59.0 | 109.0 | 75.0 |
| 26 | 128.0 | 114.0 | 71.0 | 63.0 | 111.0 | 93.0 |

*A4.15. Data used in Table 4.6.*

| Patient no. | $\alpha_2$M (base) (IU/dl) | $\alpha_2$M (24h) (IU/dl) | Hep-co II (base) (IU/dl) | Hep-co II (24h) (IU/dl) | $\alpha_1$-AT (base) (IU/dl) | $\alpha_1$-AT (24h) (IU/dl) |
|---|---|---|---|---|---|---|
| 1 | 58.0 | 35.0 | 94.0 | 60.0 | 89.0 | 128.0 |
| 2 | 49.0 | 27.0 | 95.0 | 53.0 | 93.0 | 93.0 |
| 3 | 35.0 | 25.0 | 86.0 | 53.0 | 99.0 | 128.0 |
| 4 | 38.0 | 34.0 | 88.0 | 53.0 | 80.0 | 121.0 |
| 5 | 66.0 | 22.0 | 88.0 | 53.0 | 104.0 | 72.0 |
| 6 | 84.0 | 67.0 | 52.0 | 40.0 | 134.0 | 99.0 |
| 7 | 59.0 | 33.0 | 67.0 | 40.0 | 104.0 | 99.0 |
| 8 | 62.0 | 44.0 | 52.0 | 44.0 | 146.0 | 96.0 |
| 9 | 73.0 | 49.0 | 84.0 | 50.0 | 148.0 | 205.0 |
| 10 | 71.0 | 21.0 | 57.0 | 36.0 | 97.0 | 74.0 |
| 11 | 59.0 | 42.0 | 88.0 | 54.0 | 78.0 | 50.0 |
| 12 | 44.0 | 31.0 | 103.0 | 72.0 | 103.0 | 77.0 |
| 13 | 44.0 | 24.0 | 86.0 | 42.0 | 180.0 | 87.0 |
| 14 | 45.0 | 24.0 | 75.0 | 49.0 | 106.0 | 157.0 |
| 15 | 53.0 | 29.0 | 106.0 | 129.0 | 106.0 | 129.0 |
| 16 | 147.0 | 40.0 | 69.0 | 42.0 | 109.0 | 68.0 |
| 17 | 30.0 | 30.0 | 65.0 | 46.0 | 88.0 | 92.0 |
| 18 | 85.0 | 51.0 | 97.0 | 61.0 | 103.0 | 164.0 |
| 19 | 47.0 | 41.0 | 83.0 | 47.0 | 88.0 | 123.0 |
| 20 | 66.0 | 44.0 | 100.0 | 71.0 | 123.0 | 95.0 |
| 21 | 53.0 | 33.0 | 71.0 | 63.0 | 139.0 | 123.0 |
| 22 | 47.0 | 27.0 | 99.0 | 65.0 | 119.0 | 91.0 |
| 23 | 57.0 | 25.0 | 81.0 | 32.0 | 106.0 | 75.0 |
| 24 | 52.0 | 40.0 | 93.0 | 42.0 | 101.0 | 142.0 |
| 25 | 53.0 | 36.0 | 91.0 | 60.0 | 104.0 | 95.0 |
| 26 | 50.0 | 38.0 | 97.0 | 76.0 | 135.0 | 121.0 |

*A4.16. Data used in Tables 4.5 and 4.6 and Figure 4.2*

| Patient no. | NE (base) (µg/l) | NE (24h) (µg/l) | Hct (base) (%) | Plt (base) ($10^{12}$/l) | WBC (base) ($10^{9}$/l) | Hct (24h) (%) | Plts (24h) ($10^{12}$/l) | WBC (24h) ($10^{12}$/l) |
|---|---|---|---|---|---|---|---|---|
| 1 | 98.0 | 184.0 | 42.0 | 208.0 | 4.7 | 33.0 | 128.0 | 9.3 |
| 2 | 123.0 | 340.0 | 43.0 | 249.0 | 10.5 | 27.0 | 134.0 | 11.8 |
| 3 | 59.0 | 142.0 | 38.0 | 295.0 | 8.0 | 35.0 | 92.0 | 10.8 |
| 4 | 136.0 | 142.0 | 46.0 | 219.0 | 5.4 | 38.0 | 138.0 | 7.7 |
| 5 | 115.0 | 83.0 | 49.0 | 165.0 | 5.7 | 36.0 | 78.0 | 11.1 |
| 6 | 239.0 | 247.0 | 47.0 | 229.0 | 8.1 | 25.0 | 179.0 | 9.3 |
| 7 | 153.0 | 230.0 | 35.0 | 302.0 | 6.0 | 29.0 | 247.0 | 9.4 |
| 8 | 120.0 | 1041.0 | 46.0 | 228.0 | 7.2 | 30.2 | 139.0 | 9.8 |
| 9 | 613.0 | 413.0 | 39.0 | 192.0 | 5.6 | 32.0 | 123.0 | 9.2 |
| 10 | 87.0 | 588.0 | 39.0 | 241.0 | 5.1 | 32.0 | 198.0 | 6.8 |
| 11 | 174.0 | 403.0 | 48.0 | 211.0 | 7.7 | 28.0 | 54.0 | 7.3 |
| 12 | 76.0 | 498.0 | 43.0 | 188.0 | 8.0 | 32.0 | 115.0 | 10.2 |
| 13 | 83.0 | 567.0 | 36.0 | 314.0 | 6.5 | 27.0 | 118.0 | 7.6 |
| 14 | 140.0 | 283.0 | 31.0 | 239.0 | 4.8 | 36.0 | 209.0 | 10.7 |
| 15 | 98.0 | 92.0 | 42.0 | 198.0 | 5.8 | 27.0 | 196.0 | 10.5 |
| 16 | 80.0 | 500.0 | 44.0 | 227.0 | 8.3 | 33.0 | 107.0 | 12.5 |
| 17 | 174.0 | 254.0 | 32.0 | 195.0 | 5.2 | 41.0 | 110.0 | 8.5 |
| 18 | 65.0 | 264.0 | 51.0 | 249.0 | 12.1 | 41.0 | 203.0 | 9.1 |
| 19 | 104.0 | 110.0 | 45.0 | 194.0 | 9.5 | 33.0 | 135.0 | 11.2 |
| 20 | 208.0 | 184.0 | 40.0 | 212.0 | 7.1 | 27.0 | 138.0 | 7.3 |
| 21 | 91.0 | 214.0 | 51.0 | 190.0 | 6.6 | 26.0 | 77.0 | 7.2 |
| 22 | 89.0 | 266.0 | 41.0 | 223.0 | 7.6 | 36.0 | 172.0 | 9.8 |
| 23 | 201.0 | 211.0 | 42.0 | 299.0 | 8.6 | 34.0 | 211.0 | 12.2 |
| 24 | 718.0 | 386.0 | 47.0 | 181.0 | 6.5 | 31.0 | 101.0 | 11.1 |
| 25 | 111.0 | 297.0 | 43.0 | 188.0 | 8.2 | 32.0 | 114.0 | 13.9 |
| 26 | 64.0 | 834.0 | 47.0 | 181.0 | 6.5 | 25.0 | 108.0 | 11.9 |

# A5.0. APPENDIX TO CHAPTER 5.0

*A5.1. Data used in Table 5.1 and 5.2.*

| Patient no. | Sex | Age (years) | Height (cm) | Weight (kg) | Type of surgery | Major complications |
|---|---|---|---|---|---|---|
| 1 | F | 69 | 165 | 48 | CABG | - |
| 2 | M | 52 | 180 | 94 | CABG | - |
| 3 | M | 75 | 145 | 68 | CABG | - |
| 4 | M | 59 | 183 | 88 | CABG | - |
| 5 | M | 67 | 175 | 64 | AVR | - |
| 6 | F | 40 | 150 | 57 | AVR/MVR | - |
| 7 | M | 60 | 164 | 70 | CABG | - |
| 8 | F | 79 | 153 | 59 | MVR | MODS (Died) |
| 9 | M | 60 | 180 | 81 | CABG | Haematemesis |
| 10 | F | 82 | 160 | 46 | AVR | - |
| 11 | F | 75 | 150 | 68 | AAA | MODS |
| 12 | M | 46 | 188 | 75 | Aorto-bi-fem | - |
| 13 | M | 67 | 172 | 77 | CABG | MODS (Died) |
| 14 | M | 74 | 173 | 75 | CABG | - |
| 15 | M | 64 | 187 | 99 | CABG | - |
| 16 | M | 58 | 174 | 91 | CABG | MODS (Died) |
| 17 | M | 67 | 179 | 70 | CABG | - |
| 18 | M | 46 | 174 | 56 | CABG | - |
| 19 | M | 66 | 173 | 92 | CABG | - |
| 20 | F | 62 | 150 | 56 | AAA | - |
| 21 | M | 67 | 172 | 74 | AVR | - |
| 22 | M | 71 | 168 | 82 | AAA | Respiratory failure |
| 23 | M | 43 | 175 | 80 | CABG | CVA |
| 24 | M | 79 | 167 | 74 | MVR | - |
| 25 | M | 67 | 180 | 73 | CABG | - |
| 26 | F | 77 | 128 | 55 | AAA | Paralytic ileus |

*A5.2. Data used in Table 5.3 and Figures 5.1 and 5.2.*

| Patient no. | pHi at end of surgery | IgG EndoCAb (Baseline) (%) | IgG EndoCAb (24h) (%) | IgM EndoCAb (Baseline) (%) | IgM EndoCAb (24h) (%) |
|---|---|---|---|---|---|
| 1 | 7.44 | 207.51 | 116.97 | 96.31 | 82.56 |
| 2 | 7.38 | 478.5 | 136.24 | 93.09 | 44.27 |
| 3 | 7.35 | 273.95 | 124.69 | 207.23 | 109.96 |
| 4 | 7.29 | 235.25 | 57.5 | 121.3 | 149.38 |
| 5 | 7.33 | 264.63 | 154.04 | 225.86 | 90.91 |
| 6 | 7.36 | 449.46 | 109.62 | 107.23 | 51.9 |
| 7 | 7.22 | 206.64 | 96.28 | 148.68 | 77.9 |
| 8 | 6.73 | 70.64 | 104.38 | 15.67 | 47.89 |
| 9 | 7.31 | 234.03 | 66.03 | 115.39 | 36.3 |
| 10 | 7.3 | 117.36 | 76.45 | 232.23 | 137.83 |
| 11 | 7.04 | 151.18 | 82.18 | 92.13 | 62.18 |
| 12 | 7.28 | 150.66 | 69.71 | 107.11 | 67.78 |
| 13 | 7.24 | 112.16 | 131.34 | 47.61 | 55.71 |
| 14 | 7.33 | 642.58 | 371.73 | 38.59 | 15.45 |
| 15 | 7.36 | 82.9 | 95.86 | 283.27 | 227.29 |
| 16 | 7.18 | 168.53 | 106.63 | 86.98 | 48.57 |
| 17 | 7.16 | 218.75 | 204.64 | 15.51 | 15.03 |
| 18 | 7.22 | 81.96 | 66.36 | 39.51 | 26.29 |
| 19 | 7.34 | 1600 | 608.88 | 99.49 | 34.84 |
| 20 | 7.36 | 846.69 | 430.85 | 46.69 | 37.61 |
| 21 | 7.32 | 445.25 | 57.5 | 121.3 | 149.38 |
| 22 | 7.21 | 176.4 | 102.26 | 28.33 | 14.89 |
| 23 | 7.01 | 124.73 | 60.62 | 190.41 | 88.21 |
| 24 | 7.24 | 148.87 | 74.68 | 48.87 | 23.21 |
| 25 | 7.15 | 227.29 | 317.63 | 29.99 | 21.97 |
| 26 | 7.26 | 66.35 | 77.07 | 129.53 | 105.67 |

*A5.3. Data used in Table 5.3.*

| Patient no. | Hct (Baseline) (%) | Hct (24h) (%) | Plts (Baseline) ($10^{12}$/l) | Plt (24h) ($10^{12}$/l) | WBC (Baseline) ($10^9$/l) | WBC (24h) ($10^9$/l) |
|---|---|---|---|---|---|---|
| 1 | 42 | 33 | 208 | 128 | 4.7 | 9.3 |
| 2 | 43 | 27 | 249 | 134 | 10.5 | 11.8 |
| 3 | 38 | 35 | 295 | 92 | 8 | 10.8 |
| 4 | 46 | 38 | 219 | 138 | 5.4 | 7.7 |
| 5 | 49 | 36 | 165 | 78 | 5.7 | 11.1 |
| 6 | 47 | 25 | 229 | 179 | 8.1 | 9.3 |
| 7 | 35 | 29 | 302 | 247 | 6 | 9.4 |
| 8 | 46 | 30.2 | 228 | 139 | 7.2 | 9.8 |
| 9 | 39 | 32 | 192 | 123 | 5.6 | 9.2 |
| 10 | 39 | 32 | 241 | 198 | 5.1 | 6.8 |
| 11 | 48 | 28 | 211 | 54 | 7.7 | 7.3 |
| 12 | 43 | 32 | 188 | 115 | 8 | 10.2 |
| 13 | 36 | 27 | 314 | 118 | 6.5 | 7.6 |
| 14 | 31 | 36 | 239 | 209 | 4.8 | 10.7 |
| 15 | 42 | 27 | 198 | 196 | 5.8 | 10.5 |
| 16 | 44 | 33 | 227 | 107 | 8.3 | 12.5 |
| 17 | 32 | 41 | 195 | 110 | 5.2 | 8.5 |
| 18 | 51 | 41 | 249 | 203 | 12.1 | 9.1 |
| 19 | 45 | 33 | 194 | 135 | 9.5 | 11.2 |
| 20 | 40 | 27 | 212 | 138 | 7.1 | 7.3 |
| 21 | 51 | 26 | 190 | 77 | 6.6 | 7.2 |
| 22 | 41 | 36 | 223 | 172 | 7.6 | 9.8 |
| 23 | 42 | 34 | 299 | 211 | 8.6 | 12.2 |
| 24 | 47 | 31 | 181 | 101 | 6.5 | 11.1 |
| 25 | 43 | 32 | 188 | 114 | 8.2 | 13.9 |
| 26 | 47 | 25 | 181 | 108 | 6.5 | 11.9 |

# A6.0. APPENDIX TO CHAPTER 6.0

*A6.1. Data used in Table 6.1.*

| Patient no. | Anaesthetic group | Sex | Age (years) | Height (cm) | Weight (kg) | Duration of surgery (mins) |
|---|---|---|---|---|---|---|
| 1 | Isoflurane | F | 69 | 165 | 48 | 180 |
| 2 | Enflurane | M | 79 | 167 | 74 | 270 |
| 3 | Enflurane | M | 57 | 165 | 96 | 180 |
| 4 | Propofol | M | 60 | 180 | 100 | 210 |
| 5 | Enflurane | M | 67 | 174 | 76 | 180 |
| 6 | Enflurane | M | 52 | 180 | 94 | 180 |
| 7 | Propofol | M | 58 | 178 | 70 | 200 |
| 8 | Propofol | M | 75 | 145 | 68 | 240 |
| 9 | Enflurane | M | 67 | 179 | 70 | 220 |
| 10 | Propofol | F | 82 | 160 | 46 | 160 |
| 11 | Isoflurane | M | 60 | 180 | 81 | 130 |
| 12 | Enflurane | M | 64 | 187 | 99 | 190 |
| 13 | Propofol | M | 66 | 170 | 60 | 266 |
| 14 | Isoflurane | M | 74 | 173 | 75 | 240 |
| 15 | Propofol | M | 65 | 172 | 92 | 180 |
| 16 | Enflurane | M | 57 | 180 | 82 | 200 |
| 17 | Isoflurane | M | 61 | 177 | 96 | 210 |
| 18 | Enflurane | M | 54 | 160 | 81 | 210 |
| 19 | Enflurane | M | 70 | 171 | 69 | 180 |
| 20 | Isoflurane | M | 59 | 180 | 82 | 300 |
| 21 | Propofol | M | 42 | 180 | 100 | 220 |
| 22 | Propofol | M | 69 | 172 | 78 | 180 |
| 23 | Propofol | F | 60 | 156 | 76 | 240 |
| 24 | Enflurane | F | 72 | 156 | 52 | 220 |
| 25 | Isoflurane | F | 73 | 161 | 68 | 180 |
| 26 | Isoflurane | M | 51 | 175 | 73 | 190 |
| 27 | Isoflurane | M | 68 | 161 | 76 | 230 |
| 28 | Isoflurane | M | 61 | 172 | 78 | 150 |
| 29 | Propofol | M | 55 | 181 | 81 | 180 |
| 30 | Isoflurane | M | 60 | 180 | 79 | 180 |

*A6.2. Data used in Table 6.6.*

| Patient no. | Days in ITU | Days in Hospital | Major complications | Death |
|---|---|---|---|---|
| 1 | 1 | 6 | 0 | 0 |
| 2 | 1 | 8 | 0 | 0 |
| 3 | 1 | 7 | 0 | 0 |
| 4 | 1 | 6 | 0 | 0 |
| 5 | 2 | 8 | 0 | 0 |
| 6 | 2 | 7 | 0 | 0 |
| 7 | 2 | 8 | 0 | 0 |
| 8 | 1 | 7 | 0 | 0 |
| 9 | 2 | 12 | 1 | 0 |
| 10 | 1 | 8 | 0 | 0 |
| 11 | 1 | 6 | 0 | 0 |
| 12 | 1 | 6 | 0 | 0 |
| 13 | 1 | 7 | 0 | 0 |
| 14 | 1 | 5 | 0 | 0 |
| 15 | 1 | 6 | 0 | 0 |
| 16 | 4 | 8 | 0 | 0 |
| 17 | 1 | 11 | 1 | 0 |
| 18 | 4 | 10 | 1 | 0 |
| 19 | 2 | 8 | 0 | 0 |
| 20 | 1 | 7 | 0 | 0 |
| 21 | 1 | 8 | 0 | 0 |
| 22 | 1 | 6 | 0 | 0 |
| 23 | 1 | 7 | 0 | 0 |
| 24 | 1 | 5 | 0 | 0 |
| 25 | 4 | 8 | 0 | 0 |
| 26 | 1 | 6 | 0 | 0 |
| 27 | 1 | 7 | 0 | 0 |
| 28 | 1 | 6 | 0 | 0 |
| 29 | 1 | 8 | 0 | 0 |
| 30 | 1 | 6 | 0 | 0 |

*A6.3. Data used in Tables 6.2, 6.3 and 6.4 and Figure 6.1.*

| Patient no. | HRb | HRe | SBPb (mmHg) | SBPe (mmHg) | DBP b (mmHg) | DBP e (mmHg) | MBP b (mmHg) | MBP e (mmHg) |
|---|---|---|---|---|---|---|---|---|
| 1 | 64 | 65 | 131 | 154 | 55 | 71 | 85 | 104 |
| 2 | 58 | 100 | 123 | 120 | 60 | 70 | 88 | 84 |
| 3 | 58 | 86 | 108 | 100 | 60 | 56 | 65 | 70 |
| 4 | 57 | 67 | 105 | 106 | 45 | 60 | 65 | 70 |
| 5 | 42 | 80 | 110 | 111 | 44 | 57 | 65 | 68 |
| 6 | 77 | 89 | 110 | 150 | 70 | 70 | 63 | 84 |
| 7 | 56 | 72 | 126 | 111 | 67 | 83 | 85 | 72 |
| 8 | 74 | 90 | 131 | 110 | 83 | 63 | 101 | 78 |
| 9 | 72 | 102 | 91 | 135 | 37 | 56 | 57 | 80 |
| 10 | 67 | 90 | 115 | 160 | 47 | 60 | 76 | 84 |
| 11 | 69 | 71 | 100 | 99 | 56 | 55 | 68 | 69 |
| 12 | 54 | 83 | 105 | 140 | 62 | 93 | 75 | 113 |
| 13 | 62 | 89 | 108 | 121 | 52 | 68 | 74 | 88 |
| 14 | 54 | 66 | 123 | 121 | 83 | 74 | 59 | 74 |
| 15 | 44 | 53 | 130 | 99 | 58 | 50 | 83 | 66 |
| 16 | 44 | 65 | 119 | 110 | 62 | 52 | 90 | 67 |
| 17 | 78 | 90 | 99 | 100 | 57 | 63 | 68 | 76 |
| 18 | 73 | 93 | 144 | 104 | 74 | 63 | 99 | 78 |
| 19 | 70 | 85 | 150 | 130 | 50 | 46 | 90 | 67 |
| 20 | 46 | 60 | 81 | 117 | 53 | 53 | 67 | 73 |
| 21 | 55 | 67 | 84 | 106 | 54 | 60 | 65 | 70 |
| 22 | 63 | 62 | 125 | 105 | 60 | 47 | 80 | 64 |
| 23 | 72 | 77 | 110 | 90 | 68 | 50 | 84 | 55 |
| 24 | 61 | 91 | 130 | 142 | 65 | 62 | 91 | 80 |
| 25 | 62 | 79 | 120 | 140 | 60 | 78 | 90 | 100 |
| 26 | 62 | 65 | 107 | 115 | 63 | 83 | 84 | 65 |
| 27 | 64 | 93 | 119 | 110 | 60 | 54 | 80 | 67 |
| 28 | 56 | 68 | 113 | 106 | 66 | 55 | 84 | 69 |
| 29 | 55 | 98 | 84 | 115 | 54 | 58 | 41 | 58 |
| 30 | 70 | 65 | 103 | 110 | 57 | 50 | 72 | 63 |

*A6.4. Data used in Tables 6.2, 6.3 and 6.4 and Figure 6.1.*

| Patient no. | CO b (l/min) | CO e (l/min) | SV b (ml) | SV e (ml) | CVP b (mmHg) | CVP e (mmHg) | pHi b | pHi e |
|---|---|---|---|---|---|---|---|---|
| 1 | 3.1 | 6 | 48 | 96 | 9 | 9 | 7.43 | 7.44 |
| 2 | 4 | 4 | 69 | 40 | 5 | 8 | 7.39 | 7.24 |
| 3 | 7.3 | 8.3 | 126 | 96 | 4 | 4 | 7.35 | 7.36 |
| 4 | 5.9 | 4.5 | 106 | 67 | 10 | 13 | 7.47 | 7.18 |
| 5 | 2.8 | 7 | 67 | 88 | 3 | 7 | 7.25 | 7.22 |
| 6 | 4 | 6.6 | 52 | 73 | 8 | 10 | 7.41 | 7.38 |
| 7 | 2.7 | 9 | 49 | 125 | 8 | 12 | 7.5 | 7.3 |
| 8 | 3.3 | 3.3 | 45 | 36 | 10 | 6 | 7.46 | 7.35 |
| 9 | 5.9 | 5.1 | 82 | 50 | 3 | 3 | 7.48 | 7.16 |
| 10 | 4.4 | 5.8 | 66 | 64 | 2 | 0 | 7.46 | 7.33 |
| 11 | 5 | 6.7 | 73 | 95 | 8 | 5 | 7.31 | 7.32 |
| 12 | 3.3 | 6.5 | 60 | 78 | 6 | 14 | 7.32 | 7.26 |
| 13 | 2.9 | 2.8 | 47 | 32 | 1 | 3 | 7.38 | 7.21 |
| 14 | 3.3 | 4.7 | 70 | 71 | 4 | 8 | 7.34 | 7.48 |
| 15 | 2.7 | 3.3 | 60 | 66 | 1 | 3 | 7.34 | 7.27 |
| 16 | 2.3 | 2.9 | 44 | 45 | 7 | 5 | 7.41 | 7.29 |
| 17 | 5.7 | 5.7 | 74 | 63 | 11 | 4 | 7.46 | 7.3 |
| 18 | 5.7 | 6.8 | 78 | 73 | 1 | 5 | 7.38 | 7.29 |
| 19 | 7.6 | 12.6 | 110 | 142 | 1 | 10 | 7.38 | 7.32 |
| 20 | 3.5 | 6.4 | 64 | 106 | 6 | 7 | 7.31 | 7.31 |
| 21 | 5.9 | 4.5 | 106 | 67 | 5 | 6 | 7.4 | 7.28 |
| 22 | 6.7 | 7.5 | 105 | 120 | 8 | 6 | 7.36 | 7.34 |
| 23 | 5.1 | 8 | 71 | 104 | 1 | 0 | 7.46 | 7.5 |
| 24 | 5.3 | 6.9 | 88 | 75 | 2 | 12 | 7.5 | 7.35 |
| 25 | 6.5 | 13.6 | 105 | 172 | 10 | 14 | 7.35 | 7.39 |
| 26 | 4.1 | 4 | 67 | 65 | 1 | 1 | 7.38 | 7.38 |
| 27 | 7 | 7.2 | 108 | 77 | 5 | 1 | 7.46 | 7.3 |
| 28 | 2.7 | 4.6 | 49 | 67 | 5 | 9 | 7.48 | 7.34 |
| 29 | 4.3 | 3.9 | 75 | 39 | 5 | 6 | 7.4 | 7.28 |
| 30 | 3.7 | 7.4 | 51 | 114 | 4 | 11 | 7.32 | 7.33 |

*A6.5. Data used in Tables 6.2, 6.3 and 6.4 and Figure 6.1.*

| Patient no. | $P_TCO_2$ base (kPa) | $P_TCO_2$ end (kPa) | pHa b | pHa e | $PaCO_2$ base (kPa) | $PaCO_2$ end (kPa) | $PaO_2$ b (kPa) | $PaO_2$ e (kPa) |
|---|---|---|---|---|---|---|---|---|
| 1 | 3.95 | 3.69 | 7.46 | 7.5 | 4.3 | 3.65 | 23 | 28 |
| 2 | 4.8 | 5.4 | 7.42 | 7.36 | 5.33 | 4.9 | 17.2 | 9.2 |
| 3 | 4.62 | 4.49 | 7.38 | 7.36 | 5.16 | 5.17 | 19.88 | 11.3 |
| 4 | 3.31 | 4.97 | 7.42 | 7.4 | 5.62 | 5.06 | 22 | 56 |
| 5 | 5.69 | 5.74 | 7.33 | 7.39 | 5.6 | 4.55 | 38 | 25 |
| 6 | 3.56 | 4.37 | 7.45 | 7.44 | 4.48 | 4.38 | 12.9 | 8.13 |
| 7 | 2.73 | 5.78 | 7.42 | 7.38 | 4.55 | 4.72 | 15.6 | 48 |
| 8 | 3.52 | 5.91 | 7.5 | 7.49 | 4.12 | 4.19 | 20.4 | 59.4 |
| 9 | 3.46 | 6.1 | 7.42 | 7.25 | 4.75 | 5.93 | 43.6 | 25 |
| 10 | 4.04 | 5.32 | 7.41 | 7.39 | 5.65 | 5.28 | 25 | 20 |
| 11 | 5.15 | 5.56 | 7.41 | 7.46 | 4.79 | 4.22 | 13.9 | 21.7 |
| 12 | 4.82 | 5.69 | 7.43 | 7.31 | 4.48 | 5.92 | 15.29 | 15.9 |
| 13 | 3.56 | 5.92 | 7.48 | 7.48 | 3.57 | 3.65 | 26 | 18.3 |
| 14 | 4.69 | 3.43 | 7.45 | 7.53 | 4.68 | 3.73 | 17.2 | 13.5 |
| 15 | 4.44 | 5.24 | 4.14 | 4.63 | 4.6 | 4.63 | 22 | 18 |
| 16 | 4.24 | 5.3 | 7.39 | 7.32 | 5.74 | 5.76 | 44 | 12.92 |
| 17 | 3.14 | 5.22 | 7.43 | 7.38 | 3.95 | 4.88 | 17.1 | 8.92 |
| 18 | 2.97 | 5.04 | 7.38 | 7.29 | 5.94 | 5.69 | 18 | 52 |
| 19 | 4.22 | 4.45 | 7.41 | 7.33 | 4.66 | 4.93 | 23 | 17.52 |
| 20 | 5.11 | 5.19 | 7.37 | 7.31 | 5.43 | 5.52 | 21 | 12 |
| 21 | 4.25 | 5.73 | 7.42 | 7.4 | 5.62 | 5.06 | 22.3 | 56.3 |
| 22 | 2.83 | 3.98 | 7.36 | 7.34 | 5.38 | 5.42 | 13.21 | 14.6 |
| 23 | 3.18 | 3.36 | 7.4 | 7.4 | 5.64 | 5.28 | 27 | 11.72 |
| 24 | 3.2 | 4.2 | 7.39 | 7.35 | 4.52 | 3.94 | 31 | 22 |
| 25 | 3.93 | 3.63 | 7.39 | 7.41 | 4.42 | 3.53 | 22.1 | 20.34 |
| 26 | 3.43 | 3.9 | 7.39 | 7.38 | 5.09 | 4.51 | 27 | 46 |
| 27 | 3.37 | 5.15 | 7.38 | 7.36 | 5.46 | 5.22 | 20 | 41 |
| 28 | 2.98 | 4.66 | 7.42 | 7.35 | 5.34 | 5.32 | 31 | 28.4 |
| 29 | 4.25 | 5.73 | 7.39 | 7.28 | 5.08 | 4.97 | 25 | 17 |
| 30 | 5.49 | 5.85 | 7.44 | 7.38 | 5.49 | 5.85 | 34 | 15 |

*A6.6. Data used in Tables 6.2, 6.3 and 6.4 and Figure 6.1.*

| Patient no. | HCO3 b (mmol) | HCO3 e (mmol) | BE b (mmol) | BE e (mmol) | Lactate b (mmol) | Lactate e (mmol) |
|---|---|---|---|---|---|---|
| 1 | 23 | 21.7 | 0.7 | 1.1 | 2 | 1.5 |
| 2 | 25.5 | 21 | 1.2 | -4.1 | 1.1 | 1.8 |
| 3 | 22.3 | 21.4 | -2.2 | -3.5 | 0.8 | 1.6 |
| 4 | 23 | 17 | -1 | -8.5 | 1.2 | 2.4 |
| 5 | 21.6 | 20.8 | -3.7 | -3.4 | 1.1 | 1.3 |
| 6 | 21.3 | 22.4 | 0 | -1.3 | 0.8 | 1.1 |
| 7 | 22 | 20.6 | -1.6 | -3.8 | 1.2 | 1.8 |
| 8 | 24.5 | 24 | 1.9 | 1.5 | 1.4 | 1.5 |
| 9 | 23 | 18.8 | -0.9 | -7.6 | 0.7 | 4.6 |
| 10 | 26.6 | 23.7 | 2 | -0.9 | 0.7 | 1.2 |
| 11 | 22.5 | 24.2 | -1.5 | -0.5 | 1.1 | 0.8 |
| 12 | 22 | 21.9 | -1.6 | -3.7 | 1.5 | 2.8 |
| 13 | 19.9 | 20.3 | -2.5 | -2.4 | 1.3 | 1.4 |
| 14 | 24.2 | 23.4 | 0.8 | 0.8 | 0.7 | 1.6 |
| 15 | 23 | 22.3 | -1.1 | -1.5 | 0.7 | 1.2 |
| 16 | 24.3 | 21.9 | -0.9 | -3.6 | 0.9 | 1.3 |
| 17 | 19.6 | 22 | -4 | -2.6 | 1.1 | 2.8 |
| 18 | 26 | 19.8 | 0.7 | -6.1 | 1.7 | 2.5 |
| 19 | 22 | 19.6 | -1.8 | -5.9 | 1 | 1.4 |
| 20 | 23 | 20.5 | -1.9 | -4.9 | 1.1 | 1.8 |
| 21 | 27 | 23.4 | 2.4 | -0.9 | 1.2 | 2.4 |
| 22 | 22.4 | 21.3 | -2.5 | -3.9 | 1.2 | 1.8 |
| 23 | 25.8 | 24.3 | 1.1 | -1.5 | 0.7 | 0.9 |
| 24 | 25 | 19.7 | 1.1 | -3.9 | 1.2 | 1.4 |
| 25 | 20.2 | 18.8 | -3.6 | -7.2 | 1.8 | 3.2 |
| 26 | 23.3 | 20 | -1 | -4.2 | 0.9 | 2 |
| 27 | 24.4 | 21.7 | -0.3 | -3.1 | 2.2 | 1.3 |
| 28 | 25.8 | 21.5 | 1.5 | -3.5 | 1.1 | 1.5 |
| 29 | 27 | 23 | 1.2 | 2.4 | 2.9 | 4 |
| 30 | 27.5 | 25.3 | 3.2 | 0.4 | 1.1 | 2.1 |

*A6.7. Data used in Tables 6.2, 6.3, 6.4 and 6.5.*

| Patient no. | Urine (ml) | Colloid (ml) | Crystalloid (ml) |
|---|---|---|---|
| 1 | 520 | 1200 | 1000 |
| 2 | 910 | 1200 | 1000 |
| 3 | 298 | 1000 | 1000 |
| 4 | 244 | 1400 | 1000 |
| 5 | 200 | 1200 | 1000 |
| 6 | 650 | 1000 | 2000 |
| 7 | 1500 | 1500 | 500 |
| 8 | 74 | 500 | 700 |
| 9 | 1114 | 1000 | 1000 |
| 10 | 91 | 1500 | 1000 |
| 11 | 200 | 1900 | 1000 |
| 12 | 300 | 1000 | 1000 |
| 13 | 364 | 1000 | 1000 |
| 14 | 759 | 1500 | 2000 |
| 15 | 826 | 3000 | 1000 |
| 16 | 76 | 500 | 1000 |
| 17 | 945 | 1500 | 1000 |
| 18 | 358 | 1300 | 1000 |
| 19 | 612 | 1000 | 1000 |
| 20 | 896 | 1500 | 1000 |
| 21 | 244 | 1400 | 1000 |
| 22 | 263 | 1800 | 1000 |
| 23 | 700 | 1000 | 1000 |
| 24 | 1060 | 1000 | 1000 |
| 25 | 620 | 1500 | 1000 |
| 26 | 214 | 2000 | 1000 |
| 27 | 219 | 1100 | 1400 |
| 28 | 24 | 500 | 1700 |
| 29 | 880 | 500 | 1500 |
| 30 | 148 | 1000 | 1000 |

# A7.0. APPENDIX TO CHAPTER 7.0.

*A7.1.Data used in Tables 7.1 and 7.2.*

| Patient no. | Group | Age (years) | Height (cm) | Weight (kg) | Type of surgery |
|---|---|---|---|---|---|
| 1 | PROTOCOL | 60 | 189 | 73 | MVR |
| 2 | CONTROL | 54 | 160 | 81 | CABG |
| 3 | CONTROL | 65 | 180 | 71 | CABG |
| 4 | PROTOCOL | 64 | 172 | 59 | MVR |
| 5 | CONTROL | 61 | 172 | 78 | CABG |
| 6 | CONTROL | 68 | 161 | 76 | CABG |
| 7 | PROTOCOL | 46 | 175 | 76 | CABG |
| 8 | PROTOCOL | 54 | 178 | 80.2 | CABG |
| 9 | PROTOCOL | 67 | 179 | 93 | CABG |
| 10 | CONTROL | 80 | 177 | 84 | AVR |
| 11 | PROTOCOL | 65 | 190 | 78 | CABG |
| 12 | CONTROL | 67 | 153 | 87 | MVR |
| 13 | CONTROL | 57 | 172 | 63 | CABG |
| 14 | CONTROL | 53 | 167 | 74 | CABG |
| 15 | CONTROL | 69 | 173 | 78 | CABG |
| 16 | CONTROL | 66 | 178 | 72 | CABG |
| 17 | CONTROL | 47 | 181 | 87 | CABG |
| 18 | CONTROL | 65 | 164 | 68 | AVR |
| 19 | PROTOCOL | 86 | 143 | 50 | CABG |
| 20 | CONTROL | 60 | 182 | 94.4 | CABG |
| 21 | PROTOCOL | 55 | 178 | 78.4 | CABG |
| 22 | PROTOCOL | 60 | 171 | 83 | CABG |
| 23 | CONTROL | 89 | 159 | 58 | MVR |
| 24 | CONTROL | 54 | 167 | 74 | CABG |
| 25 | CONTROL | 60 | 156 | 76 | CABG |
| 26 | PROTOCOL | 61 | 168 | 76 | CABG |
| 27 | CONTROL | 60 | 167 | 76 | CABG |
| 28 | CONTROL | 61 | 167 | 105.4 | CABG |
| 29 | CONTROL | 53 | 170 | 62 | CABG |
| 30 | PROTOCOL | 73 | 185 | 72 | CABG |

*A7.2. Data used in Tables 7.1 and 7.2.*

| Patient no. | Group | Age (years) | Height (cm) | Weight (kg) | Type of surgery |
|---|---|---|---|---|---|
| 31 | PROTOCOL | 60 | 175 | 86 | CABG |
| 32 | CONTROL | 67 | 178 | 80 | CABG |
| 33 | PROTOCOL | 62 | 168 | 60 | CABG |
| 34 | PROTOCOL | 60 | 148 | 63 | CABG |
| 35 | CONTROL | 66 | 166 | 104 | CABG |
| 36 | CONTROL | 66 | 173 | 73 | CABG |
| 37 | CONTROL | 56 | 169 | 77 | CABG |
| 38 | PROTOCOL | 53 | 158 | 74 | CABG |
| 39 | PROTOCOL | 63 | 178 | 75 | CABG |
| 40 | PROTOCOL | 71 | 178 | 71 | AVR |
| 41 | CONTROL | 72 | 151 | 68 | CABG |
| 42 | PROTOCOL | 68 | 163 | 85 | CABG |
| 43 | CONTROL | 72 | 184 | 84 | CABG |
| 44 | PROTOCOL | 64 | 179 | 74 | CABG |
| 45 | PROTOCOL | 76 | 179 | 85 | CABG |
| 46 | CONTROL | 56 | 187 | 118 | CABG |
| 47 | PROTOCOL | 68 | 175 | 78 | AVR |
| 48 | PROTOCOL | 64 | 181 | 78 | CABG |
| 49 | PROTOCOL | 78 | 151 | 58 | AVR |
| 50 | PROTOCOL | 65 | 175 | 75 | CABG |
| 51 | PROTOCOL | 65 | 157 | 61 | CABG |
| 52 | PROTOCOL | 71 | 165 | 69 | CABG |
| 53 | PROTOCOL | 58 | 175 | 84 | CABG |
| 54 | PROTOCOL | 72 | 171 | 76 | AVR |
| 55 | CONTROL | 42 | 171 | 97 | CABG |
| 56 | PROTOCOL | 44 | 156 | 70 | CABG |
| 57 | CONTROL | 58 | 167 | 76 | CABG |
| 58 | CONTROL | 74 | 185 | 71 | MVR |
| 59 | CONTROL | 59 | 148 | 71 | CABG |
| 60 | PROTOCOL | 71 | 169 | 73 | MVR |

*A7.3. Data used in Tables 7.1 and 7.8.*

| Patient no. | Duration (minutes) | By-pass (minutes) | Ao-x-clamp (minutes) | Hospital (days) | ITU (days) |
|---|---|---|---|---|---|
| 1 | 310 | 90 | 72 | 7 | 1 |
| 2 | 210 | 68 | 52 | 9 | 4 |
| 3 | 210 | 80 | 43 | 5 | 1 |
| 4 | 320 | 47 | 34 | 5 | 1 |
| 5 | 180 | 60 | 39 | 6 | 1 |
| 6 | 230 | 89 | 30 | 7 | 1 |
| 7 | 240 | 91 | 45 | 7 | 1 |
| 8 | 220 | 65 | 31 | 6 | 1 |
| 9 | 200 | 60 | 38 | 6 | 1 |
| 10 | 240 | 62 | 48 | 8 | 1 |
| 11 | 210 | 65 | 30 | 6 | 1 |
| 12 | 190 | 70 | 40 | 6 | 1 |
| 13 | 260 | 106 | 57 | 7 | 1 |
| 14 | 180 | 80 | 58 | 6 | 1 |
| 15 | 180 | 72 | 55 | 5 | 1 |
| 16 | 240 | 86 | 43 | 11 | 2 |
| 17 | 240 | 88 | 76 | 5 | 1 |
| 18 | 180 | 67 | 53 | 5 | 1 |
| 19 | 210 | 91 | 45 | 7 | 1 |
| 20 | 180 | 80 | 32 | 8 | 1 |
| 21 | 160 | 46 | 32 | 6 | 1 |
| 22 | 240 | 83 | 43 | 7 | 1 |
| 23 | 220 | 70 | 40 | 48 | 11 |
| 24 | 180 | 55 | 40 | 7 | 1 |
| 25 | 240 | 109 | 59 | 6 | 1 |
| 26 | 240 | 114 | 49 | 5 | 1 |
| 27 | 210 | 50 | 38 | 8 | 1 |
| 28 | 330 | 127 | 60 | 7 | 1 |
| 29 | 150 | 46 | 26 | 8 | 1 |
| 30 | 240 | 155 | 48 | 6 | 1 |

*A7.4. Data used in Tables 7.1 and 7.8.*

| Patient no. | Duration (minutes) | By-pass (minutes) | Ao-x-clamp (minutes) | Hospital (days) | ITU (days) |
|---|---|---|---|---|---|
| 31 | 210 | 103 | 34 | 6 | 1 |
| 32 | 210 | 100 | 48 | 7 | 1 |
| 33 | 215 | 85 | 32 | 7 | 1 |
| 34 | 170 | 68 | 32 | 7 | 1 |
| 35 | 155 | 65 | 32 | 8 | 1 |
| 36 | 280 | 74 | 42 | 7 | 1 |
| 37 | 260 | 70 | 29 | 11 | 1 |
| 38 | 210 | 70 | 55 | 5 | 1 |
| 39 | 270 | 64 | 30 | 7 | 1 |
| 40 | 150 | 62 | 40 | 7 | 1 |
| 41 | 180 | 50 | 36 | 12 | 2 |
| 42 | 248 | 82 | 46 | 5 | 1 |
| 43 | 210 | 76 | 32 | 6 | 1 |
| 44 | 240 | 96 | 50 | 5 | 1 |
| 45 | 180 | 78 | 42 | 9 | 1 |
| 46 | 285 | 70 | 40 | 37 | 4 |
| 47 | 180 | 81 | 51 | 9 | 1 |
| 48 | 210 | 88 | 40 | 5 | 1 |
| 49 | 195 | 58 | 50 | 7 | 1 |
| 50 | 240 | 90 | 36 | 5 | 1 |
| 51 | 180 | 54 | 29 | 7 | 1 |
| 52 | 230 | 64 | 42 | 7 | 1 |
| 53 | 270 | 37 | 46 | 7 | 1 |
| 54 | 240 | 55 | 30 | 7 | 1 |
| 55 | 270 | 54 | 38 | 8 | 2 |
| 56 | 270 | 76 | 48 | 5 | 1 |
| 57 | 210 | 60 | 40 | 7 | 1 |
| 58 | 180 | 50 | 40 | 21 | 2 |
| 59 | 180 | 80 | 30 | 7 | 1 |
| 60 | 230 | 65 | 35 | 6 | 1 |

*A7.5. Data used in Tables 7.5, 7.6, 7.7 and 7.8.*

| Patient no. | Minor complications | Major complications | HR b (beats/min) | HR e (beats/min) | SBP b (mmHg) |
|---|---|---|---|---|---|
| 1 | 0 | 0 | 76 | 82 | 116 |
| 2 | 1 | 0 | 73 | 93 | 144 |
| 3 | 0 | 0 | 49 | 101 | 100 |
| 4 | 0 | 0 | 95 | 73 | 97 |
| 5 | 0 | 0 | 56 | 68 | 113 |
| 6 | 0 | 0 | 63 | 93 | 125 |
| 7 | 0 | 0 | 65 | 83 | 130 |
| 8 | 0 | 0 | 48 | 87 | 94 |
| 9 | 0 | 0 | 50 | 75 | 97 |
| 10 | 1 | 0 | 62 | 69 | 102 |
| 11 | 0 | 0 | 54 | 62 | 91 |
| 12 | 0 | 0 | 50 | 69 | 114 |
| 13 | 0 | 0 | 84 | 134 | 90 |
| 14 | 0 | 0 | 64 | 71 | 116 |
| 15 | 0 | 0 | 63 | 62 | 125 |
| 16 | 1 | 1 | 83 | 84 | 92 |
| 17 | 1 | 0 | 77 | 97 | 140 |
| 18 | 0 | 0 | 66 | 87 | 117 |
| 19 | 0 | 0 | 54 | 69 | 106 |
| 20 | 1 | 0 | 53 | 58 | 104 |
| 21 | 0 | 0 | 64 | 90 | 123 |
| 22 | 0 | 0 | 55 | 82 | 107 |
| 23 | 1 | 1 | 62 | 106 | 99 |
| 24 | 1 | 0 | 58 | 72 | 110 |
| 25 | 0 | 0 | 57 | 98 | 105 |
| 26 | 0 | 0 | 87 | 95 | 113 |
| 27 | 1 | 0 | 43 | 58 | 123 |
| 28 | 1 | 0 | 70 | 87 | 116 |
| 29 | 1 | 0 | 60 | 80 | 98 |
| 30 | 0 | 0 | 80 | 80 | 140 |

*A7.6. Data used in Table 7.5, 7.6 and 7.8.*

| Patient no. | Minor complications | Major complications | HR b (beats/min) | HR e (beats/min) | SBP b (mmHg) |
|---|---|---|---|---|---|
| 31 | 0 | 0 | 80 | 97 | 140 |
| 32 | 1 | 0 | 63 | 72 | 137 |
| 33 | 0 | 0 | 68 | 79 | 114 |
| 34 | 0 | 0 | 81 | 92 | 121 |
| 35 | 0 | 0 | 65 | 86 | 101 |
| 36 | 0 | 0 | 48 | 57 | 105 |
| 37 | 1 | 1 | 73 | 115 | 139 |
| 38 | 0 | 0 | 66 | 71 | 91 |
| 39 | 1 | 0 | 48 | 69 | 103 |
| 40 | 0 | 0 | 103 | 65 | 98 |
| 41 | 1 | 1 | 61 | 63 | 150 |
| 42 | 0 | 0 | 59 | 85 | 107 |
| 43 | 1 | 0 | 55 | 85 | 95 |
| 44 | 0 | 0 | 47 | 59 | 93 |
| 45 | 0 | 0 | 53 | 64 | 126 |
| 46 | 1 | 1 | 41 | 70 | 93 |
| 47 | 1 | 0 | 67 | 72 | 127 |
| 48 | 0 | 0 | 55 | 67 | 102 |
| 49 | 1 | 0 | 84 | 73 | 120 |
| 50 | 1 | 0 | 77 | 84 | 111 |
| 51 | 0 | 0 | 54 | 66 | 106 |
| 52 | 0 | 0 | 56 | 60 | 108 |
| 53 | 0 | 0 | 66 | 87 | 98 |
| 54 | 0 | 0 | 79 | 81 | 107 |
| 55 | 1 | 0 | 105 | 106 | 120 |
| 56 | 0 | 0 | 69 | 67 | 106 |
| 57 | 0 | 0 | 69 | 91 | 86 |
| 58 | 1 | 1 | 86 | 100 | 111 |
| 59 | 0 | 0 | 52 | 78 | 94 |
| 60 | 0 | 0 | 105 | 85 | 111 |

*A7.7. Data used in Tables 7.5 and 7.6.*

| Patient no. | SBP e (mmHg) | DBP b (mmHg) | DBP e (mmHg) | MBP b (mmHg) | MBP e (mmHg) |
|---|---|---|---|---|---|
| 1 | 140 | 71 | 60 | 83 | 86 |
| 2 | 104 | 74 | 63 | 99 | 78 |
| 3 | 169 | 51 | 79 | 72 | 106 |
| 4 | 90 | 46 | 40 | 65 | 61 |
| 5 | 106 | 66 | 55 | 84 | 69 |
| 6 | 110 | 60 | 54 | 80 | 67 |
| 7 | 117 | 73 | 85 | 91 | 97 |
| 8 | 130 | 56 | 76 | 69 | 86 |
| 9 | 108 | 59 | 65 | 74 | 77 |
| 10 | 116 | 58 | 49 | 74 | 64 |
| 11 | 104 | 50 | 53 | 64 | 66 |
| 12 | 85 | 47 | 56 | 70 | 65 |
| 13 | 128 | 60 | 65 | 70 | 87 |
| 14 | 104 | 60 | 44 | 85 | 63 |
| 15 | 105 | 60 | 47 | 80 | 64 |
| 16 | 118 | 94 | 80 | 63 | 64 |
| 17 | 112 | 79 | 63 | 100 | 77 |
| 18 | 124 | 48 | 58 | 71 | 74 |
| 19 | 113 | 45 | 56 | 69 | 78 |
| 20 | 108 | 66 | 59 | 80 | 62 |
| 21 | 108 | 80 | 64 | 95 | 76 |
| 22 | 105 | 64 | 53 | 80 | 70 |
| 23 | 90 | 52 | 62 | 68 | 72 |
| 24 | 85 | 70 | 54 | 83 | 60 |
| 25 | 115 | 41 | 58 | 65 | 72 |
| 26 | 121 | 80 | 69 | 94 | 83 |
| 27 | 121 | 54 | 66 | 79 | 84 |
| 28 | 122 | 70 | 80 | 89 | 96 |
| 29 | 119 | 53 | 57 | 70 | 72 |
| 30 | 125 | 68 | 40 | 91 | 62 |

*A7.8. Data used in Tables 7.5 and 7.6.*

| Patient no. | SBP e (mmHg) | DBP b (mmHg) | DBP e (mmHg) | MBP b (mmHg) | MBP e (mmHg) |
|---|---|---|---|---|---|
| 31 | 107 | 82 | 62 | 106 | 77 |
| 32 | 116 | 58 | 53 | 84 | 65 |
| 33 | 100 | 60 | 51 | 78 | 64 |
| 34 | 100 | 82 | 60 | 98 | 73 |
| 35 | 120 | 63 | 67 | 75 | 81 |
| 36 | 108 | 60 | 54 | 76 | 70 |
| 37 | 115 | 90 | 54 | 108 | 65 |
| 38 | 90 | 57 | 58 | 70 | 70 |
| 39 | 110 | 76 | 50 | 60 | 67 |
| 40 | 121 | 57 | 65 | 69 | 80 |
| 41 | 96 | 73 | 52 | 101 | 68 |
| 42 | 104 | 54 | 56 | 69 | 72 |
| 43 | 103 | 52 | 55 | 68 | 64 |
| 44 | 111 | 50 | 73 | 66 | 86 |
| 45 | 105 | 61 | 50 | 84 | 65 |
| 46 | 105 | 52 | 62 | 61 | 75 |
| 47 | 110 | 63 | 52 | 90 | 70 |
| 48 | 106 | 64 | 54 | 73 | 69 |
| 49 | 110 | 84 | 55 | 97 | 78 |
| 50 | 120 | 64 | 57 | 79 | 72 |
| 51 | 116 | 47 | 56 | 60 | 70 |
| 52 | 110 | 71 | 60 | 83 | 73 |
| 53 | 91 | 50 | 51 | 66 | 58 |
| 54 | 90 | 70 | 54 | 80 | 68 |
| 55 | 115 | 82 | 69 | 94 | 87 |
| 56 | 118 | 61 | 73 | 79 | 90 |
| 57 | 140 | 48 | 80 | 60 | 108 |
| 58 | 123 | 57 | 56 | 70 | 74 |
| 59 | 98 | 43 | 48 | 61 | 64 |
| 60 | 126 | 71 | 70 | 80 | 88 |

*A7.9. Data used in Tables 7.5 and 7.6.*

| Patient no. | CVP b (mmHg) | CVP e (mmHg) | CO b (l/min) | CO e (l/min) | SV b (ml) |
|---|---|---|---|---|---|
| 1 | 9 | 8 | 3.6 | 7.4 | 48 |
| 2 | 1 | 5 | 5.7 | 6.8 | 78 |
| 3 | 8 | 14 | 4 | 6.6 | 83 |
| 4 | 3 | 4 | 2.3 | 6.1 | 25 |
| 5 | 5 | 9 | 2.7 | 4.6 | 49 |
| 6 | 5 | 6 | 7 | 7.2 | 108 |
| 7 | 6 | 10 | 3 | 4.7 | 47 |
| 8 | 6 | 7 | 3.2 | 5.8 | 65 |
| 9 | 7 | 5 | 4.7 | 4.6 | 93 |
| 10 | 2 | 5 | 3.3 | 7.5 | 54 |
| 11 | 4 | 8 | 4.3 | 5.9 | 79 |
| 12 | 6 | 4 | 5 | 3.4 | 99 |
| 13 | 0 | 8 | 3.1 | 3 | 37 |
| 14 | 3 | 10 | 4.2 | 3.7 | 64 |
| 15 | 8 | 6 | 6.7 | 7.5 | 105 |
| 16 | 2 | 5 | 6.9 | 4.1 | 78 |
| 17 | 10 | 2 | 6.3 | 5.3 | 82 |
| 18 | 7 | 8 | 7.5 | 9.9 | 114 |
| 19 | 4 | 5 | 5.2 | 5.6 | 96 |
| 20 | 6 | 6 | 3.2 | 4.3 | 61 |
| 21 | 11 | 9 | 4.4 | 8.3 | 64 |
| 22 | 1 | 5 | 3.4 | 8.5 | 62 |
| 23 | 9 | 7 | 3.7 | 5 | 60 |
| 24 | 8 | 10 | 2.3 | 4.2 | 40 |
| 25 | 10 | 13 | 4.3 | 3.9 | 75 |
| 26 | 10 | 9 | 5.5 | 6.3 | 63 |
| 27 | 3 | 6 | 5.4 | 5 | 126 |
| 28 | 10 | 9 | 5.8 | 4.5 | 83 |
| 29 | 6 | 2 | 6.1 | 6.3 | 101 |
| 30 | 6 | 6 | 4.8 | 8.4 | 60 |

*A7.10. Data used in Tables 7.5 and 7.6.*

| Patient no. | CVP b (mmHg) | CVP e (mmHg) | CO b (l/min) | CO e (l/min) | SV b (ml) |
|---|---|---|---|---|---|
| 31 | 9 | 3 | 4.7 | 8.1 | 59 |
| 32 | 7 | 1 | 6.1 | 5.8 | 97 |
| 33 | 4 | 3 | 4.2 | 6.6 | 68 |
| 34 | 4 | 1 | 4.4 | 7.6 | 55 |
| 35 | 6 | 7 | 4.6 | 6.6 | 71 |
| 36 | 6 | 5 | 4.2 | 4.2 | 87 |
| 37 | 16 | 9 | 3.3 | 7.4 | 45 |
| 38 | 8 | 7 | 5.7 | 11.1 | 87 |
| 39 | 1 | 3 | 2.8 | 5.1 | 58 |
| 40 | 7 | 1 | 4.6 | 4.6 | 45 |
| 41 | 3 | 6 | 4.6 | 4.7 | 76 |
| 42 | 7 | 8 | 4.4 | 6.2 | 73 |
| 43 | 8 | 14 | 4.1 | 9.7 | 75 |
| 44 | 5 | 6 | 4.4 | 5.9 | 93 |
| 45 | 5 | 2 | 6.5 | 8.5 | 118 |
| 46 | 8 | 5 | 3.2 | 6.2 | 77 |
| 47 | 6 | 3 | 6 | 5.8 | 90 |
| 48 | 6 | 10 | 3.3 | 5.7 | 55 |
| 49 | 7 | 4 | 3.4 | 4 | 40 |
| 50 | 1 | 3 | 5 | 7.3 | 65 |
| 51 | 2 | 7 | 3.2 | 4.2 | 57 |
| 52 | 10 | 5 | 3.2 | 6 | 58 |
| 53 | 2 | 1 | 7.3 | 8 | 110 |
| 54 | 8 | 8 | 6.7 | 7.8 | 84 |
| 55 | 10 | 14 | 5.6 | 5.4 | 54 |
| 56 | 8 | 6 | 4.9 | 6.2 | 70 |
| 57 | 9 | 12 | 5 | 4.3 | 73 |
| 58 | 0 | 5 | 4.6 | 5.6 | 53 |
| 59 | 2 | 5 | 3.2 | 4.7 | 61 |
| 60 | 5 | 7 | 6.7 | 7.6 | 60 |

*A7.11. Data used in Tables 7.5 and 7.6.*

| Patient no. | SV e (ml) | $P_TCO_2$ b (kPa) | $P_TCO_2$ e (kPa) | pHi b (kPa) | pHi e (kPa) |
|---|---|---|---|---|---|
| 1 | 90 | 8.35 | 7.67 | 7.18 | 7.16 |
| 2 | 73 | 4.72 | 5.94 | 7.34 | 7.27 |
| 3 | 66 | 4.64 | 7.46 | 7.33 | 7.16 |
| 4 | 84 | 5.34 | 5.7 | 7.42 | 7.39 |
| 5 | 67 | 4.7 | 5.5 | 7.48 | 7.34 |
| 6 | 77 | 4.68 | 6.13 | 7.46 | 7.3 |
| 7 | 55 | 4.12 | 5.3 | 7.48 | 7.38 |
| 8 | 67 | 7.6 | 4.2 | 7.15 | 7.45 |
| 9 | 62 | 4.85 | 5.59 | 7.48 | 7.37 |
| 10 | 108 | 5.5 | 4.65 | 7.46 | 7.37 |
| 11 | 95 | 4.6 | 4.31 | 7.47 | 7.43 |
| 12 | 49 | 5.48 | 5.57 | 7.42 | 7.36 |
| 13 | 22 | 4.61 | 5.48 | 7.53 | 7.37 |
| 14 | 62 | 4.6 | 5.1 | 7.43 | 7.3 |
| 15 | 120 | 4.2 | 4.7 | 7.49 | 7.4 |
| 16 | 53 | 3.6 | 7.35 | 7.47 | 7.25 |
| 17 | 55 | 4 | 5.5 | 7.52 | 7.28 |
| 18 | 114 | 3.66 | 4.34 | 7.5 | 7.45 |
| 19 | 81 | 4.3 | 3.64 | 7.49 | 7.49 |
| 20 | 74 | 4.17 | 4.95 | 7.45 | 7.34 |
| 21 | 92 | 3.87 | 4.46 | 7.56 | 7.5 |
| 22 | 104 | 4.64 | 4.71 | 7.42 | 7.4 |
| 23 | 47 | 4.71 | 4.96 | 7.41 | 7.11 |
| 24 | 58 | 4.28 | 5.47 | 7.49 | 7.37 |
| 25 | 39 | 4.6 | 6.11 | 7.47 | 7.18 |
| 26 | 66 | 7.3 | 5.76 | 7.28 | 7.36 |
| 27 | 85 | 4.63 | 5.46 | 7.43 | 7.33 |
| 28 | 52 | 4.56 | 6.01 | 7.48 | 7.29 |
| 29 | 78 | 4.88 | 4.16 | 7.48 | 7.36 |
| 30 | 96 | 4.6 | 3.68 | 7.48 | 7.45 |

*A7.12. Data used in Tables 7.5 and 7.6.*

| Patient no. | SV e (ml) | $P_TCO_2$ b (kPa) | $P_TCO_2$ e (kPa) | pHi b (kPa) | pHi e (kPa) |
|---|---|---|---|---|---|
| 31 | 83 | 4.4 | 4.22 | 7.46 | 7.41 |
| 32 | 81 | 5.46 | 6.82 | 7.36 | 7.29 |
| 33 | 84 | 5.35 | 5.15 | 7.4 | 7.41 |
| 34 | 82 | 4.83 | 4.34 | 7.48 | 7.41 |
| 35 | 77 | 6.25 | 6.58 | 7.36 | 7.29 |
| 36 | 74 | 8.72 | 6.75 | 7.22 | 7.22 |
| 37 | 65 | 4.31 | 6.56 | 7.46 | 7.25 |
| 38 | 156 | 6.00 | 8.46 | 7.33 | 7.11 |
| 39 | 73 | 5.06 | 4.86 | 7.43 | 7.39 |
| 40 | 72 | 5.88 | 4.75 | 7.35 | 7.43 |
| 41 | 75 | 4.51 | 6.93 | 7.48 | 7.23 |
| 42 | 85 | 4.44 | 5.38 | 7.48 | 7.34 |
| 43 | 114 | 6.82 | 7.03 | 7.43 | 7.21 |
| 44 | 100 | 4.89 | 5.37 | 7.48 | 7.38 |
| 45 | 133 | 4.54 | 5.18 | 7.49 | 7.37 |
| 46 | 89 | 5.54 | 6.05 | 7.36 | 7.3 |
| 47 | 87 | 5.42 | 4.75 | 7.41 | 7.42 |
| 48 | 86 | 5.3 | 5.55 | 7.38 | 7.36 |
| 49 | 54 | 3.24 | 3.93 | 7.48 | 7.49 |
| 50 | 86 | 4.60 | 4.97 | 7.42 | 7.40 |
| 51 | 63 | 6.02 | 4.76 | 7.36 | 7.42 |
| 52 | 100 | 4.39 | 4.79 | 7.51 | 7.43 |
| 53 | 92 | 5.21 | 5.53 | 7.42 | 7.36 |
| 54 | 96 | 5.35 | 5.11 | 7.41 | 7.4 |
| 55 | 51 | 5.23 | 6.49 | 7.42 | 7.31 |
| 56 | 89 | 4.53 | 5.25 | 7.39 | 7.35 |
| 57 | 47 | 5.38 | 7.57 | 7.37 | 7.28 |
| 58 | 56 | 6.13 | 7.38 | 7.35 | 7.2 |
| 59 | 60 | 5.39 | 5.82 | 7.3 | 7.29 |
| 60 | 90 | 5.33 | 5.01 | 7.38 | 7.36 |

*A7.13. Data used in Tables 7.5 and 7.6.*

| Patient no. | pHa b (kPa) | pHa e (kPa) | $PaCO_2$ b (kPa) | $PaCO_2$ e (kPa) | $PaO_2$ b (kPa) |
|---|---|---|---|---|---|
| 1 | 7.36 | 7.24 | 5.34 | 6.25 | 21.2 |
| 2 | 7.38 | 7.29 | 4.72 | 5.94 | 17.9 |
| 3 | 7.42 | 7.32 | 3.59 | 5.33 | 17.84 |
| 4 | 7.44 | 7.42 | 5.05 | 4.93 | 14.1 |
| 5 | 7.42 | 7.35 | 5.34 | 5.32 | 31 |
| 6 | 7.38 | 7.36 | 5.46 | 5.22 | 19.4 |
| 7 | 7.41 | 7.42 | 5.1 | 4.58 | 11.2 |
| 8 | 7.41 | 7.36 | 4.15 | 4.72 | 14.8 |
| 9 | 7.45 | 7.39 | 4.79 | 5.23 | 28.4 |
| 10 | 7.38 | 7.27 | 6.1 | 5.86 | 36.4 |
| 11 | 7.43 | 7.35 | 4.85 | 5.23 | 23.9 |
| 12 | 7.4 | 7.36 | 5.77 | 4.68 | 31 |
| 13 | 7.45 | 7.33 | 5.24 | 6.1 | 27 |
| 14 | 7.43 | 7.35 | 4.48 | 4.48 | 23.5 |
| 15 | 7.36 | 7.34 | 5.38 | 5.42 | 13.2 |
| 16 | 7.38 | 7.31 | 5.58 | 6.25 | 13.3 |
| 17 | 7.47 | 7.29 | 4.43 | 5.34 | 22.3 |
| 18 | 7.49 | 7.43 | 4.28 | 4.35 | 23.2 |
| 19 | 7.46 | 7.41 | 4.38 | 4.32 | 14.3 |
| 20 | 7.42 | 7.37 | 5.2 | 4.60 | 10.2 |
| 21 | 7.47 | 7.45 | 5.35 | 4.90 | 15 |
| 22 | 7.38 | 7.33 | 5.49 | 5.50 | 22 |
| 23 | 7.42 | 7.26 | 4.55 | 4.83 | 22.3 |
| 24 | 7.36 | 7.31 | 5.67 | 6.13 | 17.6 |
| 25 | 7.39 | 7.18 | 5.08 | 4.97 | 25 |
| 26 | 7.34 | 7.28 | 6.11 | 6.48 | 15.7 |
| 27 | 7.39 | 7.37 | 5.07 | 4.88 | 63 |
| 28 | 7.4 | 7.29 | 5.51 | 5.77 | 28.8 |
| 29 | 7.39 | 7.38 | 4.88 | 4.60 | 30 |
| 30 | 7.42 | 7.41 | 4.24 | 4.00 | 34.5 |

*7.14. Data used in Tables 7.5 and 7.6.*

| Patient no. | pHa b (kPa) | pHa e (kPa) | $PaCO_2$ b (kPa) | $PaCO_2$ e (kPa) | $PaO_2$ b (kPa) |
|---|---|---|---|---|---|
| 31 | 7.38 | 7.29 | 5.24 | 0.52 | 9.2 |
| 32 | 7.39 | 7.39 | 5.1 | 5.33 | 48 |
| 33 | 7.43 | 7.48 | 5.1 | 4.3 | 29 |
| 34 | 7.4 | 7.4 | 4.83 | 4.29 | 18.72 |
| 35 | 7.39 | 7.39 | 5.64 | 5.23 | 22.1 |
| 36 | 7.39 | 7.41 | 5.332 | 4.21 | 19.4 |
| 37 | 7.37 | 7.3 | 5.3 | 5.7 | 14 |
| 38 | 7.34 | 7.33 | 5.69 | 5.68 | 24.03 |
| 39 | 7.41 | 7.38 | 5.26 | 4.5 | 61.7 |
| 40 | 7.37 | 7.43 | 5.88 | 4.65 | 25.7 |
| 41 | 7.46 | 7.42 | 4.5 | 4.3 | 16.8 |
| 42 | 7.39 | 7.36 | 5.23 | 4.98 | 31.1 |
| 43 | 7.34 | 7.31 | 5.8 | 5.28 | 27 |
| 44 | 7.39 | 7.37 | 5.85 | 5.39 | 17.82 |
| 45 | 7.39 | 7.37 | 5.75 | 5.02 | 24.6 |
| 46 | 7.4 | 7.34 | 5.54 | 6.05 | 18 |
| 47 | 7.4 | 7.42 | 5.5 | 4.77 | 67 |
| 48 | 7.43 | 7.42 | 4.64 | 4.8 | 50 |
| 49 | 7.45 | 7.44 | 4.86 | 4.31 | 66 |
| 50 | 7.41 | 7.37 | 4.7 | 4.88 | 43 |
| 51 | 7.4 | 7.51 | 5.5 | 3.7 | 15.7 |
| 52 | 7.32 | 7.4 | 6.7 | 4.6 | 18.96 |
| 53 | 7.37 | 7.39 | 5.34 | 5.08 | 66.9 |
| 54 | 7.4 | 7.36 | 5.4 | 5.41 | 55 |
| 55 | 7.37 | 7.32 | 5.77 | 6.19 | 12.3 |
| 56 | 7.38 | 7.38 | 5.2 | 4.82 | 47.2 |
| 57 | 7.38 | 7.28 | 5.11 | 7.51 | 10.5 |
| 58 | 7.42 | 7.34 | 5.11 | 5.02 | 32.2 |
| 59 | 7.37 | 7.34 | 4.6 | 5.21 | 18.8 |
| 60 | 7.4 | 7.36 | 5.47 | 5.41 | 68 |

*A7.15. Data used in Tables 7.5 and 7.6.*

| Patient no. | $PaO_2$ e (kPa) | $HCO_3$ b (mmol) | $HCO_3$ e (mmol) | Base XS (mmol) | Base XS (mmol) |
|---|---|---|---|---|---|
| 1 | 37.5 | 22.6 | 19.6 | -2.3 | -6.8 |
| 2 | 51.6 | 25.9 | 19.8 | 0.7 | -6.1 |
| 3 | 42.8 | 17.2 | 22.2 | -6.1 | -5.1 |
| 4 | 55 | 25 | 25 | 1.8 | 0.5 |
| 5 | 28.4 | 25.8 | 21.5 | 1.5 | -3.5 |
| 6 | 41 | 24.4 | 21.7 | -0.3 | -3.1 |
| 7 | 28.7 | 23.7 | 22.8 | -0.5 | -2.1 |
| 8 | 23 | 19.5 | 21 | -4.1 | -4.6 |
| 9 | 48.7 | 24.7 | 23.3 | 1.2 | -1.2 |
| 10 | 12.8 | 26.9 | 19.7 | 1.7 | -6.5 |
| 11 | 28.02 | 23.7 | 21.1 | -0.2 | -3.9 |
| 12 | 51 | 25.8 | 22.7 | 1.1 | -1.2 |
| 13 | 26.2 | 26.9 | 23.6 | 2.8 | -2 |
| 14 | 9.22 | 22 | 18.4 | -1.4 | -6.1 |
| 15 | 14.6 | 22.4 | 21.3 | -2.5 | -3.9 |
| 16 | 14.6 | 24.3 | 23.2 | -0.5 | -2.6 |
| 17 | 19 | 24.1 | 19 | 0.6 | -6.7 |
| 18 | 16.4 | 24.5 | 21.5 | 1.9 | -2.1 |
| 19 | 19.84 | 23.2 | 20.1 | -0.2 | -3.9 |
| 20 | 17.8 | 24.6 | 19.6 | 0.5 | -4.8 |
| 21 | 30 | 29.3 | 25.3 | 5.3 | 1.5 |
| 22 | 23 | 24.2 | 21.3 | -0.5 | -4 |
| 23 | 19.2 | 21.8 | 16.6 | -2.1 | -9 |
| 24 | 14.4 | 23.7 | 22.7 | -1.4 | -3 |
| 25 | 16 | 23.3 | 17 | -1 | -8.8 |
| 26 | 29 | 24.4 | 21.9 | -1.3 | -4.5 |
| 27 | 44.5 | 22.7 | 20.7 | -1.7 | -3.9 |
| 28 | 49.3 | 25 | 21.1 | 0.5 | -4.6 |
| 29 | 28.8 | 21.9 | 20.4 | -2.4 | -3.7 |
| 30 | 18.9 | 20.7 | 19 | -2.8 | -5 |

*A7.16. Data used in Tables 7.5 and 7.6.*

| Patient no. | $PaO_2$ e (kPa) | $HCO_3$ b (mmol) | $HCO_3$ e (mmol) | Base XS (mmol) | Base XS (mmol) |
|---|---|---|---|---|---|
| 31 | 12.9 | 22.8 | 19.4 | -1.8 | -6.4 |
| 32 | 46 | 22.5 | 23.9 | -2 | -0.6 |
| 33 | 46 | 25 | 23.6 | 0.8 | 0.4 |
| 34 | 15.1 | 22.4 | 19.9 | -1.6 | -4 |
| 35 | 22.4 | 25 | 23.4 | 0.1 | -1.2 |
| 36 | 29.2 | 23.8 | 20 | -0.8 | -3.8 |
| 37 | 13.3 | 22.3 | 20.7 | -2.5 | -5 |
| 38 | 67.5 | 22.7 | 21.8 | -2.5 | -3.6 |
| 39 | 21 | 24.7 | 21 | 0.3 | -3.5 |
| 40 | 64.8 | 25.1 | 23.1 | 0 | -0.7 |
| 41 | 18 | 24.1 | 21 | 0.8 | -2.8 |
| 42 | 47 | 23.7 | 21 | -0.7 | -4 |
| 43 | 29 | 23 | 20.5 | -2.3 | -6.2 |
| 44 | 16.13 | 26.2 | 23.1 | 1.2 | -1.7 |
| 45 | 36.7 | 25.5 | 21.9 | 0.6 | 3.4 |
| 46 | 25.3 | 22.7 | 21.7 | -1.5 | -3.4 |
| 47 | 16.1 | 25.4 | 22.7 | 0.8 | -1.3 |
| 48 | 35 | 23 | 23.1 | -0.6 | -0.9 |
| 49 | 68 | 24 | 21.9 | 0.1 | -1.5 |
| 50 | 34.9 | 24.4 | 21.9 | 0.1 | -3.5 |
| 51 | 50 | 24.9 | 22.2 | 0.2 | -0.5 |
| 52 | 18 | 25.2 | 23 | -1.1 | -1.8 |
| 53 | 30.3 | 24.4 | 22.8 | -0.6 | -1.7 |
| 54 | 63 | 23.4 | 22.7 | 0.5 | -2.2 |
| 55 | 23.3 | 24.5 | 23.6 | -0.7 | -2 |
| 56 | 20.8 | 22.9 | 20.8 | -1.6 | -3.6 |
| 57 | 12.2 | 22.3 | 25.8 | -2.2 | -1.2 |
| 58 | 34 | 24.5 | 20.9 | 0.4 | -4.3 |
| 59 | 34.9 | 19.4 | 20.5 | -5.1 | -4.2 |
| 60 | 63 | 25.5 | 22.7 | 1 | -2.2 |

*A7.17. Data used in Tables 7.3, 7.5 and 7.6.*

| Patient no. | Hb b (g/dl) | Hb e (g/dl) | Lactate (mmol/l) | Lactate (mmol/l) | Crystalloid given pre-CPB (ml) |
|---|---|---|---|---|---|
| 1 | 10.4 | 10.2 | 1.5 | 2.1 | 300 |
| 2 | 12.8 | 10.4 | 1.7 | 2.5 | 500 |
| 3 | 9.7 | 10.7 | 1.8 | 3.6 | 700 |
| 4 | 12.1 | 9 | 1.1 | 2 | 200 |
| 5 | 12.5 | 8.5 | 1.1 | 1.5 | 1000 |
| 6 | 10.5 | 9.9 | 1.8 | 1.3 | 1000 |
| 7 | 11.1 | 9.1 | 1.1 | 1.9 | 1000 |
| 8 | 11.7 | 8.7 | 1.3 | 2.9 | 100 |
| 9 | 14.2 | 8.9 | 1.3 | 1.4 | 1000 |
| 10 | 11.4 | 9.2 | 1.2 | 2.5 | 1000 |
| 11 | 10.5 | 8 | 1.3 | 1.9 | 1000 |
| 12 | 8.9 | 7.7 | 1.3 | 1.9 | 0 |
| 13 | 11.1 | 7.7 | 0.7 | 2.9 | 1000 |
| 14 | 14.7 | 10.3 | 0.7 | 2.3 | 100 |
| 15 | 9.4 | 9 | 0.8 | 2.1 | 1000 |
| 16 | 11.4 | 11.2 | 0.7 | 1.4 | 1000 |
| 17 | 13.2 | 10.1 | 1.3 | 4.8 | 1000 |
| 18 | 12.4 | 8.9 | 0.8 | 1.9 | 0 |
| 19 | 8 | 7.4 | 0.7 | 1.7 | 400 |
| 20 | 13.4 | 8.8 | 1.3 | 3.5 | 300 |
| 21 | 12.4 | 8.6 | 1.5 | 2.2 | 1000 |
| 22 | 12 | 8.9 | 1.4 | 2.7 | 500 |
| 23 | 9 | 7.3 | 1.3 | 3.8 | 800 |
| 24 | 11.7 | 8.9 | 1.3 | 1.6 | 1000 |
| 25 | 11.1 | 9.1 | 2.9 | 4 | 800 |
| 26 | 13.4 | 9.1 | 2 | 1.8 | 1000 |
| 27 | 12 | 8.2 | 0.9 | 1.9 | 1000 |
| 28 | 12.6 | 7.3 | 0.8 | 1.9 | 800 |
| 29 | 11.6 | 9.5 | 1 | 3 | 1000 |
| 30 | 10.8 | 7.9 | 1.2 | 1.7 | 200 |

*A7.18. Data used in Tables 7.3, 7.5 and 7.6.*

| Patient no. | Hb b (g/dl) | Hb e (g/dl) | Lactate (mmol/l) | Lactate (mmol/l) | Crystalloid given pre-CPB (ml) |
|---|---|---|---|---|---|
| 31 | 13.7 | 8.1 | 1.5 | 2.4 | 400 |
| 32 | 12.1 | 8.8 | 1.4 | 2.6 | 1000 |
| 33 | 12.3 | 7.5 | 2.1 | 2.3 | 1000 |
| 34 | 14.2 | 8 | 1.5 | 2.3 | 1000 |
| 35 | 12.1 | 9.8 | 1.3 | 2.3 | 700 |
| 36 | 10.6 | 7.9 | 1.1 | 1.7 | 1000 |
| 37 | 11.3 | 8.3 | 1.2 | 2.5 | 1000 |
| 38 | 8.4 | 7.7 | 0.9 | 2.2 | 1000 |
| 39 | 12.3 | 8.6 | 2.3 | 2.8 | 1000 |
| 40 | 9.8 | 7.2 | 0.7 | 1.3 | 1000 |
| 41 | 11.3 | 7.1 | 1.1 | 3.3 | 1000 |
| 42 | 10.5 | 7 | 1.1 | 1.4 | 1000 |
| 43 | 9.9 | 9.8 | 0.8 | 3.5 | 1000 |
| 44 | 11.9 | 8.6 | 0.8 | 2.2 | 1000 |
| 45 | 12 | 8 | 0.8 | 2.2 | 1000 |
| 46 | 13.2 | 10.8 | 1.3 | 1.8 | 1000 |
| 47 | 9.1 | 7.7 | 1.1 | 2.1 | 1000 |
| 48 | 12.1 | 8.4 | 1.6 | 1.7 | 1000 |
| 49 | 11.3 | 9.3 | 1.3 | 1.2 | 1000 |
| 50 | 13.5 | 8.9 | 0.9 | 2.8 | 1000 |
| 51 | 10.4 | 6.7 | 0.7 | 1.5 | 800 |
| 52 | 12 | 8.4 | 1.3 | 2.2 | 400 |
| 53 | 13.2 | 7.1 | 0.8 | 2.1 | 1000 |
| 54 | 9.3 | 8.4 | 0.7 | 1.8 | 1000 |
| 55 | 14.3 | 8.3 | 1.2 | 2.5 | 1000 |
| 56 | 12.1 | 9.5 | 1.2 | 2.3 | 1000 |
| 57 | 13 | 10 | 0.9 | 2.1 | 1000 |
| 58 | 12.8 | 8.6 | 1.1 | 2.1 | 600 |
| 59 | 9 | 8.6 | 1.9 | 1.8 | 700 |
| 60 | 9.5 | 8.4 | 0.6 | 1.6 | 1000 |

*A7.19. Data used in Tables 7.3, 7.5 and 7.6.*

| Patient no. | Crystalloid given in total (ml) | Colloid given pre-CPB (ml) | Colloid given in total (ml) | Blood given in total (ml) | Urine (ml) |
|---|---|---|---|---|---|
| 1 | 300 | 1200 | 2300 | 1200 | 294 |
| 2 | 1000 | 500 | 1300 | 0 | 358 |
| 3 | 1000 | 0 | 700 | 0 | 212 |
| 4 | 200 | 600 | 1500 | 0 | 714 |
| 5 | 1700 | 500 | 500 | 0 | 24 |
| 6 | 1000 | 500 | 1000 | 0 | 219 |
| 7 | 1200 | 400 | 1400 | 400 | 315 |
| 8 | 200 | 1200 | 1500 | 300 | 605 |
| 9 | 1000 | 500 | 1000 | 0 | 781 |
| 10 | 1000 | 500 | 1000 | 500 | 252 |
| 11 | 1000 | 400 | 1200 | 800 | 275 |
| 12 | 1000 | 0 | 200 | 800 | 362 |
| 13 | 1000 | 300 | 1400 | 1600 | 222 |
| 14 | 1000 | 0 | 300 | 0 | 704 |
| 15 | 1000 | 500 | 1800 | 800 | 263 |
| 16 | 1000 | 700 | 900 | 1000 | 453 |
| 17 | 1000 | 250 | 500 | 0 | 350 |
| 18 | 1000 | 0 | 500 | 0 | 167 |
| 19 | 400 | 1200 | 1200 | 500 | 101 |
| 20 | 1000 | 0 | 500 | 0 | 146 |
| 21 | 1000 | 1000 | 1000 | 0 | 98 |
| 22 | 500 | 1200 | 1500 | 0 | 490 |
| 23 | 800 | 0 | 500 | 0 | 38 |
| 24 | 3000 | 0 | 0 | 0 | 2100 |
| 25 | 1500 | 500 | 500 | 0 | 880 |
| 26 | 1000 | 1000 | 1400 | 400 | 581 |
| 27 | 1000 | 0 | 200 | 0 | 624 |
| 28 | 1000 | 0 | 450 | 350 | 993 |
| 29 | 1000 | 0 | 500 | 0 | 486 |
| 30 | 300 | 1200 | 1700 | 0 | 961 |

*A7.20. Data used in Tables 7.3, 7.5 and 7.6*

| Patient no. | Crystalloid given in total (ml) | Colloid given pre- CPB (ml) | Colloid given in total (ml) | Blood given in total (ml) | Urine (ml) |
|---|---|---|---|---|---|
| 31 | 400 | 1700 | 1700 | 0 | 400 |
| 32 | 1000 | 700 | 800 | 0 | 675 |
| 33 | 1000 | 1000 | 1200 | 0 | 673 |
| 34 | 1000 | 1000 | 1500 | 0 | 446 |
| 35 | 1000 | 0 | 0 | 0 | 131 |
| 36 | 1000 | 300 | 900 | 1300 | 338 |
| 37 | 1000 | 750 | 1400 | 0 | 284 |
| 38 | 1800 | 1000 | 1000 | 800 | 434 |
| 39 | 1000 | 2000 | 2500 | 1000 | 641 |
| 40 | 1000 | 400 | 800 | 200 | 264 |
| 41 | 1000 | 0 | 500 | 0 | 581 |
| 42 | 1000 | 600 | 1000 | 0 | 138 |
| 43 | 1000 | 1000 | 1000 | 0 | 204 |
| 44 | 1000 | 1000 | 1500 | 0 | 268 |
| 45 | 100 | 1000 | 1500 | 0 | 96 |
| 46 | 1000 | 500 | 1500 | 0 | 300 |
| 47 | 1000 | 750 | 1000 | 0 | 420 |
| 48 | 1000 | 600 | 1000 | 0 | 140 |
| 49 | 1000 | 200 | 800 | 0 | 505 |
| 50 | 1000 | 1500 | 2000 | 0 | 212 |
| 51 | 1000 | 500 | 800 | 0 | 300 |
| 52 | 400 | 400 | 1000 | 0 | 530 |
| 53 | 1000 | 1000 | 1500 | 0 | 219 |
| 54 | 1000 | 600 | 1200 | 800 | 628 |
| 55 | 1000 | 0 | 1500 | 0 | 128 |
| 56 | 1000 | 1000 | 2100 | 400 | 873 |
| 57 | 1000 | 0 | 500 | 0 | 111 |
| 58 | 800 | 0 | 0 | 0 | 350 |
| 59 | 1000 | 0 | 1000 | 0 | 1083 |
| 60 | 1000 | 800 | 1200 | 0 | 624 |