The Haemodynamic Response to Haemorrhage and Injury in the Anaesthetised Pig, and its Consequences

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Abstract

Trauma deaths show a trimodal distribution. Fifty percent die at the scene from injuries incompatible with life, 30% die in the first few hours, and about 20% of them die "late". Most late deaths are the result of multiple organ failure (MOF). Advanced Trauma Life Support has improved the early management of trauma patients. Despite advances in intensive care the mortality from MOF has remained almost unchanged in twenty years. A better understanding of the very acute pathophysiology of trauma may provide insights into the initiation and prevention of MOF.

Young Large White pigs, anaesthetised with alphaxalone/alphadolone (15mg/kg/h), were studied in a model of haemorrhage with and without a background of brachial nerve afferent nociceptive stimulation (NS) to mimic injury. Blood loss was either 20% or 30% of total blood volume. Shed blood was reinfused after a 30 minute shock period. Haemodynamic measurements were made and oxygen transport variables calculated. Gut and femoral blood flows were measured with electro-magnetic flow probes. Hepatic portal and systemic blood was taken for assays of lactate, endotoxin, TNF-α, and IL-6.

Haemorrhage resulted in: 1) a tachycardia, 2) hypotension, which recovered during the shock phase, 3) a reduction in gut blood flow, which recovered during the shock phase. Nerve stimulation resulted in a typical "pressor response" of tachycardia and increased blood pressure.

Traumatic shock, modelled using haemorrhage and nerve stimulation, differed haemodynamically and metabolically from haemorrhagic shock. The addition of nerve stimulation had detrimental effects on the response to haemorrhage: 1) no recovery of MAP during the shock phase, 2) an immediate increase in systemic vascular resistance, 3) a greater reduction in gut blood flow, 4) gut vasoconstriction, 5) increased plasma lactate. Global oxygen consumption was unchanged despite a reduction in oxygen

delivery with haemorrhage ± NS. Oxygen extraction ratio increased in the haemorrhage groups but this did not compensate for the greater metabolic insult provided by haemorrhage + NS compared with haemorrhage alone. These haemodynamic disturbances were reversed by shed blood resuscitation.

There was no evidence of live organism translocation to the circulation or the reticulo-endothelial system in this acute model of shock. Interleukin-6 production was similar in all the groups. In contrast, endotoxin and TNF-α concentrations in hepatic portal and systemic blood was greater in experimental groups than in controls. But there were no significant differences between the different experimental groups. An inflammatory response was seen despite resuscitation with shed blood, which reversed many of the changes in haemodynamic variables.

From this study it is clear that the combination of haemorrhage plus simulated injury results in greater haemodynamic and metabolic disruption than haemorrhage alone. This needs to be considered in the development of better treatments for trauma patients. Therapies aimed at improving gut microvascular blood flow and oxygenation, gut mucosal restitution, or the host immune system all appear promising. They all require further testing in models such as this one before clinical trials are undertaken.

Declaration

I, the undersigned, declare that no portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Bernard A Foëx

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Throughout the four years, the highs and one or two lows, I have been able to count on the love and support of my parents, my brother, and my friends. They have helped me more than they can know. For that I will always be grateful.

The six months spent writing this thesis have been the happiest of my life. Thank you, Susan, for making them so.

Dedication

This study is dedicated to Pierre Foëx: a model father, a sage, and an inspiration.

Abbreviations

ABE	Arterial base excess
ANOVA	Analysis of variance
APP	Acute phase protein
BP	Blood pressure
BSA	Body surface area
BT	Bacterial translocation
CI	Cardiac index
CRP	C-reactive protein
CO	Cardiac output
DO_2	Oxygen delivery
DO_2I	Oxygen delivery index
GALT	Gut-associated lymphoid tissue
H	Haemorrhage
HR	Heart rate
HSF	Hepatocyte-stimulating factor
IFN	Interferon (α, β, γ)
IL	Interleukin (any number)
LBNP	Lower body negative pressure
LI	Limb ischaemia
LPS	Lipopolysaccharide (endotoxin)
MAP	Mean arterial pressure
MBF	Microvascular blood flow
MLN	Mesenteric lymph node
MPAP	Mean pulmonary artery pressure
OER	Oxygen extraction ratio
PAWP	Pulmonary artery wedge pressure
PEOR	Portal oxygen extraction ratio
pHi	Intramucosal pH
PmO_2	Mucosal oxygen tension
PsO_2	Serosal oxygen tension
$PsqO_2$	Subcutaneous oxygen tension
PtO_2	Transcutaneous oxygen tension
PVR	Pulmonary vascular resistance
PVRI	Pulmonary vascular resistance index
RANOVA	Repeated measures analysis of variance
SANS	Somatic afferent nerve stimulation
SaO_2	Arterial oxygen saturation
SD	Standard deviation
SE	Standard error
SMI	Skeletal muscle injury
SPF	Specific pathogen free
SpO_2	Portal venous oxygen saturation

SVI	Stroke volume index
SvO_2	Mixed venous oxygen saturation
SVR	Systemic vascular resistance
SVRI	Systemic vascular resistance index
TBV	Total blood volume
TNFα	Tumour necrosis factor alpha
VO_2	Oxygen consumption
VO_2I	Oxygen consumption index
XO	Xanthine oxidase

Chapter 1: Introduction

Opening remarks

In 1988 Yates pointed out that in the UK there were 40 deaths per day as a result of trauma, this included deaths on the roads, in the work place, at home, and from "recreational violence" (Yates 1988). Apart from the death toll he went on to comment that "more years of productive life are lost through injury than through either cancer or cardiovascular disease". Cancer and cardiovascular disease tend to affect an older age group, so that trauma affects the young to a proportionally greater extent. The commonest cause of death in men under 35 is trauma. The importance of trauma in the elderly is increasing as the population gets progressively older. Changes in the incidence and effects of trauma have recently been reviewed (Girolami et al. 1998)

Studies in the 1970s and 1980s found a trimodal distribution for trauma deaths in the United States (Lowe et al. 1983; Trunkey 1983). Deaths occurred either immediately, or within the first few hours, or much later (days even weeks after the accident).

Immediate deaths were the result of injuries incompatible with life. In the UK nearly half of the 1000 trauma deaths studied by the Royal College of Surgeons working party on the management of major injuries occurred at the scene or on arrival at hospital (Anderson et al. 1988). In the USA the problem of trauma deaths has resulted in the development of "Trauma Centres" and the Advanced Trauma Life Support (ATLS) course, which has now been adopted in the UK (American College of Surgeons 1988). It has now become an integral part of the training of all clinicians responsible for the management of trauma patients.

Reducing immediate deaths from trauma is dependent more on Government policy than changes in medical practice. Most trauma in the Western World is the result of road traffic accidents or firearms (in the USA). In earlier decades industrial accidents represented a much higher proportion of civilian trauma. Effective Government legislation relating to safety in the work place has prompted this reduction.

Deaths from highway accidents in the United States were reduced by 21% between 1968 and 1991 as a result of a highway safety campaign (Davis 1995). Measures such as the compulsory use of seat belts in motor vehicles and helmets for motor cyclists have contributed a reduction in road traffic accident deaths in England and Wales (Office of Population Censuses and Surveys 1993). There is still some debate about whether banning all alcohol consumption by drivers would reduce the incidence of accidents. A large proportion of accidents are still related to alcohol use (Levy et al. 1996). In the USA there is little doubt that the Constitutional Right to bear arms results in an excess of firearms related deaths compared to other Western Countries (The violence prevention task force of the Eastern Association for the Surgery of Trauma 1995).

In 1988 Anderson and co-workers concluded that "Preventable deaths after injury occur primarily because of missed diagnosis; failure to control haemorrhage or hypoxia, and inadequate, delayed, or non-existent surgical intervention. - We must now do more for severely injured patients". Improvements in the early management of trauma patients have been made and have resulted in a reduction in the early deaths (Regel et al. 1995), in some cases to such an extent that the trimodal distribution has disappeared (Sauaia et al. 1995). Wyatt et al., reviewing trauma deaths in SE Scotland in the early 1990s, found that 76% of deaths were immediate, but only 7% were in Trunkey's early period (Wyatt et al. 1995). The late deaths accounted for 17% of the total. They concluded that the trimodal distribution did not apply to this population, possibly because of improvements in trauma care.

The third group of trauma deaths are the late deaths: those occurring several days or even weeks after the original insult. By this stage the patients are in multiple organ failure (MOF) and despite supportive therapy fail to recover (Pape et al. 1994; Sauaia et al. 1995).

Multiple organ failure was thought to be the result of infection and sepsis leading to septic shock. It has become clear that although MOF may result from infection, for example in peritonitis and pneumonia, there are many conditions when no infective cause is ever found, such as trauma, pancreatitis or even heat stroke (Graber et al. 1971).

Despite advances in intensive care and supportive therapy for failing organs in the last twenty years there has been no discernible reduction in the mortality from MOF (Deitch 1992). Interest has been focussing on the possible causes in the hope of finding preventative measures.

The search for the aetiology of MOF

This has taken on the appearance of the search for the Holy Grail! The evolution in our understanding of the pathophysiology of MOF was summarised in 1996 (Saadia and Lipman 1996).

My interest in the pathophysiology of MOF in trauma patients dates back to 1991, when I was asked to take blood from trauma patients to track their inflammatory cytokine response. It soon became apparent that there was great variation in the pattern of injuries and the cytokine response (Foëx et al. 1993; Foëx et al. 1994). To eliminate some of the variability in the injuries the cytokine response to a standard injury, total hip replacement, was studied. This provided bone and muscle trauma, and some blood loss. None of the patients showed an increase in either tumour necrosis factor-α or in interleukin-6 (unpublished results). These results contrasted with those from other workers (Shenkin et al. 1989; Cruickshank et al. 1990; Baigrie et al. 1991; Baigrie et al. 1992; Joris et al. 1992; Baigrie et al. 1993). The majority of patients these workers studied had undergone abdominal surgery. It seemed logical to focus on the gut as a possible source for the cytokine response in trauma patients.

As Moore and his colleagues have shown, it is possible to take portal venous blood samples for several days in trauma patients (Moore et al. 1991). However, they were still constrained by variations in the mechanism and severity of the trauma. To overcome some of these problems I decided to look at an animal model. This would allow me to control any injury, to determine the volume of any blood loss, and to make detailed haemodynamic measurements, take blood samples from the portal vein, and biopsies from the reticulo-endothelial system.

Aims of this project

The overall aim of this project was to investigate the consequences of the haemodynamic response to haemorrhage and injury in an anaesthetised pig model. In particular I was interested in:

1) the effects on gut blood flow and skeletal muscle blood flow (since skeletal muscle blood flow is affected by haemorrhage)
2) the metabolic effects of the combination of haemorrhage and injury
3) whether there would be a breakdown in the gut mucosal barrier resulting in translocation of bacteria and/or endotoxin to the circulation or the reticulo-endothelial system
4) whether haemorrhage and injury would trigger an inflammatory cytokine response and if so whether the gut was the source
5) and whether such a response was triggered by haemorrhage or injury.

The physiology of haemorrhage and injury

General Introduction

This review will focus on the cardiovascular responses to haemorrhage and injury, and the way in which these responses are modified when the two insults occur simultaneously in traumatic shock. The consequences of these responses: the changes in regional and microvascular blood flows, the effects on the gut mucosa and the changes in oxygen transport will be examined as they appear to be of fundamental importance in our understanding of the long term complications of traumatic shock.

Historical Introduction

War, and the problems of assessing and treating military and civilian casualties, has been the driving force behind much of the research into the effects of haemorrhage and injury.

During World War I the Medical Research Committee of the United Kingdom set up a Special Investigation Committee to investigate the clinical phenomenon of "wound shock". This was done by observing and treating wounded soldiers on the Western Front and by experimental work on animals in the laboratory.

In World War II the UK Medical Research Council's Committee on Traumatic Shock and the US Army Board for the Study of the Severely Wounded provided some of the most detailed descriptions of the physiological responses to haemorrhage and injury and established many of the guiding principles of treatment. In the Korean War renewed efforts were made to improve our understanding of trauma and the management of casualties by the US Army Surgical Research Team. This large team operated at the "front" and was linked to facilities "behind the lines" and supported from institutions back in the US (Simeone 1984).

The US Army Surgical Research Team showed that "laboratory standard" research could be conducted, even at the front line. Its original mission was to: 1) demonstrate the practicality of field research; 2) to identify and study problems encountered in the care of combat casualties; 3) to make recommendations for improving care of combat casualties; and 4) to identify problems encountered in the Combat Theater which require further research in the laboratories of the Zone of the Interior (Simeone 1984). The Research Team added medical education to these original goals. This research effort continued during the US involvement in Vietnam.

Following the "Biological Revolution" of the 1960s interest has shifted away from traditional physiology towards cellular and molecular biology. In many countries only Army medical research teams, or their descendants, remain at the front line of research into the pathophysiology of shock and its treatment.

The Nineteenth Century and before

Lockhart-Mummery reported that in the seventeenth and eighteenth centuries shock was regarded as a complication of gunshot wounds (Lockhart-Mummery 1905). Le Dran in 1737 attributed "choc" to the commotion and agitation of the body by the bullet (Lockhart-Mummery 1905). This replaced an earlier concept of loss of vital heat or spirit (Peter Lowe, 1612, reported in Lockhart-Mummery 1905).

By the middle of last century shock was already a recognised complication of surgery and injuries. Jordan in his Hastings Prize Essay wrote "Shock may be defined in general terms as a peculiar condition of the animal system, characterised by depression of all its functions, the result of a powerful impression applied to the nervous centres, or to a portion... of the peripheral nervous expansion" (Jordan 1867). For him the causes of shock were either: 1) corporeal; or 2) psychical; or 3) a combination of both; or 4) cold. Although he thought shock was nervous in origin he recognised that shock manifested

itself "Due to secondary effects of the injury on the heart and consequently on the circulation generally".

In the last years of the century a number of theories emerged to explain the shock following surgery and injuries, Table 1.1, (adapted from Simeone 1984). These centred on collapse of some part of the nervous system.

Despite these many and varied theories it was realised that to understand shock it was necessary to understand the physiology of the circulation and particularly the physiology of blood pressure (Lockhart-Mummery 1905). This was made very much easier by the introduction of the Riva-Rocci sphygmomanometer by Harvey Cushing. This was considered a breakthrough because it was easy to operate and it enabled nurses to measure blood pressure.

It seems that the researchers of the time clung to the idea of vasomotor failure as the cause of shock even though haemorrhage, hypotension, and the importance of blood transfusion in the treatment of shock had become apparent. Lockhart-Mummery, in 1905, told his audience at the Royal College of Surgeons that "The essential factor in ... shock is a steady fall in general blood pressure" but insisted that "This fall in blood pressure results from exhaustion or fatigue of the vasomotor centres" (Lockhart-Mummery 1905). From his review of Crile's work he concluded that "Shock .... is solely due (haemorrhage excluded) to injury of the nerves of the part and is in direct proportion to the nerve supply". And yet it was well known that the usual response to nerve stimulation was an increase in blood pressure. Crile himself, in a discussion on the prevention and treatment of shock in 1910, emphasised the importance of preventing blood loss during surgery and the value of blood transfusion if other treatments failed (Crile 1910).

Table 1.1: Early theories on the origin of shock

Table 1.1

Early theories on the origin of shock

Cause	Date	Proponent
Vasomotor paralysis	1864	Mitchell, Morehouse, Keen (based on casualties of the American Civil War)
Vasoconstriction and capillary congestion	1879	Mapother
Fat embolism	1885	Groeningen
Oligaemia-haemoconcentration	1893	Sherrington, Copeman
Exhaustion by overaction (brain, liver, adrenal)	1897	Crile
Acapnia* and reduced venous return	1908	Henderson
Cardiovascular inhibition	1908	Meltzer
Adrenal exhaustion	1915	Corbett
Adrenal overactivity	1917	Bainbridge, Trevan

* Henderson showed that shock could be precipitated in thoracotomised dogs by vigorous hyperventilation (Henderson 1908). He concluded "The hypothesis is presented that acapnia (diminished CO_2 in the blood and tissues resulting from hyperpnoea and from exhalation of CO_2 from exposed viscera) is the cause of surgical shock".

World War I

Fraser and Cowell found that there were two typical blood pressure responses in wounded soldiers (Fraser and Cowell 1918): they tended to be either hypotensive or hypertensive, but very few were considered to be normotensive. Extensive study suggested that there were two types of wound shock. Primary wound shock was seen immediately or very

shortly after wounding and was usually associated with serious, often fatal injuries. Secondary shock was associated with moderately severe injuries, such as fracture of the femur, and only tended to appear some time after the injury. It could be prevented by prompt treatment (Cowell 1918).

A distinction was made between "wound shock" and haemorrhage. Cannon et al. described haematological differences between the two conditions (Cannon et al. 1918b). Shock was characterised by haemoconcentration (a high capillary red cell count), but in haemorrhage the capillary red cell count was usually much lower. Haemorrhage was regarded as a complication of shock rather than its cause.

Studies of blood volume in 14 cases of haemorrhage showed that blood pressure was only a useful indicator of blood volume below a critical level. Above this critical level of blood loss vasomotor mechanisms were able to maintain normotension. Below this level there was a direct relationship between blood pressure and blood volume (Robertson and Bock 1919a).

Cannon recognised that there was also a reduction in blood volume in wound shock (Cannon 1918b). However because many wounds were not associated with obvious haemorrhage he was unable to account for this blood loss and called it "exemia". He suggested that the hypotension of wound shock resulted from the draining of blood from essential parts of the circulation.

Hypotension in wound shock was associated with acidosis and an increase in respiratory rate (Cannon 1918a). It was noted that this acidosis could also be found in haemorrhage and after surgery. This resulted in great emphasis being placed on the use of sodium bicarbonate in the treatment of wound shock (Cannon et al. 1918a). Cannon et al. went so far as to recommend that "Large drafts of hot sweet tea made alkaline with sodium bicarbonate ... should be given whenever the shocked patient complains of thirst".

Some of the principles of resuscitation developed then seem inadequate now. But the importance of blood transfusions in the severely injured had already been understood (Fraser and Cowell 1918). The limitations of isotonic crystalloids were apparent and the advantages of hypertonic crystalloids and colloid solutions as temporising measures prior to blood transfusion were already established (Medical Research Committee 1917; Bayliss 1918). Oral and rectal fluids were used for the less critical haemorrhage cases, which still demanded prompt treatment (Robertson and Bock 1919b).

By the end of the War, Cannon was able to conclude that many of the pre-War theories on the origin of wound shock, such as Henderson's acapnia theory (Henderson 1908), and the theories of nervous exhaustion, were no longer tenable (Cannon 1918b). Instead of adrenal exhaustion it was apparent that the adrenal was overactive. Although wound shock was associated with hypotension there was no evidence for a primary cardiac defect.

The Inter-War Years

Interest in wound shock continued after World War I. Histamine had been shown to produce an oligaemic circulatory collapse not dissimilar to wound shock (Dale and Richards 1918; Dale and Laidlaw 1919). This finding combined with the results of limb trauma experiments by Bayliss and Cannon led to the concept of a toxic origin for wound shock. It was suggested that the traumatised limb released a histamine-like toxin. Once in the systemic circulation such a toxin could cause an increase in capillary permeability and a loss of plasma, which could explain the haemoconcentration found in wound shock. This resulted in considerable interest in the properties of the vascular endothelium and the microcirculation (Moon 1942).

Others came to different conclusions. Experiments by Parsons and Phemister, and others, in which they traumatised the hindlimbs of anaesthetised dogs showed that the fluid loss from wounds could account for the reduction in blood pressure (Parsons and Phemister 1930). They could find no evidence for the production of a toxin by the wound. There

was neither evidence of vasomotor failure nor evidence of fat embolism as a major factor in wound shock. They even suggested "It is preferable to speak of haemorrhage rather than shock or shock due to haemorrhage when acute loss of blood in wounds, whether closed or open, is the cause of marked circulatory embarrassment or failure" (Parsons and Phemister 1930). Blalock came to similar conclusions (1930).

By the mid 1930s the Theory of Traumatic Toxaemia had been largely discredited. In a discussion at the Royal Society of Medicine Dale went so far as to say "Whatever else it may have been, the shock following the Bayliss-Cannon limb trauma was not histamine poisoning" (Dale 1935). In the same breath he did not entirely rule out a form of toxaemia as a factor in some kinds of shock. For shock seen several hours after the initial insult he said "I am not at all sure that there has been ruled out the possibility of substances absorbed from the injured tissue, which have a long-range effect and gradually break down the permeability of the capillaries". Sixty two years on there are plenty of possible candidates.

Understanding the causes of traumatic shock was not the only priority of research during this period. There was still a need to improve its treatment. Blalock used his experiments to draw up a plan for resuscitation, which contained five essential elements (Blalock 1927):

1) control of haemorrhage,
2) sub cutaneous injection of ephedrine,
3) intra venous (i.v.) saline,
4) blood transfusion, and then
5) "repeat the procedure if condition still precarious"!

He recognised that i.v. saline should be given quickly and in large amounts and that there was little risk of "embarrassing the heart" and the "relative uselessness of drugs as contrasted to the procedures which restore the volume of blood". This echoed Bayliss's earlier conclusion "To look upon acidosis and its treatment as of secondary importance

and the maintenance of an adequate supply of oxygen by a good circulation of oxygenated blood as the essential matter. This is attained more effectively by the injection of blood or gum (gum acacia) than by drugs which constrict arterioles" (Bayliss 1918). These principles appear as relevant today as they were then.

World War II

Grant and Reeve, during the "blitz" on London also found a great variation in the physiological condition of the air-raid casualties they studied, noting that some had a normal blood pressure, while others were hypertensive, but the majority were hypotensive (Grant and Reeve 1941). Within this latter group they noticed a range of heart rates. They noted that, "In assessing the need for transfusion emphasis is placed on the severity of the injury and blood loss rather than the blood pressure". They realised that traumatic shock was the result of two distinct insults: haemorrhage and injury.

Many of the foundations of our understanding of the physiology of haemorrhage and injury were laid during and shortly after World War II. These will now be considered in more detail.

The cardiovascular response to haemorrhage

Studying the response to haemorrhage in a clinical setting is often difficult: the urgency of resuscitation makes extensive physiologic assessment impossible and the resuscitation itself will alter the responses observed. In addition haemorrhage is often associated with trauma or considerable fear (such as with bleeding oesophageal varices) all of which may alter the response to blood loss, as will be demonstrated later. Despite these problems valuable clinical observations have been made.

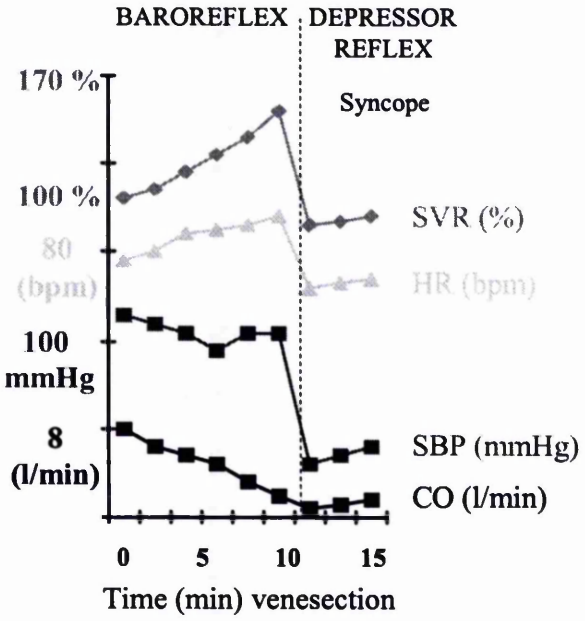
An alternative to clinical observations is to study controlled haemorrhage either in human volunteers or in animals, or to simulate haemorrhage by reducing central blood volume. The latter can be achieved by postural means (head-up tilt), the application of tourniquets around the lower limbs to produce blood sequestration, or to apply negative pressure to the lower body (LBNP) to sequester blood in the pelvis and the lower limbs.

The biphasic response to haemorrhage

During World War II extensive blood donor programmes were established. Barcroft et al. were interested in the fact that a small percentage of blood donors lost consciousness following even a small haemorrhage and that the percentage increased as the volume of venesection increased (Barcroft et al. 1944). They studied the phenomenon by venesecting volunteers and monitoring heart rate, blood pressure, cardiac output, right atrial pressure and forearm blood flow. They found that initially the response to haemorrhage was an increase in heart rate and total peripheral vascular resistance so that despite a fall in cardiac output, blood pressure was maintained (Figure 1.1). However, once about 1000 ml of blood had been removed, there was a sudden fall in blood pressure associated with a bradycardia and syncope. This was found to coincide with a profound increase in forearm blood flow and a reduction in systemic vascular resistance. These changes could be largely reversed by the reinfusion of the shed blood.

It appeared that the response to simple haemorrhage consisted of two distinct phases: an initial phase of tachycardia and increased vascular resistance maintaining arterial pressure, followed by a phase of decompensation with hypotension and bradycardia. These observations have been confirmed clinically (Sander-Jensen et al. 1986; Driscoll 1994), in simulated hypovolaemia (Secher et al. 1984; Matzen et al. 1991), and in animal experiments (Little et al. 1989; Jacobsen et al. 1990; Geerdes et al. 1993). The bradycardia of haemorrhagic shock is reviewed by Secher and Bie (Secher and Bie 1985). The precise mechanisms controlling this biphasic response to simple haemorrhage have been extensively reviewed (Kirkman and Little 1994). Only a brief outline of the reflexes involved will be provided here.

Figure 1.1: Biphasic response to haemorrhage.



Faint induced by venesection showing the biphasic response in heart rate (HR), blood pressure (SBP) and systemic vascular resistance (SVR) with a gradual reduction in cardiac output (CO) (Adapted from Barcroft et al. (1944).

The arterial baroreceptor reflex

This reflex, which depends on baroreceptors located in the aortic arch and carotid sinuses, controls blood pressure around a certain set-point. These receptors respond to the degree of stretch of the arterial wall produced by the intra-luminal pressure, rather than the pressure itself. They also show a rate sensitivity and respond to the rate of change of arterial blood pressure as well as its absolute level. Changes in pulse pressure will cause a response even if mean pressure is maintained. A relatively small haemorrhage (up to 10-15% of total blood volume (TBV)) will cause a reduction in pulse pressure and so unload the baroreceptors even if mean arterial pressure is unchanged. This unloading results in a reduction in afferent activity to the brain (via the vagus from aortic arch receptors and via the sinus nerve, a branch of the glossopharyngeal nerve, from the carotid sinus receptors). Efferent activity is consequently reduced. The efferent limb of the reflex is carried in the vagus and sympathetic nerves to the heart, and the sympathetic vasoconstrictor nerves to the blood vessels. The result is a reduction in cardiac vagal activity and an increase in sympathetic stimulation of the heart. At the same time there is an increase in sympathetic vasoconstrictor tone and an increase in total peripheral vascular resistance. As will be shown later, not all vascular beds experience the same degree of vasoconstriction. The overall effect of this reflex is to maintain arterial blood pressure and perfusion to tissues critically dependent on oxygen supply in the face of a reduction in cardiac output. When blood loss exceeds a critical volume (usually around 20% TBV) hypotension and bradycardia are seen. This is due to the activation of another reflex, referred to as the "depressor" reflex, rather than a failure of the baroreceptor reflex.

The "depressor" reflex

Severe blood loss can lead to a marked bradycardia, a reduction in peripheral resistance and consequently a fall in arterial blood pressure. This is the result of the activation of a second reflex: the "depressor" reflex, which appears to over-ride the baroreceptor reflex. The efferent limb of this "depressor" reflex is carried in the vagus (increased activation leading to bradycardia, which can be blocked by atropine (Secher et al. 1984)) and the

sympathetic vasoconstrictor nerves (decreased activation leading to vasodilatation). Until recently it was thought that the afferent limb of this reflex was carried in C-fibres originating from mechanoreceptors in the ventricular myocardium (Öberg and Thorén 1972). More recent studies have questioned the nature of the afferent pathway since a similar reflex has been reported causing a bradycardia in a cardiac transplant recipient (with denervated ventricles) (Scherrer et al. 1990). Also the "cardiac C-fibre" reflex does not appear to have the same central nervous pathway as the "depressor" response associated with severe haemorrhage (Kirkman et al. 1994) and is thus unlikely to be the same reflex.

The arterial chemoreceptor reflex

In severe haemorrhagic hypovolaemia, a third reflex seems to be important: that mediated by arterial chemoreceptors in the aortic and carotid bodies responding to hypoxia. Hypoxia results in a vagally mediated bradycardia and increased sympathetic vasoconstrictor tone in addition to an increase in respiration (possibly manifested as the air hunger of hypovolaemic shock). Details of the central pathways involved in these reflexes are covered in the review referred to above (Kirkman and Little 1994).

The triphasic response to haemorrhage

More recent animal studies (Jacobsen et al. 1990) and clinical experience (Jacobsen and Secher 1992) have suggested that with very severe haemorrhage there is an additional phase of hypotension and tachycardia after the phase of hypotension and bradycardia. In their studies with anaesthetised pigs Jacobsen et al. (Jacobsen et al. 1990) showed that with a 10% haemorrhage there was a slight increase in mean arterial pressure (MAP) and heart rate (HR). After 15% TBV had been lost, MAP fell as did HR. Further blood loss to 44% TBV resulted in a further fall in MAP but a massive increase in HR. This tachycardia was associated with increases in plasma concentrations of adrenaline and noradrenaline, indicating increased sympathetic activity. An increase in pancreatic

polypeptide was noted during the bradycardic phase, denoting an increase in parasympathetic activity, which persisted during the third or tachycardic phase. There was also an increase in aldosterone promoting the retention of sodium and hence fluid.

Jacobsen and Secher observed a similar triphasic response in a series of patients in haemorrhagic shock (Jacobsen and Secher 1992). Eighteen patients with a mean blood loss of less than 3 l (mean: 1.9 l, about 34% of estimated blood volume) had a mean HR of 83 beats min^{-1} and a mean MAP of 62 mmHg. Sixteen patients with blood loss of more than 3 l (mean: 4.0 l, corresponding to 89% of estimated blood volume) had a mean HR of 120 beats min^{-1} and a mean MAP of 52 mmHg. Six patients died from their haemorrhage with a mean blood loss of 3.1 l. These patients had a mean HR of 129 beats min^{-1} and a mean MAP of 40 mmHg. Those patients with a blood loss of less than 3 l appeared to show a relative bradycardia and hypotension while those with a greater blood loss showed a profound hypotension and tachycardia. No patients in the bradycardic phase died suggesting that this phase of haemorrhagic shock is readily reversible with prompt fluid resuscitation. However, once patients go into a phase of tachycardia and hypotension, shock may be irreversible. This third and potentially irreversible phase of haemorrhagic shock is associated with increased sympathetic activity. The precise mechanisms triggering this have not been clearly elucidated, but it may be related to the development of cerebral hypoperfusion and cerebral hypoxia.

The concept of three phases in the response to haemorrhage is not new. In 1946 Wiggers described haemorrhage progressing from: 1) a simple haemorrhagic-hypotensive state (blood loss up to 30-40% of TBV), from which spontaneous recovery was possible, to 2) an impending shock state (>40% blood loss). In this state there was greater tissue anoxia and irreversible organ damage. Only timely fluid infusion would prevent the development of, 3) an irreversible shock state (Wiggers and Ingraham 1946).

The cardiovascular response to injury

The response to injury is superficially much simpler than that to haemorrhage. Loven in 1866 found an increase in blood pressure following stimulation of the central end of a cerebro-spinal nerve (reported in Parsons and Phemister 1930). This was acknowledged as the usual response to nerve stimulation by Lockhart-Mummery despite the prevailing view that wound shock resulted from failure of the vasomotor centres (Lockhart-Mummery 1905). He recognised that in mixed nerves there were "pressor fibres" and "depressor fibres".

The response to injury consists mainly of a pressor response: an increase in blood pressure mediated by an increase in sympathetic vasoconstrictor tone producing an increase in peripheral vascular resistance (Howard et al. 1955). The injury response is accompanied by a tachycardia: there is a suppression of the baroreflex (Anderson et al. 1990) which would normally result in a bradycardia. The response to injury has been modelled in experimental animals in many different ways (see Animal Models).

A similar response is seen when the "defence area" of the brain is stimulated electrically (Coote et al. 1979; Hilton et al. 1983). Stimulation of the "defence area" provokes the "defence reaction" the features of which are: 1) a tachycardia, 2) an increase in blood pressure, 3) an increase in sympathetic efferent activity, and 4) a relative vasodilatation to skeletal muscle with vasoconstriction in the splanchnic vasculature. In contrast to the response to haemorrhage, which preserves blood flow to vital organs, the response to injury prepares the organism for "fight or flight" and so diverts blood away from the visceral organs to skeletal muscle. The similarities between these two responses has resulted in the suggestion that the response to injury is mediated via the same pathways as the defence reaction. A more detailed explanation of the central pathways can be found in Kirkman and Little (Kirkman and Little 1994).

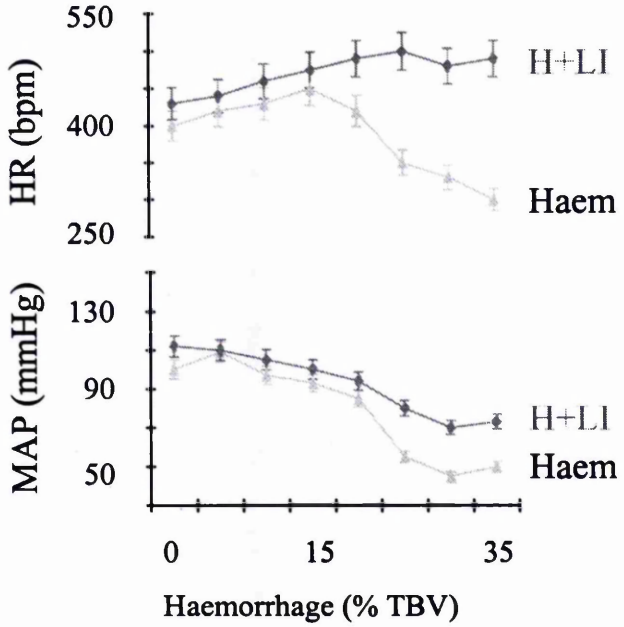
The cardiovascular response to combined haemorrhage and tissue injury.

Historically haemorrhage was regarded as a complication of wound shock. When Parsons and Phemister realised that blood loss could account for wound shock they also noticed that a minor degree of haemorrhage could be fatal if associated with an injury (Parsons and Phemister 1930). This interaction was studied later by Wang et al. (1947). In a canine model of haemorrhage and haemorrhage and soft tissue trauma there was a higher mortality in the haemorrhage and soft tissue trauma group. As the circulating volume lost was the same in both groups they concluded that another factor was involved. In further studies they found similar increases in mortality when sciatic nerve stimulation or soft tissue trauma were added to their haemorrhage model (Overman and Wang 1947). From this they deduced that afferent nerve stimulation was an important factor in the increased mortality of traumatic shock compared with simple haemorrhagic shock.

A variety of models have been used to study the interaction between haemorrhage and injury. Figure 1.2 compares the cardiovascular response to haemorrhage with and without concomitant tissue injury, modelled using hind-limb ischaemia (Little et al. 1989). A number of differences are apparent. The background limb ischaemia results in a higher resting HR and MAP. It appears that there is a re-setting of the baroreceptor reflex to a higher MAP and there is also a reduction in the sensitivity of the reflex. Coote et al. showed that somatic afferent nerve stimulation can modulate the baroreceptor reflex (Quest and Gebber 1972; Coote et al. 1979). A similar phenomenon has been recorded clinically by Anderson et al. (1990), who found that patients suffering moderate injuries, such as long bone fractures, showed a reduction in baroreflex receptor sensitivity within a few hours of their injury. The reflex had not returned to its normal sensitivity even after 14 days. Driscoll also suggested that there was a reduction in baroreflex sensitivity and a right shift in baroreflex setting in patients with significant tissue damage in addition to blood loss (Driscoll 1994). Figure 2 also shows that in those animals subjected to hind-limb ischaemia there is no bradycardia as haemorrhage progresses. Such a bradycardia is

normally mediated by the "depressor" reflex and an increased vagal tone to the heart. Thus, tissue injury also seems to interfere with this reflex pathway.

Figure 1.2: Cardiovascular response to haemorrhage and hindlimb ischaemia.



The figure shows the effects of progressive haemorrhage (Haem), and the effects of haemorrhage in the presence of bilateral hind-limb ischaemia (H+LI), on heart rate (HR), in the upper panel, and mean arterial blood pressure (MAP), in the lower panel, in the conscious rat. Values are expressed as means ± SE. Adapted from Kirkman and Little (1994)

It is clear that the cardiovascular responses to haemorrhage and injury are quite different. However, central integration of the various reflex pathways is such that the cardiovascular response, when the two conditions occur simultaneously, resembles the response to injury more than haemorrhage.

Consequences of haemorrhage and injury: regional blood flows

It is apparent from early studies on the response to haemorrhage (Barcroft et al. 1944) that in the initial phase of haemorrhage there is an increase in systemic vascular resistance (SVR), which is instrumental in maintaining arterial blood pressure in the face of a fall in cardiac output. At this time there is no change in forearm blood flow, suggesting that the increase in SVR is being caused by vasoconstriction in other vascular beds. There have been many studies of the regional blood flow responses to haemorrhage in animals, which may shed some light on which vascular beds contribute most to the global increase in vascular resistance.

Vatner studied mild and moderate haemorrhage in conscious dogs (Vatner 1974) and found that with mild haemorrhage (resulting in a tachycardia but no fall in blood pressure) there were increases in mesenteric and iliac vascular resistance, but a reduction in renal vascular resistance. With moderate hypotensive haemorrhage there was a 56% reduction in mesenteric blood flow compared to a 38% reduction in coronary blood flow. When vascular resistances were calculated it was found that mesenteric resistance had increased by 73% but had only increased by 27% in the coronary vasculature. These results suggest that the vascular response to haemorrhage results in a redistribution of blood flow in favour of the heart at the expense of other internal organs.

When haemorrhage or hypovolaemia is taken further so that there is profound hypotension, regional blood flows may alter again. Barcroft et al. found that when their volunteers became hypotensive and fainted, there was a sudden fall in SVR and a sudden increase in forearm blood flow, suggesting a sudden skeletal muscle vasodilatation (Barcroft et al. 1944). A similar skeletal muscle vasodilatation occurred when sheep were haemorrhaged until an abrupt fall in blood pressure was seen (Gunnarsson et al. 1994).

Regional vascular responses have also been studied using lower body negative pressure (LBNP) to simulate hypovolaemia (Hirsch et al. 1989). Hirsch et al. were interested in seeing whether there was any difference in regional vascular responses at different levels

of LBNP (Hirsch et al. 1989). With a 10 mmHg LBNP central venous pressure (CVP) was reduced but BP and HR were unaffected suggesting that the cardiopulmonary baroreceptors were unloaded rather than the arterial baroreceptors. In this situation there was a reduction in forearm and splanchnic blood flows but renal blood flow was maintained. When the degree of LBNP was increased so that pulse pressure fell, and so both arterial and cardiopulmonary baroreceptors were unloaded, forearm blood flow returned to control levels. However, there was a further reduction in splanchnic flow and renal blood flow finally fell, suggesting that splanchnic blood flow is more sensitive to selective unloading of cardiopulmonary baroreceptors than renal blood flow.

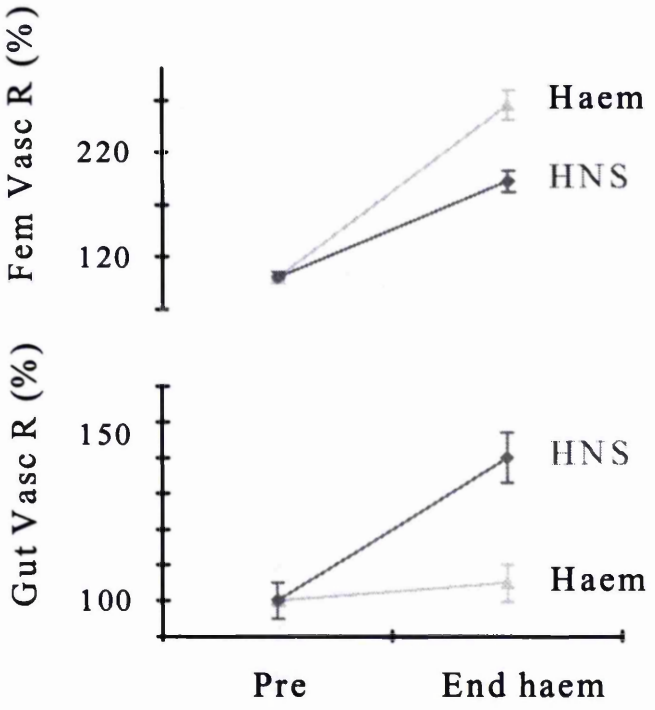
Edouard et al. used LBNP on normal volunteers to simulate normotensive hypovolaemia, that is, a mild degree of haemorrhage (Edouard et al. 1994). In this model they could produce a one-third reduction in cardiac output, which resulted in a 75% increase in SVR. They found that there was an almost equal reduction in forearm (30%) and splanchnic (33%) blood flows during simulated normotensive hypovolaemia and that these paralleled the reduction in cardiac output. However when LBNP was reversed, all cardiovascular variables returned to their pre-LBNP values except for splanchnic blood flow. This remained depressed until the end of their study period 60 min after reversal of LBNP. Their study suggested that splanchnic blood flow was particularly vulnerable to simulated hypovolaemia and that this vulnerability persisted despite apparent complete resuscitation. The results of all these studies suggest that in simple haemorrhage, blood flow to the gut is reduced from an early stage as part of the vasoconstrictor response to maintain arterial pressure.

Parallels have been drawn between the responses to injury and the defence reaction (Quest and Gebber 1972). The essential components of the regional vascular changes during the "defence reaction" are an increase in splanchnic vasoconstrictor tone and a reduction in vasoconstrictor tone to skeletal muscle, thus preparing the organism for "fight or flight" (Hilton et al. 1983).

When haemorrhage occurs on a background of somatic afferent nerve stimulation (to mimic injury) there is an alteration in the pattern of vascular response (Mackway-Jones et

al. 1994). In haemorrhage alone (30% of TBV), vascular resistance in the femoral bed increased to 265% of the pre-haemorrhage level, while resistance in the splanchnic bed hardly increased (to 105% of the pre-haemorrhage level). When 30% TBV haemorrhage was superimposed on somatic afferent nerve stimulation, femoral resistance increased much less than in haemorrhage alone (to 192%, instead of 265%). Splanchnic resistance increased much more than in haemorrhage alone (to 140%), showing that with the additional insult of somatic afferent nerve stimulation (injury), there was a relative diversion of blood away from the gut towards skeletal muscle (Figure 1.3). Thus, the gut seems to experience greater reductions in blood flow in situations of combined tissue injury and blood loss than when blood loss occurs alone.

Figure 1.3: Changes in regional vascular resistances in response to haemorrhage and somatic afferent nerve stimulation.



The figure shows the changes in femoral vascular resistance (upper panel), and gut vascular resistance (lower panel) before and after a 30% TBV haemorrhage (Haem), and after the same haemorrhage in the presence of brachial nerve stimulation (HNS). Values are expressed as means ± SE. Adapted from Mackway-Jones et al. (1994).

Consequences of haemorrhage and injury: microvascular blood flows

None of the studies described so far have examined changes in microvascular blood flow in various organs following haemorrhage. Laser Doppler flowmetry (LDF) has been used to determine microvascular blood flow following haemorrhage (Wang et al. 1990). Rats haemorrhaged to a MAP of 40 mmHg showed reductions in microvascular blood flow in the liver, spleen, kidney, small intestine and skeletal muscle, with the greatest reductions being seen in the small intestine and the kidney. Volume replacement with Ringer's Lactate could only partially restore microvascular blood flow, which continued to

deteriorate in all organs, even after resuscitation. The precise mechanisms for this reduction in flow could not be determined from the experiments but one of those suggested was the development of a tissue acidosis with hypoperfusion. Other possibilities might include an alteration in vascular tone, an alteration in α adrenergic receptors, a maldistribution of cardiac output, arterio-venous shunting, the formation of micro-emboli, or an effect of inflammatory mediators, such as cytokines.

A more detailed description of the microcirculation is beyond the scope of this review. The microcirculation is described in (Zweifach and Lipowsky 1984). Changes in the microcirculation in haemorrhage have been summarised by Zweifach (1974). It has become clear that the microcirculation plays a key role in septic shock. This is reviewed in the following chapters: (Groeneveld and Thijs 1987; Lewis 1987; Messmer 1987).

Consequences of haemorrhage and injury: gut mucosa

Do the changes in gut blood flow, which are seen with both simple haemorrhage and haemorrhage with injury have any major functional consequences? There now appears to be ample evidence that reduced gut blood flow can result in mucosal ischaemia and eventually to mucosal damage. The gut mucosa plays an important role as a barrier between the host and the gut microflora, which contains potential pathogens and toxins. Any ischaemic insult to the mucosa may result in a loss of this barrier function and the translocation of organisms or toxins from the gut lumen into the host lymphatics or circulation. Tonometers have been used for some time to measure intramucosal pH (pHi) in the gastrointestinal tract. This technique was used in a model of haemorrhagic shock in pigs (Montgomery et al. 1989) to show that pHi in the small intestine and sigmoid colon fell with only a small degree of haemorrhage (to a MAP of 80 mmHg), while gastric pHi remained unchanged. When MAP fell to 45 mmHg there were further reductions in small intestine and colon pHi as well as a drop in gastric pHi. Reinfusion of the shed blood resulted in increases in pHi back towards basal levels although only colonic pHi returned to normal. In haemorrhagic shock (Hartmann et al. 1991) gastrointestinal pHi has been compared to other measures of tissue oxygenation such as transcutaneous oxygen tension

(PtO_2) and subcutaneous oxygen tension ($PsqO_2$). Small intestine and sigmoid colon pHi and PtO_2 were both found to be sensitive indicators of blood loss. In addition PtO_2, $PsqO_2$ and pHi were all found to correlate well with total body oxygen transport.

An alternative technique for studying the gut mucosa has been to use phosphorescent tissue oxygen sensors placed on the mucosal surface. This was done in rats subjected to graded haemorrhage (Zabel et al. 1994). Mucosal oxygen tensions (PmO_2) were compared to serosal oxygen tensions (PsO_2). Even before any haemorrhage, there was a large gradient between the serosa and the mucosa, such that the mucosa appeared hypoxic compared to the serosa. Mucosal oxygen tension was found to be very sensitive to changes in blood volume: there was a fall in PmO_2 before any change in MAP or PsO_2. By the time blood loss reached 15%, PmO_2 had fallen by 40% with minimal changes in MAP and PsO_2. This provides further evidence for potential hypoxia of the gut mucosa in the face of haemorrhage.

Consequences of haemorrhage and injury: translocation of bacteria and endotoxin

If the first half of this century represented a "Golden Age" in shock research, when many of the physiological principles of shock were established, then the 1950s and 1960s marked a period of intense activity to understand why unresuscitated shock could be fatal.

One of the early theories about the cause of wound shock centred on the possibility of a toxin or toxic factor being released from the wound. The work of Parsons and Phemister, and Blalock in the 1930s suggested otherwise (Blalock 1930; Parsons and Phemister 1930). They realised the importance of fluid loss from the wound. Experience with battle casualties confirmed this. After the battle of El Alamein, Wilson wrote, "The treatment of wound shock might be summarised as full replacement of lost blood by transfusion combined with efficient control of bleeding" (Wilson 1944). He also commented that, "Severe wounding was often followed by an illness, more or less

serious and lasting at least several days, in which many factors other than blood-loss or its late effects operated" (Wilson 1944).

Interest in a possible toxin was revived by the discovery that dogs pre-treated with aureomycin, until their stool was free from E.coli and clostridia, were more resistant to haemorrhagic shock than untreated controls (Frank et al. 1952). Even at this early stage the suggestion was made that "Tissue anoxia of shock might stimulate invasion of the liver by intestinal flora".

When oral and parenteral antibiotic pre-treatments were compared, the increase in survival was repeated but there was no significant difference between the two routes of administration (Jacobs et al. 1954). The authors concluded that the gastro-intestinal flora was not the main source of the bacteria causing irreversibility in their model. They did notice that there was a higher incidence of bacteria in portal blood than in caval blood, which suggested that there was movement of bacteria out of the gut. They also suggested that this was a normal physiological process, but that normally the host's defences achieved total clearance.

That the host's defences against bacteria were weakened in haemorrhagic shock was demonstrated in a series of experiments in which dogs in various states of shock were injected with bacteria (Schweinburg et al. 1954). A shock period as short as two hours was enough to render a normally harmless dose of E.coli lethal. At the time the authors could not explain this finding, merely pointing to the known reduction in leukocyte mobilisation and walling off of inflammation in shock.

Later experiments suggested that whole blood, or even plasma, from animals in the terminal phase of haemorrhagic shock was toxic. Schweinburg et al. resuscitated dogs after two hours of haemorrhagic shock with blood removed from animals dying after 6 hours of haemorrhagic shock and found that most died (Schweinburg et al. 1957). Similar results were obtained if plasma, filtered to remove any bacteria, was used. They suggested that the whole blood, and plasma, contained a toxin. Unshocked, or normal,

recipients of this "toxic blood" showed no adverse effects, confirming that the two hour shock period was enough to weaken the recipients' antibacterial defences (Schweinburg et al. 1954).

The origin of the "toxic factor" was suggested by experiments in which antibiotics were given to the donor dogs before the six hour shock period (Schweinburg et al. 1957). Blood from donors given non-absorbable antibiotics orally and rectally for three days was much less toxic than blood from untreated animals. From this they deduced that the toxic factor originated in the gastro-intestinal tract, even commenting that "Current work to be reported later, indicates that this toxin is an endotoxin".

This toxic factor was later isolated and identified as a polysaccharide (Ravin et al. 1958). Some years earlier techniques had been developed for radiolabelling bacterial lipopolysaccharides or endotoxin (Braude et al. 1955a). Use of these techniques showed that endotoxin injected intravenously was rapidly cleared from the circulation and appeared in the liver and spleen (Braude et al. 1955b; Rowley et al. 1956). This suggested that the polysaccharide isolated by Ravin et al. might be tracked to the reticulo-endothelial system (RES).

The importance of endotoxin in the mortality from haemorrhagic shock was shown in 1959 (Fine et al. 1959). Endotoxin also seemed to be of fundamental importance in septic shock and tourniquet shock (Schweinburg and Fine 1960). So as early as the 1950s it was realised that there could be a common link in different types of shock.

The importance of the RES was shown in experiments using thorotrast. Thorotrast is a sterile colloidal suspension of 25% thorium dioxide in dextrins. Intravenous injection results in uptake by the RES, which is then unable to detoxify endotoxin for several hours. The injection of thorotrast was shown to turn a normally reversible degree of haemorrhagic shock into irreversible haemorrhagic shock in both dogs and rabbits (Fine et al. 1959).

A series of experiments were performed using coliform-free rabbits given an inoculum of ^{32}P-labelled E.coli by gavage (Ravin et al. 1960). One group was subjected to haemorrhagic shock (to a blood pressure of 50 mmHg) for five to six hours and then resuscitated with their shed blood. Fifteen minutes later they were exsanguinated and biopsies taken of the liver, spleen, and kidney. The other group acted as sham controls. Endotoxin was found in the liver, spleen, kidney, and blood of the shocked rabbits. The control animals only had detectable endotoxin in the liver. This was taken to show that the movement of endotoxin out of the gastro-intestinal tract was a normal physiological process. In normal animals this was detoxified, but in haemorrhaged animals there was a defect in the detoxification process so that endotoxin appeared in other solid organs and in the circulation. In vitro studies suggested that macrophages played an important role in this detoxification process (Rutenburg et al. 1960).

As only a fraction of the ^{32}P-labelled E.coli inoculum was recovered in any animal, it was suggested that there was passive diffusion of the endotoxin from the gut into the circulation (Ravin et al. 1960). In 1962 the term "translocation" was used by Hildebrand and Wolochow to describe the passage of particulate matter, including bacteria, from the gastro-intestinal tract to the regional lymphatic system and blood (Hildebrand and Wolochow 1962).

Nearly two decades later bacteria and endotoxin were once more in the limelight as a new generation of researchers investigated the phenomenon of bacterial or endotoxin translocation.

Berg and Garlington found no evidence for translocation of indigenous aerobic or anaerobic bacteria to the mesenteric lymph nodes of specific pathogen-free (SPF) mice (Berg and Garlington 1979). They then inoculated (intragastrically) germ-free mice with caecal flora from these SPF mice. In 65% of the mice there was translocation of organisms to the mesenteric lymph nodes (MLN). Organisms could also be cultured from some of the livers and spleens. E.coli and Lactobacillus were the commonest bacteria to translocate. No anaerobes translocated. As germ-free mice have an underdeveloped

immune system it appeared that the immune system played a role in preventing bacterial translocation.

Oral inoculation of germ-free mice with E.coli or Lactobacillus from SPF mice resulted in a 96% translocation rate to the MLN. The same inoculation of SPF mice with E.coli did not result in any translocation. However 30% of the SPF mice inoculated with Lactobacillus showed evidence of translocation. The authors suggested that the degree of bacterial translocation for any species was related to its population size. Germ-free mice had a much larger caecal E.coli population after inoculation than the SPF mice. For Lactobacillus there was much less difference, hence the increase in translocation rate compared with controls.

These experiments also suggested that the indigenous flora might have a regulatory effect. A further series of experiments compared germ-free mice inoculated orally with E.coli C25 with a similar group of mice inoculated with caecal flora from SPF mice one week after inoculation with E.coli C25 (Berg and Owens 1979). Oral inoculation with E.coli C25 resulted in 100% bacterial translocation to MLN in both groups. When caecal flora was added to the second group there was a reduction in the intestinal E.coli population, an increase in the number of anaerobic organisms in the gut, and a reduction in bacterial translocation. By day four after caecal flora inoculation there was no evidence of bacterial translocation at all. It was suggested that bacterial antagonism confined bacteria to the intestinal lumen, maybe by the formation of a layer of anaerobic organisms over the gut epithelium.

Antibiotics have been widely used to alter the gastro-intestinal flora and examine the effect on translocation. Berg used a variety of antibiotics in SPF mice and found that by reducing the number of anaerobes there was an increase in gram negative enteric bacteria and an increase in bacterial translocation (Berg 1981). When anaerobic populations returned to normal there was a reduction in the numbers of gram negative enteric bacteria and a reduction in bacterial translocation.

A similar study by Wells et al. suggested that anaerobic organisms did play a role in confining indigenous gut organisms to the gastro-intestinal tract (Wells et al. 1987). They also suggested that an intact indigenous flora helped to prevent gut colonisation by alien organisms.

The process by which organisms translocate has been investigated. It was suggested that epithelial macrophages ingested organisms and then released them, unkilled, after crossing the gut mucosa (Wells et al. 1988). However translocation of Enterococcus faecalis was observed through intact epithelial cells, rather than between them or in phagocytic cells (Wells et al. 1990). Alexander et al. examined translocation of Candida albicans, E.coli, and endotoxin in loops of small bowel in rats and guinea pigs subjected to a full-thickness burn of 50% of total body surface (Alexander et al. 1990). Using both transmission and scanning electron microscopy they found that all three probes entered intact epithelial cells. The vacuoles containing these probes did not have the normal membrane around them so there was some doubt as to whether the probes entered by normal phagocytosis. In the lamina propria most of the probes were in macrophages, but some were in intercellular spaces. These experiments showed that particles as large as Candida albicans could undergo translocation and that this occurred through intact enterocytes. Movement across the lamina propria was different as it involved macrophages and also the intercellular route.

The mechanisms which normally confine the intestinal flora to the lumen of the gut have been reviewed (Berg 1980; Wells et al. 1988).

Translocation has been demonstrated in haemorrhagic shock in rats. Baker et al. found that even a 30 minute shock period (to a MAP of 30 mmHg) was enough to induce bacterial translocation to MLNs, liver and spleen, and to result in positive blood cultures on the first day post shock (Baker et al. 1987). There was a reduction in the number of viable organisms cultured from MLNs on subsequent days. Further studies showed that there was a greater degree of bacterial translocation with longer shock periods (Baker et al.

1988). Bacterial translocation and portal endotoxaemia has also been demonstrated in a baboon model of haemorrhagic-traumatic shock (Bahrami et al. 1995).

Thermal injury has been used extensively as a model of traumatic shock (Herndon and Ziegler 1993). Burns of 15% and 30% total BSA potentiate bacterial translocation in SPF mice colonised by E.coli after decontamination with streptomycin and bacitracin. However these same burns do not result in bacterial translocation when the indigenous gut flora is intact.

Saydjari et al. showed that in pigs a 40% BSA burn resulted in a reduction in gut blood flow and bacterial translocation to MLN (Saydjari et al. 1991). If a similar reduction in gut blood flow was produced using a vascular occluder, a corresponding degree of bacterial translocation was seen. It has also been shown that if superior mesenteric arterial perfusion is maintained in a model which normally results in both a reduction in gut blood flow and an increase in intestinal mucosal permeability, then the permeability change can be prevented (Fink et al. 1991).

Alterations in intestinal permeability have been reported in a variety of clinical conditions involving blood loss (Ryan et al. 1992; Roumen et al. 1993). Whether this increase in permeability results in translocation in human clinical situations remains a controversial issue.

Consequences of haemorrhage and injury: inflammatory cytokine production

Inflammatory cytokine production has been described in models of haemorrhagic and traumatic shock (Ayala et al. 1990; Ayala et al. 1991; Deitch et al. 1994; Bahrami et al. 1995). Deitch et al. suggested that the gut acted as a cytokine-producing organ since levels of TNF-α and IL-6 were higher in portal venous blood than in systemic blood. Details of cytokine production in haemorrhage and injury appear in the section Biology of Cytokines.

Thus it appears that the vascular responses to haemorrhagic and traumatic shock can result in functionally important reductions in gut blood flow so that there is a breakdown of the gut mucosal barrier. Once this has happened bacterial or endotoxin translocation may occur with potential triggering of the production of inflammatory mediators, such as cytokines. This may represent the initiation of a chain of events leading to a process of systemic inflammation which may ultimately produce multiple organ failure.

Consequences of haemorrhage and injury: oxygen transport

The cardiovascular and regional blood flow responses to haemorrhage and injury described are accompanied by important changes in oxygen transport. Rady et al. (1991) found that in the anaesthetised pig, a 40% TBV haemorrhage resulted in a fall in oxygen delivery (DO_2I) to 55% of pre-haemorrhage DO_2I while oxygen consumption (VO_2I) was maintained. This was achieved by an increase in oxygen extraction ratio (OER) from 30% to 67% and a fall in mixed venous oxygen saturation (SvO_2) from 72% to 34%. When haemorrhage occurred on a background of SANS (to mimic injury) there was a greater reduction in DO_2I to 27% of pre-haemorrhage DO_2I. At the same time VO_2I fell to 53% of the pre-haemorrhage level despite an increase in OER from 37% to 81% and a fall in SvO_2 from 66% to 20%. Somatic afferent nerve stimulation itself was found to have no effect on either DO_2I or VO_2I, although it did produce the expected pressor response to injury (tachycardia, increase in arterial blood pressure and increase in systemic vascular resistance). A later study (Rady et al. 1993) confirmed the detrimental effect of SANS on oxygen transport in haemorrhagic shock. It also showed that skeletal muscle injury (SMI) (using two "firings" of a captive bolt to each hind-limb, avoiding all major vessels and bone) had a qualitatively similar detrimental effect. In fact, skeletal muscle injury produced a greater reduction in oxygen transport, which could not be explained by extra fluid loss into the tissues at the site of injury. They also found that similar reductions in oxygen transport resulted from a simple 40% TBV haemorrhage, a 36% TBV haemorrhage on a background of SANS, and a 29% TBV haemorrhage on a background of SMI. The precise mechanism for the deleterious effects of SANS and SMI are not

clear. It may be that SANS or injury causes an increase in critical oxygen delivery (the level of DO_2 below which VO_2 becomes supply-dependent) as has been demonstrated by Kirkman et al. (1995), which in turn may be dependent on the redistribution of blood flow away from certain metabolically active organs such as the gut towards more inactive skeletal muscle. This redistribution of blood flow may result in systemic inflammation and the metabolic anarchy caused by the release of multiple mediators which may themselves interfere with cellular metabolism.

Conclusion

In 1964 Nunn and Freeman in their paper "Problems of oxygenation and oxygen transport during haemorrhage" (Nunn and Freeman 1964) stated, "Much current research in the field of shock and haemorrhage is concerned with the biochemical mechanisms which sustain the general illness and characterise the irreversible shock state seen in animals after haemorrhage or injury". This seems to be as true now as it was then. The cardiovascular responses to haemorrhage, injury and the combination of the two insults in trauma result in functionally very significant changes in regional blood flows and hence oxygen transport. These appear to be especially important when they result in gut hypoperfusion and gut mucosal hypoxia. Any metabolically active cells such as mucosal enterocytes will suffer under these conditions. Any breakdown in their barrier function may allow the translocation of organisms or toxins which may in turn trigger the production of inflammatory mediators by the gut associated lymphoid tissue and so launch an inflammatory cascade eventually inducing multiple organ failure.

As Nunn and Freeman said "The central feature of the problem is the impairment and eventual failure of cellular oxygenation". Plus ça change, plus ça reste la même chose!

The biology of cytokines

The aims of this review section are to provide a basic introduction to the biology of cytokines and to summarise the results of studies, both laboratory and clinical, relating to the cytokine response to trauma and sepsis. This response is now regarded as playing an important role in the development of multiple organ failure: hence the measurement of TNF-α and IL-6 in the experiments described.

Introduction

Cytokines are a super family of low molecular weight glycoproteins which act as intercellular messengers. They include the interleukins, interferons, tumour necrosis factors, growth factors, transforming growth factors, colony stimulating factors and the chemokines (Table 1.2). They control and regulate a large number of essential physiological processes (Table 1.3). The considerable structural homology of these proteins within the animal kingdom reflects their importance to the proper functioning of multicellular organisms. Invertebrates possess cytokine-like proteins, suggesting that "cytokines" appeared early in the evolution of the immune system (Beck and Habicht 1996).

Table 1.2: Cytokine families

Table 1.2	**Cytokine families**		
Interleukins:	IL-1	to	IL-17
Interferons:	IFNa,	IFNb,	IFNg
Tumour necrosis factors:	TNF-α		TNF-b
Growth Factors			
Transforming growth factors			
Colony stimulating factors			
Chemokines			

Table 1.3: Physiological roles of cytokines

Table 1.3	**Physiological roles of cytokines**
Cellular control:	proliferation differentiation metabolism
Immune control:	cytotoxic cells phagocytic cells host defences against viruses and parasites
Regulation of inflammatory response and fever	
Regulation of haematopoiesis	
Wound healing and tissue remodelling	

As intercellular messengers they may act in a number of different ways:

1) autocrine communication: the cytokine interacts with the cell that produced it and modifies that cell's function. This is not necessarily a feedback process as it may regulate processes other than its own production.
2) paracrine communication: the cytokine interacts with cells adjacent to the producer cell and thus exerts very local control of cell functions.
3) endocrine communication: the cytokine passes into the systemic circulation and interacts with cells very distant to the producer cell.

Cytokines are produced by a wide range of different cell types: for example, T cells, macrophages, monocytes, fibroblasts, and endothelial cells. One cell type does not produce one specific cytokine. Different trigger stimuli acting on the same cell type may result in the production of different cytokines. Indeed T helper cells may produce several different cytokines simultaneously in response to an activating stimulus. Cytokines, unlike polypeptide hormones are synthesised de novo in response to specific stimuli (Table 1.4). There appears to be no evidence that they are ever stored intracellularly and then released. Triggers for cytokine production include processed antigens and specific antigens, viruses, double-stranded DNA, and bacterial lipopolysaccharides. All these triggers could be considered as potential threats to the well-being of the organism: hence the importance of cytokines to the body's defence mechanisms. The processes by which cytokine production is switched on are not entirely clear!

Table 1.4: Cytokines versus polypeptide hormones

Table 1.4	**Cytokines versus Polypeptide hormones**
Produced by multiple cell types	One hormone: one producer cell type
Multiple actions and duplication i.e. two cytokines having similar effects	One hormone: one action
Multiple target cells	Specific target cells/organs
Mainly local action (can be systemic)	Systemic/endocrine actions
De novo production	Storage and release
Local tissue "homoeostasis". Systemically may be deleterious	Homoeostasis

Cytokine production can be controlled at various stages:

1) transcription of genetic material
2) translation
3) during processing in endoplasmic reticulum and in the Golgi body
4) secretion

Processing in intracellular organelles may result in glycosylation, the addition of fatty acid chains, or cleavage of the protein from a precursor form to an active form. Cytokines may be secreted as either an active form or as a precursor, which is later cleaved to become active. Most cytokines are released as soluble proteins but some remain membrane-bound. In this form they may act in a paracrine fashion: interacting with receptors on adjoining cells. As proteins, cytokines are unable to penetrate cell membranes, and so, in order to have any bioactivity they must bind to receptors. Cytokine receptors are themselves proteins made up of one or more polypeptide chains. They have three domains:

1) an extracellular domain with a 3-D structure which determines both the affinity and the specificity of that receptor
2) a transmembrane domain which fixes the receptor to the cell membrane
3) an intracellular domain which interacts with second messenger systems to provide an effector mechanism.

The sensitivity of a cell to a cytokine will depend on the number of receptors on its surface and on the affinity and specificity of those receptors. Cells are thought to have 10^2 - 10^3 cytokine receptors, compared with 10^4 - 10^5 hormone or growth factor receptors (Kishimoto 1989). There may be differences in receptor numbers on different cell types: polymorphonuclear cells seem to be able to bind 2000-7000 times more interleukin-8 (IL-8) than red blood cells (Marie et al. 1997). Receptor numbers are determined by the rate at which receptors are inserted into the membrane and the rate at which they are lost by internalisation. Insertion will depend on the rate of de novo synthesis and the rate of recycling of internalised receptors. Internalisation of receptors, once ligand-binding has taken place, is an important regulatory process. By reducing the number of available receptors it may down-regulate the effect of a cytokine and render the cell temporarily insensitive to the continued presence of the cytokine. Internalisation may be an important effector mechanism in the case of interferon (IFN): allowing it to reach the cell nucleus and so have a direct effect on gene activation.

Not all receptors are membrane-bound. There are a number of soluble receptors. These may be quite specific for an individual cytokine (for example, the p55 and p75 soluble tumour necrosis factor α (TNF-α) receptors), or they may have a more general protein-binding function, for example, alpha-2-macroglobulin. These soluble receptors may act as passive carriers of cytokines, or as a circulating reservoir of the cytokine, as is the case for insulin-like growth factor-1 (IGF-1). They may also be produced to damp down the effects of a cytokine: interleukin-1 receptor antagonist (IL-Ira) is a specific antagonist of interleukin-1 (IL-1).

Second messengers are essential for the transduction of the cytokine signal. There are a number of different messenger systems (Table 1.5), which set off sequences of protein and enzyme activations resulting in the biological effects of the cytokine.

Table 1.5: Second messengers

Table 1.5	**Second messengers**
Tyrosine kinases	
G proteins	
Adenylate cyclase and cAMP	
Phospholipases	
Inositol phosphates and calcium ions	
Diacylglycerols and protein kinase C	
Arachidonic acid and prostaglandins	

At the intracellular level the main functions of cytokines are to alter gene expression and to control surface protein synthesis. Gene expression can be altered in four main ways:

1) a silent gene may be activated;
2) a gene already being expressed can be up-regulated;
3) a gene already being expressed can be down-regulated;
4) an active gene may be shut down.

Fuller details of all these processes can be found in general texts on cytokines (Clemens 1991; Thomson 1994; Nicola 1995).

Before turning to the cytokine response to experimental and clinical conditions I will look at the two cytokines which I measured in more detail.

Tumour necrosis factor

The basic molecular biology of tumour necrosis factor is well reviewed by Fiers (Fiers 1991). More general aspects of TNF-α activity can be found in the chapter by Fiers (Fiers 1993) and the review by Tracey and Cerami (Tracey and Cerami 1993). Here I will only present a brief summary.

One of the common features of chronic infection and cancer is the severe wasting known as cachexia. This was found to be mediated by a protein: cachectin, which could be purified from mouse macrophages stimulated with endotoxin. It suppressed lipopolysaccharide lipase in adipocytes after binding with high affinity receptors (Beutler et al. 1985a) and seemed to satisfy the criteria for being called a hormone. It was thought to contribute to the catabolic state induced by infection. The authors considered the relationship between cachectin and other monokines and concluded that it was different to IL-1. By the time the article was published they had already recognised some homology between cachectin and TNF-α. Cachectin was later identified as TNF-α, named after its hallmark property: cytotoxicity for certain malignant cell lines (Carswell et al. 1975).

TNF-α is a 157 amino acid polypeptide with a trimeric natural structure and a molecular mass of 52 kd. There is a related cytokine: TNF-β (previously known as lymphotoxin (LT) because it is only made by T-lymphocytes). The genes for the two cytokines are closely linked (Nedospasov et al. 1991).

TNF-α interacts with two membrane-associated receptors: TNF-R1 (55 kd) and TNF-R2 (75 kd). These receptors are found on nearly all cell types except red blood cells and unstimulated T-lymphocytes. Cells may have as few as 200 receptors or as many as 10,000 but the number of receptors is no indication of the magnitude of the response to binding. The soluble TNF-α receptors have already been described (above).

Binding of TNF-α to its receptor results in internalisation of the receptor-ligand complex. The movement of this complex was followed using gold labelled recombinant TNF-α (Mosselmans et al. 1988). Once in the cell, TNF-α was found in clathrin-coated vesicles and then in small sized endosomes, where receptor-ligand uncoupling occurred. Tumour necrosis factor-α then appeared in multivesicle bodies and then in secondary lysosomes after about 2 hours.

Tumour necrosis factor-α induces protein synthesis by gene induction and activation of transcription. The proteins synthesised may depend on the cell type and/or on the presence of appropriate co-inducers. Tumour necrosis factor-α signal transduction is thought to take place during the internalisation phase. To achieve gene induction transcription factors, such as NFκB, must be activated or at least transported into the cell nucleus. It has been suggested that reactive oxygen metabolites may activate NFκB (Schreck et al. 1991). How TNF-α does this is unclear. It may be the result of uncoupling of electron flow in the mitochondria.

Tumour necrosis factor-α, like most of the other cytokines, has a wide range of activities. Circulating TNF-α will naturally come into contact with vascular endothelial cells and these are indeed one of its primary targets. Tumour necrosis factor-α increases the inflammatory potential of these cells by increasing MHC Class 1 expression (Collins et al. 1986) and the expression of adhesion molecules. This will increase the binding of immune cells to the endothelium. There are also morphological changes such that there is an increase in vascular permeability and extravasation of the immune cells that bind to the endothelium.

Tumour necrosis factor-α creates a pro-coagulant climate by increasing the production of plasminogen activator inhibitor. Endothelial nitric oxide production is also promoted, which contributes to an increase in vasodilator tone and the hypotension seen in septic shock.

Neutrophil adhesion is further promoted by a direct action of TNF-α, related to an increase in surface expression of the glycoprotein CR3, which is one of the rare activities of TNF-α not mimicked by IL-1, (Gamble et al. 1985). Tumour necrosis factor-α also promotes neutrophil aggregation, phagocytosis, and enhances the respiratory burst (Larrick et al. 1987).

Inactive T-cells have no TNF-α receptors. Once they are activated by antigens or mitogens then TNF-α receptors are expressed and the cells proliferate in response to TNF-α binding. B-cells proliferate and mature to produce immunoglobulins in response to TNF-α in the presence of IL-2.

Fibroblasts respond to TNF-α by producing collagen and prostaglandin E2 (PGE2). Pulmonary fibrosis secondary to exposure to silica can be prevented by anti-TNF-α antibody pre-treatment (Piguet et al. 1990).

Tumour necrosis factor-α has many metabolic effects: it increases circulating triglycerides, raises lactate levels, causes acidosis, and at high doses initially elicits hyperglycaemia followed by a profound hypoglycaemia. Many of these metabolic effects can be prevented by pre-treatment with indomethacin, indicating a role for prostaglandins in the response to TNF-α.

Not all the effects of TNF-α are so obviously deleterious to its host. It does seem to have a protective effect in parasitic diseases (Playfair et al. 1984) and viral infections (Mestan et al. 1986; Wong and Goeddel 1986).

Tumour necrosis factor-α is typically associated with bacterial infection and septic shock, and as will be described, high TNF-α levels are associated with a poor outcome. There is, however, evidence that TNF-α plays a necessary role in defence against

infection (Cross et al. 1989). This is more apparent in virulent infections, when other defence mechanisms are inadequate.

Tumour necrosis factor-α has been implicated in various disease states. These are covered in the reviews cited above and others (Tracey 1991; Strieter et al. 1993). The role in sepsis will be outlined below.

In summary TNF-α is a 52 kd amino acid chain produced by a range of cell types. It has a large number of actions centred on the response to an insult, including cytotoxicity. Nearly all its actions can be performed by IL-1. It is produced very rapidly after exposure to LPS and seems to precede the production of other inflammatory cytokines such as IL-1 and IL-6.

Interleukin-6

Interleukin-6 is a 184 amino acid single chain protein with a molecular mass from 21-28 kd, depending on its cellular source. It has two potential N glycosylation sites and 4 cysteine residues. The human form of the cytokine displays 65% DNA homology and 42% protein homology with the murine.

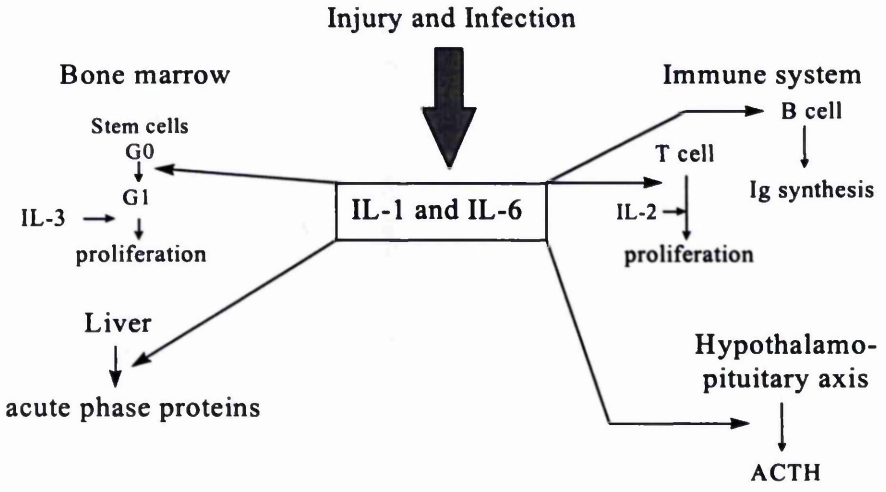
Interleukin-6 was first isolated in 1980 and called interferon-β_2 (IFN-β_2) (Weissenbach et al. 1980). During the next few years a number of factors involved in immune system and cellular regulation were isolated: B-cell stimulatory factor 2 (BSF-2), plasmacytoma growth factor (PCT-GF), human hybridoma/plasma cytoma growth factor (HPGF), hepatocyte-stimulating factor (HSF), cytotoxic T-cell differentiation factor (CDF). These were eventually found to be identical and renamed interleukin-6 (IL-6) (Van Snick 1990).

Interleukin-6 can be produced by almost any cell type, but it is not produced constitutively. It's production is triggered by bacterial lipopolysaccharide (LPS),

interleukin-1(IL-1), TNF-α, platelet-derived growth factor (PDGF), IFNβ, T-cell mitogens and T-cell antigens, and viruses. Glucocorticoids reduce IL-6 gene expression (Kishimoto 1989).

Given the history of IL-6 it is no surprise that it has many functions. These are summarised in Figure 1.4.

Figure 1.4: Coordinated response to injury and/or infection triggered by IL-1 and/or IL-6 (adapted from Van Snick (1990).



Interleukin-6 is essential for the final maturation of B-cells into plasma cells and the production of antibodies. In this capacity it acts synergistically with IL-1. IL-6 also has a role in T-cell activation: it enhances T-cell responsiveness to IL-2. Again there is a link with IL-1, which is involved in the regulation of IL-2 production (Van Snick 1990).

Interleukin-6 shows synergy with IL-3 in haematopoiesis: moving primitive stem cells into the cell cycle. It also shows synergy with classical haematopoietic growth factors.

Interleukin-6 plays a major role in the acute phase response. This can be divided into local and systemic responses. The local response is acute inflammation: the result of increased local vascular permeability and the attraction of leucocytes. The systemic response consists of fever, leucocytosis, an increase in erythrocyte sedimentation rate, increases in adrenocorticotropic hormone (ACTH) and glucocorticoids, activation of complement and clotting, a negative nitrogen balance, depletion of zinc and iron stores, and increases in plasma proteins (acute phase proteins: APPs) (Kushner 1982).

In the 1950s the liver was found to be the major source of the APPs. As their production was triggered by a remote insult it implied the action of a mediator. The search for this mediator centered on leucocytes as these were the first cells known to arrive at the site of an injury. Investigation of supernatant from monocytes and macrophages revealed the existence of a hepatocyte-stimulating factor (HSF), whose production was regulated in a dose-dependent manner by LPS.

Monocytes also produce IL-1 and TNF-α in response to LPS. They too stimulate APPs production, but unlike IL-6 they do not stimulate the full spectrum of APPs (Ritchie and Fuller 1983).

Interleukin-6 has since been shown to localise to the liver after i.v. injection. Clearance from the circulation is biphasic with an initial half life of 3 minutes and then a slower disappearance over 55 minutes. In the serum IL-6 circulates as a complex with α_2 macroglobulin.

Control of the acute phase response seems to be at the transcription level. It is this aspect of IL-6's biology which has received the most attention in the critically ill and which will be discussed in relation to trauma and sepsis. The correlation between IL-6 and C-reactive protein (CRP) was first described in burns patients in 1987 (Nijsten et al. 1987).

Interleukin-6 has close links with the nervous system. It is involved in the differentiation of neural cells and the regulation of the neuro-endocrine system. Intravenous injection of recombinant IL-6 into rats resulted in a dose-dependent increase in ACTH levels within 30 minutes (Naitoh et al. 1988). This effect of IL-6 could be blocked by the prior injection of an anti-corticotropic releasing hormone (CRH), which suggested that IL-6 had an action via CRH. Similar cytokine-neuro-endocrine links have been described for IL-1 (Lumpkin 1987).

Interleukin-6, like the other cytokines, acts via cell surface receptors. There appear to be two polypeptide subunits: one with a mass of 80 kd, the other with a mass of 130 kd. The 80 kd subunit has a low affinity binding site. The 130 kd subunit has a high affinity binding site. IL-6 binding results in an interaction between the two and signal transduction (Heinrich and Rose-John 1993). The ligand-receptor complex is then internalised. The precise mechanism of signal transduction is not clear: neither cAMP, nor cGMP, nor protein kinase C, nor inositol phosphate, nor changes in intracellular calcium seem to be responsible (Heinrich et al. 1990).

A soluble IL-6 receptor has been isolated from human urine (Novick et al. 1989). The behaviour of this soluble receptor has been investigated using an engineered form (Mackiewicz et al. 1992). This showed that the receptor-IL-6 complex increased APP gene expression in hepatoma cells (HepG2 cells). The soluble receptor acts as an agonist, in contrast to other soluble cytokine receptors, which are antagonists. The biological significance of these receptors is unclear. They may have a protective role: protecting the cytokine from proteolytic activity and thus extending its half-life.

In summary IL-6 is a 26 kd amino acid chain produced by almost any cell type under a variety of conditions. It has many actions related to cellular proliferation and differentiation. Most of these relate to the response to an insult to the organism. In this the effect on hepatocyte protein production seems to be the most important. A more detailed account of IL-6 can be found in the following reviews: (Kishimoto 1989; Heinrich et al. 1990; Van Snick 1990; Heinrich and Rose-John 1993).

The cytokine response to sepsis

There has been considerable interest in recent years in the cytokine response in the critically ill both to improve our understanding of the pathophysiology and to try to devise new therapies. For the sake of clarity I will focus on the pro-inflammatory cytokines: interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor-α (TNF-α), although a few others will need to be mentioned. The principal functions of these cytokines are summarised in Tables 1.6 - 1.10.

Table 1.6: Interleukin-1 (previously known as endogenous pyrogen)

Table 1.6	**Interleukin-1 (previously known as endogenous pyrogen)**
Mediator of host inflammatory response	
T cell activation	
B cell proliferation	
Collagen production	
Tissue repair	
Adhesion module expression	
Acute phase protein synthesis	
Pyrogen	

Table 1.7: Interleukin-2 (T cell growth factor)

Table 1.7	**Interleukin-2 (T cell growth factor)**
Autocrine growth factor for T cells	
Stimulates NK (natural killer) cell growth	
B cell growth factor	
Promotes antibody synthesis by B cells	

Table 1.8: Interleukin-6 (hepatocyte-stimulating factor)

Table 1.8	**Interleukin-6 (hepatocyte-stimulating factor)**
Acute phase protein synthesis	
B cell differentiation	
Cytotoxic T cell differentiation	
Cell proliferation	
Synergism with IL-1	

Table 1.9: Interleukin-8 (a secondary mediator of inflammation)

Table 1.9	**Interleukin-8 (a secondary mediator of inflammation)**
Neutrophil chemotaxis	
Neutrophil activation	

Table 1.10: Tumour necrosis factor (mediator of septic shock)

Table 1.10	**Tumour necrosis factor (mediator of septic shock)**
Induces IL-1, IL-6 and IL-8	
Cytokine synergism	
Pyrogen	
Acute phase protein synthesis	
Myocardial depression	
Intravascular thrombosis	
Vascular smooth muscle relaxation (via inducible nitric oxide synthase activation)	

Models of Sepsis

Many different models of sepsis have been used to study the cytokine response. The model used by Tracey et al. serves as a good example (Tracey et al. 1987). This used anaesthetised baboons given a lethal dose of E. coli such that all animals died within eight hours from an accelerated form of septic shock: a fall in blood pressure, increase in heart rate, an initial rise in cardiac output followed by a catastrophic fall associated with anuric renal failure and death from pulmonary oedema. In these animals there was a rise in TNF-α, which reached a peak about 1.5-2.5 hours after the injection of E.coli. Tumour necrosis factor-α became undetectable by 4-6 hours. If the animals were given a monoclonal anti-TNF-α antibody 1 hour before the E.coli injection there was a temporary improvement in their haemodynamic status but the 100% mortality was unchanged. Anti-TNF-α given 2 hours before the E.coli injection resulted in 100% survival at 24 hours, which strongly implicated TNF-α in the pathogenesis of shock and death following a severe bacteraemia.

The same model was used by Fong et al. to investigate the production of IL-1 and IL-6 (Fong et al. 1989). They found that there was a rise in IL-1 within 2 hours of the E.coli injection, which reached a peak at about 3 hours. The rise in IL-6 was somewhat delayed in comparison and had yet to reach a peak at 8 hours, which was the final blood sampling time in the study. The production of these cytokines could be attenuated by the prior administration of anti-TNF-α, suggesting that TNF-α was instrumental in triggering the cytokine response to severe bacteraemia.

The importance of endotoxin as a trigger for the production of cytokines has been demonstrated in studies on human volunteers (Michie et al. 1988; van Deventer et al. 1990). A bolus of endotoxin (4 ng kg^{-1}) was injected into volunteers and resulted in a rise in TNF-α starting 90 minutes after the injection. Tumour necrosis factor-α remained elevated for 180 minutes. In contrast to the baboon studies there was no elevation in IL-1 (van Deventer et al. 1990). Van Deventer et al. found that a 2 ng kg^{-1} bolus of Ecoli endotoxin resulted in a rise in TNF-α starting at 30-45 minutes and reaching a peak at 60 - 90 minutes. IL-1 was undetectable but there was a rise in IL-6. This was seen about 15 minutes after the rise in TNF-α and the peak level was reached at 120-150 minutes. Both studies found that the appearance of TNF-α in the circulation coincided with the onset of influenza-type symptoms, an increase in temperature and heart rate and a neutropenia. A neutrophilia was only seen after TNF-α had been cleared from the circulation.

The failure of both of these studies to detect the production of IL-1 is in contrast to the study by Dinarello et al. investigating the pyrogenicity of TNF-α (Dinarello et al. 1986). In this study rabbits were injected with a bolus of recombinant TNF-α at a dose of 1 mg kg^{-1}, which resulted in a monophasic fever peaking at about 50 minutes. When a much higher dose was used (10 mg kg^{-1}) the fever was found to be biphasic, with a second peak at 3.5 hours. This was found to be the result of the production of IL-1, suggesting that at high doses TNF-α triggered the production of IL-1. It may be that in the volunteer studies the concentration of TNF-α produced in response to the endotoxin was insufficient to trigger IL-1 synthesis.

Caecal ligation and puncture is a widely used model of sepsis, mimicking a visceral perforation, and so may be a more clinically valid model than direct intra-vascular injections of mono-cultured bacteria or pure endotoxin. Ertel et al. used this model in rats and found elevations in circulating endotoxin, TNF-α, IL-1 and IL-6 (Ertel et al. 1991). In contrast to those studies using a pure endotoxin or E. coli challenge, which cause a transient rise in TNF-α, Ertel et al. found that TNF-α concentrations remained elevated for several hours and then only decreased gradually despite increasing plasma endotoxin concentrations. This suggested that although the stimulus for production, namely endotoxin, was still present there was down-regulation of production by some other mediator(s).

Clinical Sepsis

There have been many studies recording the cytokine response to sepsis of various origins. Waage et al. in Norway have been particularly interested in the response in meningococcal disease (Waage et al. 1987). In 1987 they found that TNF-α was raised in these patients and that a fatal outcome was associated with a TNF-α concentration exceeding 140 pg ml^{-1}. In patients with meningococcal septic shock they found raised concentrations of IL-6, and a 50% mortality rate when IL-6 concentration exceeded 3 ng ml^{-1}. Interleukin-1 was only elevated in the most severely ill patients: they already had raised plasma concentrations of TNF-α, IL-6, as well as endotoxin. By looking at the timing of cytokine production they found that IL-6 was produced later than TNF-α (Waage et al. 1989, see also van Deventer et al. (1990).

In septic shock of various causes there is also an increase in TNF-α, which correlates with the severity of the shock and the eventual outcome (Damas et al. 1989). The presence of circulating TNF-α in septic patients was associated with a higher mortality in the study by Debets et al. (1989). They found that 25% of their patients showed detectable circulating TNF-α. The mortality from this group was 73% compared to only 34% for the group

showing no detectable TNF-α. In terms of patient characteristics, sepsis score and multiple organ failure (MOF) there was no difference between the two groups.

Interleukin-6 has been studied specifically in septic patients being admitted to an Intensive Care Unit (Hack et al. 1989). Those patients already in shock were found to have higher plasma IL-6 than those who were still normotensive. A correlation was found between IL-6 and heart rate, plasma lactate, and platelets as well as C_{3a} and C_{4a} (products of complement activation). Patients who went on to die had higher IL-6 concentrations on admission than eventual survivors. However, monitoring the course of IL-6 after admission did not provide additional information: concentrations tended to decline irrespective of outcome. In a series of 15 patients with intra-abdominal sepsis (Leindhardt et al. 1992) there was a strong correlation between the serum concentration of IL-6 and the sepsis score of Elebute and Stoner (Elebute and Stoner 1983). In contrast to the studies noted above, no correlation was found between serum TNF-α concentrations and sepsis score.

Interleukin-8 concentrations have been studied in patients with the sepsis syndrome and compared with concentrations in patients with non-septic shock. (Marty et al. 1994). Septic non-survivors had significantly higher plasma IL-8 than survivors and there was a correlation between IL-8 and IL-6 concentrations. There was no correlation between plasma IL-8 and either plasma IL-1β or plasma TNF-α concentrations. Although patients with shock from non-septic causes developed MOF at a similar rate to those with sepsis (and the mortality was equally high: 92%), IL-8 concentrations were low. All patients with MOF from whatever cause had high IL-6 concentrations. This study suggested that in sepsis a high plasma IL-8 was associated with a fatal outcome. In terms of mechanisms it suggested that in septic and non-septic conditions the signals for the production of IL-8 were not identical.

Overall it appears that in sepsis, and particularly in septic shock, there is activation of cytokine production, particularly TNF-α and IL-6. The production of TNF-α seems to precede that of IL-6. Interleukin-1 is detectable in a minority of patients: usually the most

severely ill, probably because they have sufficiently elevated concentrations of TNF-α to trigger IL-1 production. Within a given population of patients those with the higher concentrations of TNF-α and/or IL-6 seem to have a higher mortality even if other indicators of disease severity are the same.

The cytokine response to trauma

There is now good evidence that the inflammatory cytokines are involved in the response to tissue injury. This evidence comes from studies into the effects of elective surgery: a controlled form of trauma, and from studies using animal models of haemorrhagic and traumatic shock.

Elective Surgery

One of the earliest clinical studies looked at six patients undergoing elective cholecystectomy (Shenkin et al. 1989). An IL-6 response was detectable as early as 30 minutes after the initial incision. All the patients showed a response within 1.5 hours and peak IL-6 concentrations were reached at 1.5 - 4 hours after incision. Peak IL-6 concentrations were found to correlate with the duration of surgery. There were no increases in either endotoxin, IL-1, or TNF-α and it was suggested that IL-6 might be an early marker of tissue damage. Since then a number of studies have confirmed the IL-6 response to elective surgery (Cruickshank et al. 1990; Baigrie et al. 1991; Di Padova et al. 1991; Baigrie et al. 1992; Joris et al. 1992; Baigrie et al. 1993; Crozier et al. 1994; Moore et al. 1994a). The fact that peak IL-6 concentrations, in the 1989 study by Shenkin et al., correlated with duration of surgery suggested that there might be a link between the severity of surgery and the cytokine response. In a further study this group looked at the response to different types of elective surgery (Cruickshank et al. 1990). Patients undergoing minor surgery (mainly breast lumpectomy, stripping of varicose veins, and partial thyroidectomy), cholecystectomy, hip replacement, colorectal, and major vascular surgery were compared. The latter two groups of patients showed the highest peaks in IL-

6 and there was a significant correlation between the integrated IL-6 response over 48 hours and the duration of surgery, suggesting that the overall IL-6 response was related to the magnitude of tissue damage. A comparison of open and laparoscopic cholecystectomy also found that the IL-6 response was greater and lasted longer in those patients having open surgery and so greater degrees of tissue trauma (Joris et al. 1992).

Tumour necrosis factor-α is not typically raised in elective surgery. One exception is the study by Cabie et al. (Cabie et al. 1993), who found circulating plasma TNF-α in all 14 of their abdominal aortic aneurysm patients but in only two of their seven control patients (undergoing internal carotid artery surgery).

In most studies there is no detectable increase in IL-1. However Baigrie et al. did find a rise in IL-1b which preceded that of IL-6 by several hours in patients undergoing elective repair of abdominal aortic aneurysms (Baigrie et al. 1991; Baigrie et al. 1992). This IL-1b response was much smaller than that of IL-6 and of a much shorter duration. The failure to detect IL-1b in other studies may be due to sampling schedules simply missing an early very transient rise. Alternatively it may be due to the production of IL-1 inhibitors as found by Di Padova et al. (Di Padova et al. 1991).

Overall these studies of elective surgery strongly suggest that IL-6 is a sensitive marker of tissue damage.

Animal Models

Animal studies have yielded further insights into the cytokine response to trauma by allowing the response to haemorrhage and hypotension to be studied in a controlled and reproducible manner.

Ayala et al. used a mouse model of haemorrhagic shock in which the animals were bled to a mean blood pressure of 35mmHg (pre-bleed BP around 95 mmHg) and maintained at that level for 60 min before being resuscitated with their shed blood and an additional infusion of Ringer's lactate (twice the shed blood volume) (Ayala et al. 1990). In this model it was found that TNF-α increased at 30 min during the shock phase and remained elevated for 2 hours after resuscitation but had disappeared by 4 hours. Concentrations of endotoxin were checked to assess whether the stimulus for this increase might be an endotoxaemia. However endotoxin concentrations were found to be no different to those of the surgical control animals. They also noted that IL-6 levels were elevated 2 hours after resuscitation but that the control animals showed a similar IL-6 response. This suggested that the surgical preparation used on the control animals had itself elicited a cytokine response.

To investigate this the model was modified to include a laparotomy prior to haemorrhage to try to isolate the two stimuli (soft tissue trauma and then haemorrhage). In this second study (using rats) they found that plasma IL-6 levels rose after the laparotomy, but before the start of haemorrhage (Ayala et al. 1991). Tumour necrosis factor-α was undetectable before haemorrhage and reached a peak 4 hours after the onset of haemorrhage. Interestingly, plasma IL-6 continued to increase during haemorrhage and were significantly higher than in the control animals at the same time. This suggested that soft tissue trauma might be the trigger for IL-6 production and that haemorrhage might augment this response. Tumour necrosis factor-α production seemed to be determined by haemorrhage rather than trauma.

A more recent study by Schmand et al., using the Ayala mouse model, showed that the increases in IL-6 seen in simple haemorrhage, haemorrhage and trauma (laparotomy) and haemorrhage and trauma with a longer hypotensive period (90 minutes) were not significantly different but were 8-10 times higher than in the control animals (Schmand et al. 1994). They did not look at TNF-α. In contrast to the previous studies Schmand and colleagues' work suggested that the IL-6 response had been fully triggered by the simple haemorrhage model and that any further insult caused no further cytokine response.

The results of the two studies by Ayala et al. suggested that soft tissue trauma and haemorrhage/hypotension provided distinct stimuli triggering the production of quite different cytokines.

Clinical Studies

These conclusions prompted a study to see if plasma cytokine concentrations could be related to the degree of trauma or to the degree of haemorrhage in multiply injured patients (Foëx et al. 1994). Ten patients were studied. The Injury Severity Score (ISS) was used to assess the degree of trauma. The degree of haemorrhage was assessed by the blood transfusion requirement during the first 48 hours after injury. Blood was sampled as soon as possible after injury and as close to every two hours as possible for the next 48 hours. Plasma concentrations of IL-1, IL-6 and TNF-α were measured.

The patients showed a range of ISS from 9-50 and a range of transfusion requirements from 0-14 units over the 48 hours study period. Overall, five of the patients died after the end of the study period either from the sequellae of head injuries or from multi-organ failure (MOF). Plasma interleukin-1 was raised in five out of eight patients from whom enough plasma was obtained. These elevations occurred in the patients with the lowest ISS. Interleukin-6 was raised in nine of the ten patients and there was no correlation between peak plasma IL-6 and either the ISS, the degree of haemorrhage, or the eventual outcome. Tumour necrosis factor-α was raised briefly in two patients. These two patients were the most severely injured and also received the largest blood transfusions: 11 and 14

units of blood respectively. There was no characteristic pattern of cytokine production in this group of patients but it was noticeable that plasma cytokine concentrations could fluctuate widely over a short period of time. This was particularly apparent in the case of IL-1 and TNF-α, which showed very transient peaks compared to IL-6, which showed much more sustained increases.

Svoboda et al. studied a series of 42 patients with ISS > 16 (Svoboda et al. 1994). Serial measurements of IL-1b, IL-2, IL-6 and TNF-α were made on entry to the ICU then daily for 6 days and at weekly intervals until death or discharge. A slight elevation of IL-1b lasting 2-10 days was noted in 7 of the 42 patients, but IL-2 was raised in 4 patients on day 1 only. Interleukin-6 was elevated in 27 patients and there was a good correlation between IL-6 level on day 1 and the ISS (more than 90% of patients with an ISS > 25 had raised IL-6).

No correlations were found between IL-6 and Revised Trauma Score (RTS) or TRISS scores. In contrast to the ISS, RTS and TRISS are determined by physiological derangements rather than the degree of tissue injury.

If IL-6 is a marker of tissue damage it is maybe not surprising that its concentration is not related to physiological changes. Plasma interleukin-6 rapidly decreased over 48 hours in patients making a successful recovery. However, those who developed MOF and died showed huge increases in IL-6. All patients with an IL-6 concentration > 400 pg ml^{-1} subsequently died, but early IL-6 concentrations were not predictive for the development of MOF or eventual outcome. Only 4 patients showed an early elevation in TNF-α and there was no correlation with ISS or outcome. As with IL-6 those patients who developed MOF showed increases in TNF-α. The highest plasma TNF-α concentrations were seen in those patients who later died.

Increases in IL-6 were also noted by Hoch et al. in 30 trauma patients (Hoch et al. 1993). These were divided into 3 groups according to ISS (mild injury:ISS < or = to 10, moderate injury: ISS 11-24, severe injury: ISS > 25). Interleukin-1, IL-6, IL-8, TNF-α and

endotoxin concentrations were measured within 2 hours of injury, then 4 hourly for the first 24 hours and then daily until day 5.

From the outset the severely injured group had a significantly higher concentration of both IL-6 and IL-8 than the other two groups. These concentrations increased over the first 24 hours and then gradually declined. Tumour necrosis factor-α concentrations were only just raised above the detection threshold of their assay. Neither IL-1 nor endotoxin were detectable in any group. They subsequently found a correlation between further increases in IL-8 in those patients with an ISS > 25 and the extent of anatomical injury and the degree of shock (Abraham 1994). Initial plasma IL-8 was not predictive for the development of MOF.

The role of TNF-α in the cytokine response to trauma remains very unclear. One possible trigger for the production of cytokines is the translocation of bacteria or endotoxin from the gastrointestinal tract. Endotoxin is a potent stimulator of TNF-α production (van Deventer et al. 1990) and a potent stimulator of other cytokines notably IL-6. It has been suggested that the TNF-α response is too transient to be detected in most studies. To overcome this possibility Ferguson et al. obtained blood from trauma patients at the scene of the accident (Ferguson et al. 1994). In this way they were able to study TNF-α, IL-6 and IL-8 concentrations in 42 patients (mean ISS 21.7). Mean time to sampling was 81 min. They found elevated concentrations of TNF-α in 91% of patients, elevated IL-6 in 80%, and elevated IL-8 in 49% of their patients. Looking at patients whose blood samples were taken earlier than the mean time, the figures were: TNF-α raised in 80%, IL-6 in 67%, IL-8 in 32%. They concluded that TNF-α was active in the inflammatory response due to trauma and that it might be activated early and may have modulated subsequent cytokine activity.

Rabinovici et al. however found that in 100 less severely injured patients (mean ISS 12.8) TNF-α concentrations were only elevated in 35% (Rabinovici et al. 1993). Interestingly 44% of their control population of healthy volunteers showed an elevated plasma TNF-α. They did however find that in those patients developing septic complications there were

further increases in TNF-α concentrations. Plasma TNF-α was undetectable in the 25 patients studied by Meade et al. (1994).

In contrast Endo et al. found raised plasma TNF-α in 28 out of 29 patients in haemorrhagic shock (BP < 80mmhg on admission to hospital) (Endo et al. 1994). These TNF-α concentrations were higher than those in their healthy controls. They found no correlation between TNF-α concentrations and the degree of haemorrhage (as assessed by the volume of blood transfusion). This suggested that TNF-α production might be triggered by haemorrhage and hypotension but as an all or none phenomenon rather than a graded response. Surprisingly they found no difference in TNF-α concentrations between those patients developing MOF and those who did not. Far fewer patients showed a rise in IL-6 (12 of 29) and IL-8 (23 of 29). Again there was no difference according to blood transfusion requirement and there was no difference between those going into MOF and those who did not.

The results of these studies are summarised in Table 1.11.

Table 1.11: Summary of clinical trauma studies

Table 1.11 Summary of clinical trauma studies

Study	n	ISS	IL-1	IL-2	IL-6	IL-8	TNF-α
			Increased cytokine levels in patients, up to 48 hours post injury				
Foëx et al. 1994	10	9-50	5 of 8	-	9	-	2
Svoboda et al. 1994	42	>16	7	4	27	-	4
Hoch et al. 1993	8	≤10	?	-	?	?	?
	12	11-24	?	-	↑	↑	?
	10	≥25	undetected	-	6x↑	30x↑	33% samples
Ferguson et al. 1994	42	"21.7"	-	-	80% samples	49% samples	91% samples
Rabinovici et al. 1993	100	"12.8"	-	-	-	-	35
Meade et al. 1994	25	>25	undetected	-	↑	↑	undetected
Endo et al. 1994	29	haemorrhagic shock	undetected	undetected	12	23	28

? indicates that the original article did not specify the data.
↑ indicates that the original article only specified that there was an increase, without providing raw data.

The results of all these studies reveal considerable variations in the cytokine response to trauma. One of the reasons for this variability is the very heterogeneous nature of trauma patients with regard to the mechanism of injury and the types of injury sustained. This variability is not really addressed by the various scoring systems commonly used. Studying the response to elective surgery is one way of attempting to circumvent this problem.

The cytokine response to burns

Another approach has been to study the response to burns, a very specific form of trauma, and to try to correlate the magnitude of the response to the extent of the burn injury. This approach was used in 12 burn patients and revealed a good correlation between the IL-6 response and the extent of the burn (De Bandt et al. 1994). Plasma IL-6 reached a peak on day 4 after injury, which is later than for surgery or multiple trauma. Higher IL-6 concentrations were reached in those patients who became septic than in those who did not. Plasma TNF-α was raised in a smaller percentage of patients and showed no particular pattern. Plasma TNF-α was not related to the extent of the burn but TNF-α concentrations were higher in septic patients.

In contrast Cannon et al. found no correlation between cytokine concentration and burn size when they made serial measurements of IL-1b and TNF-α in 31 patients suffering 10-95% body surface area burns (Cannon et al. 1992). There was no correlation between cytokine concentration and APACHE II score but increased concentrations of IL-1b and TNF-α on day 1 did correlate with increases in body temperature. A correlation has also been found between increased plasma IL-6 concentrations and increased rectal temperature during the first 24 hours following moderate burns and scalds in children (Childs et al. 1990). Although IL-1 was originally known as endogenous pyrogen it was generally not detectable in the plasma of these pyrexial children, although it was present in blister fluid.

The question remains as to the exact trigger mechanism for the cytokine production seen in trauma and burns patients. The macrophage hypothesis of activated macrophages producing cytokines and other mediators which then result in systemic inflammation leaves the question of what activates the macrophages unanswered (Deitch 1993).

Bacterial translocation

Bacterial and/or endotoxin translocation from the gastrointestinal tract has been advanced as one possible mechanism for the activation of macrophages. This has been supported by various animal studies which have demonstrated evidence for bacterial translocation. The situation remains very controversial in the setting of human trauma.

Some groups have found little evidence for it (Moore et al. 1991; Peitzman et al. 1991), while others have found good evidence for it (Sori et al. 1988; Brathwaite et al. 1993a). Deitch et al. have suggested that, even in the absence of live translocating organisms or endotoxin in the portal and systemic circulations, the gut can still act as a cytokine generator via the activation of the gut associated lymphoid tissue (Deitch et al. 1994).

Alternatively it could be that cytokine production is triggered directly at the wound site. Evidence for this comes from both a rat model and a clinical situation (Mateo et al. 1994). Raised levels of IL-6 were found in both wound fluid and serum of rats subjected to polyvinyl alcohol sponge implantation and in fluid draining from mastectomy scars. This suggested that cytokines found in the systemic circulation might have originated at the wound site. Both IL-1 and IL-6 were found in the blisters of burned children although IL-1 was generally absent from the circulation, again suggesting local production (Childs et al. 1990).

It may be that two mechanisms act together or act independently on different cytokines: IL-6 being triggered to a greater extent at the wound site by tissue trauma while TNF-α and IL-1 might be more sensitive to changes in gut barrier function and activation of the gut associated lymphoid tissue.

Animal Models in Shock Research

Introduction

More than 11 million people attend accident and emergency departments in the UK every year. Not all of them have suffered injuries, and even fewer have suffered major trauma. But the numbers are such that it might be wondered why injury models are needed to study the effects of trauma. However trauma patients are widely scattered over many hospitals, their injuries are varied, and they do not appear at convenient times. Assembling groups of patients of similar type and severity is difficult. These limitations were recognised by Grant (1961).

The more critically injured patients, who might provide the most useful data are the most difficult to study because, as Henry Beecher wrote in the Preface to "The Physiologic Effects of Wounds" "The major consideration at all times was that our studies must not delay or otherwise interfere with the treatment of the wounded soldier" (Beecher 1952). Consequently, if the acute effects of injury are to be thoroughly investigated some sort of model is essential.

There is still a great debate about the use of animal models in biomedical research (Caplan 1983; Rowan 1997). Arguments have been put forward against the use of animals and in favour of cell, tissue and organ preparations as well as computer simulations (Barnard and Kaufman 1997). These arguments may have more validity in toxicology testing than in other types of biomedical research (Stark and Shopsis 1983). They fail to address one of the fundamental reasons for using animals: that responses to an insult such as haemorrhage or injury do not take place in isolation. They involve the whole organism. Reflex changes in one system often impinge on the workings of another.

Animal models have many limitations (Grant 1961; Little and Foëx 1996; Barnard and Kaufman 1997). Any animal model should conform to certain criteria (Held 1983). The model should:

accurately mimic the desired function or disease,
be available to multiple investigators,
be available in multiple species,
be handled easily by most investigators,
survive long enough to be functional.

In addition the following were suggested as desirable for specific types of work:

fit available animal housing facilities,
be of sufficient size to provide multiple samples,
be polytoccus so that multiple offspring are produced for each gestation.

Information gained from the physiological and biochemical study of isolated preparations may contribute to the design of the whole-animal experiments. But, there is little hope of being able to replace such research with the use of isolated organs or tissues because of the integrated nature of the responses being studied.

Trauma models

It has not always been appreciated that if the model is to yield useful results the injury and all the other conditions of the experiment must be very carefully controlled. In 1961 Stoner outlined some of the conditions which any trauma model should satisfy (Stoner 1961):

1) The site and nature of the insult should be known.
2) The insult should be reproducible.
3) The insult should be measurable. And,
4) The intensity of the insult should be controllable.

In the early years of this century there were a number of shock models. Wiggers recognised that not all were equally valuable: "The statement is frequently made that experiments rarely, if ever, reduplicate diseases in man, and that therefore only the deductions are applicable which are derived from experiments which simulate clinical conditions, and only those interpretations are valid which agree with clinical observations" (Wiggers 1941). Some, such as intra-arterial injections of fat emulsions, and massage of the stomach, bore little relation to any clinical situation. Others, such as prolonged ischaemia of limbs, and aortic compression which were not clinically relevant then, have now become relevant because of advances in clinical practice. Some were, and still are, bizarre in the extreme (Guthrie 1917).

Experimental shock models were divided into five categories by Wiggers (1950 p 103):

1) skeletal muscle trauma,
2) ischaemia, produced by compression or tourniquets,
3) general contusion,
4) burns and scalds,
5) haemorrhage.

Skeletal muscle trauma in the 1930s and 1940s usually involved hammer blows to the thigh (for example Parsons and Phemister 1930; Best and Solandt 1940; Gregersen and Root 1947). All aimed to avoid skeletal and vascular damage. Recently captive bolts have been used to the same effect (Rady et al. 1993). These models produce three potential insults: the actual muscle damage, fluid loss from the area of damage, and nociceptive afferent impulses. The importance of fluid loss from the area of injury was recognised in the 1930s (Blalock 1930; Parsons and Phemister 1930).

To these might be added models of **skeletal trauma**. An open fracture technique was first described in the rat by Cuthbertson et al. (1939) in studies of the metabolic response to injury. More recently models of combined haemorrhage and trauma have been described which include a closed fracture of the femur in the pig (Stylianos et al. 1991), and closed fractures of both femurs in the baboon (Pretorius et al. 1987).

Models of ischaemia. These were first used at the turn of the century and continue to be used. Wiggers considered these models to be "expedients that had no semblances to occurrences resulting in human shock" (Wiggers 1950 p 95). With the advent of major vascular surgery this is no longer the case and these models are now an essential tool in understanding the physiology of ischaemia and reperfusion.

Tissue ischaemia is nearly always produced in the hindlimbs using rubber-band tourniquets put in place while the animal is anaesthetised (Stoner 1961). Once the tourniquets are in place, further anaesthesia is not mandatory because an ischaemic limb rapidly loses sensation, although it should be maintained if the chosen anaesthetic does not impair the interaction between the injury and the homoeostatic mechanisms being studied. Whilst the tourniquets are in place, nociceptive afferent neural impulses are generated within the ischaemic tissues and around the site of tourniquet application. Such afferent impulses have a major impact on central control mechanisms modifying, for example, both cardiovascular and thermoregulatory reflexes. When the tourniquets are released, fluid is lost as an exudate from the circulation into the damaged tissues. Therefore a model such as hind-limb ischaemia can be used to study two of the most important factors initiating the response to injury: nociceptive afferent impulses and fluid loss from the circulation. The nociceptive barrage generated by tissue ischaemia can also be mimicked by electrical stimulation of somatic afferent nerve fibres using parameters known to activate the thin non-medullated, C-fibre afferents, which transmit nociception (Overman and Wang 1947; Kirkman et al. 1995).

The **general contusion** model was devised by Noble and Collip (1941-2). It consisted of putting a rat into a 16 inch drum and rotating it at 40 revolutions per minute. This model produced hyperaemia and congestion of the abdominal organs, tears to the liver, but few muscular or cranial haemorrhages and no fractures. Mortality was 100%, in male rats, after 700 revolutions (1400 falls). This model, while it may produce the symptoms of shock would not fulfil Wiggers assertion that "The primary aim of animal experimentation

is to reproduce diseases, not merely or necessarily the signs and symptoms." (Wiggers 1950 p 90).

Burns and scalds continue to be widely used but they represent a very specific insult in their own right and will not be considered here.

Haemorrhage. Up until the 1940s there was still some controversy as to whether haemorrhage had anything to do with shock. There was still a view that shock involved general damage to the capillary endothelium and that shock was a state in which fluid left the vascular compartment. Haemorrhage was regarded as causing no capillary damage and that tissue fluid entered the vascular compartment (Moon 1942).

Although much has been learned from models of 'simple' haemorrhage, their clinical relevance may be limited to those situations in which haemorrhage occurs in the absence of significant tissue damage (such as a penetrating injury to a major vessel or the rupture of varices).

The acceptance of Stoner's criteria has lead to a reduction in the use of models such as general contusion using the Noble-Collip drum, gastric/intestinal massage and hammer blows to the thigh. The latter have recently been re-introduced in conjunction with bilateral closed fractures of the femur (Pretorius et al. 1987).

Haemorrhage models

Haemorrhage models can be divided into those in which the animal is bled to a certain blood pressure and maintained at that level for a period of time and those in which a certain percentage of blood volume is withdrawn irrespective of the blood pressure.

Walcott devised a fixed volume model in which the animal was bled until either blood flow or respiration ceased (Walcott 1945). Before asystole 25% of the collected blood was reinfused, which resulted in blood pressure reaching about 40 mmHg. A gradual recovery followed until blood pressure reached 60 mmHg. Blood pressure remained stable for a period and then gradually deteriorated until the animal died.

The main alternative model was developed by Wiggers, who considered that percentage blood loss wasn't as important as the degree and duration of hypotension in determining the irreversibility of shock (Wiggers and Werle 1942). The model was known as the Western Reserve Method (now known as the Wiggers Method, Wiggers 1950 pp 137-139). Blood was withdrawn from a femoral artery into a reservoir until the mean arterial blood pressure was reduced to approximately 50 mm Hg. This was maintained for 90 minutes after which more blood was removed until blood pressure reached 30 mmHg. This "drastic hypotension" was maintained for another 45 minutes at which point the shed blood was reinfused. Reinfusion of the shed blood restored cardiovascular and oxygen transport values to close to pre-haemorrhage values for 30-60 minutes. A progressive deterioration followed. Mortality was described as being 82% six hours after reinfusion of the shed blood.

The controlled withdrawal of blood in distinct steps allows the relationship between oxygen delivery and oxygen consumption to be described both for the whole body and, with appropriate cannulations, for individual organs. Such techniques have been useful in the determination of the critical oxygen delivery and extraction at which oxygen consumption becomes flow dependent (Samsel and Schumacker 1992). A non-

haemorrhage technique using a progressive cardiac tamponade has also been used to reduce global (whole body) oxygen delivery (Zhang and Vincent 1993).

An advantage of the Wiggers Method is that the severity of shock can be characterised by the blood pressure during the hypotensive periods, the duration of such periods, the maximum volume of blood in the reservoir, the amount of blood taken back into the reservoir during decompensation before reinfusion, the rapidity of that 'reverse' flow and by the oxygen deficit incurred by the body during the period of hypotension (Guyton and Crowell 1961).

The levels of hypotension used in such models are close to the critical levels of autoregulation of blood flow for organs such as the brain. Blood flow cannot be autoregulated at such critical levels for long periods. Many of the haemorrhage models are studies of the necrobiotic changes in organs after a failure of oxygen transport rather than investigations of the whole body response to haemorrhage.

A feature of both model types is that they are controlled (blood loss can be regulated at the turn of a tap connected to a cannula in a major vessel). This is a very artificial situation. To overcome this limitation a number of models of uncontrolled haemorrhage were developed, such as, division of the saphenous artery (Shaftan et al. 1965), hemisection of the superficial femoral artery (Milles et al. 1966), and laceration of the abdominal aorta (Ludewig and Wangensteen 1969), and used to study the control of haemorrhage. Interest in these models has been revived. They have been used to study the efficacy of different fluids in resuscitation (Traverso et al. 1986a; Traverso et al. 1986b).

The Model

My aim was to study the acute response (homoeostatic mechanisms) to reversible traumatic shock rather than the "irreversible necrobiotic progression to death" seen in models of irreversible shock.

Trauma, particularly blunt trauma is a complex phenomenon: it consists of fluid loss from the circulation and tissue injury. Tissue injury itself has a number of components including direct damage to cells, release of cell breakdown products or "toxins" into the circulation, and stimulation of nociceptive afferent nerves.

The model had to reflect this complexity and yet be controllable, measurable and reproducible. Two components of trauma were "isolated": haemorrhage, and afferent nociceptive stimulation.

Haemorrhage

A fixed volume haemorrhage was used. Although Wiggers's Method does reproducibly produce shock the duration of the hypotensive periods seemed unrealistic in the modern clinical setting. Except in the case of an entrapment, in the UK, trauma cases should not have to wait for more than 30 minutes before any kind of intervention. Previous work showed that in general a haemorrhage of 30% of total blood volume (TBV) would produce reversible shock (Noble et al. 1946). Such a haemorrhage has been used successfully in pigs (Hannon and Bossone 1986; Weiskopf et al. 1986). The rate of haemorrhage, 1% TBV/minute, was used by Weiskopf et al. (1986).

A 30 minute shock period was selected as being clinically relevant: resuscitation in an urban setting should ensure that trauma patients are not subjected to longer periods of shock.

Nerve stimulation

Nociceptive afferent nerve stimulation has been shown to be an effective model for tissue trauma. As early as 1947 Overman and Wang showed that somatic afferent nerve stimulation could mimic the cardiovascular response to tissue injury (Overman and Wang 1947).

Use of brachial plexus stimulation goes back to a bizarre model of traumatic shock used by Guthrie (Guthrie 1917). In etherised dogs "the brachial plexus on one or both sides was exposed, ligated and cut peripheral to the ligature and further stimulated by traction, or crushing with forceps, and tetanising electrical currents. One or both sciatic nerves were prepared for stimulation chiefly to observe vasomotor reflex response. Unless pronounced symptoms of shock were soon induced, one or both of the fore limbs were about three fourths amputated, or the abdominal cavity was opened by a median incision and the intestines exposed and manipulated".

More recently Rady et al. (1993) found that brachial nerve stimulation effectively mimicked the cardiovascular response to skeletal muscle injury caused by a captive bolt.

The major advantages of nociceptive afferent nerve stimulation are that there is no additional blood or fluid loss to aggravate the effect of haemorrhage, and it avoids the release of potential "toxins"/cell breakdown products from damaged tissues. It is also reproducible and so complies with Stoner's criteria for trauma models (Stoner 1961). The target was an increase of at least a 10 mmHg in systolic blood pressure. This rise in blood pressure, known as the Loven reflex, was first noted in 1866 after stimulation of the exposed sciatic nerve by crushing or electrical stimulation (as reported in Parsons

and Phemister 1930). Blood pressure remained elevated for more than an hour if stimulation was continued for that time.

Brachial nerve rather than sciatic nerve stimulation was chosen for practical reasons. As I was interested in skeletal muscle blood flow (assessed by measuring femoral arterial flow) the femoral arteries could not be used for either blood pressure monitoring or for haemorrhaging the animal. Use of the carotids was contra-indicated as there was a risk of interfering with the carotid sinuses and so disturbing the baroreflex. This meant that the most suitable arteries were the axillary arteries. Since surgical incisions had to be made in the axillae it was relatively easy to extend these to allow exposure of the brachial plexi and so minimise the extent of tissue damage.

Laparotomy

As I was interested in the effects of haemorrhage and injury on the gut I wanted to get an indication of the blood flow to the gut. I was also interested in assessing whether there was translocation of endotoxin from the gut lumen to the portal venous circulation, and whether there was any inflammatory cytokine production from the gut-associated lymphoid tissue directly into the circulation.

To achieve these aims it was necessary to perform a laparotomy to cannulate the portal vein. The anatomy of the pig makes this relatively easy: the large bowel lies to the left of the midline, the small bowel to the right. Gentle retraction of the large and small bowel away from the midline provides direct access to the portal vein. Knowing that intestinal handling promotes translocation, blood flow was measured from a branch of the superior mesenteric artery (SMA), exposed during the dissection of a tributary of the portal vein, rather than from the SMA itself. This minimised the amount of intestinal handling required in the dissection.

Anaesthesia

Anaesthesia may greatly alter the response to shock stimuli. Dale and Laidlaw commented that i.v. histamine was rapidly fatal in rabbits and guinea pigs, but that under deep anaesthesia they would tolerate large doses (Dale and Laidlaw 1919).

Most studies of the responses to haemorrhage have been carried out on anaesthetised animals. Both the agent used and the depth of anaesthesia may affect the response. Survival time from uncontrolled ear vein haemorrhage was shortened in rabbits deeply anaesthetised with urethane compared with animals only lightly anaesthetised or awake (Downman et al. 1944). Also a number of anaesthetic agents are known to modify cardiovascular reflex activity (barbiturates can attenuate the baroreflex and the cardiovascular response to tissue injury, Redfern 1981).

The alphaxalone/alphadolone mixture was chosen as it preserves the cardiovascular and visceral responses associated with the defence reaction, in contrast to barbiturates and α-chloralose (Timms 1976; Timms 1981). These responses are similar to those seen in electrical stimulation models of injury (Quest and Gebber 1972).

Choice of species

There has been a gradual change in the species used in biomedical research. In 1960 a review of animal experimentation in general revealed that about 28% of all experiments were conducted on dogs (Schmidt-Nielsen 1961). Since then there has been a gradual reduction in the use of dogs and cats and an increase in the use of pigs and other farm animals, which attract less emotional support (Mukerjee 1997).

Pigs have increasingly been recognised as viable models for biomedical research. This is in part due to pressure to limit the use of dogs in research and in part due to the recognition of the anatomical and physiological similarities with humans. These were

outlined by Swindle (1986). Pigs have been given glowing testimonials. Phillips and Tumbleson wrote in 1986 that: "From a broad behavioral view, pigs are like people. They tend to be diurnal, sedentary, meal eaters with a fondness for alcohol and are prone to cardiovascular disease. Yet, with training and diet restriction they become fit and passable athletes, a description as appropriate for the human population." (Phillips and Tumbleson 1986)

Nearly all the shock studies reviewed by Wiggers in 1950 were performed on dogs (Wiggers 1950). As he summarised: "It has been amply demonstrated that the clinical symptomatology of shock is reproducible in dogs" (Wiggers 1950 p89). Dogs were unsuitable for this study as they are subject to intestinal haemorrhages following systemic haemorrhage. This is clearly not representative of the human response to haemorrhage.

Now nearly all the studies are performed either on rodents or on pigs or sheep. There are only a few centres where primates are still used. Primates are particularly valuable in studying the pathogenesis of "shock lung" (Adult Respiratory Distress Syndrome, ARDS) because they will breathe spontaneously under anaesthesia even in the supine position (Pretorius et al. 1987). Thus artificial ventilation, which forms part of the treatment of ARDS, can be avoided.

Rodents were not used because their size made the model impractical. Nerve stimulation without current spread would have been almost impossible, and it would have been impossible to take the sequential blood samples because of their small blood volume. Bacterial translocation and cytokine generation from the gut has already been demonstrated in the rat (Baker et al. 1988; Deitch et al. 1990; Deitch et al. 1994).

In the end pigs were chosen for their documented advantages and the ease with which they could be handled. Over the course of three years working with them I would agree with Bustad and Horstman, who wrote: "We have reached the conclusion that the pig is gregarious, perceptive, quick to learn, sensitive, very responsive to gentle care and direction, playful and courageous, and neat and clean if given a chance. They are also

one of the best experimental models for people—anatomically, physiologically, psychologically and socially. This remarkable animal deserves our respect, our enlightened stewardship and informed gentleness as well as our continued gratitude for its continuing remarkable contributions to our health and well-being." (Bustad and Horstman 1986).

Sex

Females were chosen because it was slightly easier to perform the laparotomy to cannulate the bladder. They were easier to house in a group in the animal house: males would have required more pens, which were not always available.

In some species, such as the rat, females are more resistant to trauma than males (Noble and Collip 1941-2). There is no evidence for this in swine.

Age

Young animals were used weighing 17-25 kg. This ensured that I could safely sedate them on my own and then transport them from the animal house into the laboratory.

Biological rhythm

As metabolic functions are affected by diurnal and seasonal rhythms the pigs were kept in the in an environment providing a constant temperature and humidity and a regular 12 hour day/night cycle. All the studies were started at the same time of day (sedation at about 7 am, surgery starting about 8 am).

Environmental temperature

This was not controllable in the laboratory as there was no air conditioning. Core temperature was maintained in the winter using a heating pad and lamps. In the summer there were occasions when the ambient temperature exceeded 28°C. In these circumstances surgical drapes were removed and fans were used to try to prevent excessive increases in core temperature.

Chapter 2: Methods

Experimental method

Animals

The animals used in the model were pure-bred Large White female pigs, about eight weeks old, and weighing 15-27 kg. They were kept in specially prepared pens containing sterile saw-dust but no straw. Temperature in the holding area was maintained at 17-19°C with a relative humidity of 30-45%. A 12 h light/dark cycle was operated.

Animals were given free access to water and were fed on a standard diet of Growercare 528 pellets (BOCM Pauls Ltd, Shepshed, UK), 980 g per day. This feed is enriched with:

Vitamins: A (10,000 iu/kg)
D3 (2,000 iu/kg)
E (65 iu/kg)
Sodium selenite-selenium (0.3 mg/kg)

and the growth promoters: Copper sulphate (175 mg/kg)
Tylosin phosphate (40 mg/kg)

Vitamin E is an anti-oxidant. Given that the concentration in the feed was 65 iu/kg and that each animal was given less than 1 kg/day it was not thought that this would significantly alter the inflammatory response. Any effect would have been balanced by the fact that all groups were given the same feed.

The animals were given several days to acclimatise before any studies were carried out. I visited the animals daily to check on their condition and to allow them to become

accustomed to my presence. This ensured that on the day of an experiment they could be approached and sedated with a minimum of stress.

The evening before a study, two animals were isolated in a pen with no saw-dust. This reduced the stress associated with isolation from the rest of the litter and ensured that a back-up animal was available in case of a sudden death. They were starved overnight but allowed free access to water.

Large White pigs were chosen because of their relative resistance to malignant hyperpyrexia. In preliminary studies with Landrace or Duroc pigs there was an unacceptable mortality under anaesthesia from malignant hyperpyrexia (MH).

Animals in the weight range 15-27 kg were used for ease of handling in and out of the laboratory.

Anaesthesia

Sedation

Animals were sedated with alphaxalone/alphadolone (Saffan, Pitman-Moore (UK) Ltd, Harefield, Uxbridge, Middlesex, UK) at a dose of 9-24 mg/kg given by intra-muscular injection. This was given into the back of the neck through a 19 gauge "butterfly" with a long connecting tube to avoid having to restrain the animals (Cox et al. 1975).

Transfer from the pens to the laboratory was only effected once the animals were sufficiently sedated to tolerate being picked up and placed in the transfer trolley.

Saffan

Saffan is a steroid anaesthetic, whose active constituents are alphaxalone and alphadolone acetate solubilised in saline by 20% w/v polyoxyethylated castor oil. Each millilitre of Saffan contains 12 mg of total steroids (1.2% w/v): 9 mg alphaxalone and 3 mg alphadolone acetate. Saffan is the veterinary equivalent of Althesin, which was a widely used agent in human clinical practice (Child et al. 1971; Davis and Pearce 1972; Hall 1972).

The alphaxalone/alphadolone mixture has a number of advantageous properties. It can be used successfully in pigs known to be susceptible to malignant hyperpyrexia (Hall 1972). The mixture may even have a slight protective effect when it is given before the inhalational agent halothane. Respiratory and cardiovascular depression are only seen at rates of intra-venous infusion higher than those needed to produce surgical anaesthesia (Child et al. 1971).

The alphaxalone/alphadolone mixture was chosen as it preserves the cardiovascular and visceral responses associated with the defence reaction, in contrast to barbiturates and chloralose (Timms 1976; Timms 1981). These responses are similar to those seen in electrical stimulation models of injury (Quest and Gebber 1972).

Induction

Once in the laboratory induction of anaesthesia was achieved by allowing the animals to breathe 4% halothane with oxygen and nitrous oxide (50/50) through a "snout mask". The animals were then intubated under direct vision with a 6.0 gauge cuffed rubber endotracheal tube. The cuff was inflated and the tube position checked by auscultation for air entry to both lungs.

Endotracheal intubation

Endotracheal intubation of the pig can be a difficult procedure. Some groups have elected to perform a tracheostomy for endotracheal intubation (Attinger and Cahill 1960).

In adult pigs one of the problems is the length of the snout, which makes visualisation of the vocal cords difficult. Rowson overcame this problem by using a long tubular laryngoscope (51 cm) and a metal rod in the endotracheal tube to keep it straight during intubation (Rowson 1965)

Another problem is the fact that the epiglottis is particularly long and completely obstructs the view of the larynx. Hall and Rowson both describe the need to push the epiglottis out of the way, while Davies and Hoare found that the laryngeal inlet could best be viewed with the animal on its left side (Rowson 1965; Davies and Hoare 1972; Hall 1972).

The anatomy of the adult pig larynx presents further challenges. Between the epiglottis and the larynx pigs have a "middle ventricle" into which an endotracheal tube may be passed if the cords have not been properly visualised (Riebold and Thurmon 1986). Fowler et al found that "progress of endotracheal tubes may be held up in the sharp bend that exists in the pig's larynx . This problem is neatly solved by rotating the tube through 180° during intubation" (Fowler et al. 1962). In the eight week old animals there was no need to rotate the tube through 180°, as many investigators have described (Dawson 1963; Davies and Hoare 1972). With animals weighing 40-50 kg, used in another study, this rotation was necessary.

My method involved placing the animal supine, spontaneously breathing halothane (4%) with oxygen and nitrous oxide (50/50) via a snout mask until there was sufficient muscle relaxation to fully open its jaws. A standard 18 cm straight blade laryngoscope (Longworth Scientific Instrument Co, Abingdon, Oxon, UK) was used to displace the

tongue to the left and to visualise the epiglottis. By pushing down on the soft palate with an endotracheal tube stiffened with a metal rod the tip of the epiglottis was freed. The end of the laryngoscope was hooked over the tip of the epiglottis to obtain a view of the vocal cords (Hill and Perry 1959). A plastic guide was passed between the cords and the endotracheal tube (size 6, rubber) introduced over it. If there was any resistance the endotracheal tube was withdrawn and the animal given more halothane.

Once the endotracheal tube was in place the cuff was inflated with approximately 5 ml of air and the inlet clamped. The endotracheal tube was tied in place and connected to the ventilator. End-tidal CO_2 was immediately monitored and almost invariably high so a short period of hyperventilation was used to bring it down. The position of the tube was checked by auscultation in both axillae.

This technique was found to be very effective and was performed without any assistance.

There are several reasons that may explain why intubation proved so easy in this study:

1) The animals were small. This meant that a normal laryngoscope could be used and it was easy to lift the epiglottis and so visualise the cords.

2) Halothane was used. This resulted in good muscle relaxation and more importantly good laryngeal relaxation.

3) A thin guide was passed through the cords before the tube itself.

4) The animals were exposed to a minimum of stress in the pen. Being unrestrained when given sedation they did not squeal, which might result in laryngeal oedema.

Maintenance

Ventilation continued with halothane 1-1.5% in oxygen and nitrous oxide (50/50) to achieve surgical anaesthesia. A similar anaesthetic mixture was used by Bickell et al. (Bickell et al. 1989). Tidal volume was set at 13 ml/kg and minute volume altered to give an end-tidal CO_2 in the range 32-40 mmHg.

After the end of surgery halothane was discontinued and anaesthesia was maintained by an intra-venous infusion of alphaxalone/alphadolone (15mg/kg/h). The animals continued to be ventilated with oxygen and nitrous oxide (50/50). A one hour stabilisation period was used before the experiments started.

Halothane

Halothane is often feared as an anaesthetic in pigs because of the risk of malignant hyperpyrexia (MH) (Nelson 1986). Despite this a number of groups have used it successfully (Dawson 1963; Sawyer et al. 1971; Davies and Hoare 1972; Merin et al. 1977; Bickell et al. 1989).

With nearly 90 pure-bred Large White pigs (always from the same supplier) used in a number of different studies there have only been two clear cases of MH. In a pilot study two out of six Duroc pigs died from MH.

The overall exposure to halothane was;

10-15 minutes at 4% (breathing spontaneously) to induce anaesthesia and adequate laryngeal relaxation for intubation

2-2.5 hours at 1-1.5% (ventilated) during the preparatory surgery.

In MH susceptible animals 1-2% halothane would trigger a response within 10-60 minutes (Nelson 1986). Clearly the animals I used were not particularly genetically susceptible to MH.

Surgery

Both axillary arteries were cannulated following open dissection of the axillae. Sterile 5FG 30 cm cannulae were used (Portex Ltd, Hythe, Kent, UK). By extending the incision the brachial plexus was exposed and three nerve trunks dissected. These were later used for nerve stimulation.

The left external jugular vein was exposed and cannulated with a sterile 8.5F polyurethane sheath and introducer (Baxter Healthcare Corporation, Irvine, CA, USA). This was later used to introduce a pulmonary artery flotation catheter.

The right femoral artery was exposed to allow the application of an electro-magnetic flow probe (Spectramed Inc. Oxnard, CA, USA).

A laparotomy was performed. Gentle midline retraction of the small bowel from the large bowel was used to expose the portal vein, which was cannulated through one of its minor tributaries with a 5FG 30 cm cannula (Portex Ltd, Hythe, Kent, UK). The cannula was advanced until it was seen to lie in the portal vein. This dissection was used to expose a branch of the superior mesenteric artery. An electro-magnetic flow probe was applied around it to allow some measurement of blood flow to the gut (Spectramed Inc. Oxnard, CA, USA). A compromise was made between exact reproducibility (which branch of the superior mesenteric artery was used) and the need to minimise intestinal handling and dissection. In practice only two adjacent branches were used. They were of a similar size so that only two sizes of flow probes were used.

The laparotomy was then closed with interrupted 2/0 silk sutures. Finally the bladder was cannulated via a distal midline incision to allow free drainage of urine.

Nerve stimulation

Shielded silver ring electrodes were applied to three nerve trunks from the brachial plexi. Electrical isolation of the electrodes from the surrounding tissues was ensured by lining the wound with small pieces of thin plastic film and then filling the wound cavity with silicone gel (Wacker RTV-E 604B "Silgel", Wacker Chemicals Ltd, Walton-on-Thames, Surrey, UK).

Nerves were stimulated using a Grass S88 two channel stimulator (Grass Medical Instruments, Quincy, Mass, USA) and SIU 5 stimulation isolation units (Grass Medical Instruments, Quincy, Mass, USA). One millisecond pulses were applied in 10 second trains, alternating left to right throughout the experiments, using a Digitimer D100 (Digitimer Ltd, Welwyn Garden City, Herts, UK) to gate a pair of reed switches. Alternating bilateral nerve stimulation was used to avoid the problem of "accommodation" of the nerves to electrical stimulation (Guyton 1981). The nerves were stimulated throughout the experimental period.

To determine the appropriate current setting, stimulation was started unilaterally to produce an increase in systolic blood pressure of 20 mmHg. Once this was achieved, the current was set. Stimulation was stopped to allow blood pressure to return to baseline before starting to stimulate the contralateral side. When the current was switched off blood pressure returned to baseline almost immediately. This reduction in blood pressure was also seen when the current was switched off at the end of the experiments.

Starting nerve stimulation took five to ten minutes after the control measurements had been made. Once nerve stimulation had started haemodynamic measurements were made as a further control (CNS time point) and then the thirty minute haemorrhage period was started.

Throughout the studies current was always passed through a Grass CCU I A Constant Current Unit to ensure that the stimulus remained the same. Resistance in the two sides was often different. This meant that different currents were sometimes applied to achieve the same effect.

Haemorrhage

Animals were haemorrhaged at a rate of 1% total blood volume (TBV) per minute from the left axillary artery. A rotary pump was used (Smith+Nephew Watson Marlow, Falmouth, Cornwall, UK). Shed blood was collected in sterile empty normal saline bags pre-treated with 1500u sodium heparin (Monoparin, CP Pharmaceuticals Ltd, Wrexham, Clwyd, UK).

A fixed volume haemorrhage (30% TBV) was used, as described in the Introduction (The model). In some pilot experiments there were early deaths from the combination of haemorrhage and nerve stimulation, so a lesser degree of haemorrhage, 20% TBV, was used as two additional groups. With a little more experience of the model it became apparent that animals would survive a 30% haemorrhage with nerve stimulation, so that the 30% haemorrhage and 30% haemorrhage and nerve stimulation groups were completed as well.

My calculations of volume of haemorrhage assumed a total blood volume of 75 ml/kg. This was based on a study in which blood volume was measured using carbon monoxide as a haemoglobin label (Dingley et al. 1997a; Dingley et al. 1997b). This is in line with other estimates. In their book "Essentials of Experimental Surgery" Lumley et al. quote blood volume in 30 kg pigs as being in the range 60-80 ml/kg (Lumley et al. 1990). Rady et al. estimated that in pigs weighing 15-27 kg blood volume was approximately 75 ml/kg (Rady et al. 1993).

Shed blood was re-infused at a rate of 2ml/kg/min.

Haemodynamic monitoring

A three lead ECG was recorded throughout the experiments.

Blood pressure was measured from the right axillary artery with a SensoNor 840 physiological pressure transducer (Lectromed UK Ltd, Letchworth Garden City, Herts,UK). All the blood pressure transducers were calibrated using a mercury manometer before each experiment.

Pulmonary artery pressure and pulmonary artery occlusion pressure were measured with a 5F Thermodilution Catheter (Viggo-Spectramed, Swindon, Wilts, UK), introduced via the left external jugular vein. The position of the catheter was determined by observing the characteristic wave form of the pulmonary artery pressure trace on the chart recorder.

Pressures were recorded on a six channel chart recorder (Devices Instruments Ltd, Welwyn Garden City, Herts,UK). A paper speed of 5 divisions/second (5 mm/second) was used when making measurements. The rest of the time the paper speed was 50 divisions/ 10 minutes (5 mm/minute).

Cardiac output was calculated from the thermodilution of three consecutive rapid injections of 10 ml ice cold saline (the modified Fick method) every 30 minutes. Injections were timed with the respiratory cycle. The calculations were done by a COM-1 cardiac output computer (Baxter Healthcare Ltd, Compton, Berks, UK). As animal weights were not constant, and there is no widely accepted formula for body surface area in the pig, cardiac outputs were indexed to weight. Stroke volume index, right ventricular stroke work, left ventricular stroke work, and systemic and pulmonary vascular resistance indices were also calculated (a full description of the calculations appears in a separate section).

Blood flows in the right femoral artery and the branch of the superior mesenteric artery were measured using two SP2202 blood flowmeters (Spectramed UK Ltd, Letchworth Garden City, Herts,UK).

End-tidal CO_2 was measured using a Datex Normocap CO_2 & O_2 monitor (Vickers Medical, Basingstoke, Hants,UK).

Rectal temperature was monitored using an ISA rectal probe.

Blood sampling

Arterial blood gases were taken from the control time point and then every 30 minutes throughout the experiments.

Arterial blood was also taken every hour during the observation period for assays of:

lactate
endotoxin
inflammatory cytokines (TNF-α and IL-6)

and for the determination of haematocrit.

Mixed venous blood was taken every 30 minutes for blood gas analysis.

Portal venous blood was taken every 30 minutes for blood gas analysis. Every hour during the observation period blood was taken for assays of:

lactate
endotoxin
inflammatory cytokines (TNF-α and IL-6)

Ideally cytokines would have been assayed more frequently to avoid missing any transient changes. However, I was concerned about the amount of blood being taken every hour and wanted to avoid imposing a second haemorrhage insult simply from taking blood samples. There were also financial constraints on the number of blood samples that could be analysed. No cytokine assays were performed on the 20% haemorrhage group or the 20% haemorrhage plus nerve stimulation group for this reason.

Blood gas analysis:

All analyses were performed on an ABL330 Acid Base Laboratory, coupled with an OSM3 Hemoximeter (Radiometer, Copenhagen, Denmark). The hemoximeter was set to accept pig blood. Whole body oxygen delivery and oxygen consumption were calculated and then indexed to body weight. Systemic and portal oxygen extraction ratios were calculated.

Haematocrits were determined in a standard manner: high speed centrifugation of arterial blood divided into two capillary tubes and then read with a Hawksley Micro-Haematocrit Reader

Assays

Endotoxin: Coatest (Kabi Diagnostica, Nyköping, Sweden)

Inflammatory cytokines (TNF-α and IL-6):

Coaliza TNF-α, Chromogenix AB, Mölndal, Sweden)

Coaliza IL-6, Chromogenix AB, Mölndal, Sweden)

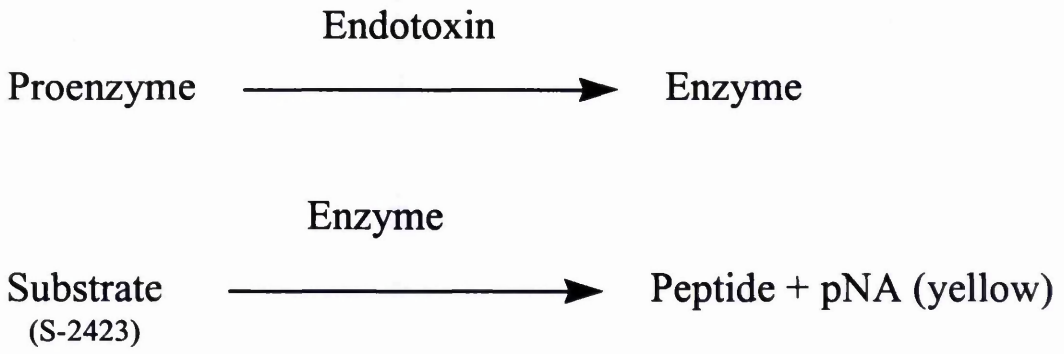
Lactate: measured fluorometrically by an "in house" enzymatic assay.

All of the assays were performed by trained laboratory technicians (Barbara Crawley and Tim Rainey), who had no interest in the results of the assays. Tim Rainey acted as a "runner" during the experiments, allowing me to remain "scrubbed" during the surgery. Barbara Crawley performed the endotoxin and cytokine assays in a totally blind manner.

Endotoxin

The principle of the test is that Gram-negative bacterial endotoxin catalyses the activation of a proenzyme in Limulus Amoebocyte Lysate (LAL), as shown in Figure 2.1. This enzyme cleaves a substrate (S-2423) resulting in the formation of yellow p-nitroaniline (pNA) dye. The reaction is stopped using acetic acid and the formation of pNA measured photometrically at 405nm. For endotoxin concentrations of 0.1-1.2 EU/ml there is a linear correlation with absorbance at 405 nm.

Figure 2.1: An outline of the Limulus Amoebocyte Lysate (LAL) test.



Blood was collected in endotoxin-free glass tubes containing dilute endotoxin-free heparin (diluted to a concentration of 25 IU/ml using endotoxin-free water) (Sigma, Dorset, UK). After centrifugation at 2000g for 10 minutes at 4°C 1ml of platelet-rich plasma was removed and stored at -70°C until assayed.

Once thawed the plasma was diluted 1:10 with sterile endotoxin-free water and heat-treated in a water bath at 75°C for 5 minutes. This procedure destroys any inhibitors which might interfere with activation and any proteolytic activities which may be present in the plasma. The samples were stored for 15 minutes at room temperature and then shaken.

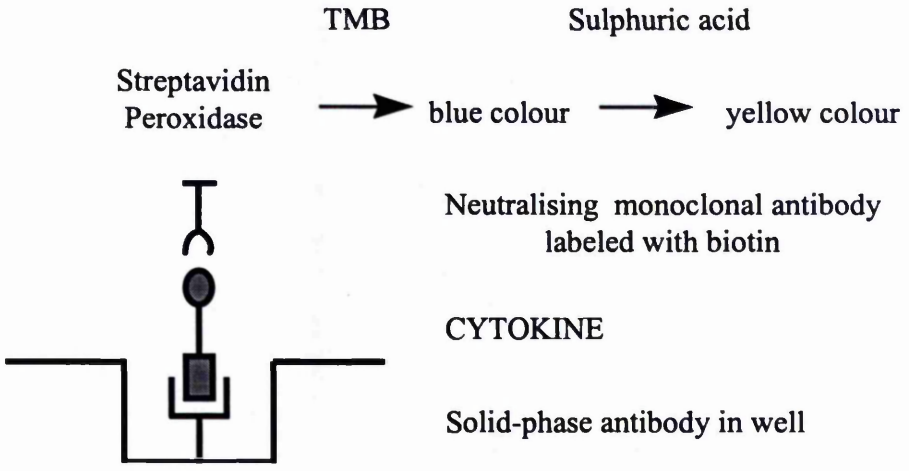
First 100 µl of the sample was added to a test tube and incubated at 37°C for 3-5 minutes before the addition of 100 µl LAL. The mixture was incubated for exactly 25 minutes and then 200 µl of substrate-buffer solution was added and the whole incubated at 37°C for 5 minutes. The reaction was stopped by adding 200 µl acetic acid. The absorbance of the samples was then read against a plasma blank in a photometer at 405 nm. Endotoxin concentrations were calculated in EUs and converted to pg/ml on the basis that 1 EU=83.3 pg/ml. The inter-assay coefficient of variation was less than 10%.

Tumour necrosis factor-α

The principle of the test is that plasma is incubated in wells coated with rabbit polyclonal anti-human TNF-α antibodies (a solid-phase antibody) so that any TNF-α present will be bound by these antibodies (Figure 2.2). A neutralising monoclonal anti-TNF-α antibody labelled with biotin is added to bind with the existing solid-phase antibody-TNF-α complex in the well.

Peroxidase conjugated streptavidin is added to bind to the biotin. The addition of a substrate (tetramethylbenzidine (TMB) dissolved in dimethyl sulfoxide) results in a blue colour reaction. The reaction is stopped using sulphuric acid, which turns the colour yellow. The intensity of the yellow is proportional to the TNF-α concentration in the sample.

Figure 2.2: Principles of the cytokine assays.



Plasma derived from the blood collected for the endotoxin assay was used to measure cytokine concentrations. The assays were carried out in polystyrene microplate wells coated with rabbit polyclonal anti-TNF-α antibody. Fifty µl of phosphate buffer and 50

μl of sample were added, mixed and incubated for 2 hours at 37°C. The wells were then washed thoroughly (4 times) and 100 μl of mouse monoclonal anti-TNF-α (labelled with biotin) added and incubated for a further 60 minutes. After washing out the wells 100 μl of peroxidase conjugated streptavidin was added and incubated for 30 minutes. After washing, 100 μl of TMB was added to initiate the colour reaction. This continued for 30 minutes after which the reaction was stopped by the addition of 100 μl sulphuric acid. Absorbance of the solution in the wells was read at 450 nm and the concentration of TNF-α in pg/ml determined from the standard curve.

At the TNF-α levels we detected the intra-assay coefficient of variation was 7.8% and the inter-assay coefficient of variation was 13.4%.

Interleukin-6

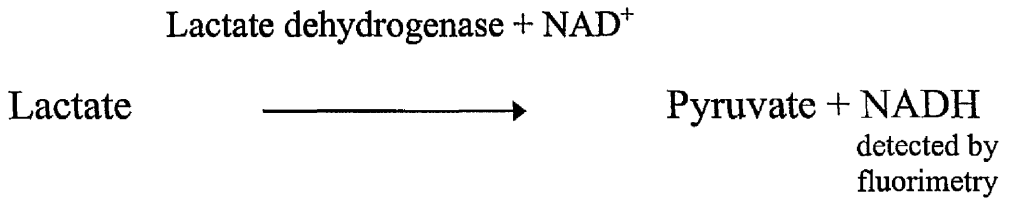
This assay follows the same principles as for TNF-α the only real difference being that the solid-phase antibodies coating the polystyrene wells were sheep polyclonal anti-IL-6 antibodies and the neutralising antibody was a mouse monoclonal anti-IL-6 antibody, labelled with biotin.

The intra-assay coefficient of variation was 1.5% at 313 pg/ml of IL-6 and 4.5% for a negative. The inter-assay coefficients of variation were 2.3% and 8.8% respectively.

Lactate

The principle of the lactate assay is that lactate dehydrogenase and NAD^+ are used to convert lactate to pyruvate and NADH (Figure 2.3). NADH can be measured fluorimetrically at a wavelength of 340 nm (Harrison et al. 1988).

Figure 2.3: An outline of the lactate assay.



This was done using a Cobas Bio Centrifugal Autoanalyser with a fluorimetric attachment (Roche UK, Welwyn Garden City, Herts, UK).

The coefficient of variation for the arterial assays performed from March 1995 to January 1996 was 4%. The coefficient of variation for the portal assays, performed in 1996, was 3.9%.

Microbiological analysis

Tissue and blood cultures were taken from a limited number of the studies. The aim was to look for evidence of the translocation of micro-organisms from the gut to the reticulo-endothelial system and the circulation.

Biopsies

Biopsies of reticulo-endothelial organs were taken for bacterial culture to determine whether there was any evidence of bacterial translocation from the gut. Mesenteric lymph nodes were biopsied at laparotomy to act as controls. After the final round of measurements the laparotomy incision was opened and another lymph node biopsied along with the spleen and liver. Small bowel and large bowel were also biopsied and cultured to check for the presence of intestinal flora.

Blood samples

Arterial blood samples (10 ml) were taken at the beginning and end of the study for bacterial culture to look for evidence of bacterial translocation to the circulation.

Culture techniques

The biopsy tissue was transferred from its pot with disposable sterile forceps into a weighed sterile bijou bottle and re-weighed. The whole was made up to 1 ml with sterile saline. The tissue was chopped as finely as possible and then mixed on a vortex mixer for one minute. Two blood agar plates, one MacConkey, one A V plate and one Neomycin plate were all inoculated using a 10μ disposable loop with a streak down the middle of the plate.

A metronidazole (MTZ) antibiotic disc was added to one blood agar plate and incubated anaerobically for 48 hours. The remaining plates were incubated at 37°C for 24 hours aerobically. The plates were then read and the organisms cultured were identified using standard laboratory techniques.

Blood was cultured for 48 hours using a BACTEC instrument. Identification of organisms was performed using standard laboratory techniques.

Protocol

1. Sedation

2. Induction of anaesthesia and intubation leading to unparalysed ventilation.

3. Surgery

4. Stabilisation period: 1 hour.

5. Control round of measurements and blood samples.

6. Start nerve stimulation.

7. Start haemorrhage.

8. Shock period: 30 minutes.

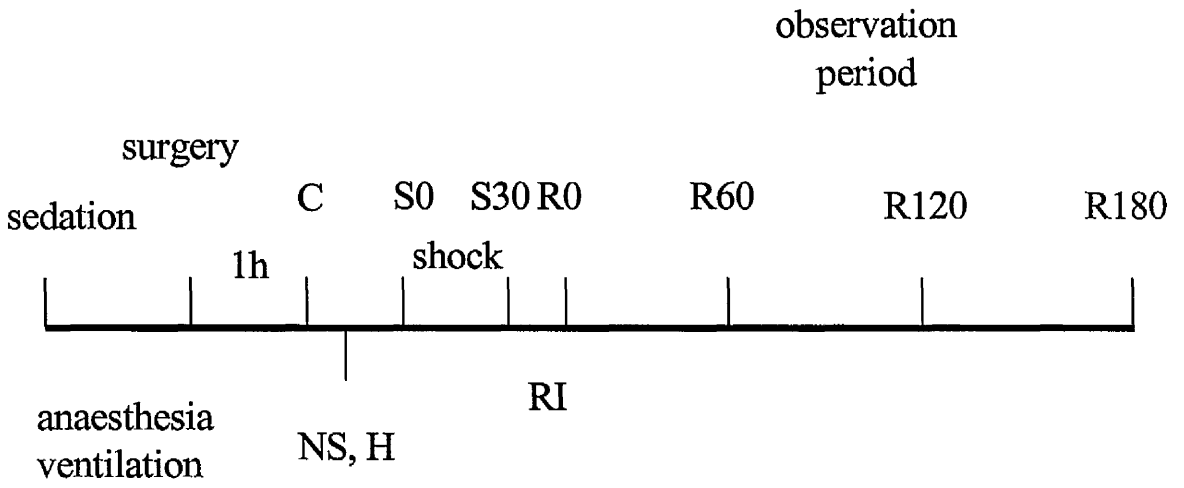
9. Re-infusion of shed blood.

10. Observation period (3 hours).

11. Final observations followed by re-opening of laparotomy for collection of tissue samples.

12. Sacrifice of animal.

Figure 2.4: Time bar showing the order of procedures and measurement time points.



NS, H = start of nerve stimulation followed by start of haemorrhage

RI = reinfusion of shed blood

Nerve stimulation was started immediately after the control measurements had been made. As explained on page 109 this took about 10 minutes, after which haemorrhage was started.

Groups

The experiments were divided into six groups. There were two groups of animals subjected to haemorrhage alone: one with a 20% TBV haemorrhage (20% Haem/H20), the other a 30% TBV haemorrhage (30% Haem/H30). Two groups were subjected to haemorrhage on a background of nerve stimulation (to mimic injury), again one group was subjected to 20% TBV (20% HNS/HNS20), the other to 30% TBV haemorrhage and nerve stimulation (30% HNS/HNS30). Two groups acted as controls: one group subjected to nerve stimulation alone throughout the experimental period (NS), and the other subjected to sham surgery with no additional insult (C).

Animal weights

All the animals were weighed after sedation and before transfer to the laboratory. Group mean weights are shown in Table 2.1 below:

Table 2.1: Animal body weights.

Body weight (kg)		
	Mean	SD
Control (n=11)	20.2	1.66
NS (n=10)	23	2.75
20% Haem (n=7)	20.4	4.20
30% Haem (n=10)	21.4	2.22
20 HNS (n=8)	19.1	0.532
30% HNS (n=11)	21	1.61

One-way analysis of variance confirmed that there was no statistically significant difference in the body weights of the different experimental groups ($F = 2.03$, df 5, $P = 0.091$).

Termination

At the end of each study the depth of anaesthesia was increased by giving 5% inhaled halothane for five minutes. The animals were then killed with an intra-venous injection of supersaturated potassium chloride.

All the experiments were performed under the Animals (Scientific Procedures) Act 1986.

Calculations

Raw data was collected and a number of derived variables calculated and then used in the analysis. Haemodynamic and oxygen transport equations were taken from Edwards et al. (Edwards et al. 1993).

1) Heart rate **(HR)** (bpm) = 60/heart period (the variable we actually measured)

2) Mean arterial pressure **(MAP)** (mmHg) = $DBP + \frac{SBP - DBP}{3}$, where

DBP = Diastolic blood pressure, and
SBP = Systolic blood pressure.

3) Mean pulmonary artery pressure **(MPAP)** (mmHg) = $DPAP + \frac{SPAP - DPAP}{3}$, where:

DPAP = diastolic pulmonary artery pressure, and
SPAP = systolic pulmonary artery pressure.

4) Cardiac index **(CI)** is usually defined as $\frac{CO(ml/min)}{BSA(m^2)}$

where:

CO = cardiac output.
BSA = body surface area.

However as there is no accepted formula for BSA in the pig, cardiac index was defined as = $\frac{CO(ml/min)}{weight(kg)}$,

and expressed in ml/min/kg.

5) Stroke volume index **(SVI)** (ml/kg/beat) = $\frac{\text{CI(ml / min/ kg)}}{\text{HR(beats / min)}}$.

6) Right ventricular stroke work **(RVSW)** (g/min)

$$= \text{SV x } (\frac{\text{MPAP} - \text{CVP}}{\text{CO}}) \text{ x } 0.0136$$

$$= \text{SVI x } (\frac{\text{MPAP} - \text{CVP}}{\text{CI}}) \text{ x } 0.0136, \text{ where}$$

CVP = central venous pressure as an index of right atrial pressure.

7) Left ventricular stroke work **(LVSW)** (g/min)

$$= \text{SV x } (\frac{\text{MAP} - \text{PAWP}}{\text{CO}}) \text{ x } 0.0136$$

$$= \text{SVI x } (\frac{\text{MAP} - \text{PAWP}}{\text{CI}}) \text{ x } 0.0136, \text{ where}$$

PAWP = pulmonary artery wedge pressure.

8) Systemic vascular resistance index **(SVRI)** (dyne.sec.cm^{-5}.kg)

$$= \frac{\text{MAP} - \text{CVP}}{\text{CI}} \text{ x } 80$$

9) Pulmonary vascular resistance index **(PVRI)** (dyne.sec.cm^{-5}.kg)

$$= \frac{\text{MPAP} - \text{PAWP}}{\text{CI}} \text{ x } 80$$

10) Oxygen delivery index **(DO_2I)** (ml/min/kg) = CI x CaO_2 x 10, where

CaO_2 = (art Hb x SaO_2 x 1.34) + (PaO_2 x 0.003) is in mlO_2/dl

11) Oxygen consumption index **(VO_2I)** (ml/min/kg) = CI x (CaO_2 - CvO_2) x 10, where

CvO_2 = (art Hb x SvO_2 x 1.34) + (PvO_2 x 0.003) is in mlO_2/dl

12) Oxygen extraction ratio **(OER)** = $\frac{CaO_2 - CvO_2}{CaO_2}$

13) Portal oxygen extraction ratio **(POER)** = $\frac{CaO_2 - CportvO_2}{CaO_2}$

14) Regional vascular resistances. These could not be calculated in the conventional manner as absolute blood flows were not measured. Mean arterial pressure was taken as the driving pressure and the percentage of baseline blood flow (femoral artery and branch of the superior mesenteric artery, respectively) was taken as an index of flow, so that:

$$\text{Resistance, } R = \frac{MAP}{Flow} \text{ (arbitrary units).}$$

Statistics

When a study involves only two groups statistical analysis can be relatively straight forward. For each measured variable the two groups can be compared using Student's t test (assuming the data are normally distributed). If there are more than two groups then multiple pairs of t tests would be needed to compare all the groups. Although this is commonly done (Godfrey 1992) it is inappropriate as it will increase the risk of finding a spurious significant difference to $1-(1-\alpha)^n$ where n is the number of comparisons. With α set at 0.05 this is approximately 0.05n. If there are 20 pairs of comparisons at least one is likely to appear significant whatever the data really suggests.

Analysis of variance

To overcome this problem analysis of variance (ANOVA) is used. This compares the variance due to differences between the groups with the variance due to differences within the groups. The ratio of these variances, the F statistic, should be 1 if the groups are drawn from the same population since both the numerator and the denominator are estimates of the population variance. The larger the F statistic the less consistent is the null hypothesis that the groups were drawn from the same population. A large F statistic shows that the variability between the group means is larger than expected from the variability within the groups (Altman and Bland 1996; Glantz 1997).

The basic principles of ANOVA will be described, as adapted from Glantz (Glantz 1997 pp293-299).

Variance within a group is the sum of the deviation of each value (x) from the mean for that group (X), divided by the number of subjects in the group minus one,

$$s^2 = \sum \frac{(x - X)^2}{n - 1}$$

$s^2 = \frac{SS}{n-1}$, where n = number of subjects in each group, and there are equal ns in each group. SS means "sum of squares".

This expression can be used to calculate the population variance from within all the groups, that is to say, for all the subjects, s^2_{wit}

$s^2_{wit} = \frac{1}{g} \cdot \sum \frac{SS}{n-1}$ (n for each group), where g = number of groups to be compared.

$$= \frac{1}{g} \cdot \frac{SS_{wit}}{n-1}$$

$$= \frac{SS_{wit}}{g(n-1)}$$

$$= \frac{SS_{wit}}{DF_{wit}}$$

= within groups mean square, known as MS_{wit}

A similar process can be applied to the variance estimated from between the groups, that is, the sum of squared deviations of the group means from the mean of all the observations, multiplied by the sample size, divided by the number of groups minus one. This sum of squares for between the groups is a measure of the variability between the groups.

The between groups variance can be expressed as

$$s^2_{bet} = ns^2_X$$

The term s^2_X is the square of the standard deviation of treatment means, that is to say:

$s^2_X = \sum \frac{(X_{eachgroup} - X_{allobs})^2}{g-1}$, where X = mean of individual values (x),

so that the between groups variance

$$s^2_{bet} = n \sum \frac{(X_{eachgroup} - X_{allobs})^2}{g-1}$$

$n \sum (X_{each\ group} - X_{all\ obs})^2$ = sum of squared deviations of the group means about the mean of all observations, otherwise known as the between groups sum of squares, or

SS_{bet} (which is a measure of the variability between the groups.

Since $g - 1 = DF_{bet}$, then the between groups variance can be written as

$$s^2_{bet} = \frac{SS_{bet}}{DF_{bet}} = MS_{bet}$$ (the between groups mean square).

The F statistic then becomes the ratio of the between groups mean square and the within groups mean square:

$$F = \frac{MS_{bet}}{MS_{wit}}$$

This can then be compared against a critical F for the appropriate numerator degrees of freedom (DF_{bet}) and denominator degrees of freedom (DF_{wit}) to asses whether the null hypothesis is tenable.

This F statistic will only indicate whether there is a significant difference between all the groups. It will not provide any information as to which pairs of groups show a difference. To investigate this, contrasts between pairs of groups are needed. This requires specific tests often known as post-hoc tests or multiple comparison post tests. There are a number of these tests, which have slightly different uses (for example, Dunnett's test for contrasting all groups with one particular group such as the control group) and have slightly different powers to detect differences (Huck and Cormier 1996 p328) . These procedures are explained by Zar (in chapter 11 1996). Zar comments that "The multiple comparison problem has received much attention in the statistical literature, yet there is no agreement as to the 'best' procedure to routinely use".

The post hoc test which I have used is the Scheffé test as it allows all groups to be compared and is conservative and so unlikely to make a Type I error (there is of course an increased risk of a type II error as a consequence (Motulsky 1995 p259).

The Scheffé Test

Scheffé was an American statistician who developed this multiple comparison procedure, which allows complex comparisons (that is, several groups can be combined and contrasted with another group). It is the most conservative of these multiple comparison procedures.

Part of the procedure is designed to correct for the increase in the risk of a Type 1 error inherent in performing multiple comparisons. Instead of using a standard F value (found from tables according to the degrees of freedom from the original ANOVA), a corrected F is calculated according to the formula:

$$F' = \sqrt{(g-1).F_{0.05(g-1,\,N-g)}}$$

where $F_{0.05(g-1,\,N-g)}$ is the F value for an α set at 0.05 and with the degrees of freedom set at g - 1, and N - g,

where there are g groups and N values in total, so that: $g - 1 = DF_{bet}$ and $N - g = DF_{wit}$.

If the Scheffé statistic, S, is greater than the corrected F, F', then the groups contrasted are said to be significantly different.

The Scheffé statistic is defined as:

$$S = \frac{\sum c_i X_i}{SE},$$

where $SE = \sqrt{MS_{wit} \sum \frac{c_i^2}{n_i}}$,

c_i = the coefficient used to multiply each group in the contrast, and $\Sigma c = 0$,

n_i = the number of values in each group, and

X_i = the mean of each group. Further details can be found in Zar (1996 pp222-225).

Repeated measures and summary measures

Many experimental and clinical studies involve the collection of serial data from all subjects.

When repeated measurements are made the data can be analysed in different ways. Data from the different groups can be compared repeatedly at each individual time point and a correction (Bonferroni) made to the P value to compensate for the fact that the test has been performed many times. This approach is not uncommon in the literature (Matthews et al. 1990). Unfortunately as the tests will be highly correlated the Bonferroni method will be too conservative and may miss real differences (Bland and Altman 1995).

There are at least two approaches to this problem. One is to find a summary measure, either some value from the series or derived from it, which can be said to represent the data/response (Matthews et al. 1990). This can then be used as if it were raw data. If measuring the concentration of an intra-venous drug the peak concentration over a certain time period might be used, or the time taken to reach the peak concentration, or the area under the curve (AUC).

For the endotoxin and cytokine data AUC was used as a summary measure as it represents the integrated or overall response: the appearance of endotoxin in the circulation, or the production of the inflammatory cytokines.

For each study an AUC was calculated and then used to calculate the group mean. These means were then analysed using a one-way ANOVA as there were more than two groups. A Bonferroni test was used for the *post hoc* contrasts (Bland and Altman 1995).

The other is to use repeated measures analysis of variance (RANOVA). In its simplest form this will be a one-way repeated measures ANOVA (also known as a one-factor within-subjects ANOVA; treatments-by-subjects ANOVA).

Repeated measures analysis of variance

In a one-way repeated measures ANOVA the within-subjects factor under scrutiny may take one of three forms: it may be the time of different measurements; it may be different treatments applied to each subject; or it may be different ways of making the same measurement.

When a variable is observed repeatedly in each subject the total variability in the observations can be partitioned into three mutually exclusive components: variability between all the subjects, variability with time, and variability within the subject's response over time.

As already stated the F statistic is calculated from a comparison of the population variance estimated from the sample means and the population variance estimated as the average of the sample variances. Or in other words:

F = between groups variance/within groups variance

Instead of using the variance the sum of squared deviations from the sample mean can be used to quantify variability, since variance and the sum of squared deviations are closely related, as demonstrated above:

variance = sum of squared deviations/degrees of freedom

Applying this notation to RANOVA the basic principles will be described as adapted from Glantz (Glantz 1997 pp302-308). If:

n = number of subjects in the study, and

t = number of time points at which observations were made

SS_{tot} = total sum of squared deviations from the grand mean of all the data

$DF_{tot} = (t\ n) - 1$ (total degrees of freedom)

Total sum of squares is partitioned into:

1) within subjects sum of squares, $SS_{wit\ subj}$ (sum of each within subject SS),

which is associated with t - 1 degrees of freedom.

As there are n subjects, $SS_{wit\ subj}$ is associated with

$DF_{wit\ subj} = n\ (t - 1)$ degrees of freedom

and

2) between subjects sum of squares, $SS_{bet\ subj}$ (sum of squared deviations of mean response of each subject about the grand mean),

which is associated with

$DF_{bet\ subj} = n - 1$

So

$$SS_{tot} = SS_{wit\ subj} + SS_{bet\ subj}$$ (Glantz 1997 p 300).

The within subjects sum of squares can be partitioned into

1) a time component (sum of squared differences between the time means and the grand mean) associated with t -1 degrees of freedom, and
2) a residual component (residual = random variation in how each individual responds at each time point) associated with DF_{res} degrees of freedom.

$$SS_{wit\ subj} = SS_{time} + SS_{residual}$$

In which case,

$$SS_{residual} = SS_{wit\ subj} - SS_{time}$$

and associated with

$$DF_{res} = DF_{wit\ subj} - DF_{time}$$

$$= n(t - 1) - (t - 1)$$
$$= (n - 1)(t - 1)$$

The estimate of the population variance from time sum of squares is:

$$MS_{time} = \frac{SS_{time}}{DF_{time}}$$

The estimate for the population variance from the residual sum of squares is:

$$MS_{res} = \frac{SS_{res}}{DF_{res}}$$

As MS_{time} and MS_{res} are estimates of the same (unknown) population variance then the null hypothesis that there is no change in experimental subjects over time can be tested by:

$$\frac{MS_{time}}{MS_{res}} = \text{F statistic}$$

If the null hypothesis is true then the F ratio will follow the F distribution with DF_{time} numerator degrees of freedom and DF_{res} denominator degrees of freedom.

As with a simple ANOVA, RANOVA may also take the form of a two or three-way repeated measures ANOVA (also known as two-way within-subjects ANOVA; two-way ANOVA with repeated measures on both factors; multiple treatments-by-subjects ANOVA). In these there are two or three repeat factors but all the subjects are treated in the same way.

In my experiments there was only one within subjects factor: the timing of the repeated measurements. In addition the subjects were divided into different groups according to their physiological insult: nerve stimulation, haemorrhage (either 20% TBV, 30% TBV), or the combination of haemorrhage and nerve stimulation.

This constitutes a two-way ANOVA with repeated measures in one factor (namely time), otherwise known as a two-way mixed ANOVA. Its general purpose is no different to that of any other ANOVA, namely to examine the sample means to see if they are further apart than would be expected by chance.

Three null hypotheses are tested:

1) that the main effect means of the first factor (in this case the group) are equal to one another
2) that the main effect means of the second factor (in this case time) are equal to one another
3) that the two factors do not interact.

As with any ANOVA the validity of the test will depend on an assumption about the data: the sphericity assumption. Huck and Cormier state that, "the sphericity assumption says that the population variances associated with the levels of the repeated measures factor, in combination with the population correlations between pairs of levels, must represent one of a set of acceptable patterns. One of the acceptable patterns is for all the population variances to be identical and for all the bivariate correlations to be identical" (Huck and Cormier 1996 p430).

Mauchley's sphericity test tests for this and should be applied to the data before analysis is performed. If the data does not conform to sphericity then the F value generated will be too large. This effect will depend on the degree to which the data violates the sphericity assumption. There are a number of ways to adjust for this problem (Huck and Cormier 1996 pp 430-432). One of these, which will be illustrated later, results in an adjustment of the degrees of freedom of the F test. The sample data are used to estimate the extent of the sphericity violation. This is expressed as a fraction: the epsilon (ε), which is used to multiply the degrees of freedom (df) values to produce adjusted dfs appropriate to the data. The two most widely used εs are the Greenhouse-Geisser ε and the Huynh-Feldt ε. The general rule is that if the Greenhouse-Geisser ε is less than 0.75 it should be used in preference to the Huynh-Feldt ε.

With a two-way mixed ANOVA only the F tests in the within subjects part of the ANOVA are based on this assumption of sphericity (that is, the main effect of time and

the group by time interaction). The F value for the between subjects factor (group) is not affected by a lack of sphericity.

Girden however comments on the fact that with multiple treatment (or time) levels then the assumption of sphericity is almost always violated (Girden 1992 p18). The problem can be solved using univariate analysis and corrections (for example, Greenhouse-Geisser or Huynh-Feldt epsilons) or by using multivariate analysis, where sphericity is not assumed.

In multivariate analysis (for example, Pillai's criterion, or Hotelling's trace criterion) the original scores are transformed into new variables and the analysis performed on these new variables (Girden 1992 pp22-25).

The actual RANOVA strategy

The experiments had a number of aims:

1) To determine the effects of haemorrhage, nerve stimulation to mimic injury, and haemorrhage combined with nerve stimulation on a variety of physiological parameters.

2) To compare two different levels of haemorrhage.

3) To compare the effects of two different levels of haemorrhage and nerve stimulation.

4) To compare each level of haemorrhage alone with the same level of haemorrhage and nerve stimulation.

The RANOVA strategy used to achieve this was divided into several stages. A preliminary analysis established whether there were overall differences between the

groups over the whole time course of the study. It also determined whether the whole data set showed any change with time using Pillai's criterion. Finally it determined whether there was a significant group by time interaction, again using Pillai's criterion. These interactions may be thought of as the patterns of the data or the shape of the data when plotted against time.

If differences between groups were identified then a number of contrast routines were performed. All the groups were contrasted first against the control group, then against the H30 group, then against the H20 group, and finally against the HNS20 group. From these contrasts only those pairs of contrasts relevant to the *a priori* aims of the study were examined, that is to say:

Each insult group vs. control group

H20 vs. HNS20

HNS20 vs. HNS30

H30 vs. H20

H30 vs. HNS30.

Hotelling's trace criterion (which is more appropriate when comparing two groups) was used to examine the null hypothesis that the group by time interactions were equal.

The final element in the RANOVA was a one-way ANOVA to look at the differences between the groups at each time point in the experiments. The Scheffé test was used to perform all the *post hoc* contrasts. The purpose of this was to identify key changes in the cardiovascular and metabolic variables. It did not appear to have very much meaning in terms of the endotoxin and cytokine data, where the overall response was far more important.

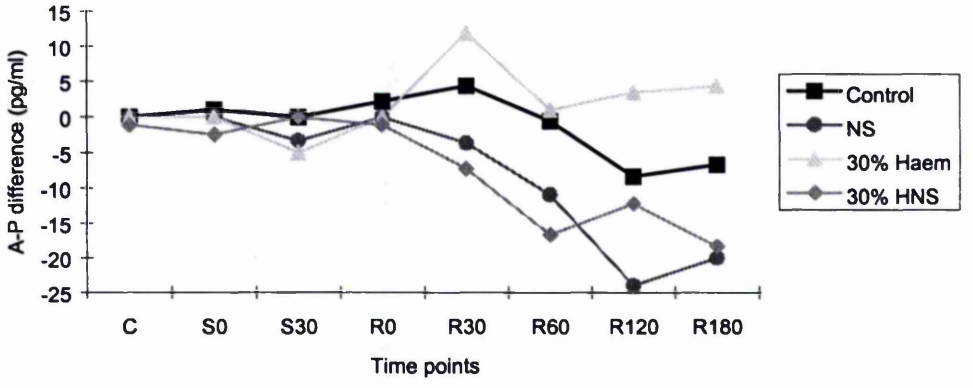
Illustrative examples

Group effect

Arterial-Portal TNF-α: there was no general change with time and there was no overall difference in the patterns of the data and so no significant difference in the group by time interactions. There was however a significant difference between the groups.

This illustrates a typical group effect:

Main effects	**F**	**df**	**Sig of F**
Group	5.59	3	**0.006**
Time	1.57	7	0.225
Group by time	1.39	21	0.172

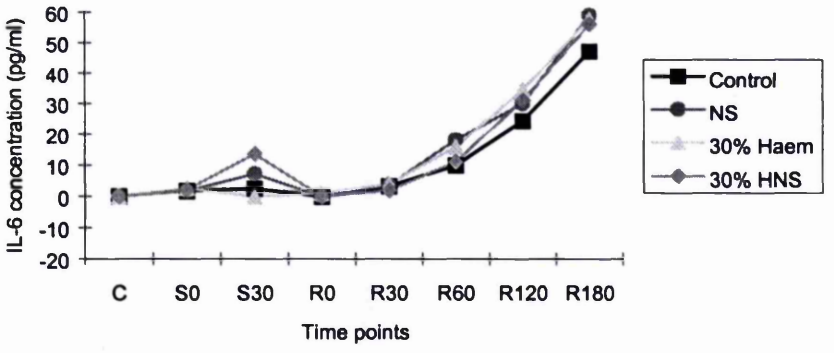


Time effect

Portal IL-6: all the groups had very similar IL-6 levels so there was no overall group difference. In addition the pattern of these levels was the same in all the groups. This resulted in no significant difference in the group by time interactions. However there was clearly a general change in IL-6 levels with time which was highly significant (sig of F = <0.0001).

This illustrates a typical time effect:

Main effects	**F**	**df**	**Sig of F**
Group	0.36	3	0.783
Time	48.51	7	**<0.0001**
Group by time	0.67	21	0.662

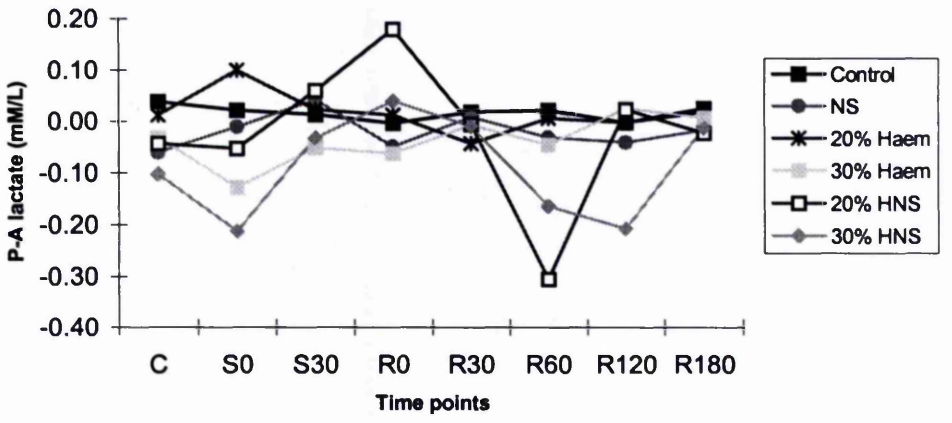


Group by time interaction effect

P-A lactate RANOVA: here there was no overall difference between the groups and no general change in the data with time. However the patterns of the data were significantly different between the groups (sig of F = 0.024).

This illustrates a typical group by time interaction effect:

Main effects	**F**	**df**	**Sig of F**
Group	0.47	5	0.795
Time	2.09	7	0.076
Group by time	1.75	35	**0.011**



Data presentation

Data for all the variables will be presented in the form of tables of the means and standard deviations (SDs) of all the groups at all the time points. These tables will also include the results of the contrasts between groups by RANOVA. In the case of endotoxin and the inflammatory cytokines the AUC will also be included along with the results of the AUC one-way ANOVA.

More detailed presentation of the results of the RANOVAs will take the form of a further set of tables presenting the F ratio, degrees of freedom, and significance of the F ratio for the overall RANOVA (including the time effect) and then the group and group by time interaction effects for the specific pair contrasts discussed above.

Finally graphs will be used to represent the trends of the data. As the means and SDs have already been presented in the tables the graphs will only include the mean data without error bars.

Statistics tables

Simplified summaries of the RANOVAs are provided:

Main effects	**F**	**df**	**Sig of F**
Group	0.72	5	0.614
Time	2.07	7	0.078
Group by time	1.61	35	**0.024**

The first column provides the value of the F statistic (the ratio of the variances). The second column shows the degrees of freedom (df) associated with the factor examined. In this example df for the group main effect was 5. As df = n-1, there were 6 groups under consideration. The final column provides the significance (or p value) of the F statistic. Those which conform to an α of 0.05 are marked in bold type.

For the contrasts:

	F	df	Sig of F
Contrasts			
Control vs NS	7.97	1	**0.007**
Contrast by time	0.41	10	0.933

The columns have the same meaning. There is no main effect of time, only the group effect, where df =1 as these are "pairwise" comparisons. In this example there are 10 (df) + 1 time points.

Univariate analysis

In this example the summary tests involving time, the within-subject factor, have a df which is not an integer. The reason is that following the Mauchley sphericity test the within + residual error matrix was found to be singular, that is to say, the variables were found to be linearly dependent on the preceding value. Using multivariate tests would not have been appropriate (Girden 1992 p26). Instead a univariate analysis was performed. To correct for the lack of sphericity the dfs for time and group by time interaction have been corrected by multiplying with the Greenhouse-Geisser Epsilon (0.309: 7 x 0.309 = 2.16, and 21 x 0.309 = 6.46). In this example the Huynh-Feldt epsilon was 0.395.

Arterial TNF- α RANOVA

Main effects	**F**	**df**	**Sig of F**
Group	2.72	3	0.07
Time	36.11	***2.16***	<0.0001
Group by time	2.32	***6.49***	0.045
Contrasts			
Control vs NS	<0.001	1	0.996
Contrast by time	1.3	***2.16***	0.285

As noted above this lack of sphericity does not affect the between-subjects main effect (the contrast between two groups).

Computer programme

All data were handled in Excel 95, which was also used to draw the graphs. All statistical analyses were performed using the SPSS for Windows software, version 6.1.3.

Chapter 3: Results

Groups:

For clarity, and to reduce the use of % signs in this section, the notation for the groups in the text will be:

C: control group subjected to sham surgery and monitored for the same time period as all the other experiments.

NS: control group subjected to sham surgery and nerve stimulation alone.

H20: haemorrhage alone group subjected to 20% TBV haemorrhage.

H30: haemorrhage alone group subjected to 30% TBV haemorrhage.

HNS20: haemorrhage and nerve stimulation group (20% TBV haemorrhage).

HNS30: haemorrhage and nerve stimulation group (30% TBV haemorrhage).

All the group mean data are shown in Tables 1-31 in the Appendix.

Haemodynamic measurements were made once nerve stimulation had been started. These are illustrated on the graphs as the time point labelled CNS. The CNS time point appears on the graphs with a similar spacing as the other time points, which occurred every 30 minutes. This is because the graphs were drawn in Excel 95, which does not allow irregular spacing on the axes.

No blood gas analyses, or assays, were performed at the CNS time point. No CNS time point appears on the graphs for the variables derived from blood gases, lactate, endotoxin, cytokines, or for haematocrit and temparature as the time point was only about 10 minutes after the control measurements.

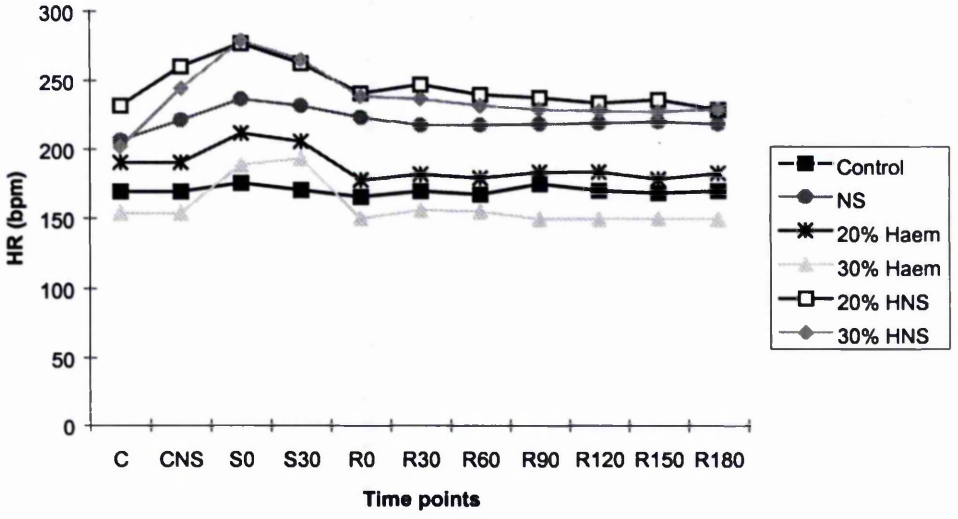
Global responses

Cardiovascular responses

Heart rate

Nerve stimulation resulted in an immediate increase in heart rate in the three NS groups (Figure 3.1). Heart rate increased by 7% in the NS group, by 12% in HNS20, and by 21% in HNS30. Haemorrhage resulted in an increase in heart rate in the haemorrhage alone groups by 11% in H20, and by 23% in H30. Heart rate increased by a further 6% in HNS20, and by a further 14% in HNS30, when haemorrhage was superimposed on nerve stimulation. Reinfusion of the shed blood resulted in a return to pre-haemorrhage heart rates, which were maintained until the end of the experiments. During this period heart rates were higher in the three NS groups than in the three non-NS groups.

Figure 3.1: Graph of heart rate data (beats per minute).



The graph shows that there was an increase in heart rate after the start of nerve stimulation and that there was a further increase with haemorrhage. Reinfusion of shed blood resulted in a return to near baseline heart rates.

Statistical analysis:

There was a statistically significant difference between the groups, a significant group by time interaction, and a significant change over time (Table 3.1). Heart rates were significantly higher in the NS and the two HNS groups than in the controls. The contrasts by time for H30, and for the two HNS groups, against the control group were statistically significant. This means that the pattern of change of heart rate was different for the H30 group compared with the control group, and was different for the two HNS groups compared with the control group. There was no pattern difference between H20 and the control group.

There were significant group differences between the HNS groups and their corresponding haemorrhage alone groups, but only HNS30 showed a statistically significant contrast by time interaction against H30.

Table 3.1: Repeated measures analysis of variance of heart rate data.

Heart rate RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	6.71	5	**<0.0001**
Time	17.79	10	**<0.0001**
Group by time	1.82	50	**0.002**
Contrasts			
Control vs NS	8.93	1	**0.004**
Contrast by time	1.34	10	0.244
Control vs H20	0.76	1	0.389
Contrast by time	0.88	10	0.561
Control vs H30	0.23	1	0.631
Contrast by time	3.40	10	**0.003**
Control vs HNS20	13.14	1	**0.004**
Contrast by time	2.56	10	**0.017**
Control vs HNS30	12.54	1	**0.001**
Contrast by time	6.26	10	**<0.0001**
H20 vs HNS20	6.22	1	**0.016**
Contrast by time	1.31	10	0.260
HNS20 vs HNS30	0.14	1	0.712
Contrast by time	1.29	10	0.270
H30 vs H20	1.71	1	0.197
Contrast by time	0.90	10	0.541
H30 vs HNS30	16.29	1	**<0.0001**
Contrast by time	3.38	10	**0.003**

There were statistically significant differences between NS and C, and between the two HNS groups and the haemorrhage alone groups.

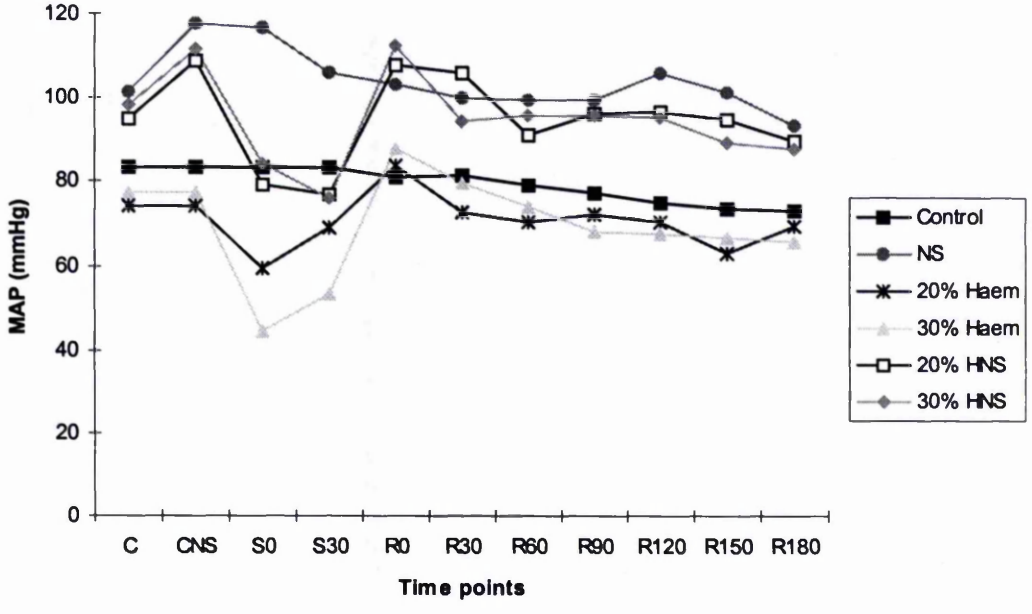
There were pattern differences: that is, significant paired contrasts by time, resulting from the haemorrhage effect compared with the control. There was a pattern difference between HNS30 and H30.

Mean arterial pressure

Nerve stimulation resulted in an immediate increase in MAP of 14-16% in the three NS groups (Figure 3.2). Mean arterial pressure remained high in NS compared with the control group throughout the experiments. The control group remained very stable, showing a drop of only 11 mmHg (from 84 mmHg, SD 12, to 73 mmHg, SD 23) over the course of the experiments. Mean arterial pressures were higher in the NS groups compared with the corresponding non-NS groups throughout the experiments.

There was a decrease in MAP with haemorrhage. In the haemorrhage alone groups there was some recovery of MAP during the 30 minute shock period. There was no recovery in the two HNS groups during this period. Reinfusion of shed blood resulted in an increase in mean arterial pressures over baseline. By R60 mean arterial pressures had decreased to baseline and then continued to decrease until R180.

Figure 3.2: Graph of mean arterial pressure data (mmHg).



The graph shows an increase in MAP with nerve stimulation and then a decrease with haemorrhage. In the two haemorrhage alone groups there was some recovery of MAP during the shock phase. In the two HNS groups there was no recovery during the shock phase. Reinfusion of shed blood restored MAP.

Statistical analysis:

The overall group difference can be attributed to significant differences in MAP between NS groups and their corresponding non-NS groups (Table 3.2). There was an overall change with time and a significant group by time interaction: that is, an overall difference in the pattern of change in MAP between the groups. The NS, H30, HNS20 and HNS30 groups showed significant contrast by time interactions against the control group because of the initial increase in MAP, when nerve stimulation was started. HNS groups showed significant contrast by time interactions against their haemorrhage alone equivalents.

Table 3.2: Repeated measures analysis of variance of mean arterial pressure data.

MAP RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	6.59	5	**<0.0001**
Time	13.83	10	**<0.0001**
Group by time	2.74	50	**<0.0001**
Contrasts			
Control vs NS	11.67	1	**0.001**
Contrast by time	3.24	10	**0.004**
Control vs H20	1.45	1	0.234
Contrast by time	1.50	10	0.175
Control vs H30	2.30	1	0.135
Contrast by time	4.83	10	**<0.0001**
Control vs HNS20	2.47	1	0.122
Contrast by time	3.78	10	**0.001**
Control vs HNS30	2.92	1	0.093
Contrast by time	7.26	10	**<0.0001**
H20 vs HNS20	6.43	1	**0.014**
Contrast by time	2.72	10	**0.012**
HNS20 vs HNS30	<0.0001	1	0.999
Contrast by time	1.90	10	0.073
H30 vs H20	0.03	1	0.871
Contrast by time	1.69	10	0.115
H30 vs HNS30	10.15	1	**0.002**
Contrast by time	5.83	10	**<0.0001**

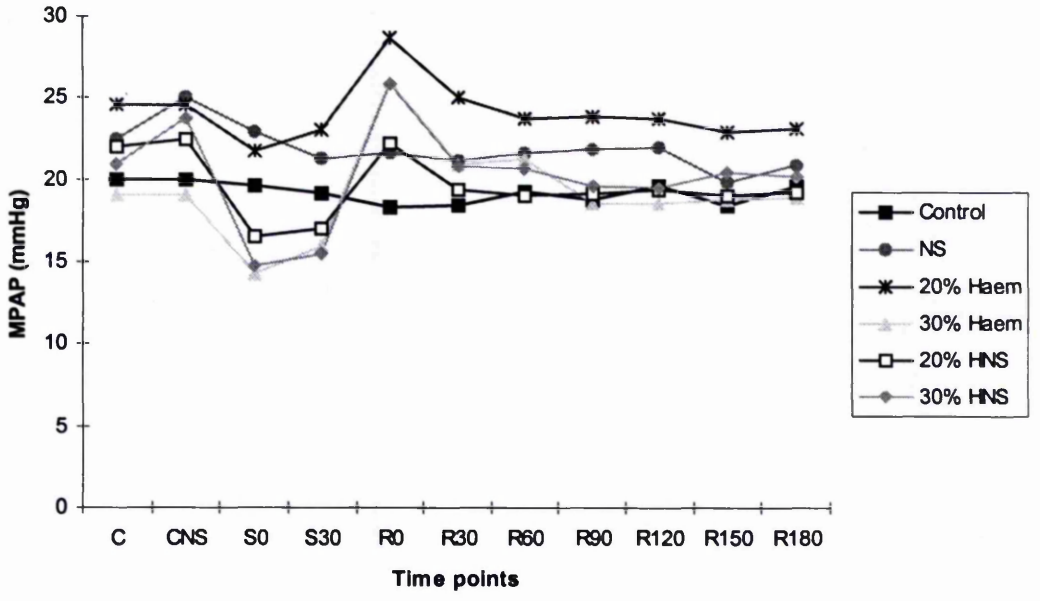
There were statistically significant group differences as a result of the addition of the nerve stimulation insult. There were group differences between NS and control, and between the HNS groups and the haemorrhage alone groups.

There were pattern differences between the haemorrhage groups and the control group (except H20) as a result of the haemorrhage phase. There were also pattern differences between the HNS and the haemorrhage alone groups.

Mean pulmonary artery pressure

Nerve stimulation resulted in an immediate increase in MPAP of 4-14% in the three NS groups (Figure 3.3). Mean pulmonary artery pressure, unlike MAP, was not higher in the NS groups compared with their non-NS counter-parts over the course of the experiments. There was a reduction in MPAP during haemorrhage in all the haemorrhage groups with a tendency for all the groups to show some recovery during the shock period. The recovery was greater in the simple haemorrhage groups compared with the HNS groups. Reinfusion of shed blood caused an elevation in MPAP above baseline levels, which only lasted until R30. For the rest of the observation phase MPAPs remained stable around baseline.

Figure 3.3: Graph of mean pulmonary artery pressure data (mmHg).



There was a reduction in MPAP with haemorrhage and only a slight recovery during the shock phase. Reinfusion of shed blood resulted in a short lived over-shoot in MPAP.

Statistical analysis:

The overall difference in groups (F = 2.53, df 5, sig. of F = 0.041) is attributable to differences between the control and NS groups, the H20 and HNS20 groups, and between the H20 and H30 groups (Table 3.3). The significant difference in group by time interaction seems to be the result of significant contrasts by time for the H30 and HNS30 against the control group. The contrast by time interaction for H20 and HNS20 against control failed to reach statistical significance (sig. of F = 0.084 and 0.061 for H20 and HNS20).

Table 3.3: Repeated measures analysis of variance of mean pulmonary artery pressure data.

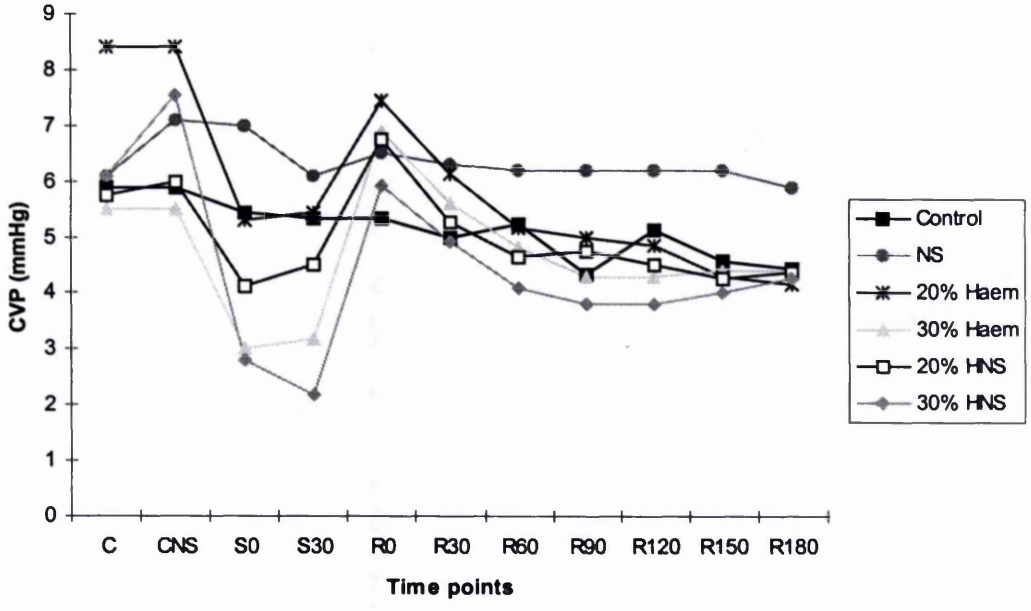
MPAP RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	2.53	5	**0.041**
Time	12.80	10	**<0.0001**
Group by time	1.64	50	**0.008**
Contrasts			
Control vs NS	3.22	1	**0.079**
Contrast by time	0.68	10	0.737
Control vs H20	6.94	1	**0.011**
Contrast by time	1.85	10	0.084
Control vs H30	<0.000 1	1	0.970
Contrast by time	4.76	10	**<0.0001**
Control vs HNS20	0.03	1	0.862
Contrast by time	2.00	10	0.061
Control vs HNS30	<0.000 1	1	0.953
Contrast by time	6.86	10	**<0.0001**
H20 vs HNS20	5.51	1	**0.023**
Contrast by time	0.57	10	0.829
HNS20 vs HNS30	0.05	1	0.822
Contrast by time	1.74	10	0.106
H30 vs H20	7.12	1	**0.010**
Contrast by time	1.06	10	0.411
H30 vs HNS30	<0.000 1	1	0.983
Contrast by time	1.70	10	0.117

Statistically significant group differences were: NS vs. C, H20 vs. C and HNS20, and H20 vs. H30.

Central venous pressure

Nerve stimulation caused an increase in CVP in the NS and HNS30 groups but not in HNS20 (Figure 3.4). There was a reduction in CVP with haemorrhage and then some recovery during the shock phase. Reinfusion of the shed blood increased CVP above baseline. Central venous pressure returned to baseline at R30 and over the rest of the experiments showed a gradual decrease.

Figure 3.4: Graph of central venous pressure data (mmHg).



There was a decrease in CVP with haemorrhage and a slight recovery during the shock phase, except in HNS30. Reinfusion of shed blood resulted in a short lived restoration of CVP and then a gradual deterioration.

Statistical analysis:

There was no overall group difference over the course of the studies (Table 3.4), but there was a significant group by time interaction as a result of the decrease in CVP with haemorrhage. All four haemorrhage groups had significantly different patterns to the control group. There was also a pattern difference between HNS20 and HNS30. In

HNS20 CVP recovered slightly during the shock phase but in HNS30 CVP was further reduced at the end of the shock phase. In view of the changes in CVP with haemorrhage it is not surprising that there was a significant time effect in the statistical analysis.

Table 3.4: Repeated measures analysis of variance of central venous pressure data.

Central venous pressure RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	0.80	5	0.556
Time	17.45	10	**<0.0001**
Group by time	2.33	50	**<0.0001**
Contrasts			
Control vs NS	*1.79*	*1*	*0.187*
Contrast by time	*2.03*	*10*	*0.055*
Control vs H20	*0.47*	*1*	*0.496*
Contrast by time	*2.33*	*10*	***0.028***
Control vs H30	*0.03*	*1*	*0.861*
Contrast by time	*4.49*	*10*	***<0.0001***
Control vs HNS20	*<0.000 1*	*1*	*0.968*
Contrast by time	*2.12*	*10*	***0.045***
Control vs HNS30	*0.13*	*1*	*0.717*
Contrast by time	*7.26*	*10*	***<0.0001***
H20 vs HNS20	*0.38*	*1*	*0.541*
Contrast by time	*0.64*	*10*	*0.773*
HNS20 vs HNS30	*0.15*	*1*	*0.703*
Contrast by time	*3.08*	*10*	***0.005***
H30 vs H20	*0.72*	*1*	*0.402*
Contrast by time	*1.68*	*10*	*0.120*
H30 vs HNS30	*0.03*	*1*	*0.855*
Contrast by time	*1.93*	*10*	*0.070*

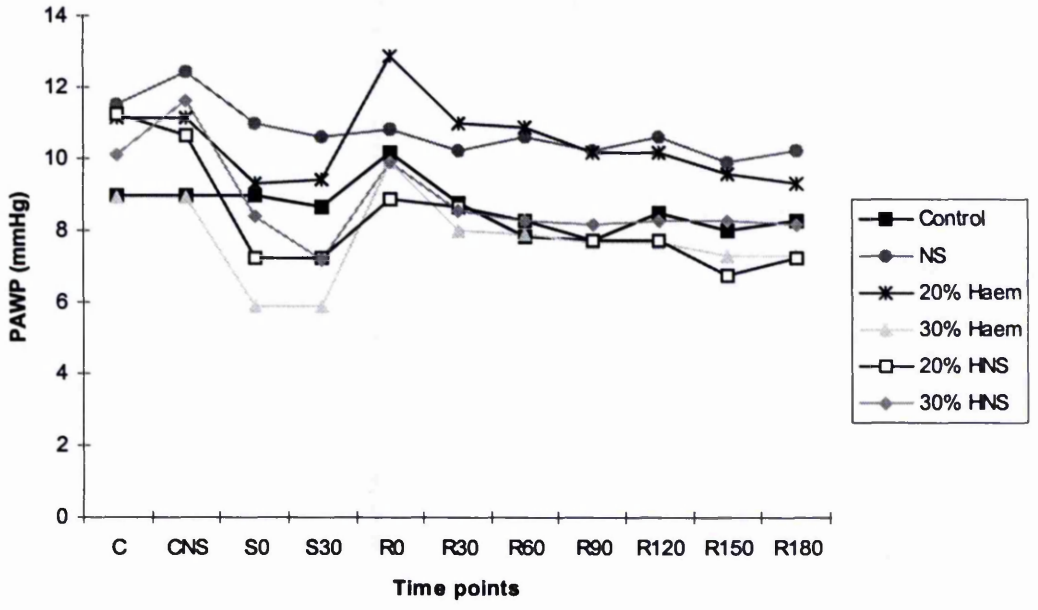
Differences between the groups did not reach statistical significance so it can be argued that further contrasts were inappropriate. The two other main effects, time and group by time interaction, were significant. Paired contrasts were performed to determine the cause of the significant group by time interaction.

There were significant paired contrasts by time because the haemorrhage groups differed from the control group. There was no significant difference between the two groups (C and NS), which did not experience blood loss.

Pulmonary artery wedge pressure

Nerve stimulation only had an immediate effect in the NS and HNS30 groups (Figure 3.5). Haemorrhage resulted in a reduction in PAWP, which was restored by reinfusion of the shed blood. After R30 there was a gradual reduction in PAWP. The NS group PAWP remained higher than that of the control group throughout.

Figure 3.5: graph of mean pulmonary artery wedge pressure data (mmHg).



Graph shows a slight increase in PAWP with nerve stimulation and then a decrease with haemorrhage. There was no recovery during the shock phase. There was a decrease in PAWP during the shock phase in HNS30. Reinfusion of shed blood restored PAWP, which gradually decreased during the observation period.

Statistical analysis:

As with CVP the difference between the groups did not reach statistical significance, but there was a difference over time and there was a significant group by time interaction

(Table 3.5). The difference in pattern was caused by the haemorrhage-induced reduction in PAWP.

Table 3.5: Repeated measures analysis of variance of pulmonary artery wedge pressure data.

PAWP RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	2.09	5	0.084
Time	11.42	10	**<0.0001**
Group by time	1.48	50	**0.031**
Contrasts			
Control vs NS	*3.59*	*1*	*0.064*
Contrast by time	*0.44*	*10*	*0.915*
Control vs H20	*1.60*	*1*	*0.212*
Contrast by time	*2.12*	*10*	***0.048***
Control vs H30	*0.83*	*1*	*0.538*
Contrast by time	*4.68*	*10*	***<0.0001***
Control vs HNS20	*0.04*	*1*	*0.850*
Contrast by time	*2.83*	*10*	***0.010***
Control vs HNS30	*0.03*	*1*	*0.873*
Contrast by time	*3.82*	*10*	***0.001***
H20 vs HNS20	*2.24*	*1*	*0.141*
Contrast by time	*1.26*	*10*	*0.286*
HNS20 vs HNS30	*0.14*	*1*	*0.706*
Contrast by time	*1.56*	*10*	*0.158*
H30 vs H20	*3.97*	*1*	*0.052*
Contrast by time	*1.02*	*10*	*0.443*
H30 vs HNS30	*0.77*	*1*	*0.385*
Contrast by time	*0.69*	*10*	*0.725*

Differences between the groups did not reach statistical significance so it can be argued that further contrasts were inappropriate.

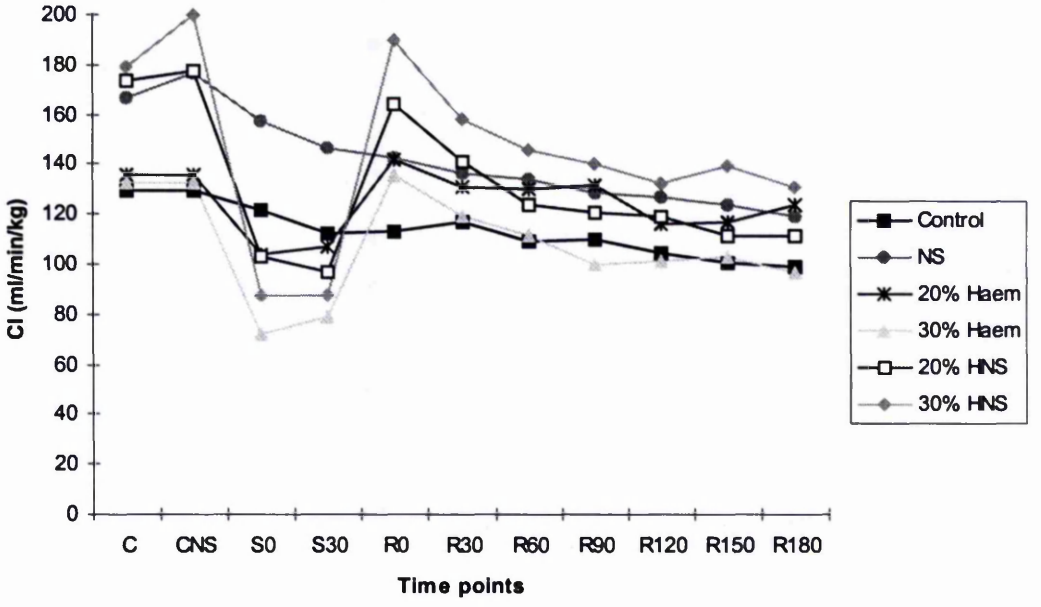
It is worth noting that that there were statistically significant contrasts by time because the haemorrhage groups differed from the control group. There was no significant difference between the control and nerve stimulation groups, which were not subjected to blood loss. There were no differences between the various haemorrhage and haemorrhage and nerve stimulation groups.

Cardiac Function

Cardiac index

The three NS groups had higher cardiac indices from the outset (Figure 3.6). The addition of the nerve stimulation insult caused a further increase in cardiac index of 2-12%. There was a 23% reduction in CI following 20% TBV haemorrhage. In the presence of nerve stimulation the same 20% TBV haemorrhage resulted in a 42% reduction in CI. A 30% TBV haemorrhage caused a greater reduction in CI (45%) than the 20% TBV haemorrhage. A 30% TBV haemorrhage on a background of nerve stimulation resulted in a 56% reduction in CI. Cardiac index was restored or even enhanced by the reinfusion of shed blood. This did not persist and by the end of the experiments all groups had cardiac indices below baseline. Cardiac index in the NS and the control group showed a gradual decrease from baseline.

Figure 3.6: Graph of cardiac index data (ml/min/kg).



Graph shows an increase in CI with nerve stimulation. Haemorrhage caused a sharp reduction in CI. There was hardly any recovery during the shock phase. There was a general decrease in CI over the course of the experiments.

Statistical analysis:

There was a significant difference between the groups (F = 4.01, df 5, sig. of F = 0.004) as both the NS and the HNS30 groups were significantly different to the control group (Table 3.6). All the haemorrhage groups had significant contrasts by time interactions versus the control group. There were significant contrast by time interactions for the haemorrhage alone groups against their respective HNS groups, and for HNS20 versus HNS30.

Table 3.6: Repeated measures analysis of variance of cardiac index data.

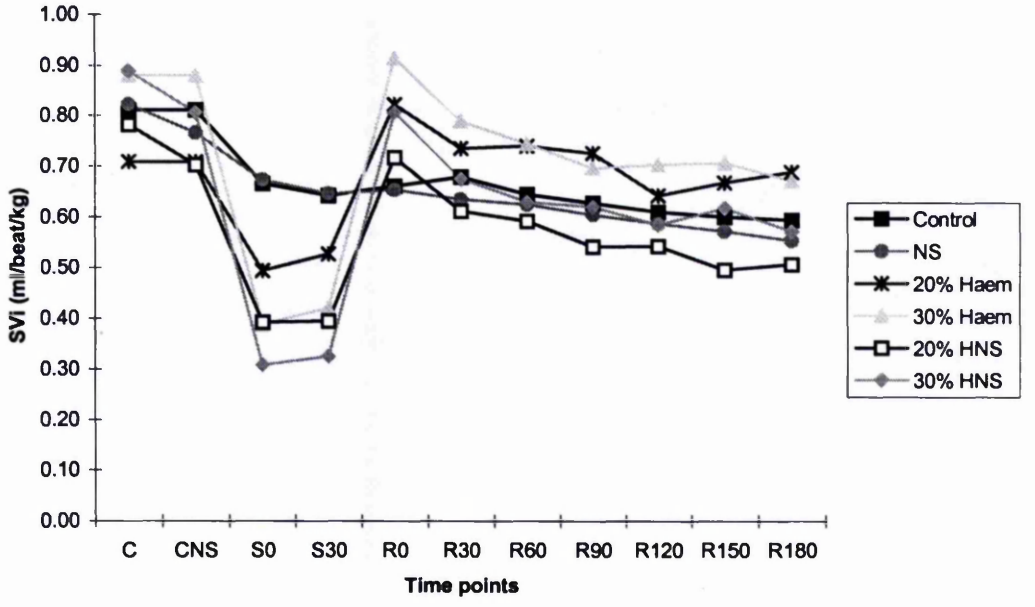
Cardiac index RANOVA			
Main effect	**F**	**df**	**Sig of F**
Group	4.01	5	**0.004**
Time	44.78	10	**<0.0001**
Group by time	2.06	50	**<0.0001**
Contrasts			
Control vs NS	7.97	1	**0.007**
Contrast by time	0.41	10	0.933
Control vs H20	0.82	1	0.369
Contrast by time	3.84	10	**0.001**
Control vs H30	0.22	1	0.641
Contrast by time	9.47	10	**<0.0001**
Control vs HNS20	1.92	1	0.172
Contrast by time	7.77	10	**<0.0001**
Control vs HNS30	9.35	1	**0.004**
Contrast by time	25.69	10	**<0.0001**
H20 vs HNS20	0.19	1	0.667
Contrast by time	2.63	10	**0.015**
HNS20 vs HNS30	1.82	1	0.183
Contrast by time	3.90	10	**0.001**
H30 vs H20	1.71	1	0.198
Contrast by time	2.04	10	0.055
H30 vs HNS30	11.87	1	**0.001**
Contrast by time	5.12	10	**<0.0001**

The NS and HNS30 groups were significantly different to C. HNS30 was significantly different to H30. All the haemorrhage groups showed a pattern difference with the control group. There were also pattern differences between the various haemorrhage groups (except between H20 and H30).

Stroke volume index

Nerve stimulation had no effect on SVI (Figure 3.7). Loss of 30% TBV resulted in a reduction in SVI to 44% of baseline, while the loss of 20% TBV resulted in a reduction in SVI to only 69%. The addition of the nerve stimulation insult resulted in a 34% reduction in SVI in HNS30 and a 50% reduction in HNS20. All four groups showed a very slight recovery during the shock phase. Stroke volume index in the two haemorrhage alone groups recovered fully with reinfusion of the shed blood but in the two HNS groups remained below baseline. All groups showed a gradual reduction in SVI until R180.

Figure 3.7: Graph of stroke volume index (ml/beat/kg).



Graph shows a decrease in SVI during haemorrhage with only a slight recovery during the shock phase. Reinfusion of shed blood restored SVI. There was an overall trend for SVI to decrease over the course of the experiments. This is clearly seen in the control and NS groups.

Statistical analysis:

The group difference seen did not reach statistical significance (F = 0.55, df 5, sig. of F = 0.736) but there was a significant change with time and a significant group by time interaction (Table 3.7). The four haemorrhage groups all had significantly different patterns to the control group.

Table 3.7: Repeated measures analysis of variance of stroke volume index data.

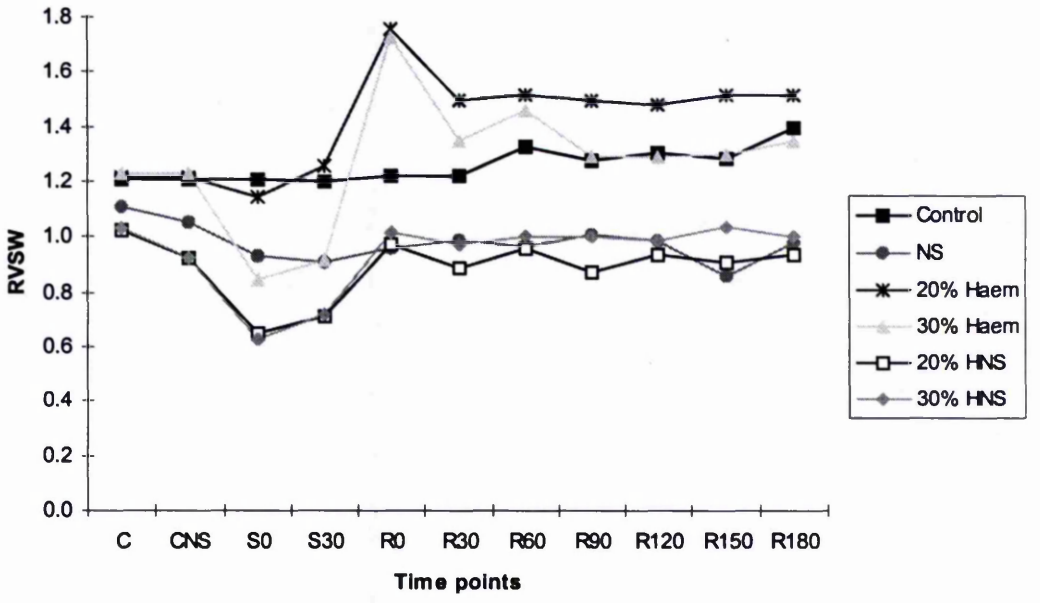
SVI RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	0.55	5	0.736
Time	40.23	10	**<0.0001**
Group by time	2.13	50	**<0.0001**
Contrasts			
Control vs NS	*0.07*	*1*	*0.796*
Contrast by time	*0.59*	*10*	*0.809*
Control vs H20	*0.02*	*1*	*0.900*
Contrast by time	*4.49*	*10*	***<0.0001***
Control vs H30	*0.31*	*1*	*0.58*
Contrast by time	*13.83*	*10*	***<0.0001***
Control vs HNS20	*0.99*	*1*	*0.325*
Contrast by time	*3.64*	*10*	***0.002***
Control vs HNS30	*0.39*	*1*	*0.535*
Contrast by time	*11.25*	*10*	***<0.0001***
H20 vs HNS20	*1.03*	*1*	*0.316*
Contrast by time	*1.49*	*10*	*0.178*
HNS20 vs HNS30	*0.18*	*1*	*0.675*
Contrast by time	*1.86*	*10*	*0.080*
H30 vs H20	*0.14*	*1*	*0.713*
Contrast by time	*3.77*	*10*	***0.001***
H30 vs HNS30	*1.33*	*1*	*0.254*
Contrast by time	*1.71*	*10*	*0.111*

There was no statistically significant difference between the groups. There was a significant change with time and a significant group by time interaction. Simple contrasts were performed to investigate these interactions. All the haemorrhage groups had significant contrasts by time with the control group: that is, showed a different pattern to the control group. There were also differences in the pattern between the two haemorrhage alone groups. There was no difference in the pattern between the control and nerve stimulation groups.

Right ventricular stroke work

Right ventricular stroke work gradually increased in the control group (Figure 3.8). In the NS group there was a slight reduction in RVSW after starting nerve stimulation. This persisted until R0. After that RVSW remained stable until the end. The loss of 30% TBV had a greater effect than 20% TBV, but there was no statistically significant difference between HNS20 and HNS30. Reinfusion of shed blood in the haemorrhage alone groups had a dramatic effect: RVSW far exceeded baseline at R0 and remained above baseline until the end. This "super recovery" was not seen in the HNS groups. RVSW was generally lower in those groups exposed to nerve stimulation than the non-NS groups.

Figure 3.8: Graph of right ventricular stroke work data (g/min).



Graph shows that nerve stimulation had no effect, but there was a reduction in RVSW with haemorrhage. Reinfusion of shed blood in the haemorrhage alone groups caused a large, short-lived overshoot in RVSW.

Statistical analysis:

There were statistically significant differences between the groups, the group by time interaction, and over time (Table 3.8). The three NS groups had significantly lower RVSWs than the control group, and the same was true for the two HNS groups in relation to the two haemorrhage alone groups. All the insult groups had significantly different patterns to the control group. There were also pattern differences between the H20 and the H30 groups and between the H30 and HNS30 groups.

Table 3.8: Repeated measures analysis of variance of right ventricular stroke work data.

RVSW RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	3.28	5	**<0.0001**
Time	10.15	10	**<0.0001**
Group by time	1.45	50	**<0.0001**
Contrasts			
Control vs NS	7.41	1	**0.008**
Contrast by time	2.65	10	**0.007**
Control vs H20	1.63	1	0.205
Contrast by time	3.99	10	**<0.0001**
Control vs H30	<0.0001	1	0.957
Contrast by time	9.69	10	**<0.0001**
Control vs HNS20	5.27	1	**0.024**
Contrast by time	2.01	10	**0.041**
Control vs HNS30	11.68	1	**0.001**
Contrast by time	8.25	10	**<0.0001**
H20 vs HNS20	9.71	1	**0.002**
Contrast by time	1.54	10	0.137
HNS20 vs HNS30	0.0002	1	0.881
Contrast by time	0.58	10	0.827
H30 vs H20	1.48	1	0.227
Contrast by time	2.62	10	**0.008**
H30 vs HNS30	11.77	1	**0.001**
Contrast by time	2.87	10	**0.004**

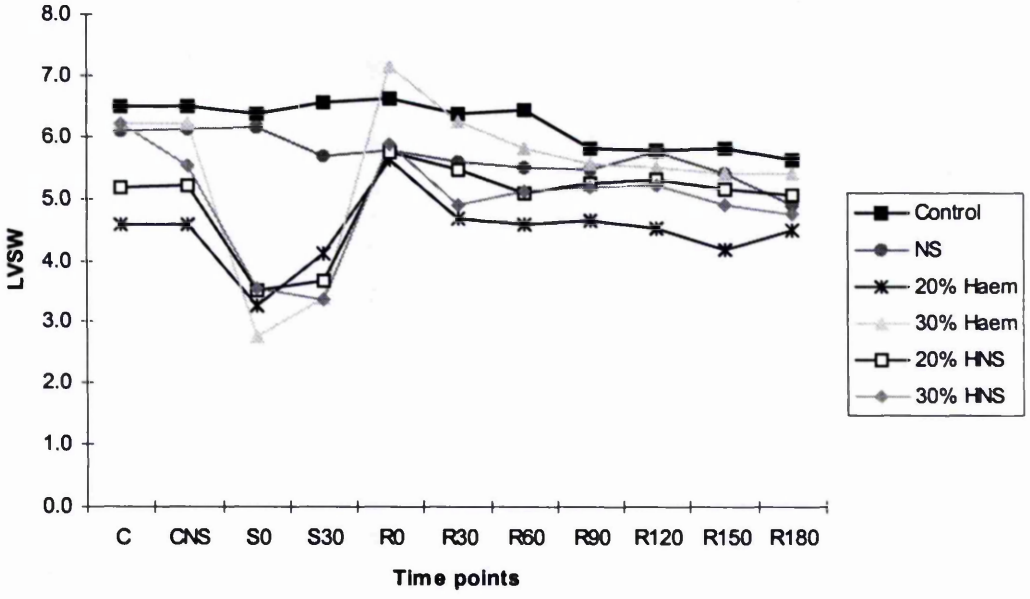
There were statistically significant group differences between the three groups with nerve stimulation and the control group. There were also pattern differences between the haemorrhage and nerve stimulation groups and their respective haemorrhage alone groups.

All experimental groups showed significant pattern differences with the control group.

Left ventricular stroke work

Nerve stimulation only resulted in a reduction in LVSW in the HNS30 group (Figure 3.9). There was very little change in LVSW in the control and the NS groups. Haemorrhage resulted in large reductions in LVSW but there was some recovery over the 30 minute shock period. Reinfusion of shed blood restored LVSW, which then remained fairly stable.

Figure 3.9: Graph of left ventricular stroke work data (g/min).



Graph shows that there was no effect of nerve stimulation but there was a large decrease in LVSW with haemorrhage. There was some recovery during the shock period in the two haemorrhage alone groups. Reinfusion of shed blood tended to restore LVSW to baseline levels.

Statistical analysis:

There was no significant difference between the groups (F = 2.18, df 5, sig. of F = 0.071) but because of the effect of haemorrhage there was a significant time effect and a

significant group by time interaction (Table 3.9). The contrast by time interactions of the four haemorrhage groups against the control group were all significant. There were significant contrast by time interactions for H30 against H20, and for H30 against HNS30.

Table 3.9: Repeated measures analysis of variance of left ventricular stroke work data.

LVSW RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	2.18	5	0.071
Time	19.32	10	**<0.0001**
Group by time	2.02	10	**<0.0001**
Contrasts			
Control vs NS	*1.07*	*1*	*0.306*
Contrast by time	*1.79*	*10*	*0.093*
Control vs H20	*9.00*	*1*	***0.004***
Contrast by time	*1.54*	*10*	*0.161*
Control vs H30	*2.27*	*1*	*0.138*
Contrast by time	*8.50*	*10*	***<0.0001***
Control vs HNS20	*4.13*	*1*	***0.048***
Contrast by time	*2.29*	*10*	***0.031***
Control vs HNS30	*4.31*	*1*	***0.043***
Contrast by time	*3.28*	*10*	***0.003***
H20 vs HNS20	*0.77*	*1*	*0.385*
Contrast by time	*1.08*	*10*	*0.401*
HNS20 vs HNS30	*0.02*	*1*	*0.879*
Contrast by time	*0.93*	*10*	*0.512*
H30 vs H20	*2.58*	*1*	*0.114*
Contrast by time	*3.25*	*10*	***0.004***
H30 vs HNS30	*0.31*	*1*	*0.582*
Contrast by time	*4.10*	*10*	***0.001***

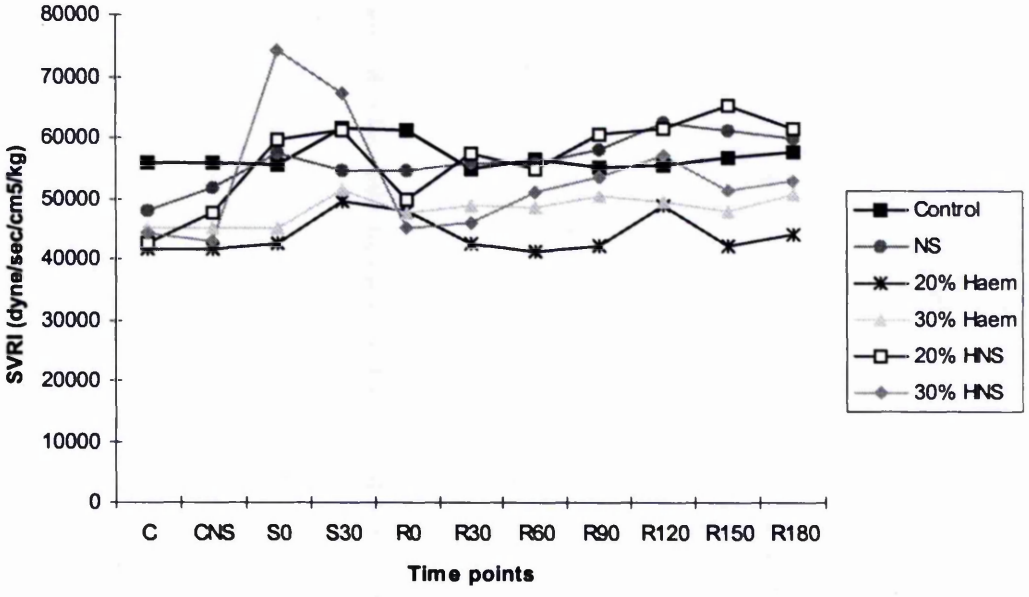
Any differences between the groups did not reach statistical significance. However as the significance of F was 0.072 and there was a significant group by time interaction the results of the simple contrasts have been shown. They show differences between the haemorrhage groups and the control group, and significant paired contrasts by time between the haemorrhage groups and the control group, for H30 vs H20, and for H30 vs HNS30.

Vascular resistance

Systemic vascular resistance index

The control group showed an increase in SVRI, which reached a maximum of 61524 resistance units (dyne.sec.cm^{-5}.kg) at S30 (Figure 3.10). There was an increase in SVRI with nerve stimulation and then a gradual increase until the end. Haemorrhage alone did not cause a major increase in SVRI and there was some recovery after reinfusion of the shed blood. Haemorrhage on a background of nerve stimulation did produce large increases in SVRI, nearly doubling it in the case of HNS30. As with the other haemorrhage groups reinfusion of the shed blood resulted in a decrease in SVRI, but in contrast SVRI increased more in the observation phase.

Figure 3.10: Graph of systemic vascular resistance index data (dyne.sec.cm^{-5}/kg).



Graph shows an increase in SVRI during the haemorrhage phase, especially in the two HNS groups. SVRI returned to near baseline with reinfusion of shed blood. There was a general increase in SVRI during the observation period in the two HNS groups.

Statistical analysis:

Neither the group differences nor the group by time interaction reached statistical significance, even though the contrast by time interactions for HNS30 against the control group, and for H30 against HNS30 did (Table 3.10). The change with time was significant.

Table 3.10: Repeated measures analysis of variance of systemic vascular resistance index data.

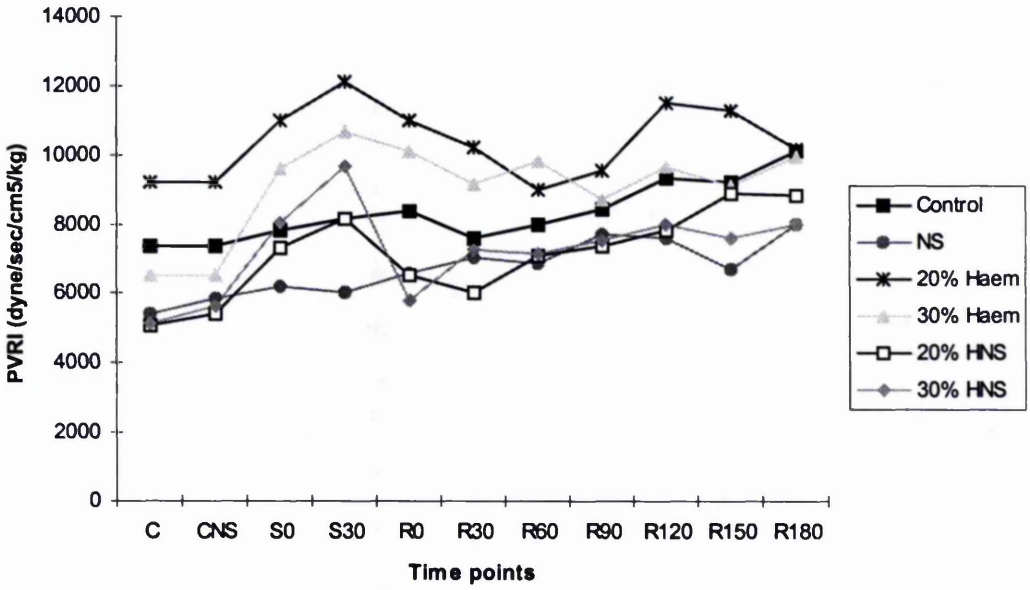
SVRI RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	1.38	5	0.249
Time	4.98	10	**<0.0001**
Group by time	1.37	50	0.066
Contrasts			
Control vs NS	*0.01*	*1*	*0.918*
Contrast by time	*1.76*	*10*	*0.100*
Control vs H20	*4.27*	*1*	*0.044*
Contrast by time	*0.56*	*10*	*0.838*
Control vs H30	*2.37*	*1*	*0.130*
Contrast by time	*0.36*	*10*	*0.203*
Control vs HNS20	*0.06*	*1*	*0.809*
Contrast by time	*1.43*	*10*	*0.203*
Control vs HNS30	*0.13*	*1*	*0.724*
Contrast by time	*5.41*	*10*	***<0.0001***
H20 vs HNS20	*2.72*	*1*	*0.106*
Contrast by time	*0.80*	*10*	*0.628*
HNS20 vs HNS30	*0.01*	*1*	*0.940*
Contrast by time	*2.69*	*10*	***0.013***
H30 vs H20	*0.44*	*1*	*0.511*
Contrast by time	*0.53*	*10*	*0.857*
H30 vs HNS30	*1.34*	*1*	*0.253*
Contrast by time	*5.37*	*10*	***<0.0001***

Any differences between the groups and in the group by time interaction did not reach statistical significance. In this situation the simple contrasts could be regarded as inappropriate. However, as the significance of F for the group by time interaction was 0.066 the results of the contrasts have been shown and they reveal that there were some significant differences in contrast by time interactions, or patterns for: H30 vs HNS30, HNS20 vs HNS30, and HNS30 vs C.

Pulmonary vascular resistance index

All groups showed a gradual increase in PVRI (Figure 3.11). This was generally progressive in the control and NS groups. The haemorrhage groups showed a sharp increase with the onset of blood loss, which continued during the shock period, and recovered with reinfusion of the shed blood.

Figure 3.11: Graph of pulmonary vascular resistance index data (dyne.sec.cm^{-5}/kg).



Graph shows an increase in PVRI in the haemorrhage phase, which continued during the shock phase, but was only partly reversed with reinfusion of shed blood. There was a general increase in PVRI over the course of the experiments.

Statistical analysis:

The differences between the groups and their group by time interactions did not reach statistical significance (Table 3.11). The changes over time did.

Table 3.11: Repeated measures analysis of variance of pulmonary vascular resistance index data.

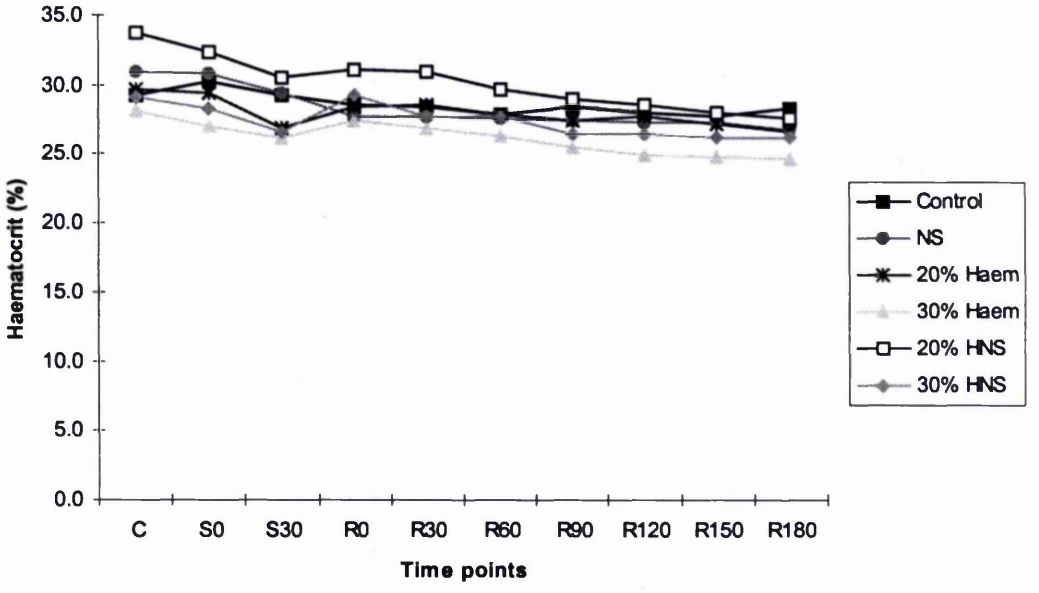
PVRI RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	2.04	5	0.092
Time	7.07	10	**<0.0001**
Group by time	1.22	50	0.174

There was no statistically significant difference between the groups and no significant group by time interaction. Simple contrasts were inappropriate and not performed.

Haematocrit

Haematocrits showed a progressive decline over the course of the experiments (Figure 3.12). The baseline range was 29.1, SD 2.5, (HNS30) to 33.7, SD 1.7, (HNS20). The range at R180 was 24.6, SD 3.5, (H30) to 28.3, SD 4.8, control group (see Table 12, Appendix).

Figure 3.12: Graph of haematocrit data (%).



Graph shows that there was a gradual decrease in haematocrit in all groups over the course of the experiments.

Statistical analysis:

No overall group differences was identified (F = 1.56, df 5, sig. of F = 0.191), nor was there a significant group by time interaction (F = 1.29, df 45, sig. of F = 0.118), Table 3.12. There was a significant overall change with time (F = 10.75, df 9, sig. of F = <0.0001): a haemodilution as fluid moved into the intra-vascular compartment from the extra-cellular space. Simple contrasts were not performed.

Table 3.12: Repeated measures analysis of variance of haematocrit data.

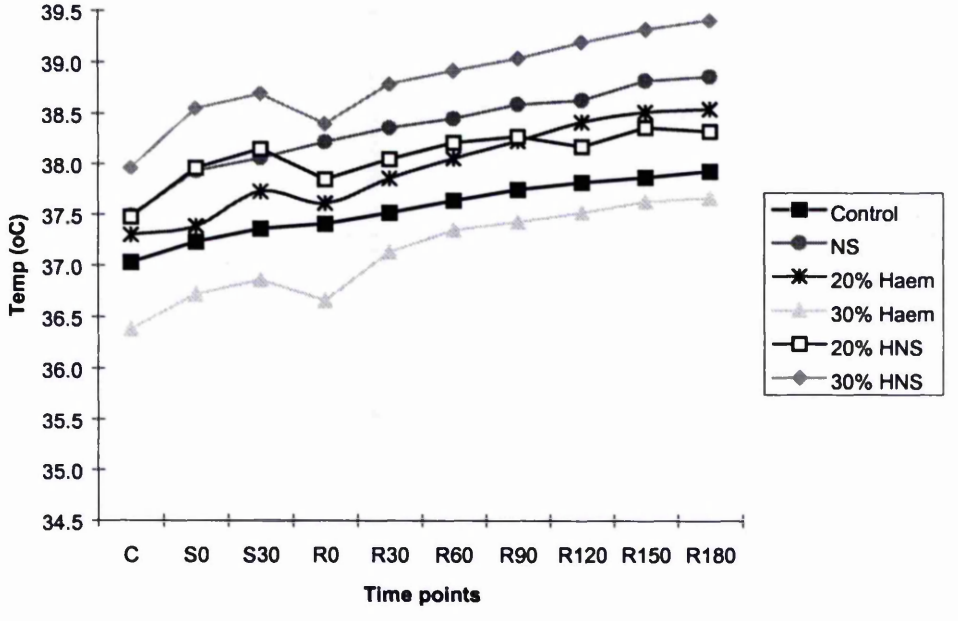
Haematocrit RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	1.56	5	0.191
Time	10.75	9	**<0.0001**
Group by time	1.29	45	0.118

As any difference between the groups was not statistically significant and there was no significant difference in group by time interaction the simple contrasts were not carried out.

Temperature

Rectal temperature was monitored in all animals throughout the studies. Our aim was to maintain body temperature to within 2 °C of normal. I was unable to maintain body temperature to within 1 °C of normal during the summer months because of the high ambient temperature in the laboratory. There was a gradual increase in body temperature (Figure 3.13). The highest temperature recorded in any animal was 42.2 °C at R180 in one of the NS animals. The lowest was 34.7 °C in one of the H30 animals at the control time point.

Figure 3.13: Graph of body temperature data (°C).



Graph shows a gradual increase in body temperature over the course of the experiments. There was no statistically significant difference between the groups.

The mean change in body temperature in each group is shown in Table 3.13i below.

Table 3.13i: Change in body temperature.

Body temperature (°C)			
	Control	**R180**	**R180 - C**
Control	37	38.17	1.17
NS	37.54	38.97	1.43
H20	37.3	38.54	1.24
H30	35.85	37.93	2.08
HNS20	37.25	38.32	1.07
HNS30	38.87	39.45	0.58

The overall mean change in body temperature was 1.26 °C.

Statistical analysis:

Repeated measures analysis of variance showed that there was no statistically significant difference in temperature between the groups, although there was an overall increase in temperature with time and the precise pattern of change in temperature was different in the different groups (Table 3.13ii).

Table 3.13ii: Repeated measures analysis of variance of body temperature data.

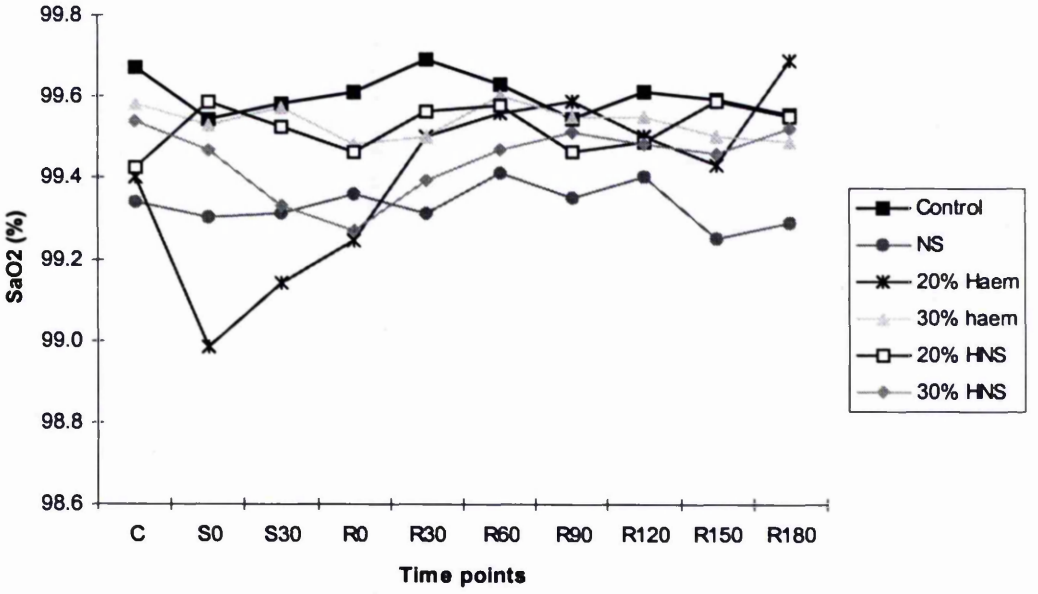
Body temperature RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	1.94	5	0.105
Time	38.05	7	**<0.0001**
Group by time	2.27	35	**<0.0001**

Oxygen transport:

Arterial oxygen saturation

As the animals were ventilated with a 50% oxygen/nitrous oxide mixture throughout the experimental period there was very little change in SaO_2 (Figure 3.14). The lowest arterial oxygen saturation (99.0%, SD 1.3) was measured at the beginning of the shock period in the H20 group.

Figure 3.14: Graph of arterial oxygen saturation data (%).



Graph shows that with the exception of the H20 group SaO_2 did not fall below 99.2% throughout the experiments.

Statistical analysis:

There were no statistically significant differences in SaO_2 between the groups (Table 3.14). There was no significant overall change with time, nor group by time interaction.

Table 3.14: Repeated measures analysis of variance of arterial oxygen saturation (%) data.

SaO_2 RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	1.96	5	0.102
Time	1.38	9	0.230
Group by time	1.08	45	0.313

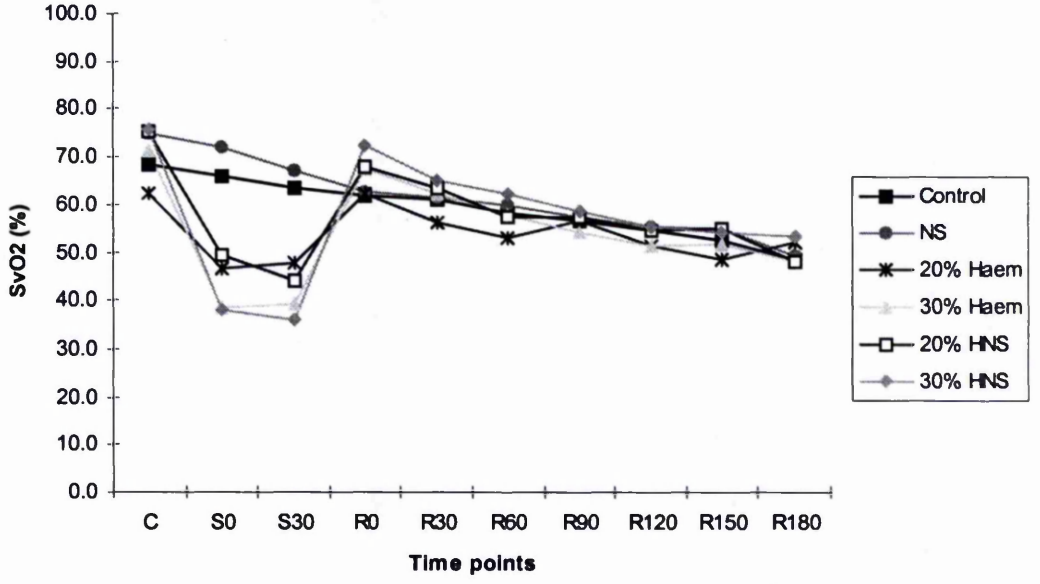
No overall group differences were detected. There was no significant change over time and the group by time interaction was not significant. No contrasts were performed.

Mixed venous oxygen saturation

Both the control and the nerve stimulation groups showed a gradual reduction in SvO_2 over the course of the experiments (Figure 3.15). The four haemorrhage groups showed a rapid reduction in SvO_2 with the onset of haemorrhage. There was a marginal recovery of saturation during the shock phase in the two haemorrhage alone groups, while there was a marginal deterioration in the two haemorrhage and nerve stimulation groups.

Reinfusion of the shed blood restored SvO_2 to baseline in the haemorrhage groups. All groups showed a gradual decrease in SvO_2.

Figure 3.15: Graph of mixed venous oxygen saturation data (%).



Statistical analysis:

Although statistically there was no overall difference between the groups there was a significant group by time interaction and a significant change over time (Table 3.15). Contrasts were performed to investigate the origin of these differences. The four

haemorrhage groups, with their sharp decrease in venous oxygen saturation during the shock phase, all had significant contrast by time interactions against the control group. There were also significant contrast by time interaction for H20 against HNS20, for H30 against H20, and for HNS20 against HNS30. The contrast by time interaction for H30 against HNS30 did not reach statistical significance ($F = 1.36$, df 9, sig. of $F = 0.243$).

Table 3.15: Repeated measures analysis of variance of mixed venous oxygen saturation (%) data.

SvO_2 RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	1.04	5	0.408
Time	50.69	9	**<0.0001**
Group by time	2.67	45	**<0.0001**
Contrasts			
Control vs NS	*0.71*	*1*	*0.403*
Contrast by time	*0.38*	*9*	*0.935*
Control vs H20	*1.45*	*1*	*0.236*
Contrast by time	*4.52*	*9*	***0.001***
Control vs H30	*0.73*	*1*	*0.398*
Contrast by time	*17.63*	*9*	***<0.0001***
Control vs HNS20	*0.01*	*1*	*0.930*
Contrast by time	*8.84*	*9*	*<0.0001*
Control vs HNS30	*0.02*	*1*	*0.901*
Contrast by time	*19.48*	*9*	***<0.0001***
H20 vs HNS20	*1.20*	*1*	*0.280*
Contrast by time	*4.33*	*9*	***0.001***
HNS20 vs HNS30	*0.04*	*1*	*0.837*
Contrast by time	*3.65*	*9*	***0.002***
H30 vs H20	*0.19*	*1*	*0.663*
Contrast by time	*5.91*	*9*	***<0.0001***
H30 vs HNS30	*0.91*	*1*	*0.346*
Contrast by time	*1.36*	*9*	*0.243*

Although the group differences were not statistically significant, there was a significant change over time, and the group by time interaction was significant.

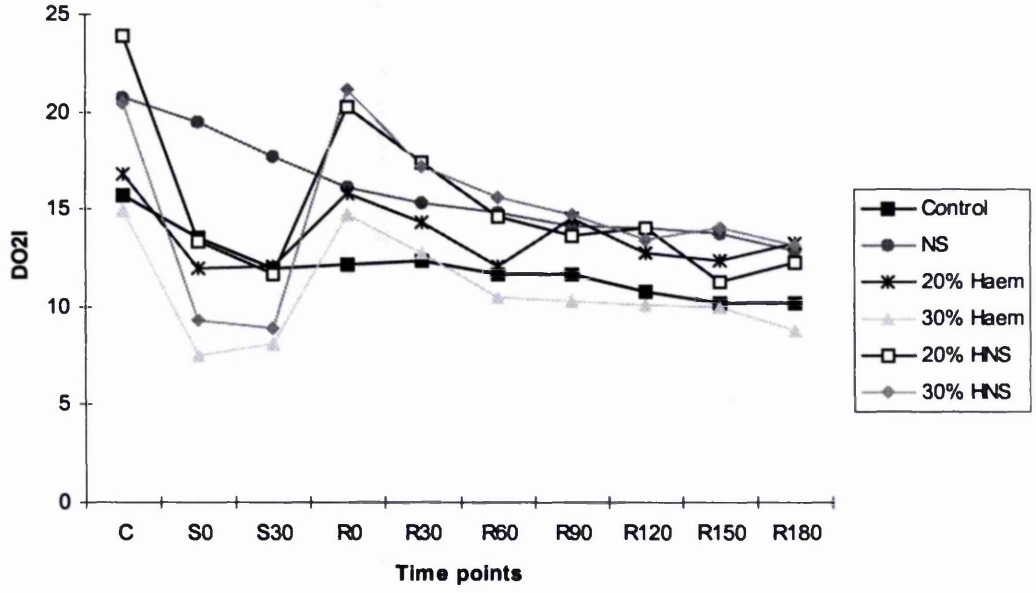
Contrasts showed that there was no pattern difference between controls and NS and that all the haemorrhage groups differed from the control group. There were also pattern differences between the two levels of haemorrhage and between H20 and HNS20.

Oxygen delivery index:

The control group showed a small but progressive reduction in oxygen delivery over the course of the experiments from 16 ml/min/kg (SD 4) at baseline to 10 ml/min/kg (SD 5) at R180, Figure 3.16. The greatest reduction was seen in the first 30 minute period. For the nerve stimulation group there was a reduction in oxygen delivery from 21 ml/min/kg (SD 7) at baseline to 13 ml/min/kg (SD 6) at R180.

All the haemorrhage groups showed reductions in oxygen delivery during the shock phase. Oxygen delivery in the two 30% blood loss groups was less than 50% of baseline at S0. There was no real recovery during the 30 minute shock period in any of the four haemorrhage groups. Reinfusion of the shed blood did increase delivery either back to baseline or very near it. After that there was a gradual decline in all four of the haemorrhage groups. During the observation phase all the haemorrhage groups had slightly higher deliveries than the control group, except for the H30 group.

Figure 3.16: Graph of oxygen delivery index data (ml/min/kg).



Graph shows a sharp reduction in DO_2I with haemorrhage. During the shock period there was no recovery. Reinfusion of shed blood resulted in a restoration of DO_2I but this was followed by a gradual decrease.

Statistical analysis:

There were significant differences between the groups, in the group by time interaction (patterns), and over time (Table 3.16). The only statistically significant group contrasts were those between the nerve stimulation group and the control group, and between the HNS30 and the H30 groups.

There were pattern differences between all the haemorrhage groups and the control group (except H20), between the two HNS groups and between HNS20 and H20.

Table 3.16: Repeated measures analysis of variance of oxygen delivery index data.

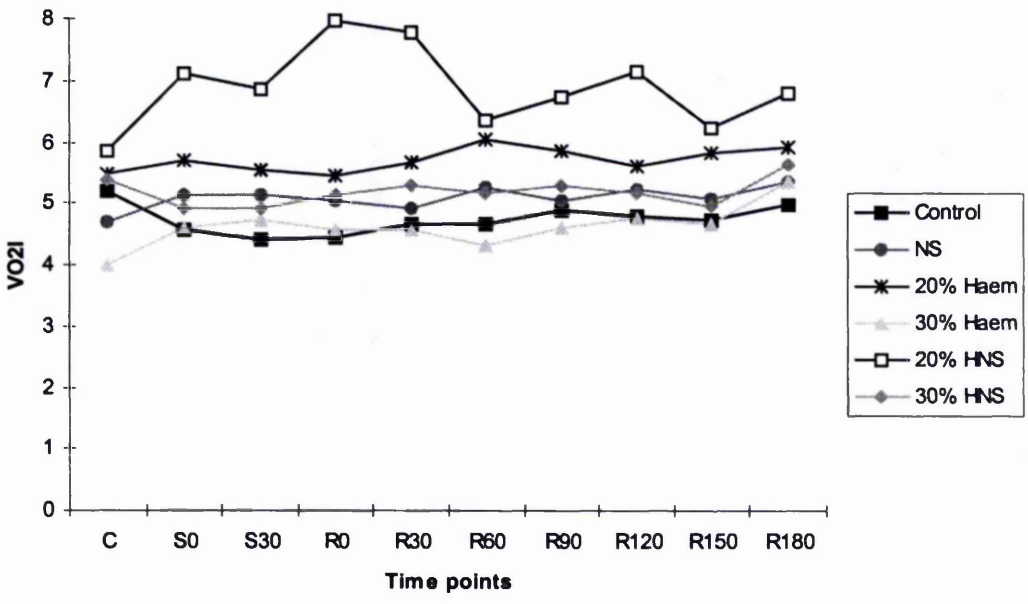
DO2 index RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	2.48	5	**0.045**
Time	27.26	9	**<0.0001**
Group by time	2.12	45	**<0.0001**
Contrasts			
Control vs NS	5.45	1	**0.024**
Contrast by time	0.55	9	0.831
Control vs H20	0.74	1	0.393
Contrast by time	1.52	9	0.173
Control vs H30	0.47	1	0.494
Contrast by time	3.23	9	**0.005**
Control vs HNS20	2.82	1	0.099
Contrast by time	4.34	9	**0.001**
Control vs HNS30	2.83	1	0.099
Contrast by time	8.66	9	**<0.0001**
H20 vs HNS20	0.55	1	0.463
Contrast by time	3.97	9	**0.001**
HNS20 vs HNS30	0.02	1	0.876
Contrast by time	2.21	9	**0.041**
H30 vs H20	2.08	1	0.156
Contrast by time	1.86	9	0.086
H30 vs HNS30	5.17	1	**0.027**
Contrast by time	1.87	9	0.086

The only statistically significant group differences were between NS and C, and between HNS30 and H30. There were pattern differences between H30, HNS20, HNS30 and C, between HNS20 and H20, and between HNS30 and HNS20.

Oxygen consumption index

The most striking feature of these results is that haemorrhage appeared to have no effect on oxygen consumption (Figure 3.17). Apart from the HNS20 group, which tended to have values a little higher than the others and show more variability, there was very little difference between the other groups or over the time course of the experiments. There was no noticeable effect of either the nerve stimulation or haemorrhage.

Figure 3.17: Graph of oxygen consumption index data (ml/min/kg).



Graph shows that VO_2I remained largely unchanged over the course of the experiments, except in the H20 group, in which there was an increase during the haemorrhage, shock, and reinfusion periods.

Statistical analysis:

There was no statistically significant overall group difference (F = 1.58. df 5, Sig. of F = 0.185) in oxygen consumption index (Table 3.17). There was no significant change with time (F = 1.31, df 9, Sig. of F 0.263), nor was there any significant group by time interaction (F = 0.86, df 45, Sig. of F = 0.726). No simple contrasts were performed.

Table 3.17: Repeated measures analysis of variance of oxygen consumption index data.

VO2I RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	1.58	5	0.185
Time	1.31	9	0.263
Group by time	0.86	45	0.726

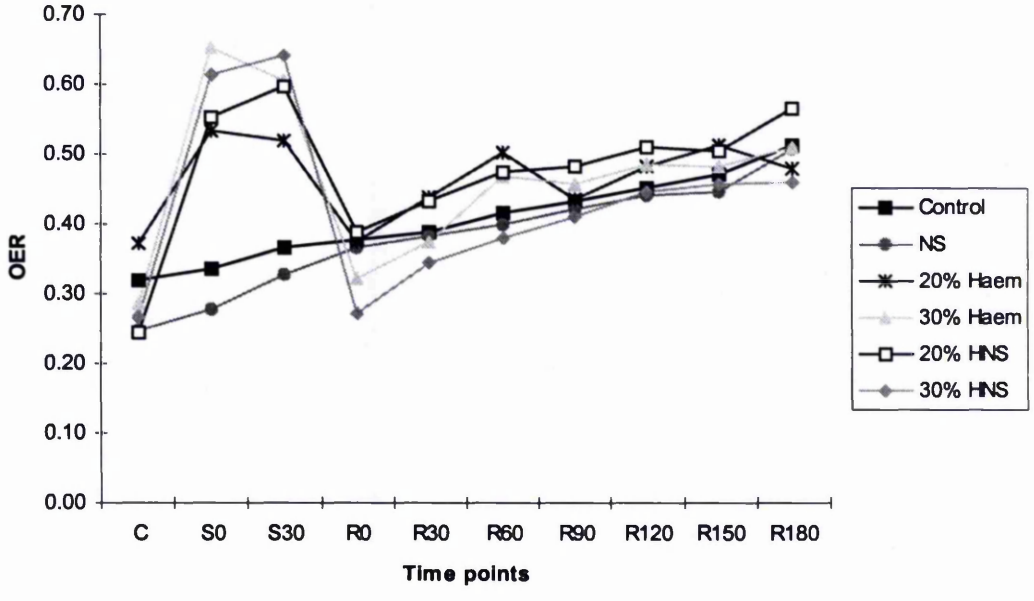
Neither the group nor the group by time interaction were statistically different so simple contrasts were not performed.

Oxygen extraction ratio

Over the course of the experiments there was a general increase in OER in all the groups (Figure 3.18). In addition all the haemorrhage groups showed a marked increase in OER with haemorrhage. This was maintained during the shock phase but returned to baseline with reinfusion of the shed blood. The progressive increase in OER started after reinfusion.

From the outset there was a range of OERs: from 0.25 to 0.37 (in both the NS and HNS20 groups, and the H20 group, respectively). At the end of haemorrhage the highest OER was 0.65 (SD 0.12), seen in H30 (see Table 18, Appendix). At the end of the experiments the range of OERs was between 0.46 (SD 0.09) in HNS30 to 0.57 (SD 0.19) in HNS20. The increase in OER in the control group was from 0.32 (SD 0.07) to 0.51 (SD 0.11).

Figure 3.18: Graph of oxygen extraction ratio data.



Graph shows a sharp increase in OER during the haemorrhage phase. OER remained elevated during the shock period. Reinfusion of shed blood returned OER to near baseline values. There was a general increase in OER during the experiments.

Statistical analysis:

There were no statistically significant overall group differences (F =1.04. df 5, sig. of F = 0.405) in oxygen extraction ratio (Table 3.18). There was a significant change with time (F = 43.39, df 9, sig. of F <0.0001), and there was a significant group by time interaction (F = 2.74, df 45, sig. of F = <0.0001). Simple contrasts were performed to verify which group by time interaction contrasts accounted for the overall difference. Not surprisingly all the haemorrhage groups showed significant contrast by time interactions against the control group. There were also significant contrasts by time for H20 against HNS20, for HNS20 against HNS30, and for H20 against H30.

Table 3.18: Repeated measures analysis of variance of oxygen extraction ratio data.

OER RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	1.04	5	0.405
Time	43.39	9	**<0.0001**
Group by time	2.74	45	**<0.0001**
Contrasts			
Control vs NS	*0.01*	*1*	*0.936*
Contrast by time	*0.41*	*9*	*0.924*
Control vs H20	*0.01*	*1*	*0.904*
Contrast by time	*4.43*	*9*	***<0.0001***
Control vs H30	*0.02*	*1*	*0.900*
Contrast by time	*11.88*	*9*	***<0.0001***
Control vs HNS20	*3.21*	*1*	*0.080*
Contrast by time	*8.23*	*9*	***<0.0001***
Control vs HNS30	*<0.0001*	*1*	*0.952*
Contrast by time	*15.01*	*9*	***<0.0001***
H20 vs HNS20	*3.09*	*1*	*0.085*
Contrast by time	*4.73*	*9*	***<0.0001***
HNS20 vs HNS30	*3.26*	*1*	*0.077*
Contrast by time	*2.74*	*9*	***0.013***
H30 vs H20	*<0.0001*	*1*	*0.995*
Contrast by time	*4.71*	*9*	***<0.0001***
H30 vs HNS30	*<0.0001*	*1*	*0.950*
Contrast by time	*1.20*	*9*	*0.322*

Differences between the groups did not reach statistical significance so it can be argued that further contrasts were inappropriate.

It is worth noting that there were statistically significant contrasts by time because the haemorrhage groups differed from the control group. There were significant contrasts by time for: H30 vs H20, H20 vs HNS20 and HNS20 vs HNS30. There was no significant difference between the control and NS groups, in which there was no blood loss.

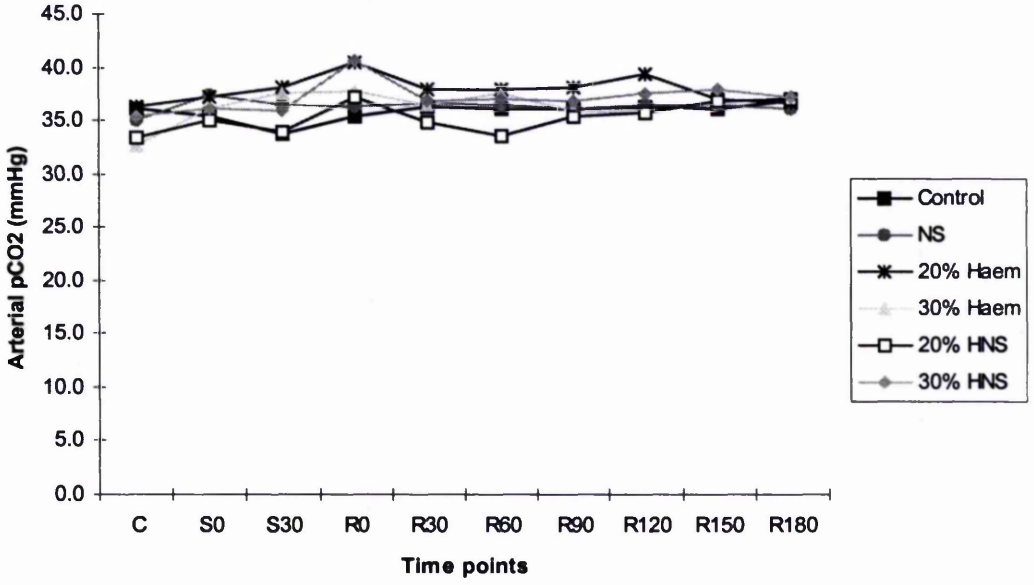
Metabolic response

Partial pressure of arterial carbon dioxide

There was a significant increase in $PaCO_2$ over the course of the experiments (F = 3.00, df 9, sig. of F = 0.008), but there was no overall difference between the groups or in the group by time interaction (Figure 3.19).

The overall range in $PaCO_2$ was from 32.6 mmHg (SD 44.0) to 40.7 mmHg (SD 9.5), see Table 19, Appendix.

Figure 3.19: Graph of partial pressure of carbon dioxide data (mmHg).



Graph shows that arterial pCO2 was well maintained in all the groups between 32-40 mmHg.

Table 3.19: Repeated measures analysis of variance of arterial partial pressure CO_2 (mmHg) data.

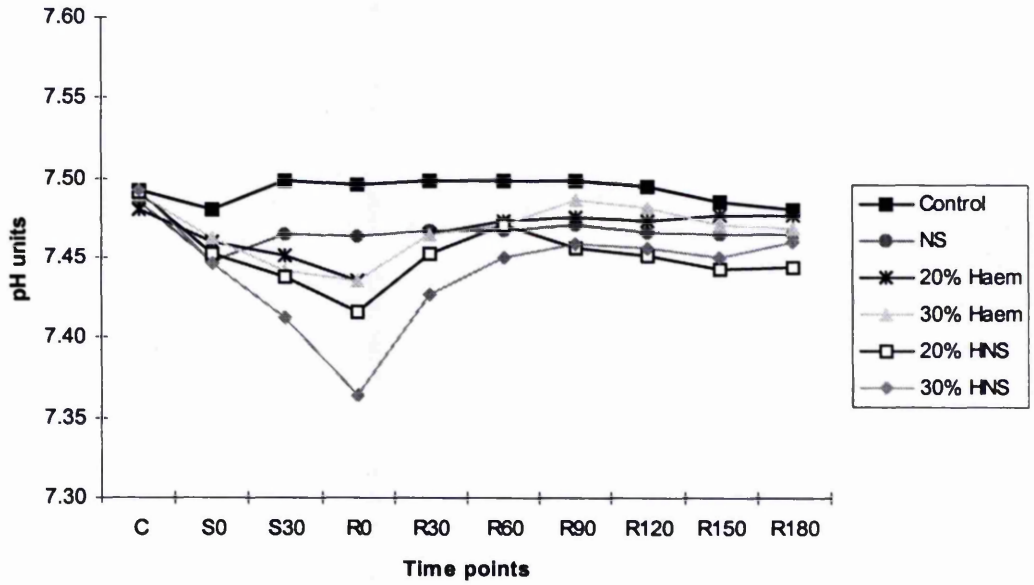
Arterial PCO_2 RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	0.80	5	0.555
Time	3.00	9	**0.008**
Group by time	1.05	45	0.396

No overall group differences were detected. There was a significant change over time, but the group by time interaction was not significant. No contrasts were performed.

Arterial pH

There was a reduction in arterial pH in all the groups over the first 30 minutes of the experiment (Figure 3.20). This corrected itself over the next 30 minutes in the control group and the nerve stimulation group. Arterial pH continued to decrease in the four haemorrhage groups until after reinfusion of the shed blood. Arterial pH then increased over the next 60-90 minutes to reach a plateau for the remaining 90 minutes of the experiment.

Figure 3.20: Graph of arterial pH data.



Graph shows that there was a reduction in arterial pH during the haemorrhage and shock phases. This was more profound in the two HNS groups. Arterial pH was restored by reinfusion of shed blood in the two haemorrhage alone groups but not in the two HNS groups.

Statistical analysis:

The differences between the groups and in the group by time interaction did not reach statistical significance (Table 3.20). The change in pH during the shock phase ensured that there was a significant overall change with time.

Table 3.20: Repeated measures analysis of variance of arterial pH data.

Arterial pH RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	1.63	5	0.169
Time	9.61	9	**<0.0001**
Group by time	0.92	45	0.612

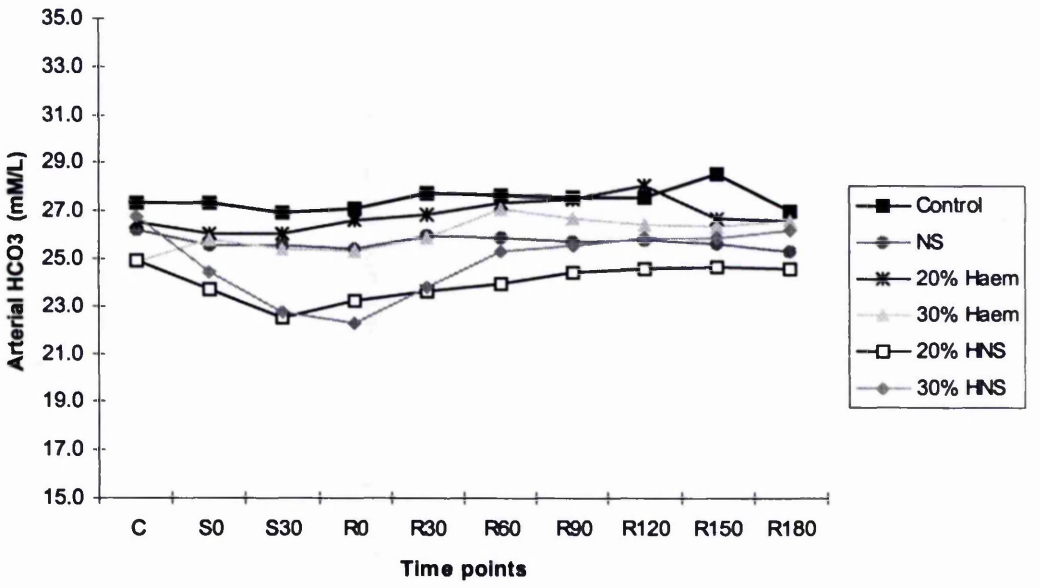
No overall group differences were detected. There was no significant change over time and the group by time interaction was not significant. No contrasts were performed.

Arterial bicarbonate

There was a general change during the experiments (Figure 3.21). The differences between the groups and between their group by time interactions were statistically significant.

The three nerve stimulation groups had significantly lower bicarbonate concentrations than the control group. There was a group difference between H20 and HNS20. The HNS30 group showed a clear reduction in bicarbonate during the shock period resulting in a significant pattern difference with the control group. There was also a pattern difference between H30 and HNS30: there was no reduction in bicarbonate during the shock phase in the H30 group.

Figure 3.21: Graph of arterial bicarbonate data (mM/L).



Graph shows that bicarbonate levels remained stable throughout the experiments, except in the two HNS groups. In the HNS groups there was a reduction in bicarbonate concentrations during the haemorrhage and shock phases. Bicarbonate concentrations returned to baseline more than one hour after reinfusion of shed blood.

Statistical analysis:

The results of the RANOVA are presented in Table 3.21. They show statistically significant differences in plasma bicarbonate between the groups, differences in pattern, because of the reduction in bicarbonate in HNS30 during the shock period, and a general change over time. Contrasts showed that plasma bicarbonate was higher in the control group than in NS, HNS20 and HNS30, and that plasma bicarbonate was higher in H20 than in HNS20.

Table 3.21: Repeated measures analysis of variance of arterial bicarbonate (mM/L) data.

Arterial HCO_3 RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	3.99	5	**0.004**
Time	7.71	9	**<0.0001**
Group by time	1.53	45	**0.024**
Contrasts			
Control vs NS	4.60	1	**0.037**
Contrast by time	0.42	9	0.916
Control vs H20	0.54	1	0.464
Contrast by time	1.62	9	0.142
Control vs H30	3.01	1	0.089
Contrast by time	1.39	9	0.224
Control vs HNS20	15.23	1	**<0.0001**
Contrast by time	0.71	9	0.697
Control vs HNS30	9.66	1	**0.003**
Contrast by time	2.52	9	**0.021**
H20 vs HNS20	8.26	1	**0.006**
Contrast by time	1.31	9	0.262
HNS20 vs HNS30	0.95	1	0.335
Contrast by time	1.35	9	0.241
H30 vs H20	0.70	1	0.407
Contrast by time	1.91	9	0.078
H30 vs HNS30	1.88	1	0.176
Contrast by time	2.28	9	**0.035**

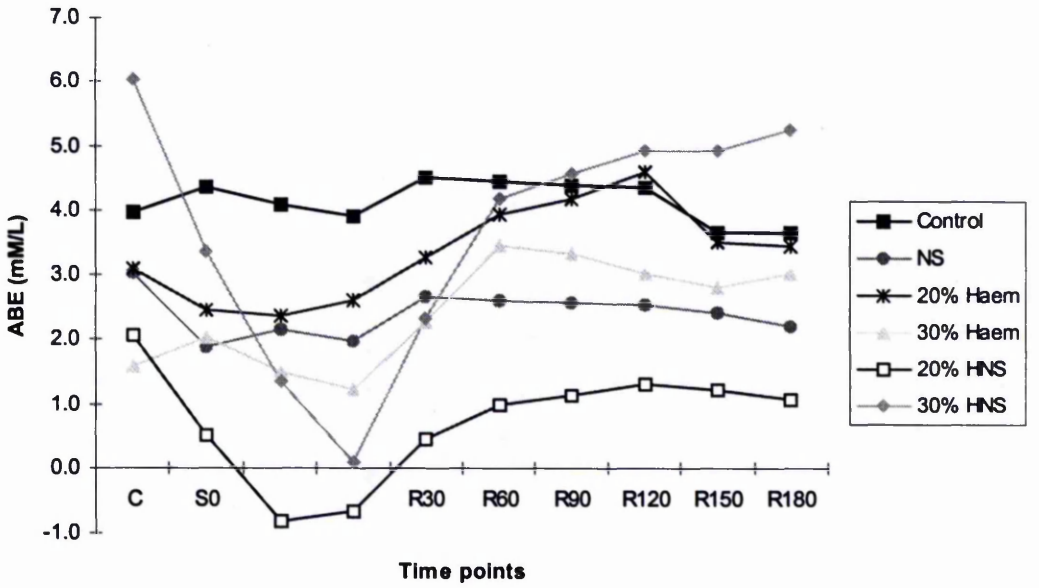
There were statistically significant differences between the groups, over time, and in the group by time interaction.

Contrasts showed that the NS and the two HNS groups were significantly different to the control group. There were pattern differences between HNS30 and control, and between HNS30 and H30.

Arterial base excess

There was considerable variation in base excess levels from the outset in the different groups and much variation within the groups (Figure 3.22). At baseline base excess in the H30 group was 1.6 mM/L (SD 2.7), while in the HNS30 group it was 6.0 mM/L (SD 7.9). The nerve stimulation group showed a reduction in base excess over the first 30 minutes of the experiment and then a slight recovery, which never reached baseline. The two HNS groups showed large reductions in base excess with haemorrhage, which continued during the shock period. Reinfusion of shed blood resulted in some recovery. The two simple haemorrhage groups tended to show a progressive increase in base excess.

Figure 3.22: Graph of arterial base excess data (mM/L).



Graph shows the dramatic reduction in arterial base excess in the two HNS groups and the very gradual recovery after reinfusion of the shed blood. There was a gradual increase in arterial base excess from baseline in the two haemorrhage alone groups. There was very little change in arterial base excess in the control and NS groups.

Statistical analysis:

No overall group differences were identified and the group by time interaction failed to reach statistical significance (F = 1.38, df 45, sig. of F = 0.071), Table 3.22. This was probably as a result of the large variation within the groups. There were clearly differences over time.

Table 3.22: Repeated measures analysis of variance of arterial base excess (mM/L) data.

Arterial base excess RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	0.58	5	0.712
Time	8.03	9	**<0.0001**
Group by time	1.38	45	0.071

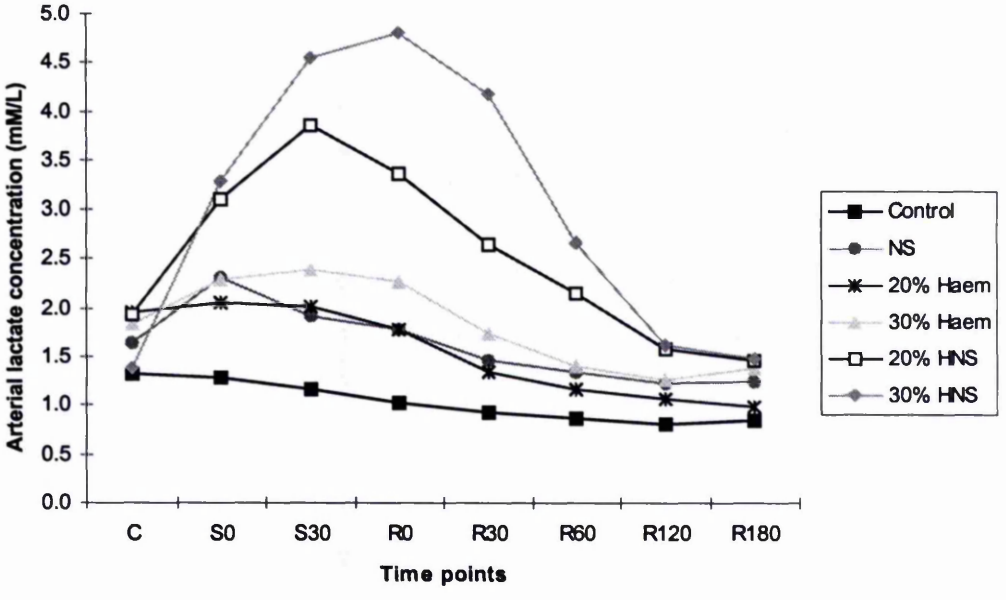
No overall group or group by time interaction differences were identified so no contrasts were performed.

Plasma Lactate

Arterial lactate

All groups had higher lactate levels at the outset than at the end (except HNS30), Figure 3.23. The two HNS groups showed a large increase in lactate with haemorrhage and during the shock phase. This persisted until 2 hours after reinfusion of the shed blood. There was a slight increase in lactate in the H30 group (from 1.8 mM/L, SD 1.2 to 2.4 mM/L, SD 1.6) but not in the H20 group.

Figure 3.23: Graph of arterial lactate data (mM/L).



Graph shows the marked increase in arterial lactate concentration in the two HNS groups. Reinfusion of shed blood did result in a return to baseline concentrations of arterial lactate two hours later. There was a general decrease in plasma lactate concentration.

Statistical analysis:

The groups were significantly different, as was the group by time interaction, and the overall change with time (Table 3.23). The group effect seems to have been because of the difference between HNS30 and the control group and H30 group, and the difference between the HNS20 and control group. There were significant pattern differences between the HNS30 group and the control, H30, and HNS20 groups (lactate continuing to increase up to R0 in HNS30).

Table 3.23: Repeated measures analysis of variance of arterial lactate (mM/L) data.

Arterial lactate RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	3.09	5	**0.017**
Time	10.61	7	**<0.0001**
Group by time	1.60	35	**0.024**
Contrasts			
Control vs NS	0.94	1	0.337
Contrast by time	0.125	7	0.295
Control vs H20	0.59	1	0.446
Contrast by time	0.36	7	0.917
Control vs H30	1.74	1	0.194
Contrast by time	0.80	7	0.590
Control vs HNS20	6.33	1	**0.015**
Contrast by time	1.54	7	0.180
Control vs HNS30	11.89	1	**0.001**
Contrast by time	6.10	7	**<0.0001**
H20 vs HNS20	2.64	1	0.110
Contrast by time	0.68	7	0.684
HNS20 vs HNS30	0.20	1	0.658
Contrast by time	2.49	7	**0.031**
H30 vs H20	0.17	1	0.681
Contrast by time	0.26	7	0.964
H30 vs HNS30	4.33	1	**0.043**
Contrast by time	3.51	7	**0.005**

There were statistically significant differences between the groups, over time, and in the group by time interaction.

Contrasts showed that the two HNS groups were significantly different to the control group. There was a significant difference between the two H30 groups. Pattern differences were between HNS30 and control, between HNS30 and H30, and between HNS30 and HNS20.

Summary of global responses

Cardiovascular

Nerve stimulation in itself caused an increase in heart rate and MAP.

Haemorrhage resulted in reductions in all functional indices. There was a tendency for the combination of haemorrhage and nerve stimulation to have a greater effect than haemorrhage alone. This was especially apparent for MAP and CI.

Following reinfusion all groups tended to behave in the same way and there was little overall change.

Systemic and pulmonary vascular resistance indices increased during the shock phase. After reinfusion there was little difference between the insult groups and the controls.

Oxygen transport

Mixed venous oxygen saturation and oxygen delivery were reduced during the shock phase but there was no change in oxygen consumption. Oxygen extraction ratio increased in the four haemorrhage groups during the shock phase, returning to control levels after reinfusion of the shed blood.

Metabolic

Arterial pH decreased with all types haemorrhage. There was great variability in arterial base excess, which tended to decrease more with HNS than with haemorrhage alone.

Arterial lactate increased significantly with HNS, but not with haemorrhage alone, during the shock phase and then returned to baseline during the observation period.

Regional responses

Cardiovascular responses

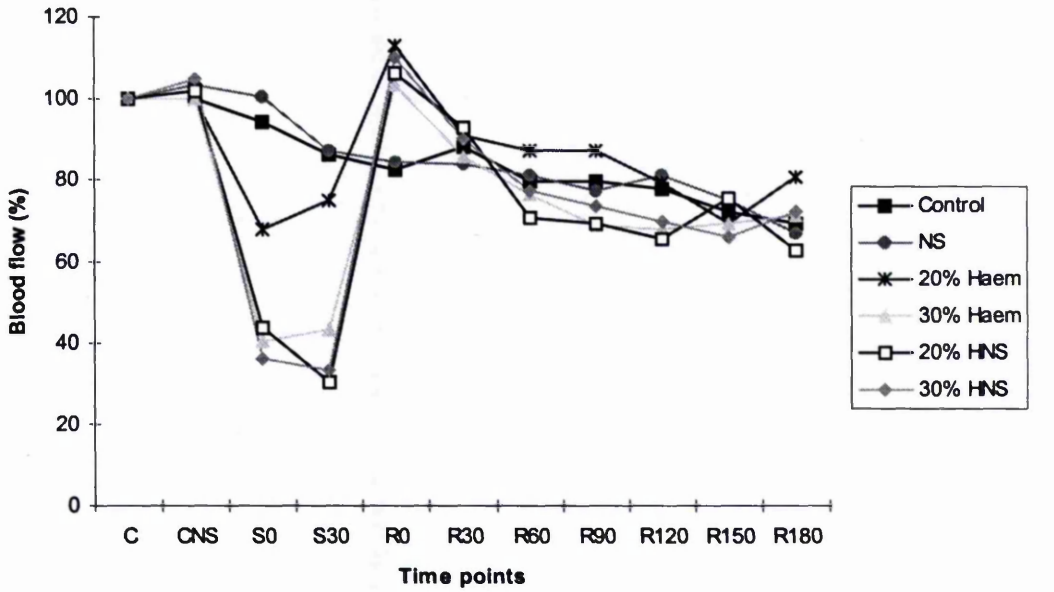
Normalised blood flows

Blood flows were measured using electro-magnetic probes placed around the right femoral artery and around a branch of the superior mesenteric artery. This gave an indication of blood flow to skeletal muscle and to the gut. Measurements were made in electrical units and were not converted into absolute flows as the aim was to see how flows changed rather than what the actual values might be. Absolute values would not have been very helpful in the case of the branch of the superior mesenteric artery as the same branch was not always used. The most easily accessible branch was used to reduce intestinal handling and so minimise operator-induced translocation. This would have resulted in artificially different flows in different animals. For this reason blood flows were normalised to baseline.

Normalised femoral blood flow

Nerve stimulation had very little impact on femoral blood flow: the immediate increase in flow ranged from 2-5% (Figure 3.24). Loss of 20% TBV reduced femoral flow to 68% (SD 23), but in the presence of NS there was a greater effect as the reduction was to 44% (SD 33). Loss of 30% TBV reduced femoral blood flow to 40% (SD 32) so that the addition of the NS insult only resulted in a further reduction to 36% (SD 27). In the two haemorrhage only groups there was a slight recovery during the shock phase, while in the two HNS groups flow continued to decrease. Reinfusion of shed blood restored flow. From R30 onwards there was a gradual reduction in flow to between 63-81% of baseline at R180.

Figure 3.24: Graph of normalised femoral blood flow data (%).



Graph shows the reduction in femoral flow with haemorrhage. There was only a slight recovery of flow during the shock phase. Reinfusion of shed blood restored flow to baseline before a gradual decrease during the observation period.

Statistical analysis:

The apparent differences between the groups did not reach statistical significance (F = 0.65, df 5, sig. of F = 0.662), although the group by time interaction was significant (F = 1.71, df 50, sig. of F = 0.005), Table 3.24. The reduction in flow with haemorrhage probably accounted for the significant group by time interaction (all the haemorrhage groups showed significant contrast by time interaction against the control group). There was no difference in pattern between the control group and NS. There was clearly a change over time.

Table 3.24: Repeated measures analysis of variance of normalised femoral blood flow data.

Normalised femoral blood flow RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	0.65	5	0.662
Time	16.69	10	**<0.0001**
Group by time	1.71	50	**0.005**
Contrasts			
Control vs NS	*0.30*	*1*	*0.584*
Contrast by time	*0.39*	*10*	*0.941*
Control vs H20	*0.07*	*1*	*0.796*
Contrast by time	*2.87*	*10*	***0.010***
Control vs H30	*1.10*	*1*	*0.300*
Contrast by time	*8.37*	*10*	***<0.0001***
Control vs HNS20	*0.02*	*1*	*0.880*
Contrast by time	*3.71*	*10*	***0.002***
Control vs HNS30	*0.38*	*1*	*0.540*
Contrast by time	*10.18*	*10*	***<0.0001***
H20 vs HNS20	*0.13*	*1*	*0.719*
Contrast by time	*1.95*	*10*	*0.071*
HNS20 vs HNS30	*0.13*	*1*	*0.721*
Contrast by time	*1.27*	*10*	*0.285*
H30 vs H20	*1.47*	*1*	*0.232*
Contrast by time	*2.68*	*10*	***0.015***
H30 vs HNS30	*0.16*	*1*	*0.688*
Contrast by time	*0.70*	*10*	*0.714*

Although the differences between the groups did not reach statistical significance the group by time interaction did. In view of this the contrasts were performed to reveal where the difference occurred. The cause of the difference was that the pattern of femoral blood flow in the haemorrhage groups was different to that in the control group. The nerve stimulation group did not differ with the control group as there was no blood loss.

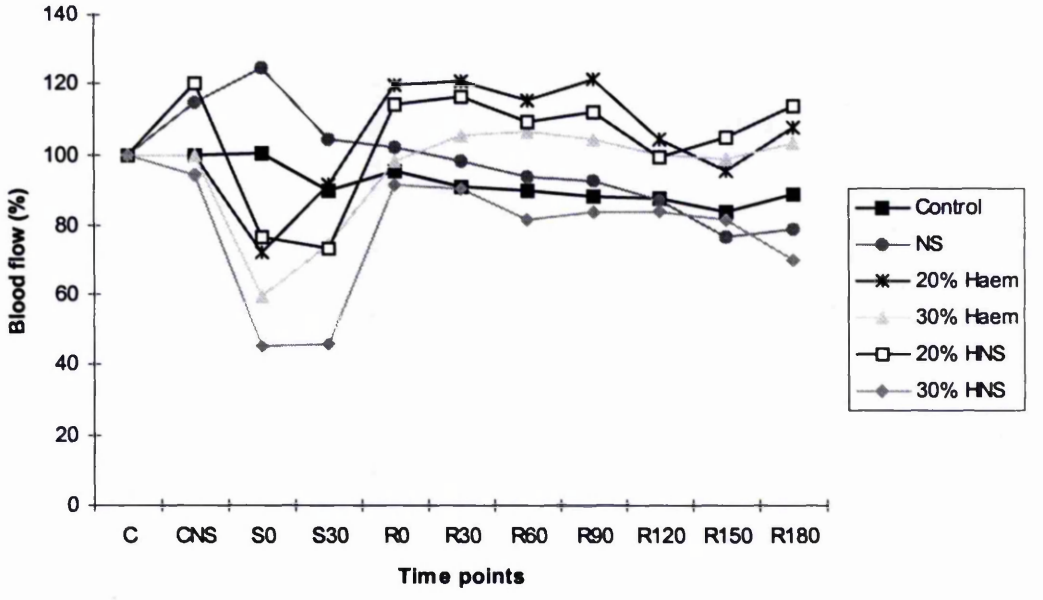
There was also a difference in the pattern of femoral blood flow between the two haemorrhage alone groups.

Normalised gut blood flow

Gut blood flow increased 14% with nerve stimulation in the NS group and 20% in the HNS20 group (Figure 3.25). In the HNS30 group there was an initial reduction in gut blood flow of 6%. Haemorrhage seemed to have less impact on gut blood flow than on femoral blood flow. In the H20 group flow was 72% (SD 8) of baseline at the end of haemorrhage and 60% (SD 14) of baseline in the H30 group. The addition of nerve stimulation to H20 made no real difference (flow of 77% baseline, SD 24). The addition of nerve stimulation had much more effect on H30 (flow fell to 45% of baseline, SD 19).

As with femoral blood flow there was some recovery of flow in the two haemorrhage alone groups during the shock period but not in the two HNS groups.
Reinfusion of shed blood restored flow to baseline levels or above. Flows remained above baseline in the two haemorrhage alone groups and the HNS20 group. Flow in the control group showed a gradual reduction to 89% of baseline (SD 23), while flow in NS reached 79% (SD 32), and in HNS30 reached 70% (SD 19) of baseline.

Figure 3.25: Graph of normalised gut blood flow data (%).



Graph shows an increase in gut blood flow with nerve stimulation in NS and HNS20. Gut blood flow decreased with haemorrhage but showed some recovery in H20 and H30. In these two groups and in HNS20 the gut showed relative hyperaemia following reinfusion of the shed blood.

Statistical analysis:

In contrast to femoral blood flow there was a group difference in the gut blood flows ($F = 3.57$, df 5, sig. of $F = 0.009$), Table 3.25. There was clearly a difference in flows over time, and a group by time interaction. The group by time interaction was the result of the haemorrhage effect, although the contrast by time for HNS20 against the control group did not reach statistical significance ($F = 2.02$, df 10, sig. of $F = 0.064$). Flows were significantly higher in HNS20 than in HNS30 and in H30 compared with HNS30.

Table 3.25: Repeated measures analysis of variance of normalised gut blood flow data.

Normalised gut blood flow RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	3.57	5	**0.009**
Time	11.62	10	**<0.0001**
Group by time	2.04	50	**<0.0001**
Contrasts			
Control vs NS	1.74	1	0.195
Contrast by time	1.48	10	0.191
Control vs H20	2.73	1	0.106
Contrast by time	2.26	10	**0.039**
Control vs H30	0.36	1	0.554
Contrast by time	3.00	10	**0.009**
Control vs HNS20	1.93	1	0.172
Contrast by time	2.02	10	0.064
Control vs HNS30	3.89	1	0.055
Contrast by time	3.89	10	**0.002**
H20 vs HNS20	0.01	1	0.922
Contrast by time	1.68	10	0.129
HNS20 vs HNS30	9.34	1	**0.004**
Contrast by time	2.46	10	**0.026**
H30 vs H20	1.29	1	0.2663
Contrast by time	1.06	10	0.418
H30 vs HNS30	6.87	1	**0.012**
Contrast by time	1.56	10	0.164

The main finding is a significant difference in the pairwise contrasts by time of the haemorrhage groups against the control group (as seen with the femoral blood flow), although for HNS20 the significance of F was 0.064.

HNS20 and HNS30 were different and had a different pattern. This did not hold for H20 and H30. There was a significant group difference between H30 and HNS30 but not a significant contrast by time. There were no statistically significant differences between H20 and HNS20.

Femoral vascular resistance

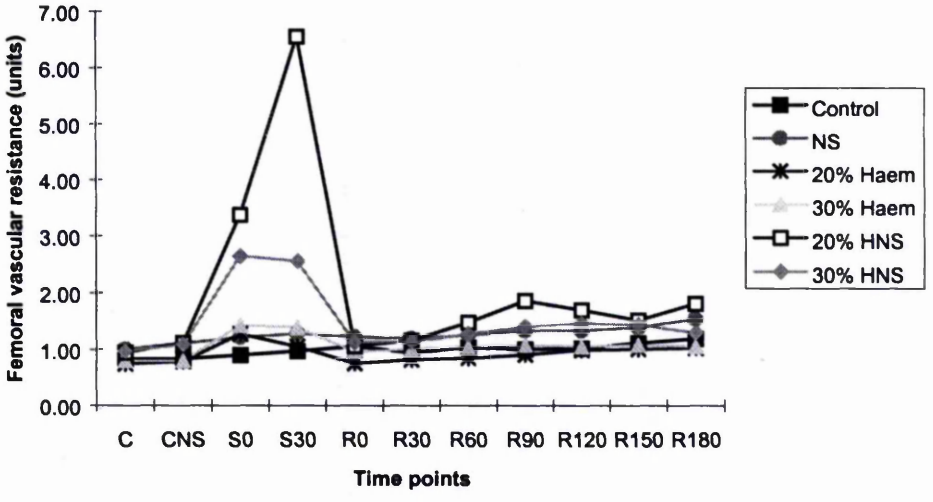
Vascular resistance was calculated as $R = \frac{MAP}{Flow}$

where Flow was expressed as the normalised flow. Thus vascular resistance is expressed in arbitrary units.

Femoral vascular resistance increased in all four haemorrhage groups following haemorrhage (Figures 3.26i and 3.26ii). There was a 202% increase in HNS20 at S0 (from CNS) and a 486% increase at S30 (from CNS). This huge increase reflects the fact that in two animals there was a massive reduction in femoral blood flow during the shock phase. Vascular resistance increased 143% in HNS30 at S0. In the two simple haemorrhage groups vascular resistance increased by 73% (H20) and 79% (H30). These increases in vascular resistance were reversed with reinfusion of the shed blood.

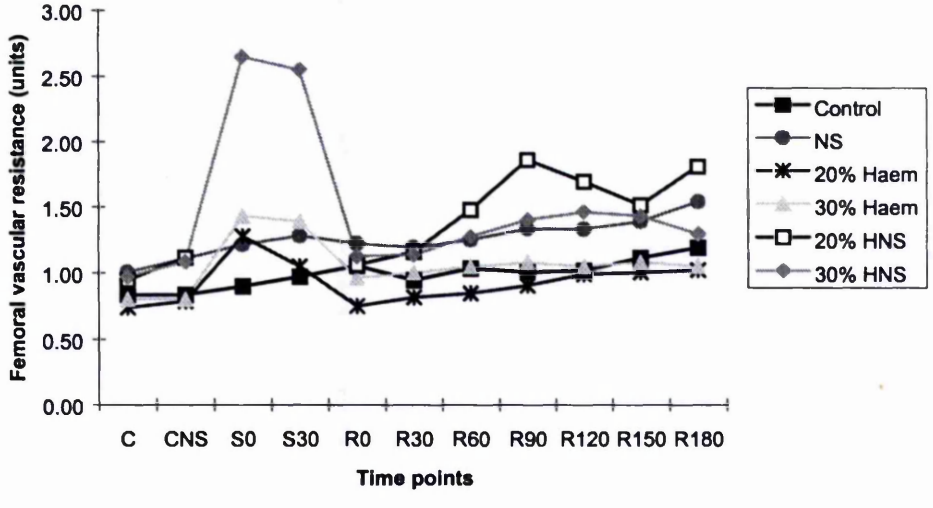
There was a general trend for an increase in vascular resistance in all the groups over the time course of the studies. Vascular resistance increased by 42% in the control group and by 52% in the nerve stimulation group.

Figure 3.26i: Graph of femoral vascular resistance (arbitrary units).



The graph shows increases in femoral vascular resistance in all the haemorrhage groups. These are especially apparent in the two HNS groups. The huge increase in resistance in HNS20 is because in two animals there was a great reduction in femoral blood flow during the shock phase. This distorts the scale of the graph. Careful examination reveals that there is also an increase in resistance in H20 and H30. This is shown more clearly in Figure 3.26ii.

Figure 3.26ii: Graph of femoral vascular resistance (arbitrary units).



In this graph the two HNS20 data points during the shock period have been omitted. With the large scale for the Y axis it is clear that there was an increase in vascular resistance in the two simple haemorrhage groups.

Statistical analysis:

There were significant main effects for groups and for time: that is, the two HNS groups were significantly different to the control group, and HNS20 and H20 were significantly different from each other, and there was a general increase in vascular resistance over time (Table 3.26).

HNS20 and HNS30 showed significant group by time interactions with the control group: that is, there was a pattern difference because in the HNS groups resistance increased in the shock phase. This was also the case for HNS20 and HNS30 because vascular resistance continued to increase in HNS20 in the shock phase but it remained almost constant in HNS30.

Table 3.26: Repeated measures analysis of variance of femoral vascular resistance.

Femoral vascular resistance RANOVA			
Main effect	**F**	**df**	**Sig of F**
Group	3.45	5	**0.01**
Time	6.07	10	**<0.0001**
Group by time	1.33	50	0.091
Contrasts			
Control vs NS	1.34	1	0.254
Contrast by time	0.47	10	0.901
Control vs H20	0.07	1	0.799
Contrast by time	0.71	10	0.712
Control vs H30	0.08	1	0.778
Contrast by time	0.84	10	0.589
Control vs HNS20	10.86	1	**0.002**
Contrast by time	3.16	10	**0.006**
Control vs HNS30	4.62	1	**0.037**
Contrast by time	2.49	10	**0.022**
H20 vs HNS20	10.79	1	**0.002**
Contrast by time	1.72	10	0.115
HNS20 vs HNS30	1.60	1	0.213
Contrast by time	2.12	10	**0.049**
H30 vs H20	0.26	1	0.614
Contrast by time	0.51	10	0.874
H30 vs HNS30	3.35	1	0.074
Contrast by time	1.61	10	0.143

The HNS20 and HNS30 groups were significantly different to C. HNS20 was significantly different to H20. The HNS20 and HNS30 groups showed a pattern difference with the control group. There was also a pattern difference between HNS20 and HNS30.

Gut vascular resistance

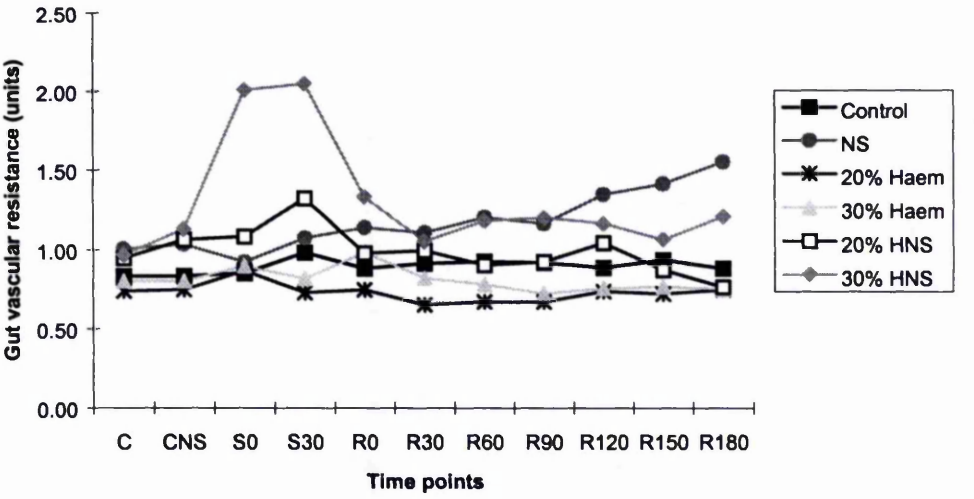
Vascular resistance was calculated as $R = \frac{MAP}{Flow}$

where Flow was expressed as the normalised flow. Thus vascular resistance is expressed in arbitrary units.

There were increases in gut vascular resistance during the shock phase in the two HNS groups (Figure 3.27). In contrast to the femoral bed there was no increase in gut vascular resistance during the shock phase in the two simple haemorrhage groups. There was no general trend for the resistance in the gut bed to increase over time except in the nerve stimulation group (resistance increased by 53%). This was the same as the increase in the femoral bed.

The greatest increase in resistance was seen in HNS30. Resistance had increased by 101% at S0 and by 111% at S30 before returning to near baseline with reinfusion of the shed blood. In HNS20 resistance increased by 39% at S30 and then returned to baseline.

Figure 3.27: Graph of gut vascular resistance (arbitrary units).



The graph shows a marked increase in gut vascular resistance in HNS30. There was also an increase in HNS20. There was no increase in the two simple haemorrhage groups. Gut vascular resistance gradually increased in NS.

Statistical analysis:

There were statistically significant differences between the groups ($F = 4.86$, df 5, sig. of $F = 0.001$) and there was a significant time effect ($F = 2.98$, df 10, sig. of $F = 0.009$), Table 3.27. Paired contrasts revealed statistically significant differences between HNS30 and the control group (sig. of $F = 0.002$), and H30 (sig. of $F < 0.001$), and HNS20 (sig. of $F = 0.011$), caused by the increase in resistance during the shock phase. This shock phase effect also resulted in significant group time interactions for these paired contrasts.

Table 3.27: Repeated measures analysis of variance of gut vascular resistance.

Gut vascular resistance RANOVA			
Main effect	**F**	**df**	**Sig of F**
Group	4.86	5	**0.001**
Time	2.98	10	**0.009**
Group by time	1.24	50	0.156
Contrasts			
Control vs NS	1.37	1	0.249
Contrast by time	0.75	10	0.670
Control vs H20	1.23	1	0.275
Contrast by time	0.412	10	0.931
Control vs H30	0.43	1	0.517
Contrast by time	0.70	10	0.717
Control vs HNS20	0.01	1	0.928
Contrast by time	0.68	10	0.735
Control vs HNS30	10.76	1	**0.002**
Contrast by time	3.82	10	**0.002**
H20 vs HNS20	1.08	1	0.305
Contrast by time	0.76	10	0.664
HNS20 vs HNS30	7.19	1	**0.011**
Contrast by time	2.73	10	**0.015**
H30 vs H20	0.27	1	0.604
Contrast by time	0.27	10	0.984
H30 vs HNS30	16.15	1	**<0.0001**
Contrast by time	3.13	10	**0.007**

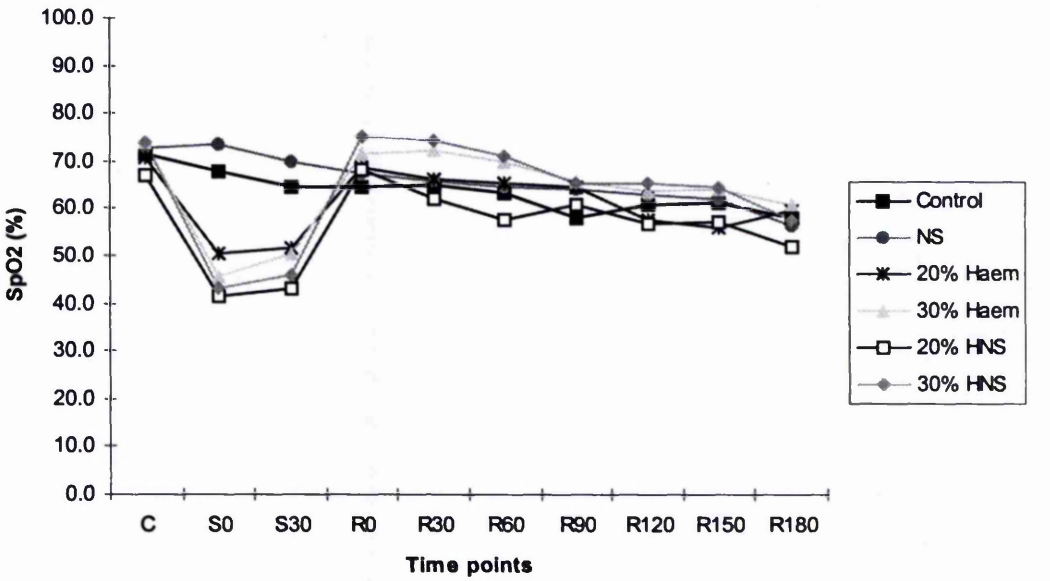
The HNS30 group was significantly different to C, H30, and HNS20. There were pattern differences between these groups.

Oxygen transport

Portal venous oxygen saturation

This showed the same pattern as the mixed venous oxygen saturation: a progressive reduction in the control and nerve stimulation groups with an additional reduction during the shock phase in the four haemorrhage groups (Figure 3.28). There was no deterioration in saturation in the shock phase in any of the haemorrhage groups, unlike SvO_2.

Figure 3.28: Graph of portal venous oxygen saturation data (%).



Graph shows the reduction in portal venous oxygen saturation with haemorrhage. There was a slight recovery during the shock phase. Saturation returned to baseline after reinfusion of shed blood.

Statistical analysis:

Although statistically there was no overall difference between the groups there was a significant group by time interaction and a significant change over time (Table 3.28). Contrasts were performed to investigate the origin of these differences. The four haemorrhage groups, with their decrease in venous oxygen saturation during the shock phase all had significant contrast by time interactions against the control group.

Significant pattern differences were also seen between H20 and HNS20 and between HNS20 and HNS30.

Table 3.28: Repeated measures analysis of variance of portal venous oxygen saturation (%) data.

SpO_2 RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	0.91	5	0.481
Time	22.00	9	**<0.0001**
Group by time	2.22	45	**<0.0001**
Contrasts			
Control vs NS	*0.24*	*1*	*0.626*
Contrast by time	*0.56*	*9*	*0.819*
Control vs H20	*0.39*	*1*	*0.535*
Contrast by time	*4.59*	*9*	***<0.0001***
Control vs H30	*0.06*	*1*	*0.811*
Contrast by time	*9.48*	*9*	***<0.0001***
Control vs HNS20	*2.51*	*1*	*0.120*
Contrast by time	*5.65*	*9*	***<0.0001***
Control vs HNS30	*0.06*	*1*	*0.814*
Contrast by time	*13.29*	*9*	***<0.0001***
H20 vs HNS20	*0.78*	*1*	*0.382*
Contrast by time	*2.29*	*9*	***0.037***
HNS20 vs HNS30	*1.77*	*1*	*0.189*
Contrast by time	*2.38*	*9*	***0.031***
H30 vs H20	*0.14*	*1*	*0.709*
Contrast by time	*1.88*	*9*	*0.089*
H30 vs HNS30	*<0.0001*	*1*	*0.991*
Contrast by time	*1.17*	*9*	*0.342*

Although the group differences were not statistically significant, there was a significant change over time, and the group by time interaction was significant.

Contrasts showed that there was no pattern difference between the control group and NS, and that all the haemorrhage groups differed from C. There were also pattern differences between the two levels of haemorrhage and nerve stimulation and between H20 and HNS20.

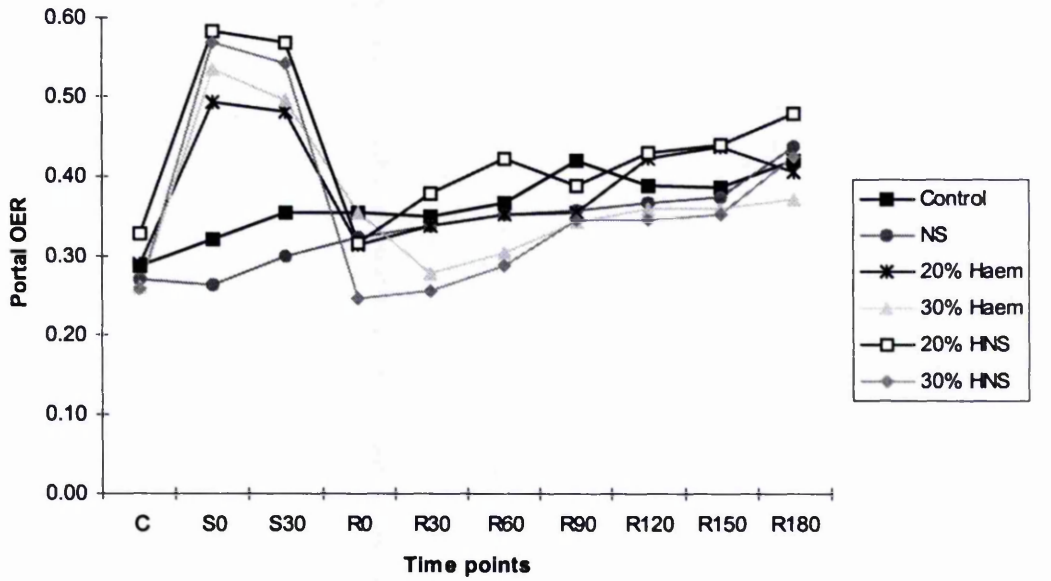
Portal oxygen extraction ratio

Portal oxygen extraction increased progressively in all groups over the course of the experiments (Figure 3.29). The four haemorrhage groups showed an additional increase with haemorrhage. Over the 30 minute shock period there was no further increase in portal oxygen extraction. Reinfusion of shed blood restored portal oxygen extraction ratios to those at baseline. Portal oxygen extraction then increased progressively until the end of the experiments, except in the H30 group in which it actually decreased from 0.35 (SD 0.24) at R0 to 0.28 (SD 0.07) at R30 and then started to increase.

Baseline portal oxygen extractions ranged from 0.26, SD 0.07, (H30 and HNS30) to 0.33, SD 0.10, (HNS20). The highest portal oxygen extraction ratio calculated was for the HNS20 group (0.58, SD 0.19), so that portal oxygen extractions were lower than systemic oxygen extractions (see Tables 18 and 29, Appendix).

At the end of the experiments portal oxygen extraction ratio ranged from 0.37, SD 0.04 (H30) to 0.48, SD 0.21 (HNS20).

Figure 3.29: Graph of portal oxygen extraction ratio data.



Graph shows the increase in OER during the haemorrhage phase. OER remained high during the shock period but returned to baseline with reinfusion of shed blood. There was an underlying increase in OER over the course of the experiments.

Statistical analysis:

There was no statistically significant overall group difference (F =1.10. df 5, sig. of F = 0.376) in oxygen extraction ratio (Table 3.29). There was a significant change with time (F = 20.70, df 9, sig. of F <0.0001), and there was a significant group by time interaction (F = 2.53, df 45, sig. of F = <0.0001). Simple contrasts were performed to verify which pairs accounted for the overall difference. Not surprisingly all the haemorrhage groups showed significant contrast by time interactions against the control group. There were also significant contrasts by time for H20 against HNS20, and for HNS20 against HNS30. The two non-haemorrhage groups did not show a significant contrast by time interaction.

Table 3.29: Repeated measures analysis of variance of portal oxygen extraction data.

Portal OER RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	1.10	5	0.376
Time	20.70	9	**<0.0001**
Group by time	2.53	45	**<0.0001**
Contrasts			
Control vs NS	*0.28*	*1*	*0.601*
Contrast by time	*0.59*	*9*	*0.795*
Control vs H20	*0.57*	*1*	*0.454*
Contrast by time	*5.34*	*9*	***<0.0001***
Control vs H30	*<0.000 1*	*1*	*0.969*
Contrast by time	*8.50*	*9*	***<0.0001***
Control vs HNS20	*2.98*	*1*	*0.092*
Contrast by time	*7.08*	*9*	***<0.0001***
Control vs HNS30	*0.0004*	*1*	*0.838*
Contrast by time	*13.18*	*9*	***<0.0001***
H20 vs HNS20	*0.75*	*1*	*0.391*
Contrast by time	*4.06*	*9*	***0.001***
HNS20 vs HNS30	*2.28*	*1*	*0.138*
Contrast by time	*2.95*	*9*	***0.011***
H30 vs H20	*0.44*	*1*	*0.509*
Contrast by time	*1.96*	*9*	*0.076*
H30 vs HNS30	*0.02*	*1*	*0.882*
Contrast by time	*0.86*	*9*	*0.564*

Differences between the groups did not reach statistical significance so it can be argued that further contrasts were inappropriate.

It is worth noting that contrasts by time were statistically significant because the haemorrhage groups differed from the control group. There was no significant difference between the control and NS groups, which did not experience blood loss.

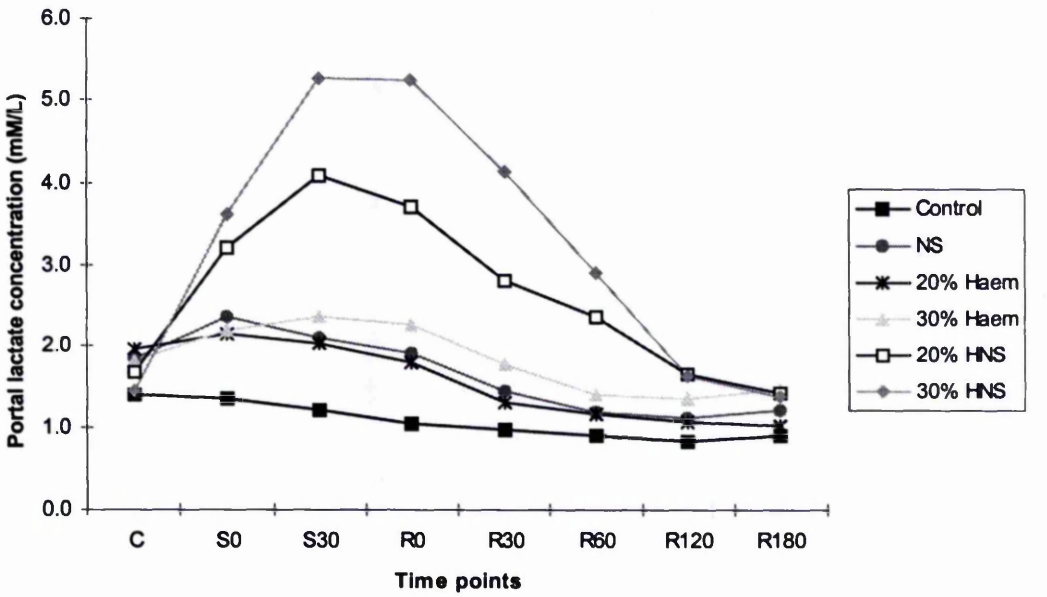
There were significant differences between some of the haemorrhage groups: H20 vs HNS20 and HNS20 vs HNS30.

Metabolic response

Portal lactate

The pattern was almost identical to that seen for arterial lactate: a tendency for all groups to show a lower lactate level at the end than at the beginning, and a large increase during the shock period in the two HNS groups (Figure 3.30).

Figure 3.30: Graph of portal lactate data (mM/L).



Graph shows the large increase in portal lactate seen in the haemorrhage and shock phases in the two HNS groups. Lactate concentrations returned to baseline two hours after reinfusion of the shed blood. There was a slight increase in lactate in H30 during the haemorrhage and shock phases.

Statistical analysis:

The reduced numbers and the high variability in peak lactate, especially in the HNS30 group (5.3 mM/L, SD 4.9) may explain why the group difference did not quite reach statistical significance ($F = 2.38$, df 5, sig. of $F = 0.055$), Table 3.30. The group by time interaction difference was not significant either ($F = 1.23$, df 35, sig. of $F = 0.187$).

Table 3.30: Repeated measures analysis of variance of portal lactate (mM/L) data.

Portal lactate RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	2.38	5	0.055
Time	10.94	7	**<0.0001**
Group by time	1.23	35	0.187
Contrasts			
Control vs NS	*0.66*	*1*	*0.420*
Contrast by time	*1.21*	*7*	*0.322*
Control vs H20	*0.48*	*1*	*0.494*
Contrast by time	*0.62*	*7*	*0.739*
Control vs H30	*1.32*	*1*	*0.258*
Contrast by time	*1.09*	*7*	*0.392*
Control vs HNS20	*5.17*	*1*	***0.028***
Contrast by time	*2.00*	*7*	*0.084*
Control vs HNS30	*9.14*	*1*	***0.004***
Contrast by time	*3.03*	*7*	***0.014***
H20 vs HNS20	*2.25*	*1*	*0.142*
Contrast by time	*0.69*	*7*	*0.679*
HNS20 vs HNS30	*0.32*	*1*	*0.574*
Contrast by time	*1.01*	*7*	*0.441*
H30 vs H20	*0.14*	*1*	*0.713*
Contrast by time	*0.58*	*7*	*0.767*
H30 vs HNS30	*3.65*	*1*	*0.063*
Contrast by time	*1.95*	*7*	*0.092*

The differences between the groups were not quite statistically significant (F = 2.38, df 5, sig. of F = 0.055) but there was a significant change over time.

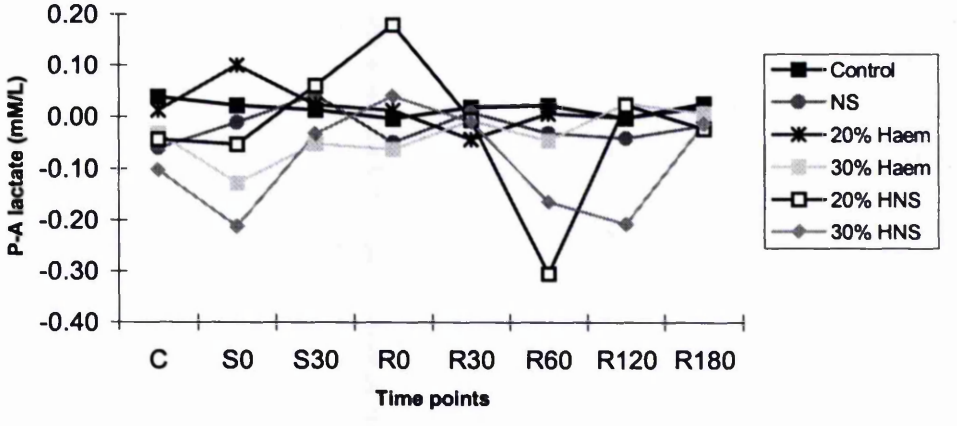
Contrasts showed that the two HNS groups were significantly different to the control group. There was a significant pattern difference between HNS30 and control.

Portal - arterial lactate

The difference in the two concentrations gives an indication of the flux of lactate across the gut (Figure 3.31). A positive difference would suggest that there was net production of lactate by the gut. A negative difference would suggest that there was net uptake or removal of lactate by the gut.

In the event portal - arterial lactate concentrations fluctuated around zero and there was no very clear trend.

Figure 3.31: Graph of portal - arterial lactate difference data (mM/L).



Graph shows there was very little difference between the portal and arterial lactate concentrations.

Statistical analysis:

The only overall difference to reach statistical significance was that in group by time interaction (Table 3.31). This is attributable to the effect of the HNS20 group, which showed a pattern significantly different to the control group, H20, and HNS30. There was also a significant pattern difference between H30 and HNS30.

Table 3.31: Repeated measures analysis of variance of portal - arterial lactate (mM/L) data.

P - A lactate RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	0.47	5	0.795
Time	2.09	7	0.076
Group by time	1.75	35	**0.011**
Contrasts			
Control vs NS	*0.17*	*1*	*0.684*
Contrast by time	*0.16*	*7*	*0.991*
Control vs H20	*<0.000 1*	*1*	*0.978*
Contrast by time	*0.29*	*7*	*0.950*
Control vs H30	*0.05*	*1*	*0.832*
Contrast by time	*0.38*	*7*	*0.908*
Control vs HNS20	*0.16*	*1*	*0.689*
Contrast by time	*8.06*	*7*	***<0.0001***
Control vs HNS30	*2.04*	*1*	*0.162*
Contrast by time	*2.29*	*7*	*0.055*
H20 vs HNS20	*0.11*	*1*	*0.739*
Contrast by time	*6.74*	*7*	***<0.0001***
HNS20 vs HNS30	*0.90*	*1*	*0.350*
Contrast by time	*6.72*	*7*	***<0.0001***
H30 vs H20	*0.03*	*1*	*0.873*
Contrast by time	*0.90*	*7*	*0.516*
H30 vs HNS30	*0.69*	*1*	*0.410*
Contrast by time	*2.59*	*7*	***0.033***

The differences between the groups and the change over time were not statistically significant but there was a significant group by time interaction.

Contrasts were performed to investigate this main effect. It appears that the HNS20 group was largely responsible: showing significant differences to the control group, H20, and HNS30. There was a significant pattern difference between H30 and HNS30.

Summary of regional responses

Cardiovascular

Loss of 30% of blood volume reduced femoral blood flow more than 20% blood loss. There was some recovery of flow in the haemorrhage alone groups, but not in HNS, during the shock period. There was no difference between the groups after reinfusion of shed blood.

There was a greater increase in femoral vascular resistance in HNS than in haemorrhage alone.

Gut blood flow was less sensitive to blood loss than femoral: gut vascular resistance increased in HNS but not in haemorrhage alone. Gut blood flow was better preserved in haemorrhage alone than in HNS. H30 had more effect than H20. With haemorrhage alone there was some recovery of flow during the shock phase. This was not seen in HNS. There was no difference between the groups after reinfusion of shed blood.

Oxygen transport

Portal venous oxygen saturation was reduced during the shock phase and then recovered after reinfusion of shed blood.

Oxygen extraction ratio increased in the four haemorrhage groups during the shock phase, returning to control levels after reinfusion of the shed blood.

Metabolic

Portal lactate showed a similar pattern to arterial lactate: that is, it increased significantly with HNS, but not with haemorrhage alone, during the shock phase and then returned to baseline during the observation period.

Endotoxin

Tables of all the group mean data can be found in Tables 32-34 in the Appendix.

Portal endotoxin

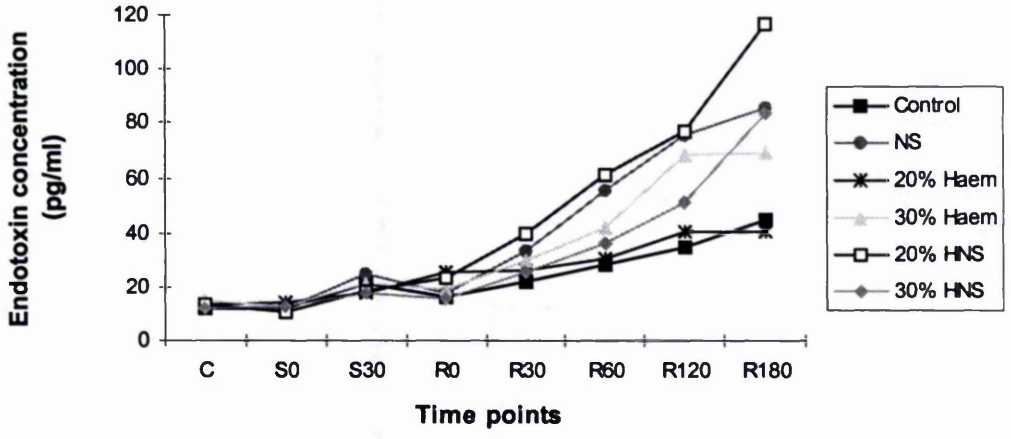
All groups showed a progressive increase in endotoxin concentration (Figure 3.32). Endotoxin was detectable from the outset in all groups (range 12-14 pg/ml). The detection threshold for the assay was 10 pg/ml. The heparinised saline we used contained 10-20 pg/ml endotoxin by this assay, so it is likely that the initial marginal level of endotoxin detected was the result of contamination of the cannula by the heparinised saline flush. The presence of detectable endotoxin contamination in drugs of biological origin has been previously reported (Haglind and Rylander 1993).

There was evidence of endotoxin translocation in the control group of animals. A mean endotoxin concentration of 12 pg/ml (SD 3) was detectable at the first sampling time. By the end of the observation period (R180) the mean plasma endotoxin had increased to 45 pg/ml (SD 20). Animals in the H20 group showed an almost identical increase in endotoxin concentration.

Loss of 30% of TBV resulted in an increase in endotoxin concentration from 15 pg/ml (SD 6) to 69 pg/ml (SD 32). Nerve stimulation alone also resulted in an increase in endotoxin concentration in the portal venous blood from a mean of 13 pg/ml (SD 3) to 85 pg/ml (SD 41) at R180.

The HNS20 group showed the greatest increase in portal endotoxin: from 14 pg/ml (SD 3) at control to 116 pg/ml (SD 47) at R180. In HNS30 the increase was from 12 pg/ml (SD 3) to 83 pg/ml (SD 42) at R180.

Figure 3.32: Graph of portal endotoxin concentration (pg/ml).



Graph shows an increase in portal endotoxin concentration in the observation period after reinfusion of shed blood. The greatest increases are in the two HNS groups, NS, and H30.

Statistical Analysis:

Analysis was conducted in two phases: one involving a summary measure of the response: the area under the curve (AUC), and the other, a repeated measures ANOVA (RANOVA) looking at all the data.

There were significant differences in AUC between the groups ($F = 4.80$, df 5, sig. of $F = 0.0011$), as shown in Table 32 in the Appendix. Further analysis using a Bonferroni test showed that the NS group had a significantly greater response than controls, and that the HNS20 group had a significantly greater response than the control group and the H20 group.

Table 3.32 shows the results of the RANOVA. There were significant differences in portal endotoxin levels between the groups ($F = 3.99$, df 5, sig. of $F = 0.004$) and a general change over time ($F = 28.14$, df 7, sig. of $F = <0.0001$). There was also a significant group by time interaction ($F = 1.11$, df 35, sig. of $F = 0.003$). This means that the groups changed over time in different ways.

The control group differed from the H30 group (F = 4.6, df 1, sig. of F = 0.037), the HNS20 group (F = 15.39, df 1, sig. of F = <0.0001), and the NS group (F = 9.41, df 1, sig. of F = 0.004). There was no statistically significant difference with the H20 group (F = 0.45, df 1, sig. of F = 0.507) or the HNS30 group (F = 2.65, df 1, sig. of F = 0.11).

Portal endotoxin concentrations were not statistically significantly different between the two haemorrhage groups (F = 1.42, df 1, sig. of F = 0.24). The HNS20 group had higher plasma endotoxin than the H20 group (F = 7.88, df 1, sig. of F = 0.007) and the HNS30 group (F = 6.13, df 1, sig. of F = 0.017). There was no significant difference between the H30 and HNS30 (F = 0.32, df 1, sig. of F = 0.572).

Only one of the simple contrasts showed a statistically significant time interaction: that between H20 and HNS20 (F = 2.46, df 1, sig. of F = 0.032).

In summary endotoxin levels changed with time and there were differences between the groups. Three groups differed significantly from the controls. There was no clear dose response at the two levels of haemorrhage. Only HNS20 showed increased endotoxin translocation with the combination of haemorrhage and nerve stimulation.

Table 3.32: Repeated measures analysis of variance of portal endotoxin data.

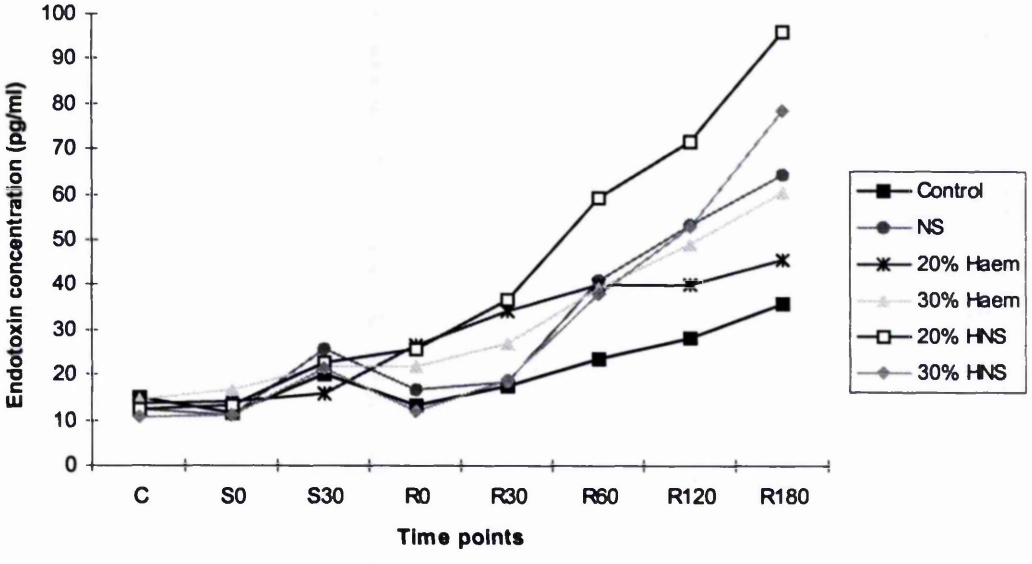
Portal endotoxin RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	3.99	5	**0.004**
Time	28.14	7	**<0.0001**
Group by time	1.11	35	**0.003**
Contrasts			
Control vs NS	9.41	1	**0.004**
Contrast by time	2.01	7	0.076
Control vs H20	0.45	1	0.507
Contrast by time	0.86	7	0.542
Control vs H30	4.6	1	**0.037**
Contrast by time	1.77	7	0.119
Control vs HNS20	15.39	1	**<0.0001**
Contrast by time	3.28	7	0.007
Control vs HNS30	2.65	1	0.11
Contrast by time	1.44	7	0.213
H20 vs HNS20	7.88	1	**0.007**
Contrast by time	2.46	7	**0.032**
HNS20 vs HNS30	6.13	1	**0.017**
Contrast by time	1.55	7	0.176
H30 vs H20	1.42	1	0.24
Contrast by time	1.11	7	0.375
H30 vs HNS30	0.32	1	0.572
Contrast by time	2.08	7	0.067

Arterial endotoxin

All groups showed an increase in endotoxin concentration over the course of the experiment (Figure 3.33). As with the portal samples, endotoxin was detectable in all control group animals. From an initial concentration of 15 pg/ml (SD 9) plasma endotoxin increased to 36 pg/ml (SD 23) at R180. The increase to 46 pg/ml (SD 20) in the H20 group was less than in the H30 group (to 61 pg/ml, SD 23). Nerve stimulation resulted in an increase from 13 pg/ml (SD 3) to 65 pg/ml (SD 24) at R180.

The combination of haemorrhage and nerve stimulation resulted in higher endotoxin concentrations than haemorrhage alone. In HNS30 endotoxin concentration reached 79 pg/ml (SD 47) at R180, while the highest concentration was seen in HNS20 (96 pg/ml, SD 48) at R180.

Figure 3.33: Graph of arterial (systemic) endotoxin concentration (pg/ml).



Graph shows an increase in arterial endotoxin concentration in all groups after reinfusion of shed blood. There was a small increase in endotoxin during the shock phase (as there was in the portal samples).

Statistical Analysis:

Area under the curve ANOVA showed significant differences between the groups (F = 4.19, df 5, sig of F = 0.003), as shown in Table 33 in the Appendix. The only significant post-hoc test was that between the control group and the HNS20 group (P = 0.001).

Table 3.33 shows the results of the RANOVA. Analysis of all the data revealed differences between the groups (F = 4.04, df = 5, sig of F = 0.004) and that there was a general change over time (F = 24.47, df = 7, sig of F = <0.0001). There was no significant group by time interaction, or pattern difference between the groups (F = 1.24, df 35, sig of F = 0.176).

All groups were significantly different to the control group but none of the comparisons between the haemorrhage groups and between the haemorrhage and the haemorrhage and nerve stimulation groups proved to be statistically significant. There was a significant difference between HNS20 and HNS30 (F = 5.89, df 1, sig of F = 0.019).

In summary arterial endotoxin levels changed with time and all the groups showed a significant difference to the control group.

Table 3.33: Repeated measures analysis of variance of arterial endotoxin data.

Arterial endotoxin RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	4.04	5	**0.004**
Time	24.47	7	**<0.0001**
Group by time	1.24	35	0.176
Contrasts			
Control vs NS	4.87	1	**0.032**
Contrast by time	1.63	7	0.155
Control vs H20	4.79	1	**0.034**
Contrast by time	1.43	7	0.221
Control vs H30	5.85	1	**0.02**
Contrast by time	1.45	7	0.212
Control vs HNS20	19.96	1	**<0.0001**
Contrast by time	3.28	7	**0.008**
Control vs HNS30	4.93	1	**0.031**
Contrast by time	1.65	7	0.15
H20 vs HNS20	2.78	1	0.102
Contrast by time	1.02	7	0.432
HNS20 vs HNS30	5.89	1	**0.019**
Contrast by time	0.949	7	0.481
H30 vs H20	0.04	1	0.842
Contrast by time	0.47	7	0.849
H30 vs HNS30	0.04	1	0.839
Contrast by time	0.61	7	0.747

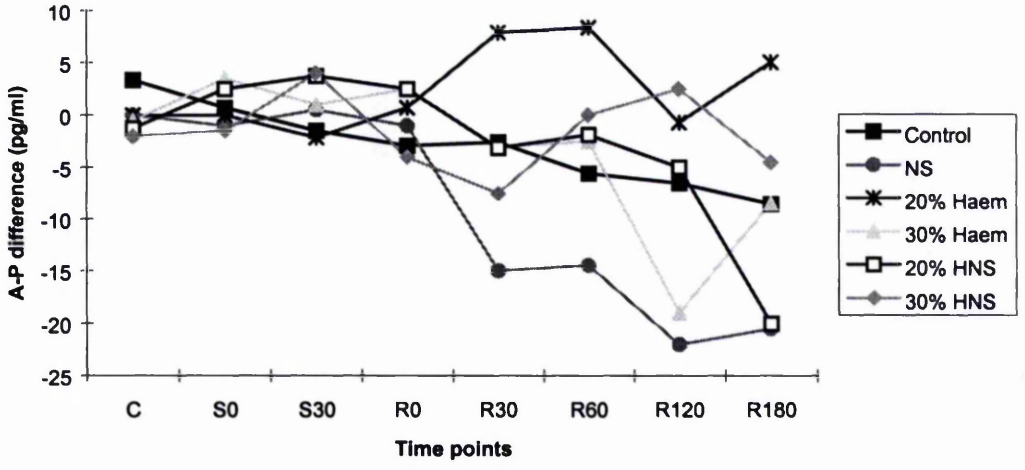
Arterial (systemic) - portal difference in endotoxin:

In all the groups the systemic - portal endotoxin differences were never greater than 25 pg/ml (Figure 3.34). From S30 onwards the control group showed a negative difference, suggesting clearance of endotoxin between the portal and the systemic circulation. The actual values were small (at R180 the difference was -9 pg/ml, SD 20), see Table 34, Appendix .

There was some fluctuation during the H20 studies with a positive difference at R30 (8 pg/ml, SD 17) and R60 (8 pg/ml, SD 8) no difference at S0 and a further positive difference at R180 (5 pg/ml, SD 22).

The H30 group showed a trend towards clearance of endotoxin: the difference was -19 pg/ml (SD 23) at R120 and -9 pg/ml (SD 21) at R180. There was clearance of endotoxin in the nerve stimulation group. There was also endotoxin clearance in HNS20 with a difference of -20 pg/ml (SD 55) at R180, although at S0, S30 and R0 the difference was positive (3 pg/ml (SD 5), 4 pg/ml (SD 12), and 3 pg/ml, SD 19). Although the difference at the end of the HNS30 studies was negative (-5 pg/ml, SD 39) it was positive at R120 (3 pg/ml, SD 12) and 0 at R60, suggesting that clearance may be a dynamic phenomenon.

Figure 3.34i: Graph of arterial - portal difference in endotoxin concentrations (pg/ml).



Graph shows fluctuations in the arterial (systemic) - portal difference. However statistical analysis revealed no significant change with time, between the groups, or between the group by time interactions.

The arterial (systemic) - portal endotoxin difference can be expressed as a percentage of the portal endotoxin level thus giving a percentage clearance. This has been calculated for all the groups at R180 and is shown in Table 3.34i:

Table 3.34i: The arterial (systemic) - portal endotoxin differences at the beginning and end of the experiments, expressed as a percentage of the portal endotoxin level, thus giving a percentage clearance.

	Percentage clearance	
	Control	R180
Control	20	-20
NS	0	-25
H20	0	12
H30	-7	-13
HNS20	-7	-17
HNS30	-17	-6

Statistical Analysis:

ANOVA of AUC showed that there was no significant difference between the groups (see Table 34 in the Appendix). RANOVA of all the data also showed no significant difference between the groups (Table 3.34ii). By the end of the studies all groups apart from H20 showed a negative arterial - portal difference suggesting that there was some clearance of endotoxin.

Table 3.34ii: Repeated measures analysis of variance of arterial - portal endotoxin data.

A - P endotoxin RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	1.18	5	0.333
Time	1.64	7	0.153
Group by time	1.03	35	0.439
Contrasts			
Control vs NS	1.29	1	0.262
Contrast by time	0.68	7	0.687
Control vs H20	1.88	1	0.178
Contrast by time	0.81	7	0.583
Control vs H30	<0.001	1	0.99
Contrast by time	1.01	7	0.439
Control vs HNS20	0.01	1	0.918
Contrast by time	0.25	7	0.298
Control vs HNS30	0.12	1	0.734
Contrast by time	1.11	7	0.373
H20 vs HNS20	1.57	1	0.217
Contrast by time	0.91	7	0.51
HNS20 vs HNS30	0.05	1	0.823
Contrast by time	0.599	7	0.753
H30 vs H20	1.92	1	0.173
Contrast by time	0.53	7	0.0806
H30 vs HNS30	0.11	1	0.736
Contrast by time	1.55	7	0.177

In summary there was no significant change in the arterial - portal endotoxin difference over the course of the studies and there was no significant difference between the groups or their group by time interactions.

Pearson correlation

A Pearson correlation was performed to test the validity of the null hypothesis that there was no relationship between the clearance of endotoxin (A - P concentration) and the portal load at the end of the experiment. A scatterplot of all the A - P endotoxin concentrations at R180 against all the portal R180 endotoxin concentrations suggested that there might in fact be a linear relationship between the two (Figure 3.34ii).

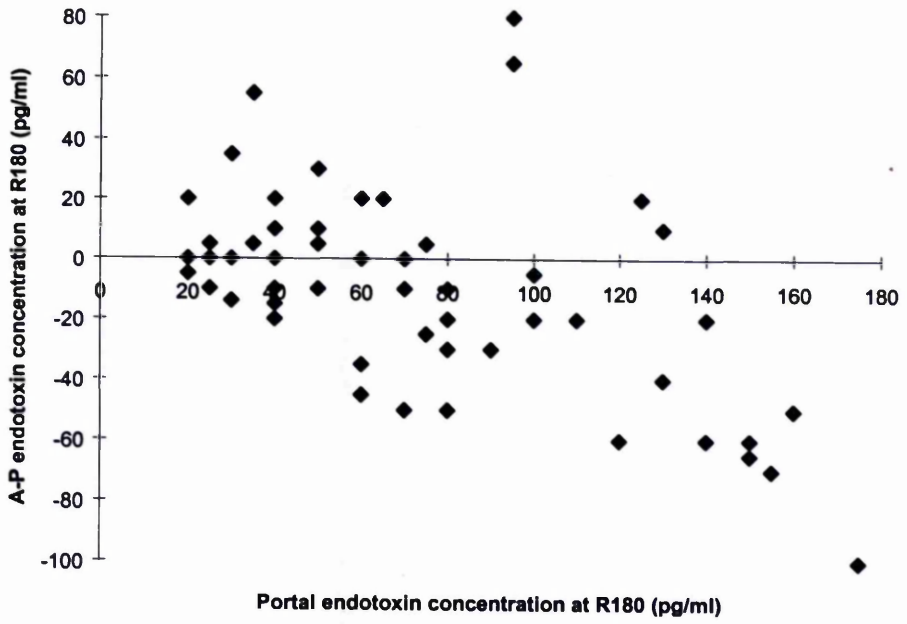
	Cases	Mean	Standard deviation
Portal conc. (pg/ml)	56	72.8	42.4
A - P conc. (pg/ml)	56	-9.9	33.3

Calculation of the Pearson **r** suggested that the null hypothesis was untenable and that there was in fact a linear relationship between clearance and load.

	Portal	**A - P**
Portal	1.0	-0.543
Cases	56	56
Significance	-	$p<0.0001$

These results suggest that there was no impairment of the liver's clearance of endotoxin even in those animals experiencing the greatest ischaemic insult and so the highest endotoxin loads.

Figure 3.34ii: Scatterplot of endotoxin "clearance" (arterial - portal concentration difference) in relation to the portal load (concentration).



A Pearson correlation revealed a Pearson **r** = -0.543, P<0.001 for "clearance" against portal load, suggesting that there was a relationship between "clearance" of endotoxin and the amount of endotoxin arriving from the gut.

Summary of endotoxin assays

Portal venous

Endotoxin levels showed a significant change with time: all groups showed an increase. There were significant differences between the groups. Endotoxin levels were higher in the NS, H30, and HNS20 groups than the controls. The HNS20 group also had significantly higher endotoxin levels than both the H20 and the HNS30 groups.

The only pattern difference was between HNS20 and H20.

Arterial (systemic)

There was a significant increase in endotoxin levels with time and all groups showed significantly higher levels than the control group. The only pattern difference was between the HNS20 group and the control group.

Arterial (systemic) - portal difference

This revealed no significant change with time, between the groups or between their group by time interactions. There may be a relationship between the "clearance" of endotoxin and the amount of endotoxin appearing in the portal circulation.

Inflammatory cytokines

Tumour necrosis factor-α

Tables of all the group mean data can be found in Tables 35-37 in the Appendix.

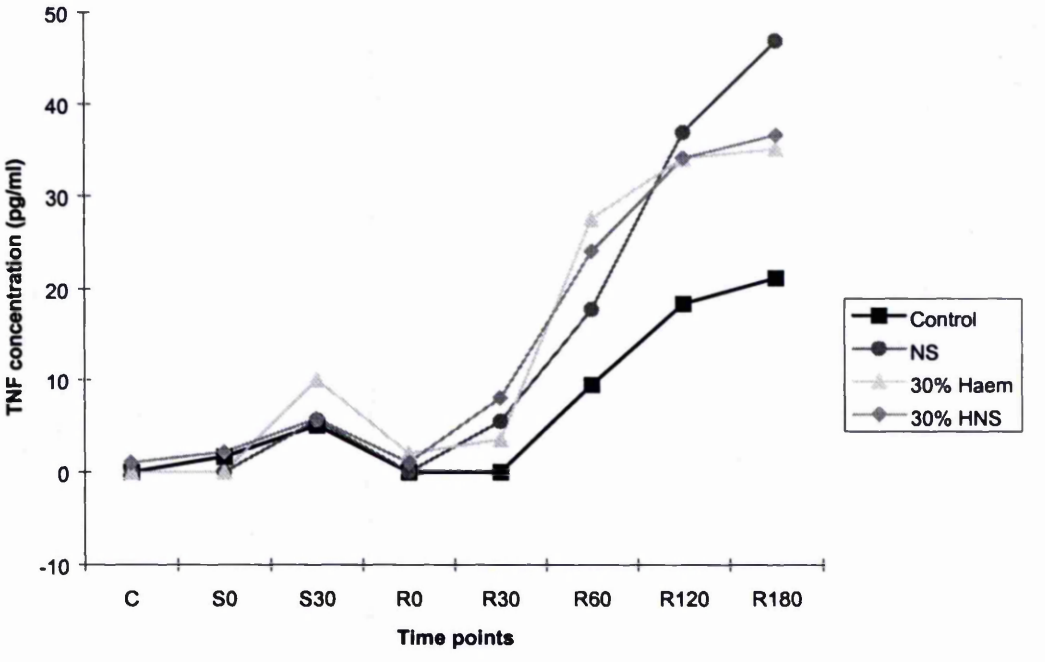
Portal TNF-α

Financial restrictions meant that no TNF-α assays were done in the two groups subjected to 20% haemorrhage. The results of the assays done in the four other groups are shown in Figure 3.35.

Tumour necrosis factor-α was undetectable throughout the study in one of the control animals. Apart from this exception TNF-α was detectable from R60, increasing to a mean of 21 pg/ml (SD 21) at R180. One of the NS animals showed no TNF-α response at all. At R30 TNF-α was only detected in two NS animals but at succeeding time points it became detectable in more and more animals to reach a mean peak concentration of 47 pg/ml (SD 28) at R180.

Haemorrhage of 30% TBV resulted in an increase in plasma TNF-α so that at R120 the mean concentration had reached 34 pg/ml (SD 9). TNF-α seemed to reach a plateau as at R180 the concentration was 35 pg/ml (SD 15). The addition of nerve stimulation to H30 did nor appear to make any difference as TNF-α concentration was 37 pg/ml (SD 20) at R180.

Figure 3.35: Graph of portal TNF-α concentration (pg/ml).



Graph shows an increase in TNF-α concentration in all groups. The increase was significantly greater in the three insult groups than the controls.

Statistical analysis:

Area under the curve was used as a summary measure of the overall TNF-α response. There was a statistically significant difference between the groups (F = 3.23, df 3, sig. of F = 0.034), see Table 35 in the Appendix. Multiple comparisons were performed using the Bonferroni test to identify differences between pairs of groups. This showed that the TNF-α response was significantly greater in the HNS30 group than in the control group. None of the other contrasts reached statistical significance.

Analysis of the full data set using RANOVA showed that the groups were significantly different (F = 3.91, df 3, sig. of F = 0.022) and that there was a significant change over time (F = 13.48, df 7, sig. of F = <0.0001), Table 3.35. There was a significant group by time interaction, meaning that the pattern of TNF-α increase was significantly different between the groups (F = 1.97, df 21, sig. of F = 0.023).

All the groups differed from the control group and all but the HNS30 group had significantly different contrast by time interactions to the control group: the shape of their curves was significantly different.

There was no difference between H30 and HNS30, nor was there a significant contrast by time interaction. This suggests that the addition of the nerve stimulation insult did not result in a greater TNF-α response than in haemorrhage alone.

In summary all groups showed an increase in TNF-α, which was significantly different to the control group. The pattern of TNF-α increase was different from the control group in all but the HNS30 group.

Table 3.35: Repeated measures analysis of variance of portal TNF-α data.

Portal TNF-α RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	3.91	3	**0.022**
Time	13.48	7	**<0.0001**
Group by time	1.97	21	**0.023**
Contrasts			
Control vs NS	9.73	1	**0.005**
Contrast by time	5.1	7	**0.003**
Control vs H30	7.4	1	**0.012**
Contrast by time	3.4	7	**0.019**
Control vs HNS30	6.49	1	**0.018**
Contrast by time	1.27	7	0.322
H30 vs HNS30	0.15	1	0.698
Contrast by time	2.12	7	0.098

Arterial TNF-α

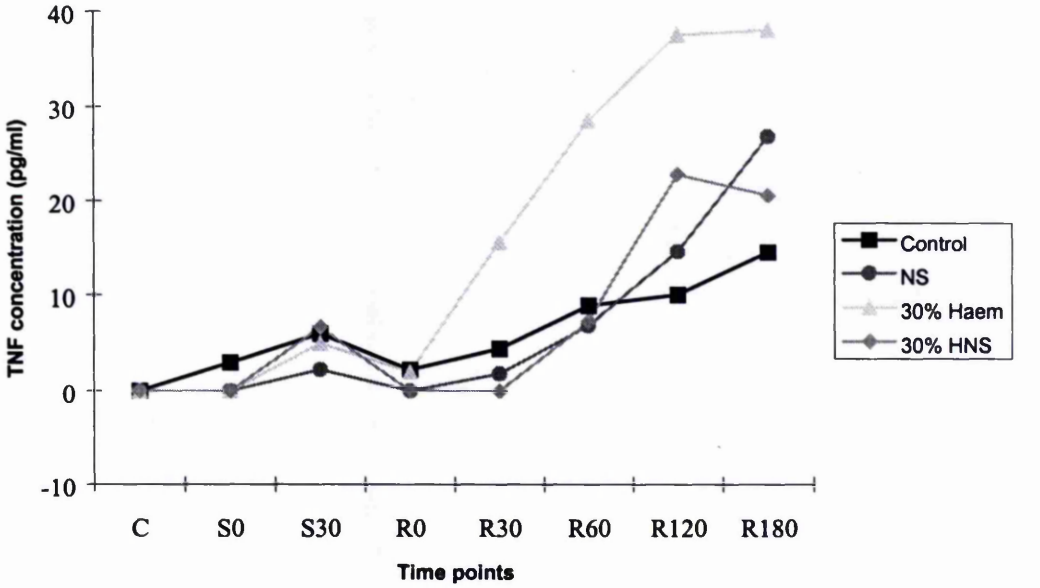
Arterial TNF-α concentrations are shown in Figure 3.36.

TNF-α was undetectable in five of the nine control studies, so the mean concentration at R180 only reached 14 pg/ml (SD 21). The TNF-α response tended to be late in the NS group. Only four animals had detectable TNF-α at R120 (mean 15 pg/ml, SD 20) but at R180 TNF-α was detectable in nine animals, giving a mean TNF-α concentration of 27 pg/ml (SD 16). The portal concentration at R180 was 47 pg/ml (SD 28).

There was a much more rapid increase in TNF-α concentration in H30: TNF-α appeared at S30 in two animals, in six animals at R30, and in all but one at R60. In one animal TNF-α was only detected at R180 and then only at a concentration of 20 pg/ml. By R180 mean TNF-α concentration was 38 pg/ml (SD 13), slightly higher than portal (35 pg/ml). In the HNS30 group assays could only be performed in nine animals and two showed no TNF-α response. TNF-α was present in all the others at R120 (mean 23 pg/ml, SD 15) and R180 (mean 21 pg/ml, SD 13).

The apparently large difference between H30 and HNS30 was caused by a combination of the rapid increase in TNF-α in H30 and the fact that two animals in the HNS30 group showed no TNF-α production at all. This illustrates the problem of variability in physiological responses in large animal models. This was apparent throughout the study.

Figure 3.36: Graph of arterial (systemic) TNF-α concentration (pg/ml).



Graph shows an increase in TNF-α concentration in all the groups. The difference between the groups did not reach statistical significance (sig. of F = 0.07).

Statistical Analysis:

Analysis of AUC showed that there was a statistically significant difference between the groups (F = 5.69, df 3, sig. of F = 0.0028), see Table 36 in the Appendix. Multiple comparisons were performed using the Bonferroni test to identify differences between pairs of groups. This showed that the TNF-α response was greater in the H30 group than in all the other groups.

When a repeated measures ANOVA was performed the groups were not statistically significantly different (F = 2.72, df 3, sig. of F = 0.07) but there was a significant change over time (F = 36.11, df 7, sig. of F = <0.0001) and a significant group by time interaction (F = 2.32, df 21, sig. of F = 0.045), Table 3.36.

None of the groups were significantly different to the control group in the simple contrast but the H30 group had a significant contrast by time interaction with the control group (F = 3.82, df 1, sig. of F = 0.026). The H30 group was significantly different to

the HNS30 group (F = 6.63, df 1, sig. of F = 0.018). Their contrast by time interactions were also significant (F = 4.09, df 7, sig. of F = 0.021)

In summary, although there was a significant increase in arterial TNF-α with time the difference between the groups was characterised by a significance of F of only 0.07. The highest levels were seen in the H30 group. There was a pattern difference between H30 and controls and between H30 and HNS30.

Table 3.36: Repeated measures analysis of variance of arterial TNF-α data.

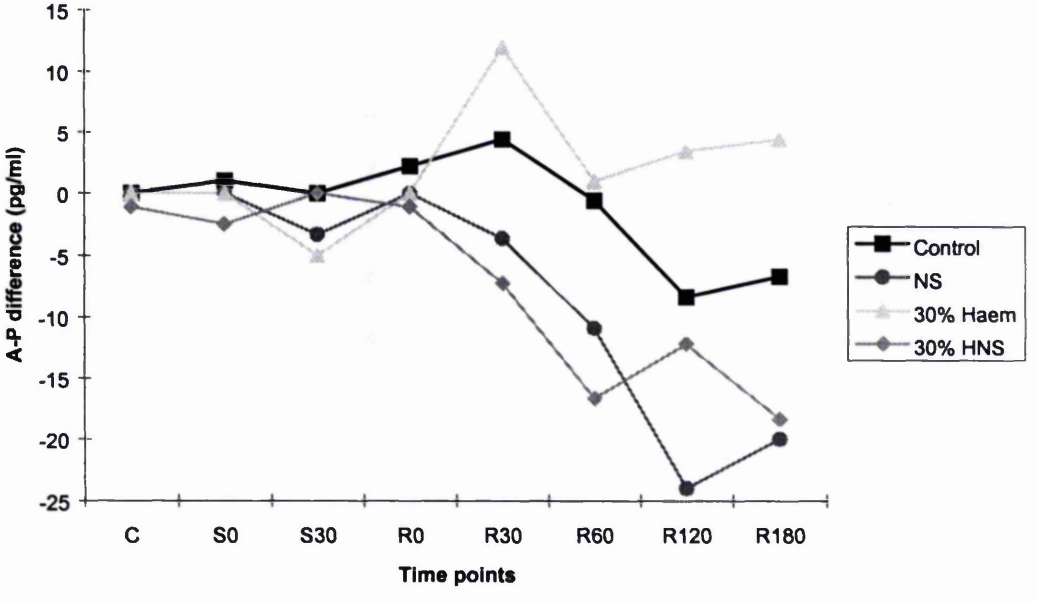
Arterial TNF-α RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	2.72	3	0.07
Time	36.11	2.16	**<0.0001**
Group by time	2.32	6.49	**0.045**
Contrasts			
Control vs NS	<0.001	1	0.996
Contrast by time	1.3	2.16	0.285
Control vs H30	4.2	1	0.053
Contrast by time	3.82	2.16	**0.026**
Control vs HNS30	0.17	1	0.688
Contrast by time	1.06	2.16	0.359
H30 vs HNS30	6.63	1	**0.018**
Contrast by time	4.09	2.16	**0.021**

Arterial (systemic) - portal TNF-α difference

This gives an indication of whether TNF-α is being taken up between the portal circulation and the systemic circulation. If there is an increase this suggests that TNF-α is being produced not only in the GALT but in other parts of the RE system such as the liver or in the lungs.

In the control group there was a negative systemic - portal difference from R60 onwards, although by R180 it had only reached 7 pg/ml (Figure 3.37i). In both the NS and the HNS30 groups there was a net negative difference, suggesting uptake of TNF-α. For NS this reached -20 pg/ml (SD 20) at R180. For HNS30 this reached -18 pg/ml (SD 21) at R180. After a brief positive difference at R30 (12 pg/ml, SD 16) the difference in the H30 group then returned close to zero (4 pg/ml, SD 12) at R180.

Figure 3.37i: Graph of arterial (systemic) - portal TNF-α concentration difference (pg/ml).



Graph shows fluctuations in the systemic - portal TNF-α concentration difference. Although there were statistically significant differences between the groups there was no overall change with time. The group by time interactions were not significantly different.

Statistical Analysis:

RANOVA showed that the groups were significantly different (F = 5.59, df 3, sig. of F = 0.006), but there was no general change with time and there was no group by time interaction (Table 3.37). Both the NS and the HNS30 groups were significantly different from the control group (F = 11.87, df 1, sig. of F = 0.003, and F = 9.29, df 1, sig. of F = 0.006, respectively), although the contrast by time interactions were not significant. There was a significant difference between the H30 group and the HNS30 group (F = 4.9, df 1, sig of F = 0.039) but the contrast by time interaction was not significant.

In summary, there were significant group differences between the NS group and the HNS30 and control groups and also between the H30 and the HNS30 groups. There was no significant difference in the group patterns and no significant change with time.

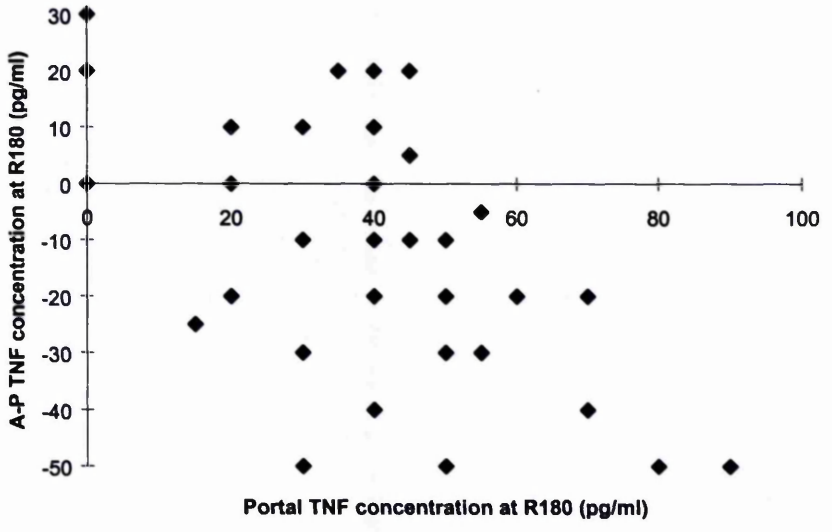
Table 3.37: Repeated measures analysis of variance of arterial-portal TNF- α data.

A-P TNF-α RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	5.59	3	**0.006**
Time	1.57	7	0.225
Group by time	1.39	21	0.172
Contrasts			
Control vs NS	11.87	1	**0.003**
Contrast by time	1.38	7	0.287
Control vs H30	0.64	1	0.433
Contrast by time	0.53	7	0.797
Control vs HNS30	9.29	1	**0.006**
Contrast by time	2.62	7	0.059
H30 vs HNS30	4.9	1	**0.039**
Contrast by time	2.14	7	0.107

Pearson correlation

A scatterplot of TNF-α clearance (A-P concentration) suggested that there might be a linear relationship between clearance and load (Figure 3.37ii). To test the null hypothesis that there was no such relationship a Pearson **r** correlation was performed.

Figure 3.37ii: Scatterplot of TNF-α "clearance" (arterial - portal concentration difference) in relation to the portal load (concentration).



A Pearson correlation revealed a Pearson **r** = -0.545, P<0.001 for "clearance" against portal load. Suggesting that there was a relationship between "clearance" of TNF-α and the amount of TNF-α arriving from the gut.

	Cases	Mean	Standard deviation
Portal conc. (pg/ml)	38	36.4	22.5
A - P conc. (pg/ml)	38	-10.6	21.9

	Portal	**A - P**
Portal	1.0	-0.545
Cases	38	38
Significance	-	p<0.001

The Pearson **r** of -0.545 suggested that the null hypothesis was untenable and that there was a linear relationship between the clearance of TNF-α and the load arriving from the portal circulation.

Interleukin-6:

Tables of all the group mean data can be found in Tables 38-40 in the Appendix.

Portal IL-6

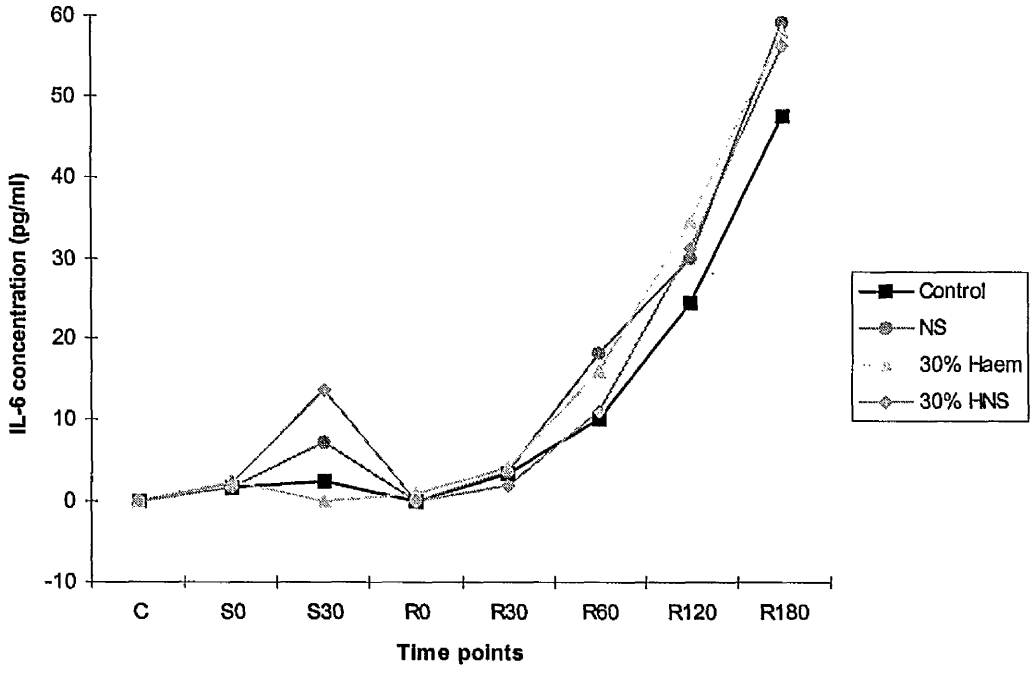
In all the groups there was a transient detection of IL-6 during the shock phase (Figure 3.38). Apart from the haemorrhage alone group all the other groups showed undetectable IL-6 at R0.

In the control group IL-6 appeared at R30 and then showed a progressive rise to reach 47 pg/ml (SD 25) at R180. One animal failed to show any IL-6 response. In the NS group one animal failed to produce IL-6. By R180 mean IL-6 concentration had reached 59 pg/ml (SD 37).

With 30% TBV haemorrhage IL-6 appeared at the same time as controls, although one animal had detectable IL-6 at R0. All showed a response by R120 (mean concentration: 35 pg./ml, SD 15) and at R180 the concentration had risen to 58 pg/ml (SD 20).

A number of problems were encountered in the HNS30 group so that assays were not done in two animals because of insufficient plasma. In another animal IL-6 was undetectable and in another it was only detected at S30 (20 pg/ml). In those animals in which IL-6 was detected the pattern of increase in plasma IL-6 seemed to be the same as for the other groups so that by R180 the mean concentration for the group was 56 pg/ml (SD 30).

Figure 3.38: Graph of portal IL-6 concentration (pg/ml).



Graph shows the increase in IL-6 over the course of the experiments. There was no difference between the groups.

Statistical Analysis:

One-way ANOVA of IL-6 AUC revealed no differences between the groups (Table 38 in the Appendix). Repeated measures ANOVA confirmed that the groups were not statistically significantly different ($F = 0.36$, df 3, sig. of $F = 0.783$) but there was a significant change over time ($F = 48.51$, df 7, sig. of $F = <0.0001$), Table 3.38. The group by time interaction was not significant ($F = 0.67$, df 21, sig. of $F = 0.662$). IL-6 production followed a similar pattern in all the groups.

Simple contrasts were not performed as there was no group difference.

In summary, there was no significant difference between the groups or their patterns (group by time interaction) but there was clearly an increase with time.

Table 3.38: Repeated measures analysis of variance of portal IL-6 data.

Portal IL-6 RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	0.36	3	0.783
Time	48.51	1.84	**<0.0001**
Group by time	0.67	5.52	0.662

Contrasts were not performed as there was no main effect for the groups at the initial RANOVA.

Arterial IL-6:

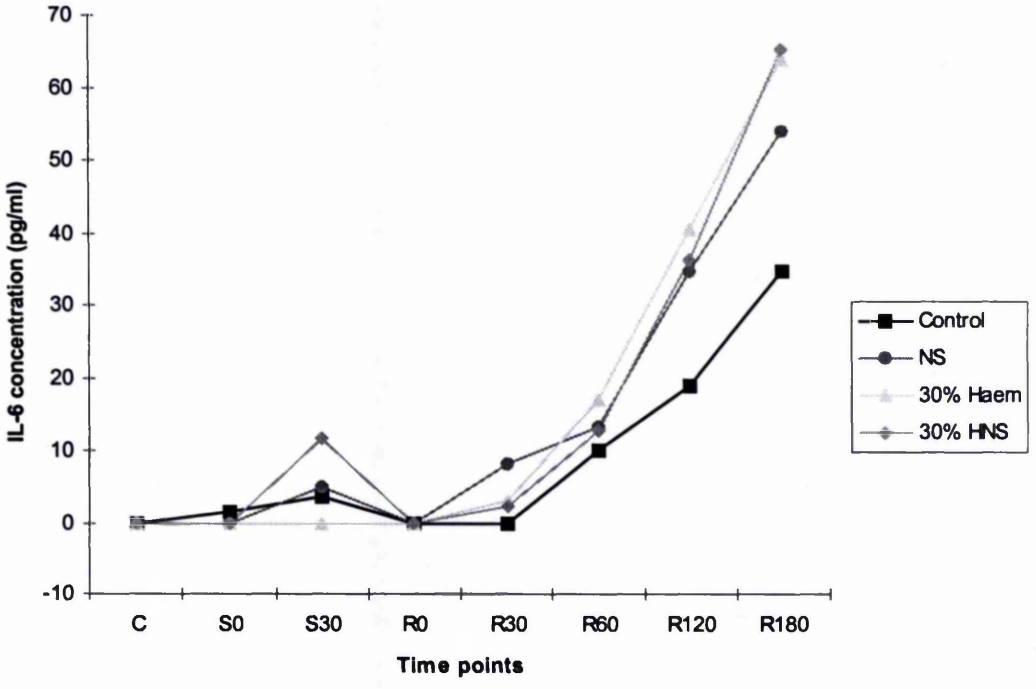
In the control group two animals showed no IL-6 response and in one IL-6 was only detectable at R180 (30 pg/ml). Apart from two animals showing some detectable IL-6 at S0 and S30, IL-6 only became detectable at R60 (10 pg/ml, SD 12). There was a gradual increase in level to 19 pg/ml (SD 20) at R120 and to 34 pg/ml (SD 23) at R180 (Figure 3.39).

In the NS group IL-6 was undetectable throughout the study in one animal. At S30 two had transiently detectable IL-6 and at R30 three animals had detectable IL-6. By R180 IL-6 was detectable in all but one of the animals resulting in a mean concentration of 54 pg/ml (SD 49).

With H30 IL-6 was undetectable during the shock period. In one animal IL-6 appeared at R30 (30 pg/ml), but as with controls most showed a response at R60 (mean for the whole group 17 pg/ml, SD 18). All showed a response at R120 (mean 41 pg/ml, SD 23) and a gradual increase to 64 pg/ml (SD 22) at R180.

Two of the HNS30 group were not assayed because of insufficient plasma. All the others had detectable IL-6 at R120 (mean 36 pg/ml, SD 18) and R180 (mean 65 pg/ml, SD 20).

Figure 3.39: Graph of arterial (systemic) IL-6 concentration (pg/ml).



Graph shows the increase in IL-6 over the course of the experiments. There was no difference between the groups.

Statistical Analysis:

One-way ANOVA of IL-6 AUC revealed no differences between the groups (Table 39 in the Appendix). Repeated measures ANOVA confirmed that the groups were not statistically significantly different ($F = 1.46$, df 3, sig. of $F = 0.7251$) but there was a significant change over time ($F = 42.05$, df 7, sig. of $F = <0.0001$), Table 3.39. The group by time interactions were not significantly different ($F = 1.52$, df 21, sig. of $F = 0.207$). Thus the pattern of IL-6 production was similar in all the groups.

Simple contrasts were not performed as there was no group difference.

In summary, all groups showed a similar increase with time but there was no significant difference between them.

Table 3.39: Repeated measures analysis of variance of arterial IL-6 data.

Arterial IL-6 RANOVA			
Main effects	**F**	**df**	**Sig of F**
Group	1.46	3	0.251
Time	42.05	1.63	**<0.0001**
Group by time	1.52	4.90	0.207

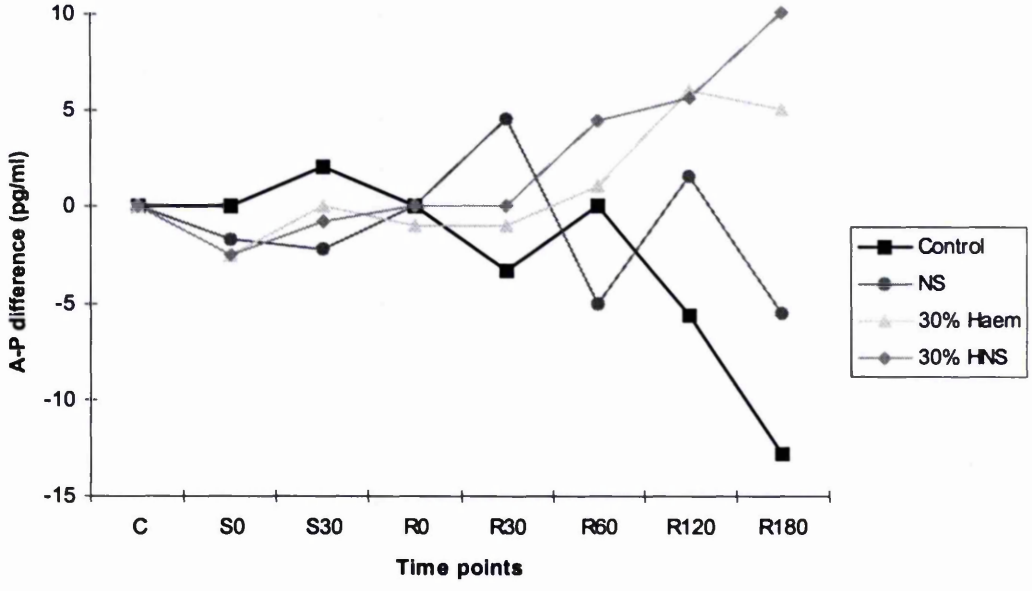
Contrasts were not performed as there was no main effect for the groups at the initial RANOVA.

Arterial (systemic) - portal IL-6 difference:

In the control group there was net uptake of IL-6, that is, a negative systemic - portal IL-6 difference (Figure 3.40I). However by R180 the systemic - portal difference was only -13 pg/ml (SD 23). For the NS group systemic - portal difference fluctuated around zero: at R0 it was 0; at R30 5 pg/ml (SD 19); at R60 -5 pg/ml (SD 23); at R120 2 pg/ml (SD 29); at R180 -5 pg/ml (SD 20).

In contrast in the H30 group arterial levels exceeded portal levels but by R180 the difference was only 5 pg/ml (SD 20). Arterial concentrations exceeded portal in the HNS30 group also. From R60 there was a positive systemic - portal difference (4 pg/ml, SD 25) suggesting additional production of IL-6. This increased to 10 pg/ml (SD 40) at R180.

Figure 3.40i: Graph of arterial (systemic) - portal IL-6 concentration difference (pg/ml).



Graph shows fluctuations in IL-6 concentration difference. There was no statistically significant difference between the groups and no significant change with time.

Statistical analysis:

Repeated measures ANOVA performed on the whole data set showed no significant differences between the groups, no group by time interaction, and no change over time (Table 3.40). Simple contrasts were not performed.

Table 3.40: Repeated measures analysis of variance of arterial-portal IL-6 data.

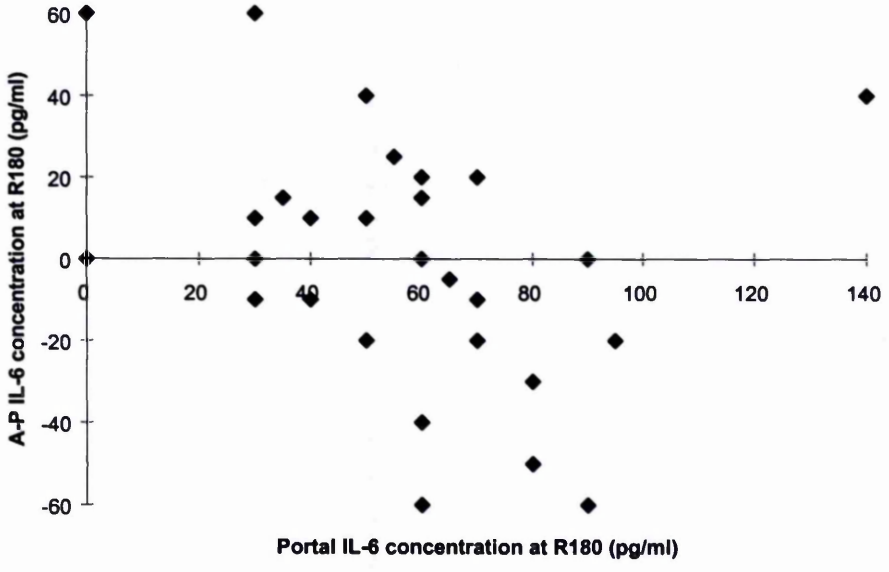
A-P IL-6			
Main effects	**F**	**df**	**Sig of F**
Group	0.3	3	0.825
Time	0.35	2.17	0.722
Group by time	0.47	6.52	0.837

In summary, there was no significant change in the arterial - portal IL-6 difference with time, and no difference between the groups or their group by time interactions.

Pearson correlation

The scatterplot of A - P IL-6 concentration at R180 against portal concentration at the same time seemed to show a relationship between clearance and the load (Figure 3.40ii). To verify the null hypothesis that there was no such relationship a Pearson correlation was performed.

Figure 3.40ii: Scatterplot of IL-6 "clearance" (arterial - portal concentration difference) in relation to the portal load (concentration).



A Pearson correlation revealed a Pearson **r** = -0.291, P = 0.081 for "clearance" against portal load. It seemed that there was no relationship between "clearance" of IL-6 and the amount of IL-6 arriving from the gut. Without the "outlier" at 140,40 the Pearson **r** = -0.496, P = 0.002!

	Cases	Mean	Standard deviation
Portal conc. (pg/ml)	37	55.1	28.8
A - P conc. (pg/ml)	37	-1.3	27.7

	Portal	**A - P**
Portal	1.0	-0.291
Cases	37	37
Significance	-	p=0.081

The Pearson **r** of -0.291 suggested that the null hypothesis was valid. This low value for **r** compared to both endotoxin and TNF-α may be due to the presence of one outlier with a portal IL-6 of 140 and a clearance of 40! Without this outlier the results are rather different:

	Cases	Mean	Standard deviation
Portal conc. (pg/ml)	36	52.8	25.4
A - P conc. (pg/ml)	36	-2.5	27.2

	Portal	**A - P**
Portal	1.0	-0.496
Cases	36	36
Significance	-	p=0.002

This suggests that as with endotoxin and TNF-α there is increased clearance in response to an increase in the load. Thus for the whole study population the reticulo-endothelial system appears to be able to respond to changes in endotoxin and the two inflammatory cytokines in the same way.

Summary of inflammatory cytokine assays

Tumour necrosis factor α

Portal venous

All the groups showed a significantly greater increase in TNF-α than the control group. The pattern of increase in the NS and the H30 groups was different to that in the control group.

Arterial (systemic)

Although the difference between the groups only reached a significance of F of 0.07 there was a significant increase in TNF-α with time. The H30 group showed the greatest response.

Arterial (systemic) - portal difference

There were significant differences between the groups (NS and HNS30 against the control group, and H30 against HNS30) but no overall change with time or in pattern.

Interleukin-6

Portal venous

All the groups showed a similar pattern of increase in IL-6 but there was no significant difference between them.

Arterial (systemic)

All the groups showed a similar pattern of increase in IL-6 but again there was no significant difference between them.

Arterial (systemic) - portal difference

There was no significant difference between the groups and no significant change with time.

Microbiology results

The results of the tissue and blood cultures will be presented by experimental group. No tissue or blood cultures were taken from the H20 group and from some of the early studies in the other groups because of difficulties in finding someone to do them, when the student, for whom this had been arranged as a degree project, pulled out. Table 3.41 shows all the results.

Control group:

There was no bacterial growth (NBG) from either of the lymph nodes or from the spleen. Coagulase negative Staphylococci were grown from half of the liver biopsies (NBG from the remainder). Coliforms were present in half of the small bowel biopsies and in three quarters of the large bowel biopsies. There was no bacterial growth from any of the blood cultures.

Coagulase negative Staphylococci (CNS) are regarded as contaminants (Turnbull et al. 1995) so there was no evidence of any bacterial translocation to either the circulation or the reticulo-endothelial system, despite the presence of potential pathogens in most of the animals (Gelfand et al. 1991).

Nerve stimulation group:

No organisms were grown from any of the reticulo-endothelial system tissue biopsies, but all the bowel biopsies grew coliforms. All the blood cultures were negative apart from contamination of one control blood sample with a species of Flavobacterium.

30% haemorrhage group:

No organisms were grown from either the lymph nodes or the spleen. Only one of the liver biopsies grew anything (a species of Flavobacterium). All of the large bowel and two thirds of the small bowel biopsies grew coliforms. There was NBG from any of the control blood samples but one of the R180 samples grew diphtheroids and one grew a species of Flavobacterium. Both were thought to be contaminants rather than evidence of translocation.

30% haemorrhage and nerve stimulation group:

Neither the lymph nodes nor the spleen grew any organisms. One of the liver biopsies grew coliforms, which were present in all the small bowel biopsies bar one and in all the large bowel biopsies. All the blood cultures were negative apart from some coagulase negative Staphylococci in one of the control blood samples.

20% haemorrhage and nerve stimulation group:

There was NBG from any of the reticulo-endothelial system organ biopsies and only one of the small bowel biopsies grew coliforms, although coliforms were present in all the large bowel biopsies. All the blood cultures were negative.

Summary of microbiology

Coliforms were present in 91% of the small and large bowel biopsies taken.

All the spleen and mesenteric lymph node biopsies were sterile. Six per cent of liver biopsies grew coliforms, 9% grew the skin contaminants coagulase negative Staphylococci.

Blood cultures only grew skin contaminants (Flavobacterium and Diphtheroids).

Table 3.41: Tissue and blood culture results.

Group	Control Lymph Node	End Lymph Node	Spleen	Liver	Small bowel	Large bowel	Blood Control	Blood R180
Control	1	1	1	1	1			
Control	1	1	1	3	1		1	1
Control	1	1	1	3	2	2	1	1
Control	1	1	1	3	2	2	1	1
Control	1	1	1	1	2	2	1	1
Control	1	1	1	1	1	1	1	1
NS	1	1	1	1	2		1	1
NS	1	1	1	1	2	2	1	1
NS	1	1	1	1	2	2	1	1
NS	1	1	1	1	2	2	1	1
NS	1	1	1	1	2	2	5	1
NS	1	1	1	1	6	6	1	1
NS	1	1	1	1	2	2	1	1
H30	1	1	1	1	1	6	1	1
H30	1	1	1	1	1	6	1	5
H30	1	1	1	1	2	2	1	1
H30	1	1	1	6	6	6	1	1
H30	1	1	1	1	2	2	1	1
H30	1	1	1	1	6	6	1	4
HNS30	1	1	1	1	1	6	3	1
HNS30	1	1	1	1	2	2	1	1
HNS30	1	1	1	1	2	2	1	1
HNS30	1	1	1	1	2	2	1	1
HNS30	1	1	1	1	2	2	1	1
HNS30	1	1	1	2	2	2	1	1
HNS20	1	1	1	1	2		1	1
HNS20	1	1	1	1	1	6	1	1
HNS20	1	1	1	1	1	2	1	1
HNS20	1	1	1	1	1	2	1	1
HNS20	1	1	1	1	1	2	1	1
HNS20	1	1	1	1	1	2	1	1
HNS20	1	1	1	1	1	6	1	1

No bacterial growth	1	**Diphtheroids**	4
Coliforms	2	**Flavobacterium**	5
Coagulase negative Staphylococci (CNS)	3	**Coliform + CNS**	6

Chapter 4: Discussion

Global responses

Group variability

One of the disadvantages of using any large animal is that there can be considerable variation in the response of individuals to any stimulus. This became apparent in some of the pilot studies, when several animals were unable to tolerate a 30% haemorrhage. This prompted the decision to add 20% haemorrhage groups as a "safety net". Several animals in the HNS20 group behaved rather differently to the others. This manifested itself with a higher VO_2I than in the other groups (Figure 3.17), and a massive increase in femoral vascular resistance (Figure 3.26i).

Without air conditioning in the laboratory it was impossible to maintain a constant core temperaturein all the experiments. This meant that in the summer some animals showed quite an increase in temperature (the highest temperature recorded was 42.2°C). In winter some of the animals cooled down more during the laparotomy, despite the use of a heated table and overheat lights. Experiments were performed in a haphazard order, rather than a strictly randomised order, because of constraints on space in the laboratory (the presence of the nerve stimulation equipment reduced the space available to other users of the laboratory). The haphazard order ensured that different experiments were performed on animals from the same litter.

In my experimental groups there was some separation of baseline values, especially for heart rate, MAP, and cardiac index. The three nerve stimulation groups had higher values. This was probably because the brachial nerves were cut to allow the placement of the ring electrodes, and to prevent distal electrical stimulation. In the three non-stimulation groups the nerves were exposed and dissected free but not cut. The aim was to try to minimise the injury stimulus in these groups. The fact that the baseline values

of these variables were different shows that this was achieved. A similar phenomenon was apparent in a previous study (Rady et al. 1991).

The inter group variation in certain variables at baseline seems to be a result of differences in the preparation (cut nerves in the NS and HNS groups vs. dissected nerves in the other groups), and differences in temperature. This problem is partly overcome by the use of repeated measures analysis of variance, which analyses pattern (group by time interaction) differences, as well as group differences, and differences over time. The main thrust of this research was to examine pattern differences in the the responses of the different groups. This was not altered by differences in baseline values.

Cardiovascular responses

Nerve stimulation, in groups NS, HNS20, and HNS30, resulted in an increase in heart rate. This was maintained throughout the time course of the studies, showing that there was continued response despite the long duration of nerve stimulation. This showed the effectiveness of the constant current device. Bleeding resulted in a further increase in heart rate. Heart rate did not increase further during the shock phase. With reinfusion of shed blood heart rate returned to baseline. A similar pattern of response was seen in the simple haemorrhage groups.

Blood loss resulted in a reduction in mean arterial pressure (MAP). The reduction in MAP was greater in H30 than in H20 (to 56% and 80% of baseline) and both groups showed a partial recovery of MAP during the shock period. In contrast there was no recovery of MAP in either of the HNS groups in the shock period, but MAP remained above 81% of baseline. Reinfusion of shed blood resulted in recovery of MAP in all four haemorrhage groups. Mean arterial pressure increased with nerve stimulation and remained elevated in NS throughout. There was only a slight general deterioration in MAP over the course of the experiments.

Mean pulmonary artery pressure, central venous pressure and pulmonary artery wedge pressure all showed similar patterns of response: an increase with nerve stimulation, a decrease with haemorrhage, and recovery when shed blood was reinfused.

Cardiac index followed this pattern, but the reduction in the two HNS groups after haemorrhage was greater than the reduction in the two simple haemorrhage groups. After reinfusion of shed blood all groups recovered. However the two HNS groups then deteriorated rapidly over the next 60 minutes. This decline was less apparent in the simple haemorrhage groups. After an initial increase there was a progressive reduction in cardiac index in the nerve stimulation group. Nearly five hours of nerve stimulation only resulted in a 29% reduction in cardiac index, confirming Henderson's comment that "Intense and prolonged stimulation of afferent nerves" was required to induce shock in a large animal (Henderson 1908). Overman and Wang concluded that "In normal animals electrical stimulation of the sciatic nerves has little effect; certainly it alone will not produce shock. Indeed, we have not been able by afferent stimulation to put any dog with a residual blood volume over 75 cc. Per kgm into fatal shock" (Overman and Wang 1947).

Stroke volume index changed in the same way as cardiac index. There was a reduction in right ventricular stroke work (RVSW) after blood loss in HNS20 and HN30, and a recovery with shed blood reinfusion. There was very little response to haemorrhage in H20, unlike H30, which did show a reduction. However both simple haemorrhage groups showed a massive increase in RVSW with reinfusion of shed blood, which was partly reversed after 30 minutes but remained above baseline. Haemorrhage had a greater effect on left ventricular stroke work (LVSW). There was a reduction in LVSW in all four haemorrhage groups, with partial recovery in H20 and H30, but no recovery in HNS20 and HNS30, during the shock period. Left ventricular stroke work returned to baseline after reinfusion of shed blood.

Systemic vascular resistance (SVR) increased with nerve stimulation. Progressive blood loss in HNS resulted in a continued increase in SVR. The greater the

haemorrhage the greater the increase in SVR. Systemic vascular resistance did not increase during the shock period (SVR decreased during the shock period in HNS30). Haemorrhage alone did not result in an immediate increase in SVR. In H20 and H30 SVR only increased by approximately 11% at S30. Resistance returned to baseline with reinfusion of shed blood. The pulmonary vascular resistance (PVR) response to haemorrhage was the same irrespective of nerve stimulation: an increase with haemorrhage, greater in the two HNS groups than in the simple haemorrhage groups, a further increase during the shock period, and then a partial recovery with reinfusion of the shed blood. There was a general increase in PVR over the course of the experiments in all groups.

The experiments confirmed the pressor effect of nociceptive afferent nerve stimulation. There was an initial increase in cardiac index, which appeared to be mediated by an increase in heart rate. MAP increased as a result of increases in systemic vascular resistance and cardiac index.

The cardiovascular response to simple haemorrhage and HNS appeared to be "volume-dependent". The interaction of haemorrhage and nerve stimulation resulted in a pattern of response different to haemorrhage alone. Mean arterial pressure was better maintained in HNS relative to haemorrhage alone: an observation made as early as 1947 (Overman and Wang 1947; Wang et al. 1947). Whereas there was some recovery of MAP during the shock phase in simple haemorrhage there was none in the HNS groups. This partial recovery seemed to be mediated by an increase in SVR, which only occurred 30 minutes after the end of haemorrhage, and the partial recovery of LVSW. In the two HNS groups SVR increased as soon as haemorrhage started and did not increase during the shock phase, possibly having reached its ceiling for that degree of haemorrhage. There was no increase in LVSW during the shock period for the two HNS groups.

Rady et al found an immediate increase in SVR following 40% blood loss (Rady et al. 1991). Maybe 20-30% TBV haemorrhage in my model was insufficient to trigger an

increase in SVR. Only after 30 minutes of shock did SVR increase. Rady's study did not follow the animals after the end of haemorrhage.

Blood loss in conscious swine produced an increase in heart rate, decrease in MAP, cardiac output, stroke volume, and left ventricular stroke work, but no change in systemic vascular resistance (Wade et al. 1989). Blood loss in this model was incremental over one hour so that total blood loss was 37.5 ml/kg (50% TBV). In contrast systemic vascular resistance did not increase significantly in nine dogs bled to a MAP of 30-35 mmHg and maintained that way for two hours and then resuscitated (Horton 1987). This was a rapid bleed and so would have been expected to result in general vasoconstriction. The presence of nerve stimulation in my study seems to have altered the response to haemorrhage in a qualitative way by promoting the increase in systemic vascular resistance.

Mean haematocrit ranged from 28.2 (SD 2.5) in H30 to 33.7 (SD 1.7) in HNS20 at baseline. There was a reduction in all groups (Figure 3.12). Haemoglobin concentrations were in the range of 8-11 g/dl. These values represent porcine anaemia but there is evidence that this is normal in pigs aged 1-2 months (Talbot and Swenson 1963; Tumbleson and Kalish 1972). In a previous study using pigs of the same age and from the same supplier haemoglobin concentrations were even lower (Mackway-Jones K, personal communication, 1994). It then became standard practice to give all newly arrived pigs an intramuscular iron injection.

There was a slight reduction in haematocrit at S30 in the four haemorrhage groups, which recovered at R0. The reduction in haematocrit was not as spectacular as that reported by Green in a canine model of haemorrhagic shock, in which haematocrit was reduced from 49 to 25 as blood pressure was reduced to 35 mmHg (Green 1961). This reduction in haematocrit was thought to represent a 7.0-8.6 ml/kg increase in circulating volume. The canine model was a more severe haemorrhage model than mine, 49% of TBV, and the dogs had high haematocrits to begin with.

A previous study found no change in plasma volume after a 26% TBV haemorrhage, although this was regarded as a threshold degree of blood loss as there were large standard errors in the measured and expected cell and plasma volumes (Deavers et al. 1958). Loss of 30% TBV, which did not result in a large increase in systemic vascular resistance, would not be expected to result in large movements of extracellular fluid. The 2-3% reduction in haematocrit with haemorrhage would indicate that 30% haemorrhage was just at or above the threshold. I made some haematocrit measurements after the first arterial cannulation, which showed that the surgical preparation, including the laparotomy, did result in a 1-2% reduction in haematocrit, so that the overall reduction in haematocrit from initial artery cannulation to S30 was approximately 5-6%. This was still much less than the 14% change reported above, but would represent a 3-4 ml/kg increase in plasma volume.

Oxygen transport

Arterial oxygen saturation remained at or above 99% in all the groups throughout the experiments. The four haemorrhage groups showed a significant reduction in mixed venous oxygen saturation (SvO_2) compared with the control and nerve stimulation groups. Reinfusion of shed blood restored SvO_2 to baseline. There was no statistically significant difference between the control and NS groups, but there was a gradual reduction in SvO_2 over the course of the experiments.

Haemorrhage resulted in a reduction in oxygen delivery (DO_2I), which did not recover during the shock phase. Oxygen delivery increased with reinfusion of shed blood. At R0 DO_2I was significantly higher in HNS20 and HNS30 than in the control group. Oxygen consumption (VO_2I) did not alter significantly over the course of the experiments and there was no statistically significant difference between the groups. Oxygen consumption was higher in HNS20 than in the other groups, especially in the shock phase and the early post-reinfusion phase, because one animal in the group more than doubled its VO_2I during that period.

As DO_2I was reduced during the shock phase but VO_2I was unaffected there was an increase in the oxygen extraction ratio (OER). OER in the four haemorrhage groups was significantly higher than NS at S0, while all but H20 were significantly higher than the control group. After reinfusion of the shed blood all groups showed a progressive increase in OER, consistent with the gradual reduction in DO_2I.

The addition of the nerve stimulation insult did not appear to alter the oxygen transport response to haemorrhage as it did not alter the pattern of response of cardiac index. A previous study suggested that VO_2I did increase in response to haemorrhage and nerve stimulation, although there was no increase with either insult alone (Rady et al. 1991).

An increase in oxygen consumption has been reported after blood loss in conscious swine (Hannon et al. 1989). This was attributed to periodic bouts of muscular activity,

which may have been a behavioural response to improve venous return. Despite an increase in respiratory rate and tidal volume, oxygen delivery decreased because of the reduction in arterial oxygen content and cardiac output. Although there was evidence of an increase in oxygen extraction this was insufficient to meet the increased demand and so an apparent oxygen debt developed.

Metabolic response

By varying minute ventilation the partial pressure of arterial carbon dioxide was maintained in the range 32-40 mmHg throughout the experiments. Bicarbonate concentrations were significantly lower in the three NS groups compared with the control group. Both HNS groups showed a reduction in bicarbonate with haemorrhage and during the shock period. This was not seen with the two simple haemorrhage groups. Over the last two hours of the experiments there were no statistically significant differences between the groups.

All groups showed an early reduction in arterial pH. In the control and NS groups pH recovered by S30, while it continued to fall in the four haemorrhage groups. This fall continued until after reinfusion of shed blood so that a full recovery was not seen until R90. Arterial base excess, although showing great variability between the groups, showed a difference in pattern between the simple haemorrhage and the HNS groups (not statistically significant). There was a small reduction in base excess in H20 at S0. In H30 base excess fell during the shock period and only increased at R30. In both these groups base excess was higher at the end of the studies than at the beginning. This was not the case for the two HNS groups. They showed a much more profound reduction in base excess during the shock period and after reinfusion of shed blood.

Arterial plasma lactate gradually decreased in the control and H20 groups. Twenty per cent blood loss seemed to have no effect on lactate metabolism, but the preparatory surgery did. Although there was a one hour stabilisation period after the preparatory surgery, which has been shown to be long enough to washout the halothane used for surgical anaesthesia (Mackway-Jones K, personal communication, 1994), this was not long enough for the animals to recover from the metabolic stress. Nerve stimulation imposed a further metabolic stress over the first 30 minutes, after which plasma lactate decreased. Plasma lactate increased with 30% blood loss and remained over 2.0 mM/L for more than an hour.

Despite the increase in oxygen extraction ratio in the HNS groups, oxygen supply appeared to be insufficient to meet the increase in metabolic demand. This was met from anaerobic metabolism so that during the shock phase arterial plasma lactate exceeded 3.0 mM/L and 4.0 mM/L in groups HNS20 and HNS30. In HNS30 the lactate concentration reached a peak at R0, that is, just after reinfusion of the shed blood. Such a "delay" or "lag" in lactate production after the end of the stimulus is also seen in high intensity exercise (Åstrand and Rodahl 1986).

The oxygen transport responses to haemorrhage and haemorrhage and nerve stimulation were similar. However the metabolic demands in the two situations seem to be very different: the combined insult seemed to make much greater demands than could be met by an increase in oxygen extraction ratio. As oxygen extraction ratios did not exceed 0.65 it may be that in HNS there is a disruption in oxygen metabolism, as has been suggested in sepsis. The "dysoxia" of sepsis has been reviewed recently (Jacobson and Singer 1996). It seems unlikely that this would be due to the presence of endotoxin in the circulation as the increase in endotoxin concentration was seen after R0, by which time the lactate concentration had reached a peak.

Fifty per cent haemorrhage in conscious swine resulted in an increase in plasma lactate from 0.6 mEq/L to 13.6 mEq/L (Hannon et al. 1990). This resembled the HNS lactate response rather than the H30 response. Apart from the difference in volume of blood loss this model probably resulted in a greater metabolic response as the animals were able to move. The authors observed muscular activity during the haemorrhage phase. They suggested that this caused an increase in oxygen consumption, which had not been reported previously (Hannon et al. 1989). The authors also calculated the "apparent oxygen debt", which increased during the haemorrhage phase to reach a peak of 8.25 ± 0.87 ml/min/kg and was reduced by resuscitation. Within the time scale of their experiments (300 minutes) oxygen debt remained above 3 ml/min/kg. This seems to reflect the fact that plasma lactate remained elevated compared with baseline, despite resuscitation (Hannon et al. 1990).

In my experiments the paradox of similar oxygen consumption in the haemorrhage and nerve stimulation studies and the haemorrhage alone studies but quite different plasma lactates can probably be resolved by the existence of an "apparent oxygen debt". As resuscitation resulted in a reduction in plasma lactate to baseline concentrations one may assume that the "apparent oxygen debt" was repaid. Åstrand and Rodahl comment that others would prefer to replace "oxygen debt" with "excess postexercise oxygen consumption" (Åstrand and Rodahl 1986 p303). In the response to haemorrhage and nerve stimulation "oxygen debt" would seem to be entirely appropriate as the very paradox that it helps to explain is the absence of "excess oxygen consumption".

Although there is increasing recognition that raised plasma lactate concentrations may not reflect anaerobic metabolism (Jacobson and Singer 1996), in my experiments I think they do. The raised plasma lactates were associated with the haemorrhage and shock phases of the experiments, and in the groups which showed no spontaneous recovery during the shock phase (the haemorrhage and nerve stimulation groups): the very groups in which anaerobic metabolism might be expected. The increase in plasma lactate preceded the increases in endotoxin and the two inflammatory cytokines. This is in direct contrast to the studies reviewed by Jacobson and Singer (1996). Another contrast with the "dysoxia" of sepsis is the fact that raised lactate concentrations in my experiments did not persist after reversal of hypovolaemia. At the very time that endotoxin and cytokine concentrations were at their highest plasma lactate concentrations had returned to baseline values. If the increases in lactate had been caused by a failure of clearance or an accelerated rate of glycolysis resulting in a build up of pyruvate, then this should have happened as a result of a septic stimulus, not before. It therefore seems likely that raised lactate concentrations in my experiments reflect anaerobic metabolism and an "oxygen debt" rather than the "dysoxia" of sepsis.

Summary for global responses

The combination of haemorrhage and nerve stimulation alters the response to haemorrhage. The main feature is the increase in plasma lactate. This may reflect a greater metabolic insult than from an equal haemorrhage without nerve stimulation. The clinical corollary would be that two patients with similar degrees of blood loss, one from a gastro-intestinal bleed, the other from trauma, will have different metabolic responses. The patient with the gastro-intestinal bleed will probably suffer less metabolic derangement than the trauma patient.

Regional responses

Cardiovascular responses

There was a significant reduction in femoral blood flow in H30, HNS20, and HNS30 at S0 compared with control and NS. There was a slight recovery of blood flow in the shock period in H20 and H30. In H20 this was such that at S30 femoral blood flow in H20 was significantly greater than in HNS20 and HNS30. Reinfusion of shed blood restored femoral blood flow in all four haemorrhage groups, so that femoral blood flows exceeded those in the control and NS groups at R0. At R30 femoral flows were similar in all groups.

These changes in femoral blood flow were not just the result of the reduction in cardiac index. There were increases in femoral vascular resistance in all four haemorrhage groups (Figure 3.26i and 3.26ii). These increases were especially large in the two HNS groups (two animals in HNS20 showed profound reductions in femoral flow and so had massive increases in vascular resistance). The two simple haemorrhage groups had very mild increases in vascular resistance in comparison.

Muscle blood flow is under neural, humoral and metabolic control (Heistad and Abboud 1974). The sympathetic α-adrenergic system mediates the vasoconstriction associated with haemorrhage. Vasodilatation can result from: 1) loss of adrenergic tone, 2) sympathetic cholinergic stimulation, 3) vasoactive mediators (such as histamine), 4) a ß-adrenergic effect of noradrenaline. In this model reinfusion of shed blood resulted in an increase in blood pressure and so activation of arterial baroreceptors, which would result in a reduction in α-adrenergic tone and so a reversal of the haemorrhage-induced vasoconstriction.

The increase in muscle vascular resistance is typical of the response to haemorrhage (Mellander and Lewis 1963; Schwinghamer et al. 1970). Most of the increase in vascular resistance is mediated via the pre-capillary resistance vessels, and to some extent the pre-capillary sphincters (although their main role is in the distribution of the remaining flow within the muscle). Post-capillary capacitance vessels are also constricted during hypovolaemia. Mellander and Lewis found that with prolonged haemorrhagic hypovolaemia there was a reduction in the reactivity of the pre-capillary mechanisms to sympathetic stimulation. Post-capillary resistance remained high so that fluid was lost from the intra-vascular compartment. The very small reductions in femoral vascular resistance seen in H20, H30, and HNS30 could not be considered in the same light as the hypotensive period was only 30 minutes. The hypotensive period used by Mellander and Lewis was 145 minutes.

The model used by Mellander and Lewis was an anaesthetised cat (Mellander and Lewis 1963). The hindlimb preparation very carefully isolated the muscle. Schwinghamer et al used a model of 25% or 50% blood loss in dogs to examine forelimb blood flow (Schwinghamer et al. 1970). They found increases in systemic and local vascular resistance and a reduction in limb weight caused by fluid moving into the vascular compartment because of the reduction in capillary hydrostatic pressure. But they found no evidence of any loss of vascular tone, despite four hours of hypotension.

Gut blood flow responses were different in the haemorrhage alone groups and the haemorrhage and nerve stimulation groups (Figure 3.25). Haemorrhage alone resulted in a reduction in gut blood flow after haemorrhage and then a partial recovery during the shock phase. This recovery was not the result of a change in gut vascular resistance, but rather followed the partial recovery of mean arterial pressure and the increase in systemic vascular resistance seen in the shock period. Gut vasoconstriction is controlled by the sympathetic α-adrenergic system in much the same way as skeletal muscle (Banks et al. 1985).

There was no recovery of gut blood flow in the HNS groups during the shock period, as there was an increase in gut vascular resistance after haemorrhage in HNS30 and an increase in resistance at S30 in HNS20. Thus haemorrhage and nerve stimulation provoked a generalised and a regional vasoconstrictor response immediately after haemorrhage, while the same degree of haemorrhage on its own only resulted in a mild systemic and femoral vasoconstriction after 30 minutes of shock. The gut was spared from vasoconstriction with haemorrhage alone. This "gut sparing" in simple haemorrhage was suggested by Mackway-Jones et al in a previous study (Mackway-Jones et al. 1994).

Reinfusion of shed blood restored gut blood flow, which was maintained until the end of the experiments. In HNS30 gut blood flow declined gradually to 70% of baseline.

Nerve stimulation resulted in an initial increase in gut blood flow, which lasted 30 minutes. This coincided with an increase in MAP, cardiac index, and SVR but not with any local vasoconstriction. Gut vascular resistance increased gradually over the course of the experiments, just as it did in the femoral vascular bed.

Two studies in dogs have shown that gut blood flow was reduced after blood loss. Vatner noticed a 56% reduction in mesenteric blood flow in "moderate hypotensive haemorrhage" (26 ml/kg) compared with a 38% reduction in coronary blood flow. Rocha-e-Silva et al observed large increases in femoral and mesenteric vascular resistances in dogs after 41-46 ml/kg blood loss, 50-60% TBV (Rocha-e-Silva et al. 1986). These dogs were bled over 15 minutes and were maintained at an MAP of 40 mmHg for 30 minutes. Femoral resistance decreased during the shock period but there was no increase in blood flow. There was a smaller decrease in mesenteric resistance during the shock phase but little improvement in blood flow.

The immediate increase in mesenteric resistance in this study may reflect the severity of the haemorrhage (50-60% TBV, over 15 minutes). In my study there was less blood loss over 30 minutes. Gut blood flow decreased to 60% of baseline in my study.

Rocha-e-Silva et al do not provide the data, but from their Figure 3 it appeared that flow was less than 25% of baseline. Renal blood flow and resistance changes were similar to those of mesenteric flow and resistance. There was less change in coronary blood flow.

Blood loss is not the only insult which causes a reduction in gut blood flow. Full thickness flame burns and endotoxaemia each result in a reduction in superior mesenteric artery flow (Morris et al. 1988; Navaratnam et al. 1990). A 30 minute intravenous infusion of E.coli endotoxin resulted in a 50% reduction in gut blood flow and an increase in mesenteric vascular resistance (Navaratnam et al. 1990). Vascular resistance peaked 45 minutes after the end of the infusion then returned to baseline 45 minutes later. There was a further increase in mesenteric and systemic vascular resistance 2-5 hours post infusion. At the same time there was a decrease in non-mesenteric resistance. There seemed to be a diversion of blood from the gut to the non-gut systemic circulation in the post infusion period. By 30 hours mesenteric flow, vascular resistances and cardiac index had returned to baseline. All six sheep in the endotoxaemia group had positive mesenteric lymph node bacterial cultures. Only one positive culture was found in the control group.

Changes in gut blood flow have been induced by complete or partial occlusion of the superior mesenteric artery (SMA) to investigate the mechanisms involved in gut mucosal barrier failure. A canine model of partial SMA occlusion resulted in a 50%-60% reduction in gut blood flow for one hour (Sheng et al. 1992). After release of the SMA occluder animals were allowed to recover and observed for 72 hours. Six of the group of ten animals died before that time. Post-mortem examination showed ischaemic damage to the ileal and caecal mucosa: subepithelial oedema, haemorrhages and erosions, and infiltration by neutrophils and lymphocytes. Bacteria were found in the lamina propria, hepatic sinusoids and alveoli. There were also pathological changes in the liver, lungs, and heart.

The authors suggested that gut ischaemia and reperfusion promoted gut mucosal barrier failure and bacterial translocation. They found a reduction in circulating sodium

dismutase (an anti-oxidant) and an increase in plasma malondialdehyde (a marker of oxygen free radical production) after ischaemia-reperfusion, which persisted until 72 hours. Gut ischaemia-reperfusion was also associated with increases in plasma C5a and thromboxane B_2 (TXB_2). It was suggested that gut ischaemia-reperfusion resulted in the production of oxygen free radicals, the activation of complement, and metabolism of arachidonic acid to thromboxane A_2 (TXB_2 is its stable metabolite). These three pathways could all contribute to the tissue damage seen and organ failure. The gut ischaemia-reperfusion in my studies was not associated with an increase in plasma TXB_2 (Foëx et al. 1997), possibly because the hypoperfusion period was only half an hour compared with their one hour model. In rats 20 minutes of total ischaemia resulted in total depletion of mucosal ATP (Blum et al. 1986), and 30 minutes of ischaemia resulted in structural and functional changes (Robinson et al. 1981).

The progressive endotoxaemia seen in my nerve stimulation group may have been partly responsible for the gradual increase in gut vascular resistance. The endotoxaemia in the control group may not have reached a threshold to cause gut vasoconstriction. There was no progressive increase in resistance in the haemorrhage groups even though they showed a progressive endotoxaemia after reinfusion of shed blood.

Oxygen transport and metabolism

Changes in portal venous oxygen saturation were similar to changes in mixed venous oxygen saturation: all the haemorrhage groups showed a reduction with haemorrhage, no recovery during the shock phase, and then a return to baseline levels with reinfusion of shed blood (Figure 3.28).

As I did not measure absolute gut blood flow I was unable to calculate gut oxygen delivery and consumption. A reduction in delivery can be assumed from the reduction in systemic oxygen delivery and the relative reduction in gut blood flow with haemorrhage. An increase in gut oxygen consumption was reported in a pig model of normotensive faecal peritonitis (Rasmussen and Haglund 1992). Peritonitis was associated with a reduction in cardiac index, oxygen delivery and blood flow in the portal vein. There was an increase in systemic vascular resistance and oxygen consumption. Gut oxygen consumption increased before the reduction in delivery. In this model there was no change in arterial or portal lactate concentrations.

Global and regional oxygen transport responses to peritonitis seemed to be quite different to those associated with haemorrhage and injury.

Portal oxygen extraction ratio increased in the four haemorrhage groups to 0.48-0.58 during the shock period and then returned to baseline after reinfusion of the shed blood. Thus portal oxygen extraction ratio increased less than systemic oxygen extraction ratio. A lower gut than systemic maximum OER has been reported in a canine model of progressive haemorrhage (Nelson et al. 1987). Nelson et al used progressive haemorrhage to reduce oxygen delivery to a segment of small intestine, and systemically, to examine the effect on oxygen consumption and extraction (Nelson et al. 1987). They were particularly interested in the relationship between oxygen delivery and consumption and the critical point at which consumption became supply-dependent. Critical oxygen delivery (DO_{2crit}) in the gut was higher than systemic DO_{2crit} (34.3 ± 11.3 ml/kg/min vs. 7.9 ± 1.9) and occurred at a systemic DO_2 (9.7 ± 2.7) greater than the

systemic DO_{2crit}. The critical oxygen extraction ratio (OERcrit) at DO_{2crit} was lower in the gut than systemic OER_{crit} (0.63 ± 0.09 vs. 0.69 ± 0.12). Maximum OER in the gut was also lower than systemic maximum OER. The gut was more susceptible to supply-dependence of oxygen consumption and had less capacity to compensate by increasing extraction.

The pattern of plasma portal lactate was similar to arterial lactate: there was a much greater response in the HNS groups than in the haemorrhage groups. Again it appeared that the increase in portal oxygen extraction ratio was insufficient to meet the metabolic demands made by the combined insult of haemorrhage and nerve stimulation.

Plasma portal - plasma arterial lactate was calculated to get an indication of the lactate flux across the gut itself. There were no statistically significant group or time effects, but there was a significant group by time interaction because of the contrasting pattern seen in HNS20. In the other groups there was very little difference between the portal and arterial concentrations of lactate.

What does P-A concentration really mean? Does it mean anything at all? Although concentrations only were measured these may be useful markers of the actual lactate load, which would be the "Gold standard". Arterial flow to the gut will be reduced with haemorrhage. As portal venous flow will also be reduced one may be able to assume that flows in and out of the gut are about the same unless the gut becomes a blood reservoir, which is unlikely in simple haemorrhage or HNS.

Concentration in and out then represent the flux across the gut. Arterial lactate concentration represents systemic lactate, that is to say, lactate coming from the gut and any other lactate producing organs such as skeletal muscle. As the flux is nearly zero the gut does not appear to be producing additional lactate.

The role of the liver cannot really be assessed as there were no post liver measurements. Arterial lactate is not useful as this will include lactate from muscle. The liver may well be metabolising lactate but this may be replaced by lactate from muscle.

If this line of argument is correct then it would appear that the gut is more tolerant of reductions in blood flow than skeletal muscle. It has already been suggested that with haemorrhage there is an increase in muscle activity (Hannon et al. 1989). In an HNS situation it would not be surprising if this were exaggerated thus causing muscle metabolism to become anaerobic.

If the gut is not so vulnerable to reductions in blood flow then in my model there may not be very much gut damage. There must be some damage as in the insult groups there was a tendency for higher concentrations of endotoxin if not both of the inflammatory cytokines. This damage must be the result of the reduction in blood flow and thus should be anaerobic/ischaemic.

Summary for regional responses

The regional responses to haemorrhage are altered in the presence of nociceptive afferent nerve stimulation. The increase in femoral resistance was much greater when blood loss occurred on a background of nerve stimulation. Blood loss alone did not cause a gut vasoconstriction. Gut vasoconstriction did occur when blood loss and nerve stimulation were combined. The combination of these insults appeared to be particularly deleterious to gut blood flow, although there was little evidence of functional deterioration, such as translocation of live organisms.

Endotoxin

Portal

All experimental groups showed an increase in portal endotoxin concentrations (Figure 3.32). There were some significant differences between the groups. The control and H20 groups showed the lowest endotoxin concentrations. Endotoxin concentrations were higher in the NS, H30 and HNS20 groups than in the control group. Endotoxin concentrations were higher in the HNS20 group than in H20.

The increase in portal endotoxin concentration in the control group shows that there was translocation of endotoxin with no haemorrhagic or nociceptive insult. Twenty per cent haemorrhage did not result in more endotoxin translocation than surgical preparation, which included the cannulation of central vessels but also a laparotomy and a degree of intestinal handling to cannulate the portal vein. It has long been known that vigorous intestinal handling could cause shock, indeed gastric massage was included in the second category of Wiggers's classification of shock models: "drastic techniques that induced shock more certainly and more rapidly", (than the techniques that simulated adverse events in man) (Wiggers 1941).

Wiggers described a study, using ten dogs, in which shock was induced by exteriorisation of the intestine and repeated manipulation for 1-3.25 hours (!) (Wiggers 1950 p66-67). Eight of the dogs were described as being in a shocked state (increased heart rate, increased respiratory rate, weakness, apathy, reduced responsiveness to external stimuli) 1-2 hours after closure of the abdomen and recovery from the anaesthesia. Four were hypotensive with a mean arterial pressure of 50-60 mmHg. In all four animals died. This may have been an early demonstration of endotoxin translocation as a result of intestinal handling.

Bowel exteriorisation in Sprague-Dawley rats resulted in positive mesenteric lymph node cultures (MLN) in 94% of cases (Guzman-Stein et al. 1987). In contrast femoral fracture resulted in positive MLN cultures in only 23% of cases. Anaesthesia was not associated with positive cultures.

Cabie et al detected endotoxin in portal blood after bowel manipulation in 36% of patients undergoing aortic surgery (Cabie et al. 1993). Endotoxin was undetectable in control patients undergoing internal carotid surgery. The detection threshold for their endotoxin assay was 12 pg/ml.

The blood loss in my H30 group did provide a greater stimulus for endotoxin translocation than mere intestinal handling in the control group. A similar degree of translocation was seen in HNS30 and in the nerve stimulation group. So nociceptive afferent nerve stimulation could have an adverse effect on gut mucosal integrity. Although there was no period of gut ischaemia to account for this it has already been established that mediators can be released from sites of injury, which mediate an endotoxaemia (Cuevas et al. 1974).

The highest endotoxin concentrations were seen in the HNS20 group. Although 20% TBV haemorrhage alone did not have a significant effect, the addition of nerve stimulation seemed to produce a significant breakdown in the gut mucosal barrier. It was surprising that endotoxin levels were higher in the HNS20 group than the HNS30 group.

As there were differences in endotoxin concentrations between the groups translocation is unlikely to be an all-or-none phenomenon, such as opening a channel. Rather there may be degrees of breakdown of the gut mucosal barrier.

Arterial

Arterial endotoxin concentrations increased with time in all groups, including the control group (Figure 3.33). All the experimental groups had significantly higher endotoxin concentrations than the control group. There was no statistical difference between the different insults. A 20% TBV haemorrhage did seem to result in a significant systemic endotoxaemia compared with the control group. It may be that there were different mechanisms by which endotoxin reached the systemic and portal circulations (see below). There seemed to be more endotoxin translocation in the HNS20 group than in HNS30, despite the fact that HNS30 resulted in a greater reduction in gut blood flow.

Arterial endotoxin concentrations tended to be lower than portal. This could mean that there was some clearance of endotoxin passing through the liver. As the time course of endotoxin appearance in the systemic and portal circulations was the same it may be that there was also translocation from the gut to the lymphatics and on to the systemic circulation. In rats subjected to haemorrhagic shock the lymphatic route seemed to be the primary route for bacterial translocation within six hours of resuscitation with shed blood (Deitch et al. 1994).

Cabie et al found that there was no difference in the portal and arterial endotoxin concentrations in their abdominal aortic aneurysm patients (Cabie et al. 1993). They concluded that there was translocation of endotoxin to the lymphatics and on to the systemic circulation.

Endotoxin concentrations showed their greatest increase after reinfusion of the shed blood rather than during the shock period. To ensure that this was not an artefact related to storage of the shed blood during the shock period an aliquot of blood was taken from the bags and assayed for endotoxin. Endotoxin was detectable but only in concentrations similar to those seen before the shock period.

The systemic increase in endotoxin appeared to be endogenous. It seems likely that endotoxin translocation increased following a period of relative gut ischaemia and reperfusion. In abdominal aortic aneurysm patients endotoxin concentrations were higher in both the portal and systemic circulations after the aortic cross clamp was released and so after a period of intestinal ischaemia (Cabie et al. 1993).

Control and NS animals also showed a progressive increase in endotoxin concentrations, despite the absence of a period of gut hypoperfusion (intestinal ischaemia). A number of studies have shown that endotoxin itself promotes the translocation of bacteria (Deitch and Berg 1987; Deitch et al. 1987), and has a deleterious effect on the gut mucosa (Deitch et al. 1989b) and on gut permeability (Deitch et al. 1991). It may be that once intestinal handling has resulted in an initial translocation of endotoxin the cycle of systemic endotoxaemia and increased gut permeability perpetuates itself. This would manifest itself as an ever increasing endotoxin concentration in both the portal and systemic circulations.

The mechanism by which endotoxin causes gut mucosal damage and increases gut permeability has been studied (Deitch et al. 1989a; Deitch et al. 1989b). Intra-peritoneal (i.p.) injection of E.coli endotoxin resulted in bacterial translocation in three groups of mice (controls, endotoxin hyporesponsive, and complement deficient) (Deitch et al. 1991). As early as 2 hours after endotoxin challenge there was evidence of an increase in ileal (but not jejunal) permeability, even though there was no evidence of any microscopic damage to the mucosa until 24 hours (villous oedema).

Bacterial translocation to MLNs was evident in all groups. As one group was complement deficient the authors suggested that complement activation was not needed in endotoxin-induced bacterial translocation. Mediators from macrophage activation were also thought unimportant in this process because bacterial translocation was seen: 1) in the endotoxin hyporesponsive mice, and 2) when an anti-TNF antibody was given before the endotoxin challenge. (Anti-TNF antibody pre-treatment did reduce plasma TNF concentrations).

Pre-treatment with allopurinol (a competitive inhibitor of xanthine oxidase, XO) did prevent endotoxin-induced bacterial translocation and increased ileal permeability (Deitch et al. 1991). It seemed that endotoxin promoted bacterial translocation by a direct effect on gut permeability, which was mediated by xanthine oxidase. The role of xanthine oxidase is described in the General discussion below.

Arterial (systemic) - portal difference

Figure 3.34i shows that although the statistical analysis revealed no significant main effects a number of trends were apparent. Until R0 there was no difference between the systemic and portal endotoxin concentrations. During this part of the experiments endotoxin was near the detection limit of the assay (12 pg/ml) so no difference was expected. During the observation phase (R0 to R180) there was a progressively negative systemic - portal difference in the control, NS, H30, and HNS20 groups. This negative difference may mean that there was some clearance of endotoxin from the portal circulation.

In H20 and HNS30 there was a slight positive systemic - portal difference. Instead of clearance there was an increase in endotoxin from the portal to the systemic circulations, or contamination from one of the cannulae used for blood sampling.

Alternatively this may simply be a consequence of endotoxin translocation directly to the lymphatics and the systemic circulation before passing through the liver. Although there may be clearance of endotoxin from the fraction of endotoxin passing directly into the portal circulation this is masked by the endotoxin, which initially bypasses that element of the reticuloendothelial system (RES).

Table 3.34i shows that endotoxin clearance ranged from 6-20%, with the exception of the H20, which showed a net increase in endotoxin of 12%. The similarity in clearance in the other groups suggests that despite changes in gut blood flow, which seem to result

in mucosal barrier breakdown, these changes do not result in hepatic RE function failure.

The Pearson correlation calculation ($\mathbf{r} = -0.543$, $P<0.001$), by taking all the endotoxin data may provide a more integrated picture of endotoxin handling. It showed that when all the data were taken into account there was a relationship between the "clearance" of endotoxin and the amount of endotoxin arriving from the gut. So overall there was no disruption of RES function in this model of shock.

This does not take into account that the systemic endotoxin concentration also represents some of the endotoxin arriving directly from the gut via the peritoneal cavity or the lymphatics. To represent this a factor ***k*** *could be added to the portal endotoxin concentrations on the X axis, thus shifting all the points to the right, which would tend to maintain the relationship if* ***k*** *were constant, or exaggerate it if* ***k*** *increased in proportion with the arterial concentration from which it was derived!*

General discussion

The experiments of Fine and co-workers in the 1950s and 1960s showed that endotoxin of gut origin might have a role in the mortality from various types of experimental shock (see Introduction: the physiology of haemorrhage and injury). The development of reliable assays for endotoxin in the late 1960s and early 1970s made clinical measurement of endotoxaemia a realistic proposition (Reinhold and Fine 1971).

Endotoxaemia was documented in 13 patients (Caridis et al. 1972b). In five cases the endotoxaemia was associated with failure of the RES (either liver failure, with or without failure of the spleen, usually splenectomy) and was fatal. Liver failure was commonly associated with gastro-intestinal haemorrhage. In these cases the endotoxaemia was not linked to a septic focus. In eight cases there was an identifiable septic focus. Eradication of the septic focus resulted in a resolution of the endotoxaemia and recovery.

Further analysis of clinical cases emphasised the link between gastro-intestinal haemorrhage, mucosal damage and endotoxaemia, irrespective of the presence of a septic focus (Woodruff et al. 1973). Where there was no septic focus it was suggested that the endotoxin was of gut origin. Many experimental studies have pointed to the gut as the source for an endotoxaemia, as summarised below.

Cuevas and Fine showed that chemical peritonitis in rabbits resulted in endotoxaemia (Cuevas and Fine 1972b). As the only source of endotoxin was the gut, with its indigenous flora including Gram negative bacteria, they suggested that the endotoxaemia was of gut origin.

Experimentally 25-30% BSA immersion burns in rabbits resulted in an endotoxaemia 90 - 120 minutes after the scald (Cuevas et al. 1974). Endotoxin was found in the liver and lungs, and bacterial cultures were positive. The gastro-intestinal tract was found to be oedematous and haemorrhagic.

The authors demonstrated that the endotoxaemia did not originate from the burn itself by taking blood samples from the venous drainage of the burn. Endotoxin appeared in these blood samples 30 minutes later than in aortic blood samples. They also noted that the endotoxaemia was associated with the presence of endotoxin in the peritoneal cavity.

In a later series of experiments the rabbits supplied were deficient in Gram negative enteric bacteria (Cuevas et al. 1974). Following the same size burn there was no endotoxaemia despite the associated hypovolaemia. This strongly implicated the gut as the source of the endotoxin.

What was the mechanism leading from a skin burn to endotoxaemia? If blood draining from the burn site was collected and given to healthy rabbits they became endotoxaemic and suffered a vascular collapse as if they had been burned (Cuevas et al. 1974). This

collapse was similar to that seen with infusions of the vasoactive amines bradykinin, serotonin, and histamine (Cuevas and Fine 1973). It was suggested that these or other vasoactive substances might be produced by the burn and result in an increase in gut permeability to endotoxin and hence an endotoxaemia (if the gut contained Gram negative organisms). Henry Dale's 1935 comment: "I am not at all sure that there has been ruled out the possibility of substances absorbed from the injured tissue, which have a long-range effect and gradually break down the permeability of the capillaries" seems particularly prescient (Dale 1935).

Gut ischaemia has been linked directly with endotoxaemia. Occlusion of the superior mesenteric artery (SMA) in the rabbit resulted in a systemic endotoxaemia within five minutes (Cuevas and Fine 1971). The endotoxin concentration remained elevated until the animals died seven or eight hours later. If the SMA was only occluded for one hour and then blood flow was restored there was a much greater increase in systemic endotoxin concentration and the model was more rapidly fatal. Decontamination of the gut before SMA occlusion prevented the endotoxaemia in some animals.

The endotoxaemia associated with SMA occlusion could be reduced by intermittent peritoneal lavage (Caridis et al. 1972a). It appeared that gut ischaemia resulted in a very rapid increase in gut permeability to endotoxin, so that endotoxin passed into the peritoneal cavity, from where it was immediately absorbed into the systemic circulation.

This was thought to explain why endotoxin appeared more rapidly in the systemic circulation than in the portal circulation after SMA occlusion (Cuevas and Fine 1972a). The lymphatic route was not regarded as a viable explanation in this model because of the speed with which endotoxin appeared in the systemic circulation after SMA occlusion (Cuevas and Fine 1971). One hour after release of the occlusion systemic and portal endotoxin concentrations were the same. Gut reperfusion seems to play an important role in the development of a portal endotoxaemia. My results point in the same direction. Breakdown of the gut mucosal barrier may not depend only on gut ischaemia but also on the processes triggered by reperfusion after ischaemia. There has

been a lot of interest in the biochemical mediators of gut mucosal injury. One of these is xanthine oxidase, which has been implicated in the increased microvascular permeability and the gut mucosal lesions seen with ischaemia and reperfusion (reviewed in Granger 1988). Xanthine oxidase reduces molecular oxygen to superoxide and hydrogen peroxide. These oxidants are mediators in the microvascular and mucosal damage. Superoxide can also reduce ferric iron to ferrous, which in turn can react with hydrogen peroxide to form hydroxyl radicals, which may mediate the lipid peroxidation associated with reperfusion of the small intestine.

Xanthine oxidase does not seem to be important in bacterial translocation following intestinal bacterial overgrowth. Allopurinol had no effect on bacterial translocation in mice mono-associated with E.coli C25 (Deitch et al. 1989a), but did have an effect on haemorrhage-induced translocation. A 30 minute haemorrhagic shock period in rats (30 mmHg) followed by shed blood resuscitation resulted in a 61% incidence of bacterial translocation to the mesenteric lymph nodes at 24 hours (Deitch et al. 1988). In the sham-shock group the incidence of bacterial translocation was 7%. The incidence of bacterial translocation was reduced to 14% in the allopurinol treated group and to 10% in rats fed a tungsten-enriched diet (tungsten inactivates xanthine oxidase). Neither intervention had any effect on the gram negative flora of the gut (Deitch et al. 1989a), but both of these agents reduced the degree of mucosal damage seen following haemorrhage.

Xanthine oxidase may be an important mediator of the translocation seen in my experiments. It would be interesting to see whether it played a role in the increased plasma endotoxin in the nerve stimulation group.

Summary for endotoxin

The experiments provide evidence for endotoxin translocation in response to haemorrhage, nerve stimulation, and the combination of the two insults. Intestinal handling also resulted in translocation. It is assumed that translocation was the result of mucosal damage, either from ischaemia or from local crushing (handling).

The experiments did not distinguish between translocation to the portal circulation and translocation directly to the systemic circulation via the lymphatics.

Inflammatory cytokines

Tumour necrosis factor-α

Cytokine assays were not performed in the two groups subjected to 20% TBV haemorrhage.

Portal TNF-α

All groups showed some TNF-α response (Figure 3.35). There was a very slight but transient increase in concentration during the shock phase. In all groups TNF-α re-appeared between R30 and R60, that is, 30-60 minutes after reinfusion of shed blood in the haemorrhage studies and 120-150 minutes after the start of nerve stimulation in the NS group. As the main effect in the haemorrhage groups was seen after reinfusion of shed blood (at R60 and after) assays were performed on aliquots of blood from the storage bags. These confirmed that TNF-α was not being produced as a result of the storage process (blood was only stored for 30 minutes). Portal TNF-α levels gave a hint of reaching a plateau around R120-R180.

Statistical analysis showed that the TNF-α concentrations were higher in the three insult groups than in the control group (Table 3.35). The control group did show an increase in portal TNF-α concentration. There was no statistical difference between H30 and HNS30, despite the apparent difference in physiological insult.

The similarity between the three insult groups shows that the stimulus for TNF-α production in these groups was of the same magnitude. The precise nature of the inflammatory stimulus cannot be determined from these studies. One might speculate that it is the translocation of endotoxin since all three insult groups showed evidence of

greater translocation than the controls. Although the degree of endotoxin translocation varied between these groups the TNF-α response was almost the same. In contrast to endotoxin translocation maybe there is a certain "all or none" quality to the TNF-α response once a certain degree of endotoxin is present.

Arterial TNF-α

As with portal concentrations the increase in systemic TNF-α was seen at and after R60 (Figure 3.36). The control group showed the lowest mean concentrations as TNF-α was undetectable in five of the animals. The highest plasma TNF-α concentrations were seen in the H30 group. Tumour necrosis factor-α concentrations seemed to reach a plateau at R180 in both the haemorrhage groups.

Although the group effect part of the RANOVA did not quite reach statistical significance (sig. of F = 0.07) the paired contrasts were still performed. These showed that there were significant group by time interactions between H30 and the control group (a plateau in H30), and between H30 and HNS30 (in HNS30 there was a slight reduction in TNF-α concentration at R180). Tumour necrosis factor-α concentrations were significantly higher in the H30 group than in the HNS30 group (sig. of F = 0.018), despite the apparently greater physiological insult in the latter. The contrasts between the control group and NS, and between the control group and HNS30 were not statistically significant.

Arterial (systemic) - portal TNF-α difference

Apart from group H30 the portal levels were higher than systemic. This may mean that most TNF-α was produced in the gut associated lymphoid tissue in response to a local inflammatory stimulus, such as the translocation of endotoxin. The negative systemic - portal difference showed that there was some TNF-α uptake either in the liver or in the lungs.

The scatterplot (Figure 3.37ii) showed a relationship between portal TNF-α concentration and "clearance", when all the data were entered. This was confirmed by a Pearson **r** = -0.545, P<0.001.

Clearance of TNF-α has been studied in human volunteers. After a bolus injection of endotoxin serum TNF-α reached a peak in 90-180 minutes but was then cleared within a few hours (Michie et al. 1988; van Deventer et al. 1990). Intravenously administered recombinant TNF-α has a short half-life (six to seven minutes) and is fully cleared from the circulation in 20-30 minutes (Beutler et al. 1985b). Radiolabelled TNF-α injected into mice was later recovered from liver (31%), skin (30%), the gastro-intestinal tract (9%), and the kidney (8%). Only 2% was found in the lungs, and 1% in the spleen (Beutler et al. 1985b). The skin and lungs represented non-saturable sites for TNF-α uptake. This may explain the fact that in another study of TNF-α biodistribution the kidneys, liver, and lungs contained the highest TNF-α/gram tissue (Palladino et al. 1987).

Thus in the control and NS groups the liver would have been the most likely site for TNF-α uptake from the portal circulation. The lungs would only come into play after saturation of the liver binding sites.

General discussion

It is unlikely that just the surgery itself provoked TNF-α production since the control group underwent exactly the same surgical preparation but had lower portal TNF-α concentration than the other groups. Haemorrhage in itself is unlikely to be the sole trigger as the TNF-α response in the nerve stimulation alone group was similar to that in the two haemorrhage groups. This contrasts with the results of Ayala et al, who found that in rodents haemorrhage was a potent trigger for TNF-α production but tissue trauma from surgery was not (Ayala et al. 1991). Nerve stimulation may have elicited a

TNF-α response by causing greater endotoxin translocation than seen by the surgery alone. In mice TNF-α production was associated with haemorrhage in the absence of any increase in endotoxin compared with controls (Ayala et al. 1990).

Ayala et al suggested that haemorrhage, rather than tissue trauma or endotoxaemia, was a stimulus for TNF-α production in rodents (Ayala et al. 1990; Ayala et al. 1991). Deitch et al went further, suggesting that the gut might act as a cytokine-generating organ in haemorrhagic shock (Deitch et al. 1994). Rats were subjected to haemorrhagic shock (MAP 30 mmHg) for 30, 60, or 90 minutes and then resuscitated with their shed blood and observed for six hours. The mesenteric lymph channel draining the MLN complex was cannulated to collect lymph for bacterial culture and TNF-α and IL-6 measurement. When the animals were sacrificed venous and cardiac blood samples were taken for the same bacterial cultures and cytokine measurements.

TNF-α concentrations were higher in portal blood than cardiac blood in the 60 minute and 90 minute shock groups. The 30 minute shock group did not show significant TNF-α production compared with the control group. There was evidence of bacterial translocation to the MLN in all three shock groups but blood, liver and spleen samples taken at six hours were all sterile. This suggested that there was bacterial translocation but that the organisms failed to reach the systemic circulation. The higher portal TNF-α concentrations suggested that TNF-α was produced in the gut-associated lymphoid tissue, which was exposed to bacteria, rather than the rest of the reticulo-endothelial system, which was not.

The gut-associated lymphoid system was also implicated in the higher portal than systemic TNF-α concentrations found by Cabie et al (Cabie et al. 1993). They found elevated TNF-α concentrations in all their elective abdominal aortic aneurysm patients after manipulation of the bowel and after reperfusion. Portal concentrations were significantly higher than systemic. They concluded that the source of the TNF-α was likely to be gut-associated macrophages. The portal-systemic difference suggested that

there was considerable clearance of TNF-α across the liver. In a similar group of patients Baigrie et al have been unable to detect TNF-α (Baigrie et al. 1992; Baigrie et al. 1993).

Tumour necrosis factor-α has been detected in a number of studies of trauma patients (Table 1.10). The results are not very consistent. In three studies TNF-α was detectable in only a small proportion of patients, or barely detectable at all (Hoch et al. 1993; Foëx et al. 1994; Svoboda et al. 1994). In contrast Ferguson et al showed an early increase in plasma TNF-α concentrations in more than 90% of their patients (Ferguson et al. 1994). In one study plasma TNF-α was raised in a third of the trauma patients but was also raised in 44% of the controls (Rabinovici et al. 1993), while TNF concentrations were the same in injured patients and controls in another (Moore et al. 1991). Endo et al found raised plasma TNF-α concentrations in haemorrhagic shock patients, but no correlation with the degree of blood loss (Endo et al. 1994), while Foëx et al found TNF-α in the two patients with the highest ISS and the most blood loss (Foëx et al. 1994).

Trauma patients developing MOF tend to show a cytokine response similar to those with non-traumatic septic shock: gradually increasing plasma concentrations of inflammatory cytokines including TNF (Rabinovici et al. 1993; Svoboda et al. 1994). High plasma TNF concentrations have been reported in studies of septic patients, for example (Waage et al. 1987; Damas et al. 1989; Debets et al. 1989; Endo et al. 1992). In general the highest concentrations are seen in those patients who develop MOF and die, although Endo et al found no difference in TNF-α concentrations in patients developing MOF after haemorrhagic shock and those who did not (Endo et al. 1994).

Clinical studies do not answer the question of whether tissue trauma, haemorrhage, endotoxin or bacterial translocation are the triggers for TNFα production in trauma patients. This is partly because the studies have not tried to measure all of these variables, but also because TNF may only be detectable transiently, and because of the great variability in trauma patients. Mechanisms of injury, the injuries and their

management, the degree of blood loss and fluid replacement, complications: all may vary in any study.

Interleukin-6

Cytokine assays were not performed in the two groups subjected to 20% TBV haemorrhage.

Portal

All four groups showed an IL-6 response after reinfusion of shed blood (Figure 3.38). Assays for IL-6 performed on aliquots of blood taken from the storage bags confirmed that the increase in IL-6 was endogenous rather than a storage phenomenon. Interleukin-6 tended to appear slightly later than TNF- α and to reach higher concentrations. There was no tendency to reach a plateau level towards R120-R180.

There were no statistical differences between the groups or in the pattern of IL-6 production, although there was a slight separation of the lines for the insult groups from the control group at R180 (Figure 3.38). All the groups were subjected to the same basic trigger for IL-6 production and neither haemorrhage nor nerve stimulation had any major additional effect.

Ayala et al found that the surgical preparation in their mouse model resulted in some IL-6 production (Ayala et al. 1990). They then developed a rat model, which included a midline laparotomy, as a tissue trauma insult, before the haemorrhage (Ayala et al. 1991). Interleukin-6 levels were raised after laparotomy, even before any haemorrhage, unlike TNF-α. The addition of haemorrhage to the model resulted in further production of IL-6. It appeared that there were two distinct triggers for IL-6 production, the most important being tissue trauma.

Arterial

All four groups showed an increase in systemic IL-6 concentrations (Figure 3.39). There was no significant group effect on RANOVA but the control group had consistently lower mean IL-6 concentrations than the insult groups. This was probably because two control animals showed no IL-6 response throughout. At R180 there seemed to be some separation of the haemorrhage groups from the NS group (Figure 3.39). There was no evidence that the IL-6 response was reaching a plateau at R180.

Arterial (systemic) - portal difference

Although there was no significant group or time effect on RANOVA, Figure 3.40i shows that there was a negative systemic - portal difference in the control and NS groups and a positive systemic - portal difference in the two haemorrhage groups.

These results show that some clearance took place either in the liver or the lungs of the control and NS groups. Interleukin-6 is well known as a hepatocyte-stimulating factor (see Biology of cytokines) so the liver is its major target organ. Interleukin-6 clearance has been reported to be biphasic: consisting of an initial rapid phase (corresponding to a half-life of about three minutes) followed by a slower phase (corresponding to a half-life of about 55 minutes) (Castell et al. 1988). Eighty per cent of the radiolabelled IL-6 injected into rats in this study was recovered from the liver. The only other significant recovery was from the kidney (10-15%). There was an insignificant amount of recovery from the spleen, lungs, and thymus. Thus the liver probably accounted for any clearance in my experiments. In contrast to the experimental situation, where a single bolus injection of IL-6 was given, there seems to have been continuous production of IL-6.

In the haemorrhage groups the positive systemic - portal difference shows that there was additional IL-6 production beyond the portal circulation. This would constitute

systemic inflammation. Endothelial cells, fibroblasts and monocytes/macrophages are all sources of IL-6 in systemic inflammation (Heinrich et al. 1990). Endothelial cells and fibroblasts are preferentially stimulated by IL-1 and TNF-α, while monocytes/macrophages are preferentially stimulated by endotoxin. As endotoxin concentrations increased systemically it may be that this triggered IL-6 production beyond the gut-associated lymphoid tissue.

The scatterplot of IL-6 "clearance" (Figure 3.40ii) did not immediately suggest a relationship between clearance and IL-6 load from the portal circulation. However once the outlying data point had been removed the corrected Pearson **r** (-0.496, P = 0.002) suggested that there might be a relationship between the two.

General discussion

High IL-6 concentrations have been found in trauma patients (Moore et al. 1991; Hoch et al. 1993; Ferguson et al. 1994; Foëx et al. 1994; Meade et al. 1994; Svoboda et al. 1994). In some there was a correlation between the IL-6 response and the severity of injury (Hoch et al. 1993; Svoboda et al. 1994), in others none was found (Foëx et al. 1994). In some there was a link with MOF and death (Svoboda et al. 1994), in others not (Moore et al. 1991; Foëx et al. 1994).

As with TNF-α the very varied nature of trauma patients makes it very difficult to uncover the factors involved in triggering the cytokine response. Surgical patients can at least be divided into groups according to relatively well defined degrees of tissue trauma so that the IL-6 response can be assessed in relation to tissue damage.

Many studies have reported increased IL-6 levels following surgery (Shenkin et al. 1989; Cruickshank et al. 1990; Di Padova et al. 1991; Hall and Desborough 1992; Joris et al. 1992; Crozier et al. 1994). Several studies have suggested that the IL-6 response to surgery depends on the degree of tissue trauma. Cruickshank et al found a gradation of IL-6 responses according to the severity of surgery (Cruickshank et al. 1990). The

introduction of laparoscopic surgery has offered the possibility of comparing two surgical procedures with different degrees of tissue trauma. As expected laparoscopic surgery has been shown to result in an attenuated response compared with open surgery (Joris et al. 1992).

Baigrie et al have compared portal and systemic venous IL-6 levels in abdominal aortic aneurysm surgery (Baigrie et al. 1993). They found that by the time the laparotomy incision was closed portal IL-6 levels were eight times higher than systemic. The portal - systemic ratio increased with time, so they suggested that the liver was effectively clearing IL-6. The authors suggested that, as they were sampling from the inferior mesenteric vein draining mainly the colon, and there was no correlation between endotoxin and IL-6, the colon was the major source of the IL-6, possibly as a result of occult cellular injury.

Cabie et al (Cabie et al. 1993), who compared abdominal aortic aneurysm surgery with surgery to the internal carotid, found similar IL-6 levels in both groups. This was despite the fact that the aneurysm patients were subjected to some bowel manipulation and so might be expected to exhibit occult cellular injury. The authors felt that the IL-6 response was a general response to surgery and contrasted this with the TNF-α response, which was largely confined to the aneurysm patients.

Van Deventer et al found that there was a temporal relationship between the appearance of TNF-α and IL-6 after a bolus injection of endotoxin in human volunteers: the increase in IL-6 seemed to follow the increase in TNF-α by 15 minutes (van Deventer et al. 1990). They suggested that TNF-α might stimulate the release of IL-6 from monocytes/macrophages or endothelial cells. My studies suggest that TNF-α levels started to increase before IL-6, and were reaching a plateau, but that IL-6 levels were continuing to increase at R180. Thus TNF-α could be implicated in the IL-6 response. It seems unlikely that TNF-α was the only factor since the IL-6 response was very similar in all the groups while the TNF-α response in the control group was attenuated compared with the insult groups. Fong et al found that an anti-TNF-α antibody, given

to baboons before an overwhelming bacteraemia, attenuated but did not abolish the IL-6 response, suggesting that there was a factor other than TNF-α involved in the IL-6 response (Fong et al. 1989).

Summary for inflammatory cytokines

Tumour necrosis factor-α was produced in all four groups assayed. In contrast to rodent studies, my experiments do not suggest that haemorrhage is the main stimulus for TNF-α production.

The role and significance of TNF-α in clinical sepsis seems clear. The same is not true for trauma.

Interleukin-6 was produced in all four groups assayed. Surgical trauma may have been the main stimulus for IL-6 as the concentration curves were almost identical. Clinical studies have shown a relationship between the severity of surgical and traumatic insults and IL-6 response.

Microbiology discussion

The culture results show that coliforms were present in the gastro-intestinal tract of 29/32 of the animals from which biopsies were taken (91%). All the spleen and mesenteric lymph node (MLN) biopsies were sterile. Coagulase negative Staphylococci (CNS) were grown from three liver biopsies (9%) and coliforms from two (6%). One control blood culture grew CNS and another grew Flavobacterium. Of the blood cultures taken at the end of the experiments one grew Flavobacterium and one grew Diphtheroids.

Although coliforms were present in the majority of the animals studied there was no evidence of live organisms in the MLN or the spleen. Coliforms were found in the liver of one animal in each of the H30 and HNS30 groups. The only organisms found in blood cultures: CNS, Diphtheroids, and Flavobacterium are all regarded as contaminants (Turnbull et al. 1995). The rate of contamination (up to 9%) was comparable with that reported by other investigators (Gelfand et al. 1991).

These results provide no support for the concept of bacterial translocation within the first three hours after a haemorrhagic or traumatic insult. Can these results be reconciled with the results of previous studies?

It may be that there really was no translocation in this model. The disruption to gut blood flow resulting from 20-30% TBV haemorrhage and/or nerve stimulation may not have caused enough damage to the gut mucosa to sufficiently break down the gut mucosal barrier. Reinfusion of the shed blood seemed to restore gut blood flow fairly effectively. This, combined with only a 30 minute shock period, may have ensured that the ischaemic insult was only temporary and fully reversible.

If gut mucosal biopsies had been taken and examined histologically it might have been possible to evaluate whether there was any mucosal damage at the end of the studies.

Gelfand et al. found minimal mucosal damage following a 40% TBV haemorrhage and a six hour shock period (Gelfand et al. 1991). They too found no evidence of bacterial translocation from cultures of portal and systemic blood, or MLNs.

Chiu et al. showed an inverse relationship between superior mesenteric artery blood flow and intestinal mucosal damage in mongrel dogs (Chiu et al. 1970). Complete occlusion of the artery for 30 minutes resulted in grade 3 mucosal damage, that is "Massive epithelial lifting down the sides of villi. A few tips may be denuded". An equal amount of mucosal damage was seen after three hours in the group with a superior mesenteric artery flow rate of 5 ml/kg/min, which is approximately a third of the normal flow rate. In my study gut blood flow was never less than 40% of baseline.

Rodent studies have suggested that bacterial translocation still occurs across an intact mucosa. Wells et al used immunofluorescence, light and electron microscopy to show E.faecalis translocation to MLN, liver, and spleen across an intact gastro-intestinal epithelium in a mouse model of E.faecalis intestinal overgrowth (Wells et al. 1990). They found bacteria adherent to epithelium, bacteria in vacuoles in intact epithelial cells, but no bacteria between the cells. They concluded that bacterial overgrowth resulted in bacterial translocation and occurred across intact epithelium rather than between cells. This contrasted with the view that macrophages ingested bacteria and carried them across the mucosa, and released them, unkilled, on the other side (Alexander et al. 1990).

My studies finished three hours after the reinfusion of shed blood. This may have been too soon to detect bacterial translocation. Had the animals been monitored for 24 hours rather than three hours and the biopsies taken then, the results might have been very different. Although Gelfand et al. found no evidence of translocation at six hours in their model of 40% TBV haemorrhage, a later study found translocation to the mesenteric lymph nodes in all animals at 48 hours (Morales et al. 1992).

The study by Morales et al. included a 40% TBV haemorrhage, a two hour shock period and then resuscitation with either whole blood or Ringer's lactate. One group of pigs was killed at 8.5 hours, at which time there was no evidence of translocation either to MLNs or portal blood. At 48 hours there was evidence of translocation to the MLNs, liver and spleen in the haemorrhage groups and the control group. Portal blood cultures were only positive in one animal in each of the haemorrhage groups.

The authors suggested that bacterial translocation was the result of the stress of the preparation of the animals (anaesthesia and the intestinal handling needed to cannulate the portal vein), rather than any change in gut blood flow induced by a 40% TBV haemorrhage. As there was no evidence of translocation in the animals sacrificed at 8.5 hours it was thought unlikely that the 48 hour results were caused by contamination.

Sheep have been subjected to a similar degree of intestinal handling and instrumentation to measure blood flow in the superior mesenteric artery, but did not show such bacterial translocation (Morris et al. 1988). Forty per cent BSA flame burns and smoke inhalation caused a 42% reduction in blood flow in the superior mesenteric artery 16 hours later. Despite resuscitation, to maintain haemodynamic values and urine output to within 10% of pre-injury baseline, at 48 hours all nine animals had positive bacterial cultures of mesenteric lymph nodes, liver, kidney, and lung, and all but one had positive spleen cultures. Only one of the three control animals had a positive liver and lung culture. In this study there was no evidence that the stress of instrumentation resulted in bacterial translocation.

In a two-stage shock model Turnbull et al. looked for bacterial translocation in porcine endotoxaemia, haemorrhagic shock, and haemorrhagic shock followed by endotoxaemia (Turnbull et al. 1995). There was no evidence of gut-derived bacterial translocation in serial blood cultures in endotoxaemia or simple haemorrhage, even when the animals were followed for five days. There was some evidence of haematogenous spread of gut-derived bacteria in a small proportion of blood cultures taken from animals subjected to haemorrhage and then endotoxaemia.

Translocation to mesenteric lymph nodes was seen in all three groups. In only one of the six animals in the endotoxaemia group did organisms progress to the solid organs cultured. In the haemorrhage alone group bacteria translocated to solid organs in three of the five animals, suggesting that the mesenteric lymph node barrier had been breached. Five of the six animals in the haemorrhage and endotoxaemia group showed positive tissue cultures. This suggested that pigs previously subjected to haemorrhagic shock were less able to withstand a second stressor (endotoxin).

This provides an example of the "Two-hit Theory" for development of MOF (Deitch 1992). In this theory an initial insult primes the host so that after a second insult the response is magnified and so has consequences, which either of the insults alone would not have caused. This echoes the findings of Schweinburg et al. who found that dogs subjected to a haemorrhagic insult were much less tolerant of a later bacterial insult (Schweinburg et al. 1954). The mechanisms by which trauma might prime cells have been reviewed elsewhere (Friese et al. 1994). Moore et al. have suggested that the post-ischaemic gut may act as a priming bed for circulating neutrophils (Moore et al. 1994b). These primed neutrophils enter the systemic circulation and then reach vascular beds, such as the lung. A second insult, such as a low dose of endotoxin, will then activate them. Activated neutrophils will adhere to the endothelium, cross into the tissues and release reactive oxygen metabolites, causing tissue damage and eventually organ dysfunction and failure.

The insults in my model, whether nerve stimulation or haemorrhage alone, or in combination, would only provide the "first hit" in this paradigm. As a "first hit" it is clearly insufficient to cause bacterial translocation. It could, however, be sufficient to prime the host (neutrophils) so that a subsequent insult, such as a further bleed or the endotoxaemia, which was present, might then result in activation and the real organ damage, including a breakdown of the gut mucosa. In this scenario bacterial translocation would be a sign of secondary organ damage. It might perpetuate the inflammatory response rather than initiate it.

Translocation may have been undetectable because the reticulo-endothelial system managed to neutralise those organisms that did pass the gut mucosal barrier. There is evidence to suggest that organisms may cross the gut mucosal barrier but remain undetected in tissue cultures. Using a technique such as immunofluorescence may reveal the presence of bacterial breakdown products within lymph nodes. Brathwaite et al. found evidence of E.coli translocation to the mesenteric lymph nodes in trauma patients with indirect immunofluorescence using a mouse monoclonal antibody to E.coli beta-galactosidase (Brathwaite et al. 1993a). In these same 20 patients only one had a positive MLN culture and only three had positive portal venous blood cultures. It has been suggested that the mechanism of bacterial translocation in trauma patients is one of phagocytosis since E.coli beta-galactosidase was found only in the cytoplasm of macrophages within the mesenteric lymph nodes (Brathwaite et al. 1993b).

Reed et al. compared MLN culture with electron microscopy in the detection of bacterial translocation in 16 patients with abdominal trauma (Reed et al. 1994). One patient showed evidence of translocation by culture alone, three by culture and microscopy, and nine by microscopy alone. They concluded that translocation was commoner than indicated by tissue cultures. As there seemed to be no link between translocation and the severity of injury, haemorrhagic shock, the development of infection, or the length of hospital stay they also suggested that this translocation might be an epiphenomenon of doubtful clinical relevance.

Bacterial translocation in man: the controversy

Bacterial translocation is well documented in rodents (Baker et al. 1987; Deitch et al. 1987; Baker et al. 1988; Deitch et al. 1990). Enteric organisms have been cultured from rats bled to a mean arterial pressure of 30 mmHg and maintained until 80% of the shed blood had been returned or a maximum period of seven hours, and then resuscitated with the remaining blood and crystalloids (Koziol et al. 1988).

That such organisms originated in the gastro-intestinal tract was elegantly demonstrated by Sori et al. (Sori et al. 1988). They used the same haemorrhagic shock model used by Koziol et al., but before haemorrhage the rats were fed carbon 14-labelled E.coli. Seven of the 14 shocked animals were found to have increased plasma carbon-14 activity compared to controls. They had a 100% mortality at 80 hours, and blood cultures grew E.coli. The seven animals which showed no evidence of translocation had an 83% survival rate.

Translocation in humans remains controversial. Moore et al. inserted portal vein catheters in 20 trauma patients undergoing emergency laparotomy to take serial portal venous blood samples (Moore et al. 1991). Over the course of five days 212 portal venous blood cultures were taken but only eight were positive. Seven of these cultures were considered to have grown contaminants. Only one systemic blood culture was positive and this came from a patient with a known Staphylococcal pneumonia. Endotoxin assays performed over the first 48 hours were also negative. Although 30% of these patients went on to develop MOF the investigators were not convinced that MOF was caused by gut-derived organisms.

Evidence of bacterial translocation was sought in twenty five blunt trauma patients and four patients requiring laparotomy for primary gastro-intestinal disease (Peitzman et al. 1991). None of the patients had perforated a hollow viscus or an intra-abdominal infection. A mesenteric lymph node was excised and cultured from each patient. All the trauma patient cultures were negative (despite the fact that 28% suffered major

infectious complications with enteric pathogens). Three of the patients having surgery for gastro-intestinal disease had positive cultures. The authors did suggest that: 1) lymph nodes might have been obtained before sufficient gut compromise had occurred; 2) that the gut's immune defences had been able to neutralise any translocating organisms; and 3) that preoperative antibiotics had killed any organisms crossing the gut mucosal barrier.

If endotoxaemia is taken as an index of translocation then it has been shown to occur on the day of injury and to increase until day four in burns patients (Winchurch et al. 1987). No endotoxaemia was found in two series of trauma patients (Moore et al. 1991; Hoch et al. 1993), while only 7% of patients in haemorrhagic shock had raised endotoxin concentrations (Endo et al. 1994).

Rush et al. (1988) did find evidence of bacterial translocation in trauma patients very shortly after their injury. Positive blood cultures were found in 13 of 50 trauma patients. The incidence of positive cultures was related to blood pressure on admission: in the 18/50 patients with a systolic blood pressure (SBP)<80 mmHg: ten (56%) had positive blood cultures, in the 7/50 patients with SBP 80-110 mmHg: two (28%) had positive cultures, and in the 25/50 patients with SBP>110 mmHg: only one (4%) had a positive culture. Of the 18 patients with SBP<80 mmHg 16 died. Whether these patients provide evidence for bacterial translocation as part of the pathophysiology of trauma is debatable. Their average admission blood pressure was 45 mmHg. Bacterial translocation in these patients may simply have been an agonal event.

Bacterial translocation: concluding remarks

Bacterial translocation, as a concept, was not even new in the 1950s. Matthews refers to the idea in his classic text "British Mammals" (Matthews 1952). He reports Carlier's observation that white cells were present in large numbers all along the alimentary canal in hibernating hedgehogs (Carlier 1893). Carlier himself suggested that this movement of white cells was to combat the invasion of bacteria from the gut when body temperature fell during hibernation. So the concept of bacteria from the gut invading the host dates back at least to last century. Now one may suggest that with hibernation there is a reduction in gut blood flow, caused by a general slowing of the circulation and a lack of digestive activity, which results in a breakdown of the gut mucosal barrier.

There can be little doubt that bacterial translocation occurs in rodents. It has even been suggested that translocation of live, and/or killed bacteria may be a normal biological process, which regulates local and systemic immunity by exposing immature immune cells in the gut-associated lymphoid tissue to the antigens present in the gut (Wells et al. 1988). These immature cells may reach the systemic circulation and return to the gut mucosa as mature T or B cells.

In large mammals the evidence is mixed but a number of investigators have demonstrated translocation in swine (Morales et al. 1992; Turnbull et al. 1995), sheep (Morris et al. 1988; Navaratnam et al. 1990), and baboons (Bahrami et al. 1995).

From the evidence in man one may suggest that finding live organisms in the circulation or the reticulo-endothelial system is a rare, possibly agonal, event. Immunofluorescence and microscopy have suggested that organisms may translocate across the gut mucosal barrier but that they are then killed by the secondary defences.

Summary for microbiology

Culture of small and large bowel biopsy material showed that coliforms were present in the gastro-intestinal tracts of the animals used. The experiments did not provide evidence for bacterial translocation, either to the circulation or to the reticulo-endothelial system.

Although bacterial translocation has been demonstrated in several species of mammal, its occurrence and significance in man remains controversial.

Therapeutic implications

Research into surgical and wound shock has been largely motivated by clinical problems (recovery from surgery, the management of battle casualties, and now mainly civilian victims of accidents and urban violence).

Even in the last century clinicians were investigating possible treatments for shock. Jordan, in his Hastings Prize essay of 1867, recommended artificial respiration and heat as the two most important treatments for the patient in shock (Jordan 1867). Under his supervision a series of experiments were performed to assess the possible benefits of chloroform in shock. These experiments involved microscopic observation of the circulation in the webs of a group of frogs given chloroform and a control group. The shock model was primitive in the extreme: their heads were "bashed in" with a hammer. Jordan admitted that the results were far from clear, but he felt that there seemed to be some circulatory advantage in the animals given chloroform before this catastrophic insult.

The insult in my study was far from catastrophic but there were a number of almost immediate physiological changes. There was a reduction in gut blood flow, worse in haemorrhage and nerve stimulation than in haemorrhage alone, which was mediated by vasoconstriction. This was associated with a lactic acidosis in the haemorrhage and nerve stimulation groups. There was also early evidence of endotoxin translocation and the generation of a systemic inflammatory response.

What would the consequences of this response have been? Would it have resulted in MOF, or would the inflammation have resolved spontaneously? This might be answered by monitoring the animals for longer after shed blood reinfusion. In a pilot group of animals this was done for a further two hours to see whether there was any further change in endotoxin and cytokine concentrations. Plasma endotoxin and IL-6

concentrations continued to increase, but plasma TNF-α concentrations reached a plateau and had already decreased at the end of the experiments. These findings are consistent with the time course of TNF-α production in response to a bolus injection of endotoxin (Michie et al. 1988; van Deventer et al. 1990).

It may be that there is an immediate inflammatory response but that with prompt and adequate resuscitation it resolves spontaneously. Complications may only arise in trauma patients if there is no resolution of this immediate response or if there is a second insult ("Two-hit Theory"). The second insult may be obvious, such as a further bleed, or may be part of the treatment, such as delayed definitive treatment of fractures. It has also been suggested that with spontaneous resolution of the immediate inflammatory response there is a "knock-on" period of immune suppression, which would make the patient more susceptible to infection (Moore and Moore 1996).

What then are the options for improving the management of trauma patients?

Advanced Trauma Life Support emphasises the importance of securing the airway, ensuring adequate oxygenation and maintaining the circulation (American College of Surgeons 1988). Even with these measures there will be disruption of gut blood flow, a lactic acidosis, and an inflammatory response. Some of these disturbances might be addressed specifically.

Fluid resuscitation may alter the inflammatory response. In a mouse model of peritonitis giving subcutaneous serum or normal saline has been shown to reduce the expression of TNF-α and IL-1β mRNA (Wilson et al. 1996). It would be interesting to see whether the cytokine response was affected by the prompt reinfusion of shed blood in my experiments. In haemorrhage it seems that reperfusion after a period of ischaemia is a greater inflammatory stimulus than a long period of pure ischaemia, so the results from this peritonitis model may not apply to haemorrhagic shock.

As part of the resuscitation, measures may be taken to enhance gut blood flow, or more importantly to maximise gut oxygenation.

Dopexamine hydrochloride has been studied because of its inotropic properties and its ability to vasodilate certain vascular beds. It binds to peripheral dopamine 1 (DA_1) receptors, which have been found in the mesenteric vasculature. Rat studies have shown dopexamine binding to all three layers of the superior mesenteric artery in vitro (Amenta et al. 1991). In vivo studies reported in the same article showed that there was a dose-related increase in mesenteric blood flow associated with a reduction in mesenteric vascular resistance. After blocking both the DA_1 receptors and $ß_2$ adrenoceptors neither of these effects was seen.

Reductions in mesenteric vascular resistance and a consequent increase in mesenteric blood flow has been shown in anaesthetised dogs (Brown et al. 1985). In a dog model of haemorrhagic shock (150 minutes at a mean arterial pressure of 40-45 mmHg), followed by reinfusion of shed blood over 15 minutes, a dopexamine infusion started 30 minutes before reinfusion restored renal and mesenteric blood flows to baseline, which did not happen in the control group (Lokhandwala and Jandhyala 1992).

An improvement in gastric pHi has been reported in ten critically ill patients given a one hour infusion of dopexamine, which was taken as an indication of an improvement in splanchnic oxygenation (Smithies et al. 1994).

Gut blood flow may be improved in another way. Allopurinol, an inhibitor of xanthine oxidase, was given to rats as a bolus and then an infusion after one hour of haemorrhagic shock to a blood pressure reduced to 50% of baseline, (Flynn et al. 1997). The animals were resuscitated with their shed blood and Ringer's lactate. Intravital microscopy showed that during haemorrhagic shock blood flow in the A1 arterioles of the distal ileum was reduced to 13-18% of baseline. After resuscitation mean arterial pressure and cardiac output were restored. In the controls A1 flow remained below baseline at 120, 150 and 180 minutes. In the allopurinol treated group A1 arteriolar

flow exceeded baseline after resuscitation and then returned to baseline. At 180 minutes A1 arteriolar flow in the allopurinol treated group exceeded that in the control group. There was no vasodilatation. The authors suggested that xanthine oxidase generated superoxide may have contributed to persistent blood flow deficits in the gut microcirculation.

Therapies to improve oxygenation of the gut could be very valuable. They might prevent ischaemic damage to the gut mucosa and prevent the spread of organisms crossing the mucosal barrier, as hypoxia has been linked to impairments in host defences and bacterial killing (Knighton et al. 1984; Knighton et al. 1990).

Blood flow in the microvasculature has been improved by adding low molecular weight heparin to resuscitation fluids in haemorrhagic shock (Chaudry et al. 1991). This type of improvement in blood flow has been shown to improve gut function. In a rat model of trauma-haemorrhage adding heparan sulphate (danaparoid sodium) to the Ringer's lactate used in resuscitation resulted in a restoration of gut absoptive capacity (D-xylose absorption).

Further work on the same model, using fluorescent oxygen sensors on the mucosa and serosa in the caecum and colon to measure PO_2, showed that haemorrhage resulted in a greater reduction in gut mucosal PO_2 than in gut serosal PO_2 (Zabel et al. 1995). Resuscitation with shed blood and crystalloids did not restore mucosal PO_2 to baseline, unlike mean arterial pressure and serosal PO_2, which were restored, unless heparan was added to the resuscitation fluids. Heparan not only seemed to improve blood flow but by improving mucosal oxygenation, improved mucosal function. It may be that improved mucosal oxygenation would also reduce bacterial translocation by maintaining the mucosal barrier and maintaining the bactericidal capacity of gut mucosal macrophages.

The role of xanthine oxidase, oxygen and hydroxyl radicals, in translocation has already been discussed. There may be a place for enzyme inhibitors, such as allopurinol in the

treatment of the critically ill, but there is controversy about the value of prostaglandin inhibitors, such as ibuprofen. A recent trial reported some encouraging results (Bernard et al. 1997b), but also drew criticisms (Zimmerli and Widmer 1997), which the original investigators acknowledged (Bernard et al. 1997a).

An alternative approach might be to try to stabilise some of the cells, which generate the inflammatory mediators. This has been done successfully in asthma for many years by giving sodium cromoglycate, nedocromil, or corticosteroids to stabilise mast cell membranes. As the gut also contains mast cells such an approach might work in the gut. The effectiveness of cromolyn, which stabilises peritoneal-type mast cells, quercetin, which stabilises mucosal-type mast cells, and dexamethasone was recently assessed in a canine model of terminal ileum ischaemia-reperfusion (Szabó et al. 1997). Ischaemia-reperfusion resulted in an increase in mucosal permeability to a marker: sodium fluorescein. The increase in permeability was attenuated by all three pre-treatments, although there was no effect on the extent of histological damage. Mast cells in the gut may contribute to the functional changes in the mucosa. The local application of mast cell stabilising drugs may provide another line of investigation into new treatments for the critically ill.

If some mucosal damage is an inevitable consequence of reductions in gut blood flow and gut oxygenation then maybe one way of minimising the secondary insult of translocation and inflammation would be to accelerate healing of any damage. Within 18 hours after a 90 minute episode of ischaemia, which resulted in denudation of ileal villi, the gut mucosa of rats had healed (Park and Haglund 1992). Mucosal repair seemed to be mediated by cell migration rather than cell division at the site of injury, as thymidine incorporation was limited to the bottom of the crypts.

This mucosal repair process is called "restitution". In this adjacent cells flatten, send out lamellipodia and migrate over the denuded basal lamina and so cover the defect. Its great evolutionary advantage over cell division is that it can begin within minutes and may rapidly cover a large defect (Kvietys 1996).

As the ischaemic insult in my model was short it is likely that any mucosal damage was minor. As resuscitation was rapid and apparently fairly successful it is not difficult to imagine that enough cells were left in good working order to allow restitution to take place, minimising further damage, and effecting a rapid repair of any defect.

It is apparent that a number of cytokines can improve restitution: endothelial growth factor (EGF), transforming growth factor-α (TGF-α), IL-1β, IFN γ (Dignass and Podolsky 1993). They seem to work through TGF-β_1 as restitution was impaired by a specific anti-TGF-β_1 antibody. Collagen and collagen synthesis have also been shown to be important in the restitution process (Moore et al. 1992).

In future studies the presence of TGF-β in portal venous blood might be used as an indicator of mucosal damage. The role of TGF-ß could be investigated by either perfusing the gut with TGF-β or an anti TGF-β monoclonal antibody and assessing the effect on endotoxin translocation and the inflammatory response. If perfusion with TGF-ß did promote mucosal healing and reduce endotoxin translocation and the inflammatory response then it might provide another treatment in the critically ill.

One of the products of the biotechnology revolution of the last two decades has been the monoclonal antibody (Hawkins et al. 1992; Russell et al. 1992). Monoclonal antibodies have been developed to endotoxin and various inflammatory cytokines and mediators (reviewed in Foëx and Shelly 1996). Initial trials of anti-endotoxin were greeted with considerable enthusiasm and optimism for the future of therapy for the critically ill (Wolff 1982; Fisher and Bellingan 1991; Hinds 1992). Even in the early days there were problems in deciding which patients should receive this potentially expensive treatment in addition to standard intensive care (Bone 1991). In the end the U.S. Food and Drug Administration (FDA) refused a product license for HA-1A (the anti-endotoxin antibody developed by Centocor), although product licences were approved in some European countries.

Many trials of monoclonal antibodies in septic patients have been disappointing and been carefully analysed (Warren et al. 1992; Wenzel 1992; Fisher et al. 1993; Fisher et al. 1994a; Reinhart et al. 1996), others have been more positive (Dhainaut et al. 1994; Fisher et al. 1994b). The result has been a more cautious approach to therapies designed to modulate the immune response in sepsis (Bone 1993). In contrast to sepsis there may be a place for using some of these treatments in the trauma patients. Trauma might have the advantage that the monoclonals could be given before the inflammatory response has fully developed.

Initial efforts at immunotherapy were largely directed at the initiating mediators of the inflammatory response. It now appears that in sepsis treatment was probably being given too late: the inflammatory cascade was well underway and the neutralisation of any isolated element could not reverse the metabolic anarchy. An alternative approach could be to focus on a final common pathway: the endothelium of the end organs damaged in MOF? The importance of endothelial cells in the inflammatory process has been reviewed (Pober and Cotran 1990).

Moore and Moore have suggested that nutrition could be used to optimise the immune response in the critically ill (Moore and Moore 1996). This could be of particular benefit during the post-acute phase of immunosuppression, days after the initial insult. During this phase patients might also benefit from other treatments, which enhance immune function. Flutamide, an androgen receptor blocker has been shown to restore splenocyte, and splenic and peritoneal macrophage functions (proliferation and interleukin-1, -2, and -3 production) in male mice subjected to midline laparotomy trauma and 90 minutes of haemorrhagic shock (Wichmann et al. 1997).

Whether or not any of these putative treatments ever reaches clinical trials may not matter. By investigating their actions much has been learnt about the biochemical, immunological and metabolic consequences of haemorrhage, trauma and sepsis. It has become apparent that these responses are extremely complex with multiple interactions, redundancy, and synergisms. Altering the responses is proving to be difficult,

particularly at the proximal end of the inflammatory cascade, so that more attention is being focussed on events at the distal end and at the failing organs.

The controversy surrounding the clinical relevance of bacterial translocation, the effects of monoclonal antibodies, and various biochemical pathway inhibitors is a potent reminder that the results of in vitro and animal experiments cannot be assumed to translate into clinical breakthroughs.

Final thoughts

Despite their many limitations, animal models are an essential tool in the quest to improve our understanding of the pathophysiological processes that may occur in the critically ill. Animal models allow us to study the effects of specific isolated insults and then intervene to uncover the underlying mechanisms and pathways.

There is no doubt that there is a need for increasing our knowledge of the early phase of the response to injury as a number of preventable deaths occur at this time (Anderson et al. 1988). However the other challenge lies in reducing the later deaths due to sepsis, multiple organ failure and the systemic inflammatory response. It is hoped that better early treatment will reduce or even prevent such problems. A better understanding of the chronic shock state is needed. Achieving this in the laboratory setting is difficult. If the original insult is to be severe enough to elicit more than a transient late response the animal must be resuscitated with fluids and maintained, most probably under sedation/anaesthesia, in an intensive care facility where mechanical and pharmacological treatments are available. Increasingly complex models are now being used to try to replicate more closely the far more complex conditions encountered in clinical practice (such as gunshot wounds combined with haemorrhagic shock (Yao et al. 1995), and sequential haemorrhagic and endotoxic shock (Turnbull et al. 1995).

These models present considerable practical difficulties and as with all models can only provide definite information about the model itself. Whether the same processes occur clinically requires careful clinical observation. As the controversy surrounding anti-endotoxin monoclonal antibodies has illustrated, deducing therapeutic interventions from animal studies can be a very unreliable business (Warren et al. 1992). Animal studies must be viewed very critically: they may provide new ideas but clinical trials and clinical experience remain the final arbiter in therapeutic break-throughs.

This study has shown that in an animal model the response to haemorrhage is altered by the presence of nociceptive afferent stimulation to mimic injury. This altered response carries an additional metabolic cost. A systemic inflammatory response is seen very rapidly after the physiological disruption caused by haemorrhage with or without associated "injury". This may be the start of the process which results in multiple organ failure.

There are two main ways in which this model may be used: 1) to try to answer the question of whether the acute inflammatory response does result in multiple organ failure, and 2) to set up intervention studies to attempt to alter the physiological and inflammatory responses. The first option would require monitoring the animal for several days. If the animal was allowed to recover then the model would have to be significantly altered: the brachial nerves could not be cut. To use the same model the animal would have to remain anaesthetised for the entire period. It would then have to be treated almost like an intensive care patient, with the attendant cost in drugs and manpower. The second option offers considerable scope as the previous section demonstrated, and is the most likely to be taken.

Summary

Final summary

Introduction

Trauma deaths show a trimodal distribution. Late deaths account for about 20% of all trauma deaths. Most of these deaths are the result of multiple organ failure (MOF). Despite advances in intensive care the mortality from MOF has remained unchanged.

A better understanding of the initiation and pathophysiology of MOF after trauma might enable us to prevent its onset or at least improve the treatment.

Methods

Young Large White pigs were studied in a model of haemorrhage with and without a background of brachial nerve stimulation to mimic injury.

Gut and femoral blood flows were measured with electro-magnetic flow probes.

Portal and systemic blood was taken for assays of lactate, endotoxin, TNF-α, and IL-6.

Results

The addition of nerve stimulation altered the response to haemorrhage: there was no recovery of MAP during the shock phase and there was an immediate increase in systemic vascular resistance.

There was a similar reduction in oxygen delivery whether haemorrhage was associated with nerve stimulation or not. Oxygen consumption did not increase in the experimental groups.

Oxygen extraction ratio increased similarly in the four haemorrhage groups but this did not compensate for the greater metabolic insult provided by haemorrhage and nerve stimulation compared with haemorrhage alone.

Haemorrhage and nerve stimulation caused gut vasoconstriction, while haemorrhage alone did not.

There was evidence of endotoxin translocation in all the groups but there was more in the experimental groups than the controls.

There was TNF-α and IL-6 production in all the groups.

There was no evidence of live organism translocation to the circulation or the reticulo-endothelial system in this acute model of shock.

Discussion

The presence of nerve stimulation (injury), alters the response to haemorrhage. It results in a greater metabolic disruption, which may not be apparent from simple clinical measurements.

Endotoxin translocation appeared to be the result of intestinal handling (in the control and NS groups) and of changes in gut blood flow, mediated either by a reduction in cardiac output or increases in vascular resistance or a combination of both.

In contrast to rodent studies, haemorrhage did not appear to be the main trigger for TNF-α production.

The IL-6 responses appeared to be dependent on the preparatory surgery rather than the blood loss or nociceptive afferent stimulation.

There is still considerable controversy over the clinical relevance of bacterial translocation.

Advances in our understanding of the pathophysiology of haemorrhagic/traumatic and septic shock are stimulating many new approaches to the clinical management of these conditions.

References

Abraham, E. (1994). "Reply to letter by Botha et al." Critical Care Medicine **22**: 895.

Alexander, J., S. Boyce, G. Babcock, L. Gianotti, M. Peck, D. Dunn, T. Pyles, P. Childress and S. Ash (1990). "The process of microbial translocation." Annals of Surgery **212**: 496-512.

Altman, D. G. and J. M. Bland (1996). "Comparing several groups using analysis of variance." British Medical Journal **312**: 472-473.

Amenta, F., A. Ricci, P. Napoleone, S. Vyas and M. Lokhandwala (1991). "Anatomical localization of the binding and functional characterization of responses to dopexamine hydrochloride in the rat mesenteric vasculature." Pharmacology **42**: 211-222.

American College of Surgeons (1988). Advanced Trauma Life Support Provider Manual. Chicago.

Anderson, I. D., R. A. Little and M. H. Irving (1990). "An effect of trauma on human cardiovascular control: baroreflex suppression." Journal of Trauma **30**: 974-981.

Anderson, I. D., M. Woodford, F. T. de Dombal and M. Irving (1988). "Retrospective study of 1000 deaths from injury in England and Wales." British Medical Journal **296**: 1305-1308.

Åstrand, P.-O. and K. Rodahl (1986). Textbook of Work Physiology. New York, McGraw-Hill Book Company.

Attinger, E. O. and J. M. Cahill (1960). "Cardiopulmonary mechanics in anesthetised pigs and dogs." American Journal of Physiology **198**: 346-348.

Ayala, A., M. M. Perrin, D. R. Meldrum, W. Ertel and I. H. Chaudry (1990). "Hemorrhage induces an increase in serum TNF which is not associated with elevated levels of endotoxin." Cytokine **2**: 170-174.

Ayala, A., P. Wang, Z. F. Ba, M. M. Perrin, W. Ertel and I. H. Chaudry (1991). "Differential alterations in plasma IL-6 and TNF levels after trauma and haemorrhage." American Journal of Physiology **260**: R167-R171.

Bahrami, S., G. Schlag, Y.-M. Yao and H. Redl (1995). Significance of translocation/endotoxin in the development of systemic sepsis following trauma and/or haemorrhage. Bacterial endotoxins:Lipopolysaccharides from genes to therapy. J. Levin, C. R. Alving, R. S. Munford and H. Redl. New York, Wiley-Liss: 197-208.

Baigrie, R., P. Lamont, D. Kwiatkowski, M. Dallman and P. Morris (1992). "Systemic cytokine response after major surgery." British Journal of Surgery **79**: 757-760.

Baigrie, R., P. Lamont, S. Whiting and P. Morris (1993). "Portal endotoxin and cytokine responses during abdominal aortic surgery." American Journal of Surgery **166**: 248-251.

Baigrie, R. J., P. M. Lamont, M. Dallman and P. J. Morris (1991). "The release of interleukin-1B precedes that of interleukin-6 in patients undergoing major sugery." Lymphokine and Cytokine Research **10**: 253-256.

Baker, J. W., E. A. Deitch, R. Berg and M. La (1987). "Hemorrhagic shock impairs the mucosal barrier, resulting in bacterial translocation from the gut and sepsis." Surgery **38**: 73-74.

Baker, J. W., E. A. Deitch, M. Li, R. D. Berg and R. D. Specian (1988). "Hemorrhagic shock induces bacterial translocation from the gut." Journal of Trauma **28**: 896-906.

Banks, R., R. Gallaven, M. Zinner, G. Bulkley, S. Harper, D. Granger and E. Jacobson (1985). "Vasoactive agents in control of the mesenteric circulation." Federation Proceedings **44**: 2743-2749.

Barcroft, H., O. G. Edholm, J. McMichael and E. P. Sharpey-Schafer (1944). "Posthaemorrhagic fainting study by cardiac output and forearm flow." Lancet: i,489-491.

Barnard, N. and S. Kaufman (1997). "Animal research is wasteful and misleading." Scientific American **276**(February): 64-66.

Bayliss, W. (1918). "Gum injections in "Shock"." Journal of Physiology **52**: xvii.

Beck, G. and G. S. Habicht (1996). "Immunity and the invertebrates." Scientific American **Nov**: 42-46.

Beecher, H., for the Board for the Study of the Severely Wounded (1952). The Physiological Effects of Wounds. Washington,D.C., Office of the Surgeon General, Department of the Army.

Berg, R. (1980). "Mechanisms confining indiginous bacteria to the gastrointestinal tract." The American Journal of Clinical Nutrition **33**: 2472-2484.

Berg, R. (1981). "Promotion of the translocation of enteric organisms from the gastrointestinal tracts of mice by oral treatment with penicillin, clindamycin, or metronidazole." Infection and Immunity **33**: 854-861.

Berg, R. and A. Garlington (1979). "Translocation of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs in a gnotobiotic mouse model." Infection and Immunity **23**: 403-411.

Berg, R. and W. Owens (1979). "Inhibition of translocation of viable Escherichia coli from the gastrointestinal tract of mice by bacterial antagonism." Infection and Immunity **25**: 820-827.

Bernard, G., A. Wheeler and B. Christman (1997a). "Ibuprofen in patients with sepsis." New England Journal of Medicine **337**: 710.

Bernard, G., A. Wheeler and J. Russell (1997b). "The effects of ibuprofen on the physiology and survival of patients with sepsis." New England Journal of Medicine **336**: 912-918.

Best, C. and D. Solandt (1940). "Studies in experimental shock." The Canadian Medical Association Journal **43**: 206-209.

Beutler, B., J. Mahoney, N. Le Trang, P. Pekala and A. Cerami (1985a). "Purification of cachectin, a lipoprotein lipase-suppressing hormone secreted by endotoxin-induced RAW 264.7 cells." Journal of Experimental Medicine **161**: 984-995.

Beutler, B., I. Milsark and A. Cerami (1985b). "Cachectin/tumor necrosis factor: production, distribution, and metabolic fate in vivo." Journal of Immunology **135**: 3972-3977.

Bickell, W., S. Bruttig and C. Wade (1989). "Hemodynamic response to abdominal aortotomy in the anesthetized swine." Circulatory Shock **28**: 321-332.

Blalock, A. (1927). "Mechanism and treatment of experimental shock." Archives of Surgery **15**: 762-798.

Blalock, A. (1930). "Experimental shock; the cause of the low blood pressure produced by muscle injury." Archives of Surgery **20**: 959-996.

Bland, J. M. and D. G. Altman (1995). "Multiple significance tests: the Bonferroni method." British Medical Journal **310**: 170.

Blum, H., J. Summers, M. Schnall, C. Barlow, J. Leigh, B. Chance and G. Buzby (1986). "Acute intestinal ischemia studies by phosphorus nuclear magnetic resonance spectroscopy." Annals of Surgery **204**: 83-88.

Bone, R. C. (1991). "Monoclonal antibodies to endotoxin. New allies against sepsis?" Journal of American Medical Association **266**: 1125-1126.

Bone, R. C. (1993). "Monoclonal antibodies to tumor necrosis factor in sepsis: help or harm?" Critical Care Medicine **21**: 311-312.

Brathwaite, C. E. M., S. E. Ross, R. Nagele, A. J. Mure, K. F. O'Malley and F. A. Garcia-Perez (1993a). "Bacterial translocation occurs in humans after traumatic injury: evidence using immunofluorescence." Journal of Trauma **34**: 586-589.

Brathwaite, C. E. M., S. E. Ross, R. Nagele, A. J. Mure, K. F. O'Malley, C. G. Relum and R. Burns (1993b). "Bacterial translocation in trauma patients: evidence suggesting phagocytic transport of intestinal organisms." Critical Care Medicine **21**: A162.

Braude, A., F. Carey, D. Sutherland and M. Zalesky (1955a). "Studies with radioactive endotoxin. I. The use of Cr^{51} to label endotoxin of Escherichia coli." Journal of Clinical Investigation **34**: 850-857.

Braude, A., F. Carey and M. Zalesky (1955b). "Studies with radioactive endotoxin. II. Correlation of physiologic effects with distribution of radioactivity in rabbits injected with lethal doses of E.coli endotoxin labeled with radioactive sodium chromate." Journal of Clinical Investigation **34**: 859-866.

Brown, R., J. Farmer, J. Hall, R. Humphries, S. O'Connor and G. Smith (1985). "The effects of dopexamineon the cardiovascular system of the dog." British Journal of Pharmacology **85**: 609-619.

Bustad, L. and V. Horstman (1986). Pigs: From BC to 2000 A.D. : From outhouse to penthouse. Swine in Biomedical Research. M. Tumbleson. New York, Plenum Press. **1**: 3-16.

Cabie, A., J. C. Farkas, C. Fitting, C. Laurian, J. M. Cormier, J. Carlet and J. M. Cavaillon (1993). "High levels of portal TNF-α during abdominal aortic surgery in man." Cytokine **5**(5): 448-453.

Cannon, J. G., J. S. Friedberg, J. A. Gelfand, R. G. Tompkins, J. F. Burke and C. A. Dinarello (1992). "Circulating interleukin - 1B and tumor necrosis factor-a concentrations after burn injury in humans." Critical Care Medicine **20**: 1414-1419.

Cannon, W. (1918a). "Acidosis in cases of shock, hemorrhage and gas infection." Journal of American Medical Association **70**: 531-535.

Cannon, W. (1918b). "A consideration of the nature of wound shock." Journal of American Medical Association **70**: 611-617.

Cannon, W., J. Fraser and E. Cowell (1918a). "The preventive treatment of wound shock." Journal of American Medical Association **70**: 618-621.

Cannon, W., J. Fraser and A. Hooper (1918b). "Some alterations in distribution and character of blood in shock and hemorrhage." Journal of American Medical Association **70**: 526-531.

Caplan, A. (1983). Beastly conduct: ethical issues in animal experimentation. The role of animals in biomedical research. J. Sechzer. New York, New York Academy of Sciences. **406:** 159-169.

Caridis, D., P. Cuevas and J. Fine (1972a). "Treatment of acute ischemia of the intestine by peritoneal lavage in the rabbit." Surgery Gynaecology and Obstetrics **135**: 199-202.

Caridis, D., R. Reinhold, P. Woodruff and Fine.J (1972b). "Endotoxaemia in man." Lancet **1**: 1381-1386.

Carlier, E. (1893). "Contribution to the histology of the hedgehog." Journal of Anatomy and Physiology **27**: 85-111, 169-178, 354-360, 508-518.

Carswell, E., L. Old, R. Kassel, S. Green, N. Fiore and B. Williamson (1975). "An endotoxin-induced serum factor that causes necrosis of tumors." Proceedings of the National Academy of Sciences of the USA **72**: 3666-3670.

Castell, J., T. Geiger, V. Gross, T. Andus, E. Walter, T. Hirano, T. Kishimoto and P. Heinrich (1988). "Plasma clearance, organ distribution and target cells of interleukin-6/hepatocyte-stimulating factor in the rat." European Journal of Biochemistry **177**: 357-361.

Chaudry, K., G. Singh and M. Rana (1991). "Low molecular weight heparin (LMWH) administration during resuscitation of hemorrhagic shock improves renal microvasculature (abstract)." Circulatory Shock **34**: 25.

Child, K. J., J. P. Currie, B. Davies, M. G. Dodds, D. R. Pearce and D. J. Twissell (1971). "The pharmacological properties in animals of CT1341 - A new steroid anaesthetic agent." British Journal of Anaesthesia **43**: 2-12.

Childs, C., R. Ratcliffe, I. Holt, R. Little and S. Hopkins (1990). The relationship between interleukin-1, interleukin-6 and pyrexia in burned children. The physiological and pathological effects of cytokines. C. Dinarello, M. Kluger, M. Powanda and J. Openheim. New York, Wiley-Liss**:** 295-300.

Chiu, C.-J., A. McArdle, R. Brown, H. Scott and F. Gurd (1970). "Intestinal mucosal lesion in low-flow states." Archives of Surgery **101**: 478-483.

Clemens, M. (1991). Cytokines. Oxford, Bios Scientific Publishers.

Collins, T., L. Lapierre, W. Fiers, J. Strominger and J. Pober (1986). "Recombinant human tumor necrosis factor increases mRNA levels and surface expression of HLA-A,B antigens in vascular endothelial cells and dermal fibroblasts in vitro." Proceedings of the National Academy of Sciences of the USA **83**: 446-450.

Coote, J. H., S. M. Hilton and J. F. Perez-Gonzalez (1979). "Inhibition of the baroreceptor reflex on stimulation in the brain stem defence centre." Journal of Physiology **288**: 549-560.

Cowell, E. (1918). "The inititation of wound shock." Journal of American Medical Association **70**: 607-610.

Cox, J. E., S. H. Done, P. Lees and J. R. Walton (1975). "Preliminary studies of the actions of alphaxalone and alphadalone in the pig." The Veterinary Record **87**: 497-498.

Crile, G. (1910). "Discussion on the prevention and treatment of shock." British Medical Journal **2**: 758-759.

Cross, A., J. Sadoff, N. Kelly, E. Bernton and P. Gemski (1989). "Pretreatment with recombinant murine tumor necrosis factor alpha/cachectin and murine interleukin-1 protects mice from lethal bacterial infection." Journal of Experimental Medicine **169**: 2021-2027.

Crozier, T. A., J. E. Müller, D. Quittkat, M. Sydow, W. Wuttke and D. Kettler (1994). "Effect of anaesthesia on the cytokine responses to abdominal surgery." British Journal of Anaesthesia **72**: 280-285.

Cruickshank, A. M., W. D. Fraser, H. J. G. Burns, J. van Damme and A. Shenkin (1990). "Response of serum interleukin-6 in patients undergoing elective surgery of varying intensity." Clinical Science **79**: 161-165.

Cuevas, P. and J. Fine (1971). "Demonstration of a lethal endotoxaemia in experimental occlusionof the superior mesenteric artery." Surgery Gynaecology and Obstetrics **133**: 81-83.

Cuevas, P. and J. Fine (1972a). "Rate of absorption of endotoxin from the intestine in non-septic shock." Journal of the Reticuloendothelial Society **11**: 535-538.

Cuevas, P. and J. Fine (1972b). "Role of intestinal endotoxin in the death from peritonitis." Surgery Gynaecology and Obstetrics **134**: 953-957.

Cuevas, P. and J. Fine (1973). "Production of fatal endotoxic shock by vasoactive substances." Gastroenterology **64**: 285-291.

Cuevas, P., M. Ishiyama, S. Koizumi, P. Woodruff, A. Kaufman and J. Fine (1974). "Role of endotoxaemia of intestinal origin in early death from large burns." Surgery Gynaecology and Obstetrics **138**: 725-730.

Cuthbertson, D., J. McGirr and J. Robertson (1939). "The effect of fracture of bone on the metabolism of the rat." Quarterly Journal of Experimental Physiology **29**: 13-25.

Dale, H. (1935). "Discussion on traumatic shock." Proceedings of the Royal Society of Medicine **28**: 1493-1495.

Dale, H. and P. Laidlaw (1919). "Histamine shock." Journal of Physiology **52**: 355-390.

Dale, H. and A. Richards (1918). "The vasodilator action of histamine and of some other substances." Journal of Physiology **52**: 110-166.

Damas, P., A. Reuter, P. Gysen, J. Demonty, M. Lamy and P. Franchimont (1989). "Tumor necrosis factor and interleukin-1 serum levels during severe sepsis in humans." Critical Care Medicine **17**: 975-978.

Davies, D. C. and M. N. Hoare (1972). "Endotracheal intubation in the pig." The Veterinary Record **84**: 151.

Davis, B. and D. R. Pearce (1972). "An introduction to Althesin (CT1341)." Postgraduate Medical Journal **June Suppl.**: 13-17.

Davis, J. H. (1995). "Editorial." Journal of Trauma **38**: 162.

Dawson, J. B. (1963). "Anaesthesia for the experimental pig." British Journal of Anaesthesia **35**: 736-739.

De Bandt, J. P., S. Chollet-Martin, A. Hernvann, N. Lioret, L. Desroys Du Roure, S.-K. Lim, M. Vanbourdolle, J. Guechot, R. Saizy, J. Giboudeau and L. Cynober (1994). "Cytokine response to burn injury: relationship with protein metabolism." Journal of Trauma **36**: 624-628.

Deavers, S., E. Smith and R. Huggins (1958). "Critical role of arterial pressure during hemorrhage in the dog on release of fluid into the circulation and trapping of red cells." American Journal of Physiology **195**: 73-76.

Debets, J. M. H., R. Kampmeijer, M. P. M. H. van der Linden, W. A. Buurman and C. J. van der Linden (1989). "Plasma tumor necrosis factor and mortality in critically ill septic patients." Critical Care Medicine **17**: 489-494.

Deitch, E. (1992). "Multiple organ failure - Pathophysiology and potential future therapy." Annals of Surgery **216**: 117-134.

Deitch, E. and R. Berg (1987). "Endotoxin but not malnutrition promotes bacterial translocation from the gut in burned mice." Journal of Trauma **27**: 161-166.

Deitch, E., R. Berg and R. Specian (1987). "Endotoxin promotes the translocation of bacteria from the gut." Archives of Surgery **122**: 185-190.

Deitch, E., W. Bridges, J. Baker, J.-W. Ma, L. Ma, M. Grisham, D. Granger, R. Specian and R. Berg (1988). "Hemorrhagic shock-induced bacterial translocation is reduced by xanthine oxidase inhibition or inactivation." Surgery **104**: 191-198.

Deitch, E., L. Ma, J.-W. Ma, M. Grisham, D. Granger, R. Specian and R. Berg (1989a). "Inhibition of endotoxin-induced bacterial translocation in mice." Journal of Clinical Investigation **84**: 36-42.

Deitch, E., M. Taylor, M. Grisham, L. Ma, W. Bridges and R. Berg (1989b). "Endotoxin induces bacterial translocation and increases xanthine oxidase activity." Journal of Trauma **29**: 1679-1983.

Deitch, E. A. (1993). "Cytokines yes, cytokines no, cytokines maybe?" Critical Care Medicine **21**: 817-819.

Deitch, E. A., W. Bridges, L. Ma, R. Berg, R. D. Specian and D. N. Granger (1990). "Hemorrhagic shock-induced bacterial translocation:the role of neutrophils and hydroxyl radicals." Journal of Trauma **30**: 942-951.

Deitch, E. A., R. D. Specian and R. D. Berg (1991). "Endotoxin-induced bacterial translocation and mucosal permeability:role of xanthine oxidase, complement activation, and macrophage products." Critical Care Medicine **19**: 785-791.

Deitch, E. A., D. Xu, L. Franko, A. Ayala and I. H. Chaudry (1994). "Evidence favoring the role of the gut as a cytokine-generating organ in rats subjected to hemorrhagic shock." Shock **1**: 141-146.

Dhainaut, J.-F. A., A. Tenaillon, Y. Le Tulzo, B. Schlemmer, J.-P. Solet, M. Wolff, L. Holzapfel, F. Zeni, D. Dreyfuss, J.-P. Mira, F. de Vathaire, P. Guinot and t. B. S. S. Group (1994). "Platelet-activating factor receptor antagonist BN52021 in the treatment of severe sepsis: a randomised, double-blind, placebo-controlled, multicenter clinical trial." Critical Care Medicine **22**: 1720-1728.

Di Padova, F., C. Pozzi, M. J. Tondre and R. Tritapepe (1991). "Selective and early increase of IL-1 inhibitors, IL-6 and cortisol after elective surgery." Clinical and Experimental Immunology **85**: 137-142.

Dignass, A. and D. Podolsky (1993). "Cytokine modulation of intestinal epithelial cell restitution: central role of transforming growth factor ß." Gastroenterology **105**: 1323-1332.

Dinarello, C. A., J. G. Cannon, S. M. Wolff, H. A. Bernheim, B. Beutler, A. Cerami, I. S. Figari, M. A. Palladino and J. V. O'Connor (1986). "Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1." Journal of Experimental Medicine **163**: 1433-1450.

Dingley, J., B. Foëx and R. Little (1997a). "Repeatability of a bedside system for measuring circulating blood volume using the carbon monoxide method." Critical Care Medicine **In press**.

Dingley, J., B. A. Foëx and R. A. Little (1997b). "Repeatability of a bedside system for measuring circulating blood volume using the carbon monoxide method." Clinical Intensive Care **8**: 107 A41.

Downman, C., C. MacKenzie and B. McSwiney (1944). "The effects of acute haemorrhage on the peripheral blood pressure in unanaesthetised and in anaesthetised rabbits." Journal of Physiology **103**: 350-357.

Driscoll, P. (1994). Changes in systolic blood pressure, heart rate, shock index, rate pressure product and tympanic temperature following blood loss and tissue damage in humans. Leeds, University of Leeds.

Edouard, A. R., A.-C. Degrémont, J. Duranteau, E. Pussard, A. Berdeaux and K. Samii (1994). "Heterogeneous regional vascular responses to simulated transient hypovolaemia in man." Intensive Care Medicine **20**: 414-420.

Edwards, J., W. Shoemaker and J.-L. Vincent (1993). Standard equations. Oxygen transport: principles and practice. J. Edwards, W. Shoemaker and J.-L. Vincent. London, W.B. Saunders Company Ltd**:** 357-359.

Elebute, E. and H. Stoner (1983). "The grading of sepsis." British Journal of Surgery **70**: 29-31.

Endo, S., K. Inada, Y. Inoue, Y. Kuwata, M. Suzuki, H. Yamashita, S. Hoshi and M. Yoshida (1992). "Two types of septic shock classified by the plasma levels of cytokines and endotoxin." Circulatory Shock **38**: 264-274.

Endo, S., K. Inada, Y. Yamada, T. Takakuwa, T. Kasai, H. Nakae, M. Yoshida and M. Ceska (1994). "Plasma endotoxin and cytokine concentrations in patients with hemorrhagic shock." Critical Care Medicine **22**: 949-955.

Ertel, W., M. H. Morrison, P. Wang, Z. F. Ba, A. Ayala and I. H. Chaudry (1991). "The complex pattern of cytokines in sepsis." Annals of Surgery **214**: 141-148.

Ferguson, K., A. Cardellio, V. Tonapi, J. L. Rodriquez and R. F. Maio (1994). "TNF is involved in the systemic cytokine response to trauma." Academic Emergency Medicine 1(Society of Academic Emergency Medicine Annual Meeting Abstracts): Poster.

Fiers, W. (1991). "Tumor necrosis factor." FEBS Letters **285**: 199-212.

Fiers, W. (1993). Tumour necrosis factor. Humoral factors. E. Sim. Oxford, Oxford University Press**:** 65-120.

Fine, J., S. Rutenburg and F. Schweinburg (1959). "The role of the reticulo-endothelial system in hemorrhagic shock." Journal of Experimental Medicine **110**: 547-569.

Fink, M. P., K. L. Kaups, H. Wang and H. R. Rothschild (1991). "Maintenance of superior mesenteric arterial perfusion prevents increased intestinal mucosal permeability in endotoxic pigs." Surgery **110**: 154-161.

Fisher, C. and G. Bellingan (1991). "Immunotherapy of sepsis syndrome: A comparison of the available treatments." Klinische Wochenschrift **69**(Suppl XXVI): 162-167.

Fisher, C., J.-F. Dhainaut, S. Opal, J. Pribble, R. Balk, G. Slotman, T. Iberti, E. Rackow, M. Shapiro, R. Greenman, H. Reines, M. Shelly, B. Thompson, J. LaBrecque, M. Catalano, W. Knaus and J. Sadoff (1994a). "Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome." Journal of American Medical Association **271**: 1836-1843.

Fisher, C. J., S. M. Opal, J.-F. Dhainaut, S. Stephens, J. L. Zimmerman, P. Nightingale, S. J. Harris, R. M. H. Schein, E. A. Panacek, J.-L. Vincent, G. E. Foulke, E. L. Warren, C. Garrard, G. Park, M. W. Bodmer, J. Cohen, C. Van der Linden, A. S. Cross, J. C. Sadoff and t. C. S. S. S. Group (1993). "Influence of an anti-tumour necrosis factor monoclonal antibody on cytokine levels in patients with sepsis." Critical Care Medicine **21**: 318-327.

Fisher, C. J., G. J. Slotman, S. M. Opal, J. P. Pribble, R. C. Bone, G. Emmanuel, D. Ng, D. C. Bloedow, M. A. Catalano and t. I.-R. S. S. S. Group (1994b). "Initial evaluation of human recombinant interleukin-1 receptor antagonist in the treatment of sepsis syndrome: a randomized, open-label, placebo-controlled multicenter trial." Critical Care Medicine **22**: 12-21.

Flynn, W., D. Pilati and E. Hoover (1997). "Xanthine oxidase inhibition after resuscitated hemorrhagic shock restores mesenteric blood flow without vasodalation." Shock **8**: 300-304.

Foëx, B., W. Lamb, T. Roberts, P. Brenchley, S. Brear and I. Macartney (1994). "The inflammatory cytokine response to multiple injury." Intensive Care Medicine **20**(Supplement 1): S62.

Foëx, B., J. Quinn, R. Little, M. Shelly and G. Slotman (1997). "Differences in eicosanoid and cytokine production between injury/hemorrhagic shock and bacteremic shock in the pig." Shock **8**: 276-283.

Foëx, B. A., W. R. Lamb, T. E. Roberts, S. G. Brear, I. Macartney, M. Hammer and P. E. C. Brenchley (1993). "Early cytokine response to multiple injury." Injury **24**: 373-376.

Foëx, B. A. and M. P. Shelly (1996). "The cytokine response to critical illness." Journal of Accident and Emergency Medicine **13**: 154-162.

Fong, Y., K. J. Tracey, L. L. Moldawer, D. G. Hesse, K. B. Manogue, J. S. Kenney, A. T. Lee, G. C. Kuo, A. C. Allison, S. F. Lowry and A. Cerami (1989). "Antibodies to cachectin/tumor necrosis factor reduce interleukin 1B and interleukin 6 appearance during lethal bacteremia." Journal of Experimental Medicine **170**: 1627-1633.

Fowler, J., D. W. Hill, R. L. Morgan, J. F. Nunn, B. Weaver and R. F. Woolmer (1962). "Anaesthesia for the irradiated pig; a study in remote control." British Journal of Anaesthesia **34**: 327-331.

Frank, H., S. Jacob, F. Schweinburg, J. Goddard and J. Fine (1952). "Traumatic shock. XXI. Effectiveness of an antibiotic in experimental hemorrhagic shock." American Journal of Physiology **168**: 430-436.

Fraser, J. and E. Cowell (1918). "Clinical study of blood pressure in wound conditions." Journal of American Medical Association **70**: 520-526.

Friese, R. S., T. F. Rehring, M. Wollmering, E. E. Moore, L. L. Ketch, A. Banerjee and A. H. Harken (1994). "Trauma primes cells: editorial review." Shock **1**: 388-394.

Gamble, J., J. Harlan, S. Klebanoff and M. Vadas (1985). "Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor." Proceedings of the National Academy of Sciences of the USA **82**: 8667-8671.

Geerdes, B. P., K. L. Frederick and M. J. Brunner (1993). "Carotid baroreflex control during hemorrhage in conscious and anesthetized dogs." American Journal of Physiology **265**: R195-R202.

Gelfand, G. A. J., J. Morales, R. L. Jones, P. Kibsey, M. Grace and S. M. Hamilton (1991). "Hemorrhagic shock and bacterial translocation in a swine model." Journal of Trauma **31**: 867-874.

Girden, E. R. (1992). ANOVA Repeated Measures. Newbury, Ca, SAGE Publications.

Girolami, A., B. Foëx and R. Little (1998). "Changes in the causes of trauma in the last 20 years." Trauma **In press**.

Glantz, S. A. (1997). Primer of Biostatistics. New York, McGraw-Hill.

Godfrey, K. (1992). Comparing the means of several groups. Medical Uses of Statistics. J. C. B. III and F. Mosteller. Boston, BEJM Books: 233-257.

Graber, C. D., R. B. Reinhold, J. G. Breman, R. A. Harley and G. R. Henningar (1971). "Fatal heat stroke. Circulating endotoxin and gram negative sepsis as complications." Journal of American Medical Association **216**: 1195-1196.

Granger, D. (1988). "Role of xanthine oxidase and granulocytes in ischemia-reperfusion injury." American Journal of Physiology **255**: H1269-H1275.

Grant, R. (1961). "Comments." Federation Proceedings **20**(2, Part III, Supplement 9): 48-49.

Grant, R. T. and E. B. Reeve (1941). "Clinical observations on air-raid casualties." British Medical Journal **Aug 30, Sept 6**: 293-297, 329-332.

Green, H. (1961). "Physiology of peripheral circulation in shock." Federation Proceedings **20**(2 Part III Supplement 9): 61-68.

Gregersen, M. I. and W. S. Root (1947). "Experimental traumatic shock produced by muscle contusion with a note on the effects of bullet wounds. A study of the clinical signs of shock in the dog and the role of blood volume reduction in the development of the shock syndrome." American Journal of Physiology **148**: 98-123.

Groeneveld, A. and L. Thijs (1987). Systemic microvascular permeabilityin septic shock. Septic Shock. J.-L. Vincent and L. Thijs. Berlin, Springer-Verlag: 43-50.

Gunnarsson, U., H. Hjelmqvist and M. Rundgren (1994). "Haemodynamic responses to hypotensive haemorrhage in conscious sheep with emphasis on renal and femoral blood flow." Experimental Physiology **79**: 957-965.

Guthrie, C. (1917). "Experimental shock." Journal of American Medical Association **69**: 1394-1398.

Guyton, A. (1981). Textbook of Medical Physiology. Philadelphia, WB Saunders Company.

Guyton, A. and J. Crowell (1961). "Dynamics of the heart in shock." Federation Proceedings **20**(2, Part III, Supplement 9): 51-60.

Guzman-Stein, G., M. Bonsack, J. Liberty, J. P. Delaney and C. W. Wells (1987). "Intestinal handling facilitates enteric bacterial translocation." Surgical Forum **38**: 75-76.

Hack, C. E., E. R. DeGroot, R. J. F. Felt-Bersma, J. H. Nuijens, R. J. M. Strack Van Schijndel, A. J. M. Eerenberg-Belmer, L. G. Thijs and L. A. Aarden (1989). "Increased plasma levels of interleukin-6 in sepsis." Blood **74**: 1704-1710.

Haglind, E. and R. Rylander (1993). "Endotoxin contamination of superoxide dismutase and catalase." Circulatory Shock **39**: 80-81.

Hall, G. M. and J. P. Desborough (1992). "Interleukin-6 and the metabolic response to surgery (Editorial)." British Journal of Anaesthesia **69**: 337-338.

Hall, L. W. (1972). "Althesin in the larger animal." Postgraduate Medical Journal **June Supp**: 55-58.

Hannon, J. and C. Bossone (1986). The conscious pig as a large animal model for studies of hemorrhagic hypotension. Swine in Biomedical Research. M. Tumbleson. New York, Plenum Press. **3:** 1413-1428.

Hannon, J. P., C. E. Wade, C. A. Bossone, M. M. Hunt, R. I. Coppes and J. A. Loveday (1990). "Blood gas and acid-base status of conscious pigs subjected to fixed-volume hemorrhage and resuscitated with hypertonic saline dextran." Circulatory Shock **32**: 19-29.

Hannon, J. P., C. E. Wade, C. A. Bossone, M. M. Hunt and J. A. Loveday (1989). "Oxygen delivery and demand in conscious pigs subjected to fixed-volume hemorrhage and resuscitated with 7.5% NaCl in 6% dextran." Circulatory Shock **29**: 205-217.

Harrison, J., A. Hodson, A. Skillen and R. Stappenbeck (1988). "Blood glucose,lactate, pyruvate, glycerol, 3-hydroxybutyrate and acetoacetate measurements in man using a centrifugal analyser with a fluorimetruc attachment." Journal of Clinical Chemistry and Clinical Biochemistry **26**: 141-146.

Hartmann, M., A. Montgomery, K. J"nsson and U. Haglund (1991). "Tissue oxygenation in hemorrhagic shock measured as transcutaneous oxygen tension, subcutaneous oxygen tension, and gastrointestinal intramucosal pH in pigs." Critical Care Medicine **19**: 205-210.

Hawkins, R., M. Llewelyn and S. Russell (1992). "Adapting antibodies for clinical use." British Medical Journal **305**: 1348-1352.

Heinrich, P., J. Castell and T. Andus (1990). "Interleukin-6 and the acute phase response." Biochemical Journal **265**: 621-636.

Heinrich, P. and S. Rose-John (1993). Interleukin-6. Humoral Factors. E. Sim. Oxford, Oxford University Press**:** 47-64.

Heistad, D. and F. Abboud (1974). "Factors that influence blood flow in skeletal muscle and skin." Anesthesiology **41**: 139-156.

Held, J. (1983). "Appropriate animal models." Annals of the New York Academy of Sciences **406**: 13-19.

Henderson, Y. (1908). "Acapnia and shock. - 1. Carbon dioxid as a factor in the regulation of the heart rate." American Journal of Physiology **21**: 126-156.

Herndon, D. N. and S. T. Ziegler (1993). "Bacterial translocation after thermal injury (Review)." Critical Care Medicine **21**: S50-S54.

Hildebrand, G. and H. Wolochow (1962). "Translocation of bacteriophage across the intestinal wall in the rat." Proceedings Society for Experimental Biology and Medicine **109**: 183-185.

Hill, K. J. and J. S. Perry (1959). "A method for closed-circuit anaesthesia in the pig." The Veterinary Record **71**: 296-299.

Hilton, S. M., J. M. Marshall and R. J. Timms (1983). "Ventral medullary relay neurones in the pathway from the defence areas of the cat and their effect on blood pressure." Journal of Physiology **345**: 149-166.

Hinds, C. J. (1992). "Monoclonal antibodies in sepsis and septic shock." British Medical Journal **304**: 132-133.

Hirsch, A. T., D. J. Levenson, S. S. Cutler, V. J. Dzau and M. A. Creager (1989). "Regional vascular responses to prolonged lower body negative pressure in normal subjects." American Journal of Physiology **257**: H219-H225.

Hoch, R. C., R. Rodriguez, T. Manning, M. Bishop, P. Mead, W. C. Shoemaker and E. Abraham (1993). "Effects of accidental trauma on cytokine and endotoxin production." Critical Care Medicine **21**: 839-845.

Horton, J. (1987). "Cardiocirculatory function in the intoxicated shocked dog: Acid base derangements." Circulatory Shock **22**: 23-34.

Howard, J. M., C. P. Artz and R. R. Stahl (1955). "Hypertensive response to injury." Annals of Surgery **141**: 327-336.

Huck, S. W. and W. H. Cormier (1996). Reading Statistics and Research. New York, Harper Collins.

Jacobs, S., H. Weizel, E. Gordon, H. Korman, F. Schweinburg, H. Frank and J. Fine (1954). "bacterial action in development of irreversibility to transfusion in hemorrhagic shock in the dog." American Journal of Physiology **179**: 523-531.

Jacobsen, J. and N. H. Secher (1992). "Heart rate during haemorrhagic shock." Clinical Physiology **12**: 659-666.

Jacobsen, J., S. Sofelt, S. Sheikh, J. Warberg and N. H. Secher (1990). "Cardiovascular and endocrine responses to haemorrhage in the pig." Acta Physiologica Scandinavica **138**: 167-173.

Jacobson, D. and M. Singer (1996). The cell, the mitochondrion, oxygen and sepsis. Yearbook of Intensive Care and Emergency Medicine. J.-L. Vincent. Berlin, Springer-Verlag: 263-274.

Jordan, F. (1867). "Shock after surgical operations and injuries." British Medical Journal: 73-76, 136-137, 164-167, 192-193, 219-224, 257-261, 281-282.

Joris, J., I. Cigarini, M. Legrand, N. Jacquet, D. De Groote, P. Franchimont and M. Lamy (1992). "Metabolic and respiratory changes after cholecystectomy performed via laparotomy or laparoscopy." British Journal of Anaesthesia **69**: 341-345.

Kirkman, E. and R. Little (1994). Central control of cardiovascular responses to injury. Brain control of responses to trauma. N. Rothwell and F. Berkenbosch. Cambridge, Cambridge University Press: 202-238.

Kirkman, E., T. Shiozaki and R. A. Little (1994). "Methiothepin antagonism does not attenuate the bradycardia associated with severe haemorrhage in the anaesthetized rat (abstract)." British Journal of Pharmacology **112**: 231.

Kirkman, E., H. Zhang, H. Spapen, R. A. Little and J. L. Vincent (1995). "Effects of afferent neural stimulation on critical oxygen delivery: a hemodynamic explanation." American Journal of Physiology **269**: R1448-R1454.

Kishimoto, T. (1989). "The biology of Interleukin-6." Blood **74**: 1-10.

Knighton, D., V. Fiegel, T. Halverson, S. Schneider, T. Brown and C. Wells (1990). "Oxygen as an antibiotic: The effect of inspired oxygen on bacterial clearance." Archives of Surgery **125**: 97-100.

Knighton, D., B. Halliday and T. Hunt (1984). "Oxygen as an antibiotic: The effect of inspired oxygen on infection." Archives of Surgery **119**: 199-204.

Koziol, J. M., B. F. Rush, S. M. Smith and G. W. Machiedo (1988). "Occurrence of bacteremia during and after hemorrhagic shock." Journal of Trauma **28**: 10-16.

Kushner, I. (1982). "The phenomenon of the acute phase response." Annals of the New York Academy of Sciences **389**: 39-48.

Kvietys, P. R. (1996). "Restitution of gastrointestinal epithelium." News in Physiological Science **11**: 248.

Larrick, J., D. Graham, K. Toy, L. Lin, G. Senyk and B. Fendly (1987). "Recombinant tumor necrosis factor causes activation of human granulocytes." Blood **69**: 640-644.

Leindhardt, D. J., G. L. Carlson, W. R. Lamb, P. E. C. Brenchley, R. A. Kay, K. A. Shipley and M. Mughal (1992). "The cytokine response to abdominal sepsis." The Proceedings of the Nutrition Society **51(2)**: 113A.

Levy, R., C. Hebert, B. Munn and R. Barrack (1996). "Drug and alcohol use in orthopaedic trauma patients: A prospective study." Journal of Orthopaedic Trauma **10**: 21-27.

Lewis, D. (1987). Disturbances in microcirculatory regulation in septic shock. Septic Shock. J.-L. Vincent and L. Thijs. Berlin, Springer-Verlag: 26-34.

Little, R. A. and B. A. Foëx (1996). Animal models in critical care research. Current Topics in Intensive Care. R. P. Dellinger, H. Burchardi, G. J. Dobb and J. Bion. London, WB Saunders. **3:** 148-165.

Little, R. A., H. W. Marshall and E. Kirkman (1989). "Attenuation of the acute cardiovascular responses to haemorrhage by tissue injury in the conscious rat." Quarterly Journal of Experimental Physiology **74**: 825-833.

Lockhart-Mummery, J. (1905). "The physiology and treatment of surgical shock and collapse." Lancet **1**: 696-703.

Lokhandwala, M. and B. Jandhyala (1992). "Effects of dopaminergic agonists on organ blood flow and function." Clinical Intensive Care **3 (Suppl)**: 12-15.

Lowe, D., H. Gately, J. Goss, C. Frey and C. Peterson (1983). "Patterns of death, complication, and error in the management of motor vehicle accident victims: implications for a regional system of trauma care." Journal of Trauma **23**: 503-509.

Ludewig, R. and S. Wangensteen (1969). "Aortic bleeding and the effect of external counterpressure." Surgery Gynaecology and Obstetrics **128**: 252-258.

Lumley, J., C. Green, P. Lear and J. Angell-James (1990). Essentials of Experimental Surgery. London, Butterworths.

Lumpkin, M. (1987). "The regulation of ACTH secretion by IL-1." Science **238**: 452-454.

Mackiewicz, A., H. Schooltink, P. Heinrich and S. Rose-John (1992). "Complex of soluble human IL-6 receptor/IL-6 up-regulates expression of acute phase proteins." Journal of Immunology **149**: 2021-2027.

Mackway-Jones, K., B. Foëx, E. Kirkman and R. A. Little (1994). "Modification of the haemodynamic response to blood loss by somatic afferent stimulation (abstract)." Shock **2**(Supplement): 19.

Marie, C., C. Fitting, C. Cheval, M. R. Losser, J. Carlet, D. Payen, K. Foster and J. M. Cavaillon (1997). "Presence of high levels of leukocyte-associated interleukin-8 upon cell activation and in patients with sepsis syndrome." Infection and Immunity **65**(3): 865-871.

Marty, C., B. Misset, F. Tamion, C. Fitting, J. Carlet and J. M. Cavaillon (1994). "Circulating interleukin-8 concentrations in patients with multiple organ failure of septic and nonseptic origin." Critical Care Medicine **22**(4): 673-679.

Mateo, R. B., J. S. Reichner and J. E. Albina (1994). "Interleukin-6 activity in wounds." American Journal of Physiology **266**: R1840-R1844.

Matthews, J. N. S., D. G. Altman, M. J. Campbell and P. Royston (1990). "Analysis of serial measurements in medical research." British Medical Journal **300**: 230-235.

Matthews, L. (1952). British Mammals. London, Collins.

Matzen, S., G. Perko, S. Groth, D. B. Friedman and N. H. Secher (1991). "Blood volume distribution during head-up tilt induced central hypovolaemia in man." Clinical Physiology **11**: 411-422.

Meade, P., W. C. Shoemaker, T. J. Donnelly, E. Abraham, M. A. Jagels, H. G. Cryer, T. E. Hugli, M. H. Bishop and C. C. J. Wo (1994). "Temporal patterns of hemodynamics, oxygen transport, cytokine activity, and complement activity in the development of adult respiratory distress syndrome after severe injury." Journal of Trauma **36**: 651-657.

Medical Research Committee (1917). "Memorandum upon surgical shock and some allied conditions." British Medical Journal, Lancet **1,1**: 381-383, 502-505.

Mellander, S. and D. Lewis (1963). "Effect of hemorrhagic shock on the reactivity of resistance and capacitance vessels and on capillary filtration transfer in cat skeletal muscle." Circulation Research **13**: 105-118.

Merin, R. G., P. D. Verdouw and J. W. Jong (1977). "Dose-dependent depression of cardiac function and metabolism by halothane in swine (sus scrofa)." Anaesthesiology **46**: 417-423.

Messmer, K. (1987). Microcirculatory changes in endotoxinemia and septic shock. Septic Shock. J.-L. Vincent and L. Thijs. Berlin, Springer-Verlag: 35-42.

Mestan, J., W. Digel, S. Mittnacht, H. Hillen, D. Blohm, A. Möller, H. Jacobsen and H. Kirchner (1986). "Antiviral effects of recombinant tumour necrosis factor in vitro." Nature **323**: 816-819.

Michie, H. R., K. R. Manogue, D. R. Spriggs, A. Revhaug, S. O. O'Dwyer, C. A. Dinarello, A. Cerami, S. M. Wolff and D. W. Wilmore (1988). "Detection of

circulating tumor necrosis factor after endotoxin administration." New England Journal of Medicine **318**: 1481-1486.

Milles, G., C. Koucky and H. Zacheis (1966). "Experimental uncontrolled arterial hemorrhage." Surgery **60**: 434-442.

Montgomery, A., M. Hartmann, K. J"nsson and U. Haglund (1989). "Intramucosal pH measurement with tonometers for detecting gastrointestinal ischemia in porcine hemorrhagic shock." Circulatory Shock **29**: 319-327.

Moon, V. (1942). "The vascular and cellular dynamics of shock." The American Journal of the Medical Sciences **203**: 1-18.

Moore, C. M., J. P. Desborough, H. Powell, J. M. Burrin and G. M. Hall (1994a). "Effects of extradural anaesthesia on interleukin-6 and acute phase response to surgery." British Journal of Anaesthesia **72**: 272-279.

Moore, E. E., F. A. Moore, R. J. Franciose, F. J. Kim, W. L. Biffl and A. Banergee (1994b). "The postischemic gut serves as a priming bed for circulating neutrophils that provoke multiple organ failure." Journal of Trauma **37**: 881-887.

Moore, F. and E. Moore (1996). Early postinjury nutrition: Does it make a difference? Yearbook of Intensive Care and Emergency Medicine. J. Vincent. Berlin, Springer-Verlag: 250-259.

Moore, F. A., E. E. Moore, R. Poggetti, O. J. McAnena, V. M. Peterson, C. M. Abernathy and P. E. Parsons (1991). "Gut bacterial translocation via the portal vein: a clinical perspective with major torso trauma." Journal of Trauma **1991**: 629-636.

Moore, R., J. Madri, S. Carlson and J. Madara (1992). "Collagens facilitate epithelial migration in restitution of native guinea pig intestinal epithelium." Gastroenterology **102**: 119-130.

Morales, J., P. Kibsey, P. D. Thomas, M. J. Poznansky and S. M. Hamilton (1992). "The effects of ischaemia and ischaemia reperfusion on bacterial translocation, lipid peroxidation, and gut histology: studies on haemorrhagic shock in pigs." Journal of Trauma **33**: 221-226.

Morris, S. E., N. Navaratnam, C. M. Townsend and D. N. Herndon (1988). "Bacterial translocation and mesenteric blood flow in a large animal model after cutaneous thermal and smoke inhalation injury." Surgical Forum **39**: 189-190.

Mosselmans, R., A. Hepburn, J. Dumont, W. Fiers and P. Galand (1988). "Endocytic pathway of recombinant murine tumor necrosis factor in L-929 cells." Journal of Immunology **141**: 3096-3100.

Motulsky, H. (1995). Intuitive Biostatistics. Oxford, Oxford University Press.

Mukerjee, M. (1997). "Trends in animal research." Scientific American **276**(February): 70-77.

Naitoh, Y., J. Fukata, G. Tominaga, Y. Nakai, S. Tamai, K. Mori and H. Imura (1988). "Interleukin-6 stimulates the secretion of adrenocorticotropic hormone in conscious, freely moving rats." Biochemical Biophysical Research Communications **155**: 1459.

Navaratnam, R. L. M., S. E. Morris, D. L. Traber, J. Flynn, L. Woodson, H. Linares and D. N. Herndon (1990). "Endotoxin (LPS) increases mesenteric vascular resistance (MVR) and bacterial translocation (BT)." Journal of Trauma **30**: 1104-1115.

Nedospasov, S., I. Udalova, D. Kuprash and T. RL (1991). "DNA sequence polymorphism at the human tumor necrosis factor (TNF) locus.
Numerous TNF/Lymphotoxin alleles tagged by two closely linked microsatellites in the upstream region of the lymphotoxin (TNF-ß) gene." Journal of Immunology **147**: 1053-1059.

Nelson, D., C. King, S. Dodd, P. Shumacker and S. Cain (1987). "Systemic and intestinal limits of oxygen extraction in the dog." Journal of Applied Physiology **63**: 387-394.

Nelson, T. E. (1986). Malignant hyperthermia: a pharmacogenetic disease of man and pigs. Swine in Biomedical Research. M. E. Tumbleson. New York, Plenum Press. **1**: 261-272.

Nicola, N., Ed. (1995). Guidebook on Cytokines and their Receptors. Oxford, Sambrook & Tooze Publication at Oxford University Press.

Nijsten, M., E. de Groot, H. Ten Duis, H. Klasen, C. Hack and L. Aarden (1987). "Serum levels of interleukin-6 and acute phase responses." Lancet **2**: 921.

Noble, R. and J. Collip (1941-2). "A quantitative method for the production of experimental traumatic shock without haemorrhage in unanaesthetized animals." Quarterly Journal of Experimental Physiology **31**: 187-200.

Noble, R., M. Gregersen, P. Porter and A. Buckman (1946). "Blood volume in clinical shock.II. The extent and cause of blood volume reduction in traumatic, hemorrhagic, and burn shock." Journal of Clinical Investigation **25**: 172-183.

Novick, D., H. Engelmann, D. Wallach and M. Rubinstein (1989). "Soluble cytokine receptors are present in normal human urine." Journal of Experimental Medicine **170**: 1409-1414.

Nunn, J. F. and J. Freeman (1964). "Problems of oxygenation and oxygen transport during haemorrhage." Anaesthesia **19**: 206-216.

Öberg, B. and P. Thorén (1972). "Increased activity in left ventricular receptors during hemorrhage or occlusion of caval veins in the cat. A possible cause of the vaso-vagal reaction." Acta Physiologica Scandinavica **85**: 164-173.

Office of Population Censuses and Surveys (1993). Mortality statistics - causes (England and Wales). London, HMSO.

Overman, R. R. and S. C. Wang (1947). "The contributory role of the afferent nervous factor in experimental shock: sublethal hemorrhage and sciatic nerve stimulation." American Journal of Physiology **148**: 289-295.

Palladino, M., M. Shalaby, S. Kramer, B. Ferraiolo, R. Baughman, A. Deleo, D. Crase, B. Marafino, B. Aggarwal, I. Figari, D. Liggitt and J. Patton (1987). "Characterization of the antitumor activities of human tumor necrosis factor-α and the comparison with other cytookines: induction of tumoor-specific immunity." Journal of Immunology **138**: 4023-4032.

Pape, H.-C., D. Remmers, W. Kleemann, J. Goris, G. Regel and H. Tscherne (1994). "Posttraumatic multiple organ failure - a report on clinical and autopsy findings." Shock **2**: 228-234.

Park, P.-O. and U. Haglund (1992). "Regeneration of small bowel mucosa after intestinal ischaemia." Critical Care Medicine **20**: 135-139.

Parsons, E. and D. Phemister (1930). "Haemorrhage and "shock" in traumatized limbs." Surgery Gynaecology and Obstetrics **51**: 196-207.

Peitzman, A. B., A. O. Udekwu, J. Ochoa and S. Smith (1991). "Bacterial translocation in trauma patients." Journal of Trauma **31**: 1083-1086.

Phillips, R. W. and M. E. Tumbleson (1986). Models. Swine in Biomedical Research. M. E. Tumbleson. New York, Plenum Press. **1**: 437-440.

Piguet, P., M. Collart, G. Grau, A.-P. Sappino and P. Vassalli (1990). "Requirement of tumour necrosis factor for development of silica-induced pulmonary fibrosis." Nature **344**: 245-247.

Playfair, J., J. Taverne and N. Matthews (1984). "What is tumour necrosis factor really for?" Immunology Today **5**: 165-166.

Pober, J. and R. Cotran (1990). "The role of endothelial cells in inflammation." transplantation **50**: 537-544.

Pretorius, J., G. Schlag, H. Redl, W. Botha, D. Goosen, H. Bosman and A. van Eeden (1987). "The "lung in shock" as a result of hypovolemic-traumatic shock." Journal of Trauma **27**: 1344-1353.

Quest, J. A. and G. L. Gebber (1972). "Modulation of baroreceptor reflexes by somatic afferent nerve stimulation." American Journal of Physiology **222**: 1251-1259.

Rabinovici, R., R. John, K. M. Esser, J. Vernick and G. Feuerstein (1993). "Serum tumor necrosis factor-alpha profile in trauma patients." Journal of Trauma **35**: 698-701.

Rady, M. Y., E. Kirkman, J. Cranley and R. A. Little (1993). "A comparison of the effects of skeletal muscle injury and somatic afferent nerve stimulation on the response to hemorrhage in anesthetised pigs." Journal of Trauma **35**: 756-761.

Rady, M. Y., R. A. Little, J. D. Edwards, E. Kirkman and S. Faithfull (1991). "The effect of nociceptive stimulation on the changes in hemodynamics and oxygen transport induced by hemorrhage in anesthetised pigs." Journal of Trauma **31**: 617-622.

Rasmussen, I. and U. Haglund (1992). "Early gut ischemia in experimental fecal peritonitis." Circulatory Shock **38**: 22-28.

Ravin, H., D. Rowley, C. Jenkins and J. Fine (1960). "On the absorption of bacterial endotoxin from the gastro-intestinal tract of the normal and shocked animal." Journal of Experimental Medicine **112**: 783-792.

Ravin, H., S. Schweinburg and J. Fine (1958). "Host resistance to hemorrhagic shock. XV. Isolation of toxic factor from hemorrhagic shock plasma." Proceedings Society for Experimental Biology and Medicine **99**: 426-431.

Redfern, W. (1981). Effects of limb ischaemia on the cardiac component of the baroreceptor reflex in the unanaesthetized rat - afferent, central and efferent mechanisms. North Western Injury Research Centre. Manchester, University of Manchester.

Reed, L. L., M. Martin, R. Manglano, B. Newson, F. Kocka and J. Barrett (1994). "Bacterial translocation following abdominal trauma in humans." Circulatory Shock **42**: 1-6.

Regel, G., P. Lobenhoffer, M. Grotz, H. C. Pape, U. Lehmann and H. Tscherne (1995). "Treatment results of patients with multiple trauma: an analysis of 3406 cases treated between 1972 and 1991 at a German level 1 trauma centre." Journal of Trauma **38**: 70-78.

Reinhart, K., C. Wiegand-Löhnert, F. K. Grimminger, M, S. Withington, D. Treacher, J. Eckart, S. Willats, C. Bouza, D. Krausch, F. Stockenhuber, J. Eiselstein, L. Daum, J. Kempeni and t. M. F. S. S. Group (1996). "Assessment of the safety and efficacy of the monoclonal anti-tumor necrosis factor antibody-fragment, MAK 195F, in patients with sepsis and septic shock: A multicenter randomized, placebo-controlled, dose-ranging study." Critical Care Medicine **24**: 733-742.

Reinhold, R. and J. Fine (1971). "A technique for quantitative measurement of endotoxin in human plasma." Proceedings Society for Experimental Biology and Medicine **137**: 334-340.

Riebold, T. W. and J. C. Thurmon (1986). Anesthesia in Swine. Swine in Biomedical Research. M. E. Tumbleson. New York, Plenum Press. **1:** 243-254.

Ritchie, D. and G. Fuller (1983). "Hepatocyte-stimulatory factor: a monocyte-derived acute phase regulatory protein." Annals of the New York Academy of Sciences **408**: 490-500.

Robertson, O. and A. Bock (1919a). "Blood volume in wounded soldiers.I. Blood volume and related blood changes after hemorrhage." Journal of Experimental Medicine **29**: 139-153.

Robertson, O. and A. Bock (1919b). "Blood volume in wounded soldiers.II. The use of forced fluids by the alimentary tract in the restoration of blood volume after hemorrhage." Journal of Experimental Medicine **29**: 155-171.

Robinson, J., V. Mirkovitch, B. Winistorfer and F. Saegesser (1981). "Response of the intestinal mucosa to ischemia." Gut **22**: 512-527.

Rocha-e-Silva, M., G. A. Negraes, A. M. Soares, V. Pontieri and L. Loppnow (1986). "Hypertonic resuscitation from severe hemorrhagic shock: patterns of regional circulation." Circulatory Shock **19**: 165-175.

Roumen, R. M. H., T. Hendricks, R. A. Wevers and R. J. A. Goris (1993). "Intestinal permeability after severe trauma and hemorrhagic shock is increased without relation to septic complications." Archives of Surgery **128**: 453-457.

Rowan, A. (1997). "The benefits and ethics of animal research." Scientific American **276**(February): 63.

Rowley, D., J. Howard and C. Jenkin (1956). "The fate of P^{32} - labelled bacterial lipopolysaccharides in laboratory animals." Lancet **i**: 366-367.

Rowson, L. E. A. (1965). "Endotracheal intubation in the pig." The Veterinary Record **77**: 1465.

Russell, S., M. Llewelyn and R. Hawkins (1992). "Principles of antibody therapy." British Medical Journal **305**: 1424-1428.

Rutenburg, S., F. Schweinburg and J. Fine (1960). "In vitro detoxification of bacterial endotoxin by macrophages." Journal of Experimental Medicine **112**: 801-807.

Ryan, C. M., M. L. Yarmush, J. F. Burke and R. G. Tompkins (1992). "Increased gut permeability early after burns correlates with extent of burn injury." Critical Care Medicine **20**: 1508-1512.

Saadia, R. and J. Lipman (1996). "Multiple organ failure after trauma." British Medical Journal **313**: 573-574.

Samsel, R. and P. Schumacker (1992). Oxygen delivery and uptake: insight from anomal studies. Yearbook of Intensive Care and Emergency Medicine. J.-L. Vincent. Berlin, Springer-Verlag**:** 271-280.

Sander-Jensen, K., N. H. Secher, P. Bie, J. Warberg and T. W. Schwartz (1986). "Vagal slowing of the heart during haemorrhage:observations from 20 consecutive hypotensive patients." British Medical Journal **292**: 364-366.

Sauaia, A., F. A. Moore, E. E. Moore, K. S. Moser, R. Brennan, R. A. Read and P. T. Pons (1995). "Epidemiology of trauma deaths: a reassessment." Journal of Trauma **38**: 185-193.

Sawyer, D. C., W. V. Lumb and H. L. Stone (1971). "Cardiovascular effects of halothane, methoxyflurane, pentobarbital, and thiamylal." Journal of Applied Physiology **30**: 36-43.

Saydjari, R., G. I. J. M. Beerthuizen, C. M. Townsend, D. N. Herndon and T. C. Thompson (1991). "Bacterial translocation and its relationship to visceral blood flow, gut mucosal ornithine decarboxylase activity, and DNA in pigs." Journal of Trauma **31**: 639-644.

Scherrer, U., S. Vissing, B. Morgan, P. Hanson and R. G. Victor (1990). "Vasovagal syncope after infusion of a vasodilator in a heart-transplant recipient." New England Journal of Medicine **322**: 602-604.

Schmand, J., A. Ayala and I. H. Chaudry (1994). "Effects of trauma, duration of hypotension, and resuscitation regimen on cellular immunity after hemorrhagic shock." Critical Care Medicine **22**: 1076-1083.

Schmidt-Nielsen, B. (1961). "Choice of experimental animals in research." Federation Proceedings **20**: 902-906.

Schreck, R., P. Rieber and P. Baeuerle (1991). "Reactive oxygen intermediates as apparently widely used messengers in the activation of the NFkB transcription factor and HIV-1." The EMBO Journal **10**: 2247-2258.

Schweinburg, F. and J. Fine (1960). "Evidence for a lethal endotoxemia as the fundamental feature of irreversibility in three types of traumatic shock." Journal of Experimental Medicine **112**: 793-800.

Schweinburg, F., H. Frank and J. Fine (1954). "Bacterial factor in experimental hemorrhagic shock. Evidence for development of a bacterial factor which accounts for irreversibility to transfusion and for the loss of the normal capacity to destroy bacteria." American Journal of Physiology **179**: 532-540.

Schweinburg, F., P. Shapiro, E. Franck and J. Fine (1957). "Host resistance to hemorrhagic shock. IX. Demonstration of a circulating lethal toxin in hemorrhagic shock." Proceedings Society for Experimental Biology and Medicine **95**: 646-650.

Schwinghamer, J., G. Grega and F. Haddy (1970). "Skin and muscle circulatory responses during prolonged hupovolaemia." American Journal of Physiology **219**: 318-326.

Secher, N. H. and P. Bie (1985). "Bradycardia during reversible haemorrhagic shock - a forgotten observation?" Clinical Physiology **5**: 315-323.

Secher, N. H., K. Sander-Jensen, C. Werner, J. Warberg and P. Bie (1984). "Bradycardia during severe but reversible hypovolaemic shock in man." Circulatory Shock **14**: 267-274.

Shaftan, G., C. Chiu, C. Dennis and B. Harris (1965). "Fundamentals of physiologic control of arterial hemorrhage." Surgery **58**: 851-856.

Sheng, Z.-Y., Y.-L. Dong and X.-H. Wang (1992). "Bacterial translocation and multiple system organ failure in bowel ischemia and reperfusion." Journal of Trauma **32**: 148-153.

Shenkin, A., W. D. Fraser, J. Series, F. P. Winstanley, A. C. McCartney, H. J. G. Burns and J. Van Damme (1989). "Serum interleukin 6 response to elective surgery." Lymphokine Research **8**: 123-127.

Simeone, F. (1984). "Studies of trauma and shock in man: William S Stone's role in the military effort (1983 William S Stone Lecture)." Journal of Trauma **24**: 181-187.

Smithies, M., T. Yee, L. Jackson, R. Beale and D. Bihari (1994). "Protecting the gut and the liver in the critically ill: Effects of dopexamine." Critical Care Medicine **22**: 789-795.

Sori, A. J., B. F. Rush, T. W. Lysz, S. Smith and G. W. Machiedo (1988). "The gut as a source of sepsis after haemorrhagic shock." American Journal of Surgery **155**: 187-192.

Stark, D. and C. Shopsis (1983). "Developing alternative assay systems for toxicity testing." Annals of the New York Academy of Sciences **406**: 92-103.

Stoner, H. (1961). "Critical analysis of traumatic shock models." Federation Proceedings **20**(2, Part III, Supplement 9): 38-48.

Strieter, R. M., S. L. Kunkel and R. C. Bone (1993). "Role of tumor necrosis factor alpha in disease states and inflammation." Critical Care Medicine **21**: S447-S463.

Stylianos, S., G. Wakabayashi, J. A. Gelfand and B. H. Harris (1991). "Experimental hemorrhage and blunt trauma do not increase circulating tumor necrosis factor." Journal of Trauma **31**: 1063-1067.

Svoboda, P., I. Kantorova and J. Ochmann (1994). "Dynamics of interleukin 1, 2 and 6 and tumor necrosis factor alpha in multiple trauma patients." Journal of Trauma **36**: 336-340.

Swindle, M. M. (1986). Porcine models in surgical research:an overview. Swine in Biomedical Research. M. E. Tumbleson. New York, Plenum Press. **1**: 235-242.

Szabó, A., M. Boros, J. Kaszaki and S. Nagy (1997). "The role of mast cells in mucosal permeability changes during ischemia-reperfusion injury of the small intestine." Shock **8**: 284-291.

Talbot, R. and M. Swenson (1963). "Normochromic, microcytic anemia of baby pigs." Am. J. Vet. Res. **24**: 39-41.

The violence prevention task force of the Eastern Association for the Surgery of Trauma (1995). "Violence in America: a public health crisis-the role of firearms." Journal of Trauma **38**: 163-168.

Thomson, A., Ed. (1994). The Cytokine Handbook. London, Academic Press.

Timms, R. J. (1976). "The use of the anaesthetic steroids alphaxalone-alphadolone in studies of the forebrain in the cat." Journal of Physiology **256**: 71-72.

Timms, R. J. (1981). "A study of the amygdaloid defence reaction showing the value of Althesin anaesthesia in studies of the functions of the fore-brain in cats." Pflugers Archiv **391**: 49-56.

Tracey, K. (1991). "Tumor necrosis factor (cachectin) in the biology of septic shock syndrome." Circulatory Shock **35**: 123-128.

Tracey, K. J. and A. Cerami (1993). "Tumor necrosis factor: An updated review of its biology." Critical Care Medicine **21**: S415-S422.

Tracey, K. J., Y. Fong, D. G. Hesse, K. R. Manogue, A. T. Lee, G. C. Kuo, S. F. Lowry and A. Cerami (1987). "Anticachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia." Nature **330**: 662-664.

Traverso, L., S. Hollenbach, R. Bolin, M. Langford and L. DeGuzman (1986a). "Fluid resuscitation after an otherwise fatal hemorrhage: II. Colloid solutions." Journal of Trauma **26**: 176-182.

Traverso, L., W. Lee and M. Langford (1986b). "Fluid resuscitation after an otherwise fatal hemorrhage. I: Crystalloid solutions." Journal of Trauma **26**: 168-175.

Trunkey, D. D. (1983). "Trauma." Scientific American **249**: 20-27.

Tumbleson, M. and P. Kalish (1972). "Serum biochemical and hematological parameters in crossbred swine from birth through eight weeks of age." Can. J. Comp. Med. **36**: 202-209.

Turnbull, R., J. Talbot and S. Hamilton (1995). "Hemodynamic changes and gut barrier function in sequential hemorrhagic and endotoxic shock." Journal of Trauma **38**: 705-712.

van Deventer, S. J. H., H. R. Büller, J. W. ten Cate, L. A. Aarden, C. E. Hack and A. Sturk (1990). "Experimental endotoxaemia in humans: analysis of cytokine release and coagulation, fibrinolytic and complement pathways." Blood **76**: 2520-2526.

Van Snick, J. (1990). "Interleukin-6: an overview." Annual Review of Immunology **8**: 253-278.

Vatner, S. F. (1974). "Effects of haemorrhage on regional blood flow distribution in dogs and primates." Journal of Clinical Investigation **54**: 225-235.

Waage, A., P. Brandtzaeg, A. Halstensen, P. Kierulf and T. Espevik (1989). "The complex pattern of cytokines in serum from patients with meningococcal septic shock." Journal of Experimental Medicine **169**: 333-338.

Waage, A., A. Halstensen and T. Espevik (1987). "Association between tumour necrosis factor in serum and fatal outcome in patients with meningococcal disease." Lancet **i**: 355-357.

Wade, C. E., J. P. Hannon, C. A. Bossone, M. M. Hunt, J. A. Loveday, R. Coppes and V. L. Gildengorin (1989). "Resuscitation of conscious pigs following hemorrhage: comparative efficacy of small volume resuscitation." Circulatory Shock **29**: 193-204.

Walcott, W. (1945). "Standardization of experimental hemorrhagic shock." American Journal of Physiology **143**: 254-261.

Wang, P., J. G. Hauptman and I. H. Chaudry (1990). "Hemorrhage produces depression in microvascular blood flow which persists despite fluid resuscitation." Circulatory Shock **32**: 307-318.

Wang, S. C., R. R. Overman, J. W. Fertig, W. S. Root and M. I. Gregersen (1947). "The relation of blood volume reduction to mortality rate in hemorrhagic and traumatic shock in dogs." American Journal of Physiology **148**: 164-173.

Warren, H. S., R. L. Danner and R. S. Munford (1992). "Anti-endotoxin monoclonal antibodies." New England Journal of Medicine **326**: 1153-1157.

Weiskopf, R., M. Bogetz, I. Reid, M. Roizen and L. Keil (1986). Cardiovascular,endocrine and metabolic responses of conscious swine to hemorrhage. Swine in Biomedical Research. M. Tumbleson. New York, Plenum Press. **3:** 1405-1411.

Weissenbach, J., Y. Chernajovsky, M. Zeevi, L. Shulman, H. Soreq, U. Nir, D. Wallach, M. Perricaudet, P. Tiollais and M. Revel (1980). "Two interferon mRNAs in human fibroblasts: *In vitro* translation and *Eschereichia coli* cloning studies." Proceedings of the National Academy of Sciences of the USA **77**: 7152-7156.

Wells, C., R. Jechorek and S. Erlandsen (1990). "Evidence for the translocation of Enterococcus faecalis across the mouse intestinal tract." Journal of Infectious Diseases **162**: 82-90.

Wells, C., M. Maddaus, C. Reynolds, R. Jechorek and R. Simmons (1987). "The role of anaerobic flora in the translocation of aerobic and facultatively anaerobic intestinal bacteria." Infection and Immunity **55**: 2689-2694.

Wells, C., M. Maddaus and R. Simmons (1988). "Proposed mechanisms for the translocation of intestinal bacteria." Review of Infectious Diseases **10**: 958-979.

Wenzel, R. P. (1992). "Anti-endotoxin monoclonal antibodies - a second look." New England Journal of Medicine **326**: 1151-1152.

Wichmann, M., M. Angele, A. Ayala, W. Cioffi and I. Chaudry (1997). "Flutamide: A novel agent for restoring the depressed cell-mediated immunity following soft-tissue trauma and hemorrhagic shock." Shock **8**: 242-248.

Wiggers, C. (1941). "Applicability of experimental results to the shock problem in man." Journal of American Medical Association **117 (2)**: 1143-1147.

Wiggers, C. (1950). Physiology of Shock. New York, The Commonwealth Fund.

Wiggers, C. and J. Werle (1942). "Exploration of a method for standardizing hemorrhagic shock." Proceedings Society for Experimental Biology and Medicine **49**: 604-606.

Wiggers, H. and R. Ingraham (1946). "Hemorrhagic shock: definition and criteria for its diagnosis." Journal of Clinical Investigation **25**: 30-36.

Wilson, M., M. Chou, D. Spain, P. Downard, Q. Qian, W. Cheadle and R. Garrison (1996). "Fluid resuscitation attenuates early cytokine mRNA expression after peritonitis." Journal of Trauma **41**: 622-627.

Wilson, W. (1944). "State of men severely wounded in battle." Lancet **i**: 587-591.

Winchurch, R. A., J. N. Thupari and A. M. Munster (1987). "Endotoxemia in burn patients: Levels of circulating endotoxins are related to burn size." Surgery **102**: 808-812.

Wolff, S. M. (1982). "The treatment of gram-negative bacteremia and shock." New England Journal of Medicine **307**: 1267-1268.

Wong, G. and D. Goeddel (1986). "Tumour necrosis factors α and ß inhibit virus replication and synergize with interferons." Nature **323**: 819-822.

Woodruff, P., D. O'Carroll, S. Koizumi and J. Fine (1973). "Role of the intestinal flora in major trauma." Journal of Infectious Diseases **128**(Supplement): S290-S294.

Wyatt, J., D. Beard, A. Gray, A. Busuttil and C. Robertson (1995). "The time of death after trauma." British Medical Journal **310**: 1502.

Yao, Y.-M., Z.-Y. Sheng, H.-M. Tian, Y.-P. Wang, Y. Yu, X.-B. Fu, L.-R. Lu, D.-W. Wang and Y.-Y. Ma (1995). "Gut-derived endotoxaemia and multiple system organ failure following gunshot wounds combined with hemorrhagic shock: an experimental study in the dog." Journal of Trauma **38**: 742-746.

Yates, D. W. (1988). "Action for accident victims." British Medical Journal **297**: 1419-1420.

Zabel, D., H. Hopf and T. Hunt (1995). "Transmural gut oxygen gradients in shocked rats resuscitated with heparan." Archives of Surgery **130**: 59-63.

Zabel, D. D., K. Kramer and T. K. Hunt (1994). "Transmural gut tissue oxygen tensions during graded hemorrhage." Critical Care Medicine **22(1)**: A185.

Zar, J. (1996). Biostatistical Analysis. Upper Saddle River, NJ, Prentice-Hall International Inc.

Zhang, H. and J.-L. Vincent (1993). "Oxygen extraction is altered by endotoxin during tamponade-induced stagnant hypoxia in the dog." Circulatory Shock **40**: 168-176.

Zimmerli, W. and A. Widmer (1997). "Ibuprofen in patients with sepsis." New England Journal of Medicine **337**: 710.

Zweifach, B. (1974). "Mechanism of blood flow and fluid exchange in microvessels. Hemorrhagic hypotension model." Anesthesiology **41**: 157-168.

Zweifach, B. and H. Lipowsky (1984). Pressure-flow relations in blood and lymph microcirculation. Microcirculation. E. Rnekin and C. Michel. Bethesda, American Physiological Society. **IV, Part 1:** 251-307.

Postscript

My initial interest in doing this research was to gain a greater understanding of the pathophysiology of trauma. In 1991 and 1992, when I first began to look at the cytokine response to trauma, and the part it might play in the development of MOF, inflammatory cytokines and other inflammatory mediators seemed of the utmost importance. It was natural to concentrate on these in the design of the project.

Since then it has become clear that there are both inflammatory and anti-inflammatory cytokines, and that both may be important in the response to a septic challenge. My examiners, for this thesis, were keen for me to comment on these anti-inflammatory mechanisms/mediators, and whether they might have affected the results of this study, and the management of MOF. This section has been added to cover, in outline, some of these developments.

The principal anti-inflammatory cytokines are Interleukin-4 (IL-4), Interleukin-10 (IL-10), and Interleukin-13 (IL-13) (Cavaillon 1994; Marie et al. 1996; Marie and Cavaillon 1997).

In the last few years interest has started to focus, not just on extrinsic modulation of the inflammatory response in sepsis, but also on endogenous anti-inflammatory mechanisms (Dinarello 1995; van der Poll and Lowry 1995).

It has been suggested that Interleukin-10 (IL-10) be added to the arsenal of potential therapies for sepsis (Gérard et al. 1993). This idea is based largely on in vitro and animal studies. Interleukin-10 is an 18.5 kDA protein made up of 160 amino acids produced by T cells (CD4+, CD8+), macrophages/monocytes, B cells, keratinocytes, mesangial cells, and certain tumour cells (Goldman and Marchant 1996).

Interleukin-10 was originally called cytokine synthesis inhibitory factor (CSIF) because of its ability to suppress cytokine synthesis by Th1 cells. Recombinant mouse IL-10 has been shown to be very effective in suppressing TNF-α production by mouse peritoneal macrophages exposed to endotoxin (Bogdan et al. 1991). Inhibition of inflammatory cytokine (IL-1, IL-6, IL-8, TNF-α, GM-CSF, and G-CSF) synthesis has been seen when IL-10 was added to human monocytes activated with endotoxin (de Waal Malefyt et al. 1991). The potential therapeutic benefit was demonstrated by studies, which showed a reduction in inflammatory cytokine production, and in mortality, in murine models of endotoxaemia (Gérard et al. 1993; Howard et al. 1993).

It is apparent that IL-10 is produced in sepsis along with the inflammatory cytokines (Lin et al. 1994; Marchant et al. 1994; Gomez-Jimenez et al. 1995). Patients in septic shock were found to have higher IL-10 concentrations than those with sepsis but not in shock (Marchant et al. 1994). There was no difference between those patients whose sepsis was caused by gram negative or gram positive organisms.

The clinical importance of IL-10 in the course of a septic patient is unclear: Gomez-Jimenez et al. found no difference in IL-10 concentrations in survivors compared with non-survivors (Gomez-Jimenez et al. 1995). In trauma patients, Sherry et al. found that plasma IL-10 was associated with hypotension on admission, and the development of sepsis (Sherry et al. 1996). Detection of IL-10 was not related to the severity of injury. In contrast, in another study, higher IL-10 concentrations were seen in patients with an ISS greater than 25 than in those with a lower ISS (Neidhardt et al. 1996). In this same group of 401 patients higher IL-10 concentrations were measured in those patients who became septic and those who developed multiple organ dysfunction syndrome (MODS). The authors suggested that IL-10 might actually be involved in the pathogenesis of sepsis and MODS after injury. This seems to run counter to the ideas generated by laboratory studies.

Whether IL-10 played any part in modifying the inflammatory response in my studies is open to debate. Interleukin-10 is detected later than the inflammatory cytokines. In

vitro studies have shown that IL-10 only becomes detectable at 6-7 hours after an endotoxin stimulus (de Waal Malefyt et al. 1991; Marchant et al. 1994): at about the time when the inflammatory cytokines reached their peak concentrations. Given the time course of my studies it seems unlikely that IL-10 would have been detectable, even at R180.

Interleukin-4 and IL-13 also have anti-inflammatory properties (Cavaillon 1994; Marie et al. 1996).

Another cytokine, which may have a beneficial effect in sepsis, and the response to major trauma, is Interleukin-11 (IL-11). Although it was described as a haematopoietic growth factor it is now apparent that one of its potential therapeutic effects is to limit injury to the gut mucosa and to promote regeneration of the epithelial layer after insults such as chemotherapy. The potential benefits of IL-11 in sepsis have been recently reviewed (Opal and Keith 1996).

There is good laboratory evidence for a protective effect of the endogenous inhibitor of IL-1, Interleukin-1 receptor antagonist (IL-1ra), in endotoxaemia (Ohlsson et al. 1990; Fischer et al. 1992). The role of IL-1ra and IL-1 inhibitory activity in clinical situations is less clear. Elective surgery resulted in an increase in IL-1 inhibitory activity over a 24 hour period, but there was still an increase in IL-6 and in the C-reactive protein, mostly after IL-1 inhibitory activity had returned to baseline. An increase in IL-1ra was seen to follow the increase in soluble TNF receptors (sTNF-R) in 22 trauma patients (Cinat et al. 1995). However, concentrations of sTNF-Rs, IL-1ra and a soluble IL-2 receptor were higher in non-survivors than in survivors. The authors suggested that these receptors might be markers of the severity of the insult, rather than contributors to the mortality. Although inflammatory cytokines were undetectable, it does not appear that they had a very protective function.

Interleukin-1 receptor antagonist (IL-1ra) has already been the subject of clinical trials in septic patients (Fisher et al. 1994a; Fisher et al. 1994b). Although there was no effect

on 28 day all cause mortality, there was an increase in survival time in patients with one or more organ dysfunction.

The role of sTNF-Rs seems equally ambiguous. In experimental endotoxaemia they are protective (Lesslauer et al. 1991). They are produced in trauma patients (Cinat et al. 1994; Cinat et al. 1995), but in both studies concentrations were higher in non-survivors than in survivors. If they had a protective anti-inflammatory function one might expect that the non-survivors would be those patients *unable* to produce the receptors, rather than those who produced the most. Interestingly, sTNF-R concentrations decreased during episodes of infection or hypoxia in individual patients (Cinat et al. 1994). Maybe these reductions in sTNF-R concentrations do indicate a loss of endogenous anti-inflammatory activity during secondary episodes of sepsis. This may form part of the basis of the "two-hit" model for the development of MOF.

There are clearly endogenous anti-inflammatory mechanisms. Their mode of action is not fully understood, but we have yet to harness them to any great therapeutic benefit in trauma and sepsis (Eichacker and Natanson 1996; Martin et al. 1996).

References

Bogdan, C., Y. Vodovotz and C. Nathan (1991). "Macrophage deactivation by interleukin-10." Journal of Experimental Medicine **174**: 1549-1555.

Cavaillon, J. M. (1994). "Cytokines and macrophages." Biomedicine and Pharmacotherapy **48**(10): 445-453.

Cinat, M., K. Waxman, N. Vaziri, K. Daughters, S. Youseefi, G. Scannell and G. Tominaga (1995). "Soluble cytokine receptors and receptor antagonists are sequentially released after truama." Journal of Trauma **39**: 112-120.

Cinat, M. E., K. Waxman, G. A. Granger, W. Pearce, C. Annas and K. Daughters (1994). "Trauma causes sustained elevation of soluble tumour necrosis factor receptors." Journal of American College of Surgery **179**: 529-537.

de Waal Malefyt, R., J. Abrams, B. Bennett, C. Figdor and J. de Vries (1991). "Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an

autoregulatory role of IL-10 produced by monocytes." Journal of Experimental Medicine **174**: 1209-1220.

Dinarello, C. (1995). Traditional and novel approaches to interleukin-1 antagonism. Yearbook of Intensive Care and Emergency Medicine. J.-L. Vincent. Berlin, Springer-Verlag: 437-448.

Eichacker, P. and C. Natanson (1996). Rethinking the anti-inflammatory approach to septic shock. Yearbook of Intensive Care and Emergency Medicine. J.-L. Vincent. Berlin, Springer-Verlag: 165-176.

Fischer, E., M. Marano, K. Van Zee, C. Rock, A. Hawes, W. Thompson, L. DeForge, J. Kenney, D. Remick, D. Bloedow, R. Thompson, S. Lowry and L. Moldawer (1992). "Interleukin-1 receptor blockade improves survival and hemodynamic performance in *Escherichia coli* septic shock, but fails to alter host response to sublethal endotoxemia." Journal of Clinical Investigation **89**: 1551-1557.

Fisher, C., J.-F. Dhainaut, S. Opal, J. Pribble, R. Balk, G. Slotman, T. Iberti, E. Rackow, M. Shapiro, R. Greenman, H. Reines, M. Shelly, B. Thompson, J. LaBrecque, M. Catalano, W. Knaus and J. Sadoff (1994a). "Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome." Journal of American Medical Association **271**: 1836-1843.

Fisher, C. J., G. J. Slotman, S. M. Opal, J. P. Pribble, R. C. Bone, G. Emmanuel, D. Ng, D. C. Bloedow, M. A. Catalano and t. I.-R. S. S. S. Group (1994b). "Initial evaluation of human recombinant interleukin-1 receptor antagonist in the treatment of sepsis syndrome: a randomized, open-label, placebo-controlled multicenter trial." Critical Care Medicine **22**: 12-21.

Gérard, C., C. Bruyns, A. Marchant, D. Abramowicz, P. Vandenabeele, A. Delvaux, W. Fiers, M. Goldman and T. Velu (1993). "Interleukin10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia." Journal of Experimental Medicine **177**: 547-550.

Goldman, M. and A. Marchant (1996). Interleukin-10 production during septic shock. Yearbook of Intensive Care and Emergency Medicine. J.-L. Vincent. Berlin, Springer-Verlag: 105-110.

Gomez-Jimenez, J., M. Martin, R. Sauri, R. Segura, F. Esteban, J. Ruiz, X. Nuvials, J. Boveda, R. Peracaula and A. Salgado (1995). "Interleukin-10 and the monocyte/macrophage-induced inflammatory response in septic shock." Journal of Infectious Diseases **171**: 472-475.

Howard, M., T. Muchamuel and S. Andrade (1993). "Interleukin 10 protects mice from lethal endotoxaemia." Journal of Experimental Medicine **177**: 1205-1208.

Lesslauer, W., H. Tabuchi, R. Gentz, M. Brockhaus, E. Schlaeger, G. Grau, P. Piguet, P. Pointaire, P. Vassilli and H. Loetscher (1991). "Recombinant soluble tumor necrosis factor receptor proteins protect mice from lipopolysaccharide-induced lethality." European Journal of Immunology **21**: 2883-2886.

Lin, R., M. Astiz, J. Saxon, D. Saha and E. Rackow (1994). "Relationship between plasma cytokine concentrations and leukocyte functional antigen expression in patients with sepsis." Critical Care Medicine **22**: 1595-1602.

Marchant, A., J. Devière, B. Byl, D. De Groote, J.-L. Vincent and M. Goldman (1994). "Interleukin-10 production during septicaemia." Lancet **343**: 707-708.

Marie, C. and J. M. Cavaillon (1997). "Le retrocontrole negatif de l'inflammation: role des cytokines antiinflammatoires Negative feedback in inflammation: The role of antiinflammatory cytokines." Bulletin de l'Institut Pasteur **95**(1): 41-54.

Marie, C., C. Pitton, C. Fitting and J. M. Cavaillon (1996). "Regulation by anti-inflammatory cytokines (IL-4, IL-10, IL-13, TGFbeta) of interleukin-8 production by LPS- and/or TNFalpha- activated human polymorphonuclear cells." Mediators of Inflammation **5**(5): 334-340.

Martin, C., X. Viviand and F. Potié (1996). Immunotherapy of severe sepsis and septic shock: is there a future? Yearbook of Intensive Care and Emergency Medicine. J.-L. Vincent. Berlin, Springer-Verlag: 177-193.

Neidhardt, R., M. Keel, U. Steckholzer, A. Safret, U. Urigenthum, O. Trentz and W. Ertel (1996). Interleukin-10 (IL-10) plasma levels from injured patients correlate with severity of injury and clinical outcome. 56th Annual Meeting of the American Association for the Surgery of Trauma, Houston, Texas, American Association for the Surgery of Trauma.

Ohlsson, K., P. Bjork, M. Bergenfeldt, R. Hageman and R. Thompson (1990). "Interleukin-1 receptor antagonist reduces mortality from endotoxin shock." Nature **348**: 550-552.

Opal, S. and J. Keith (1996). Potential role for human Interleukin-11 in bacterial sepsis. Yearbook of Intensive Care and Emergency Medicine. J.-L. Vincent. Berlin, Springer-Verlag: 111-118.

Sherry, R., J. Cué, J. Goddard, J. Parramore and J. DiPiro (1996). "Interleukin-10 is associated with the development of sepsis in trauma patients." Journal of Trauma **40**: 613-617.

van der Poll, T. and S. Lowry (1995). Endogenous mechanisms regulating TNF and IL-1 during sepsis. Yearbook of Intensive Care and Emergency Medicine. J.-L. Vincent. Berlin, Springer-Verlag: 385-397.

Appendix

The Appendix contains a table of the group means and standard deviations for each of the measured and calculated variables referred to in the text. The numbering is the same as that used in the Results and Discussion chapters, so the data for heart rate is found in Table 1 of this Appendix, and in Figure 3.1 (graph), and Table 3.1 (statistical table), of the Results.

Tables in this Appendix contain a column labelled RANOVA, which points out the statistically significant paired contrasts. The symbols for these contrasts are the same as those used within the tables to point out the statistically significant one-way ANOVA results. The one-way ANOVA was used to examine any differences between the groups at individual time points.

The tables for endotoxin and the two cytokines have an additional column for the results of the Area Under the Curve (AUC) analysis.

Legend

* = P<0.05 vs. Control

£ = P<0.05 vs. NS

+ = P<0.05 vs. H20

= P<0.05 vs. H30

@ = P<0.05 vs. HNS20

$ = P<0.05 vs. HNS30

Table 1: Heart rate (bpm). Means and standard deviations.

			Control		Shock (min)		Reinfusion (min)							
Group		**n**	**C**	**CNS**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**	**RANOVA**
Control	Mean	11	169	*169*	175	170	165	170	167	175	170	168	170	
	SD		49	*49*	55	55	52	56	54	56	58	62	67	
NS	Mean	10	206	221 * #	237	232	223	218	218	218	219	220	219	*
	SD		37	26	24	40	48	52	55	56	58	58	60	
H20	Mean	7	190	*190*	212	206	178	182	179	183	184	178	183	
	SD		51	*51*	54	52	53	49	51	47	47	48	49	
H30	Mean	10	154	*154*	189	193	150	156	155	149	149	150	149	
	SD		35	*35*	35	26	25	33	35	35	35	36	39	
HNS20	Mean	8	232 #	260 * #	277* #	263 *	241 * #	247 #	240 #	238 #	234	236	229	* +
	SD		58	50	61	64	61	71	75	61	62	56	56	
HNS30	Mean	11	202	244 * #	279 * #	266 *	238 * #	237 #	232	229 #	228 #	227	230	* #
	SD		48	36	46	47	38	34	32	44	47	50	51	

One-way ANOVA: * = P<0.05 vs. Control, + = P<0.005 vs. H20, # = P<0.05 vs. H30.

Table 2: Mean arterial pressure data (mmHg). Means and standard deviations.

			Control		Shock (min)		Reinfusion (min)							
Group		**n**	**C**	**CNS**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**	**RANOVA**
Control	Mean	11	84	-	84 #	83	81	81	79	78	75	74	73	
	SD		12	-	15	16	19	20	20	23	21	21	23	
NS	Mean	10	102 + #	118 * + #	117 + # @	106 #	103	100	100	100	106	101	93	*
	SD		9	10	13	27	28	33	31	40	39	44	44	
H20	Mean	7	74	-	59	69	84	73	71	72	70	63	69	
	SD		19	-	24	20	16	22	21	18	16	19	21	
H30	Mean	10	78	-	44	53	88	80	74	68	68	67	66	
	SD		15	-	15	14	22	22	24	23	22	23	22	
HNS20	Mean	8	95	109 * + #	79	77	108	106	91	96	97	95	90	+
	SD		9	15	26	23	16	22	25	13	17	22	15	
HNS30	Mean	11	98 + #	112 * + #	84 #	76	113	94	96	96	96	89	88	#
	SD		10	13	34	34	25	22	22	25	24	20	27	

One-way ANOVA: * = P<0.05 vs. Control, + = P<0.05 vs. H20, # = P<0.05 vs. H30.

Table 3: Mean pulmonary artery pressure (mmHg). Means and standard deviations.

			Control		Shock (min)		Reinfusion (min)						
Group		**n**	**C**	**CNS**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**	**RANOVA**
Control	Mean	11	20	-	20	19	18	18	19	19	20	18	20	
	SD		7	-	6	5	5	4	5	3	3	4	3	
NS	Mean	10	23	25	23 # $	21	22	21	22	22	22	20	21	*
	SD		3	4	2	3	4	4	4	4	4	5	4	
H20	Mean	7	25	-	22	23 $	29 * # $	25	24	24	24	23	23	* # @
	SD		5	-	5	3	6	4	3	3	4	4	4	
H30	Mean	10	19	-	14	16	26	21	21	19	19	19	19	
	SD		4	-	5	4	6	6	6	6	7	5	4	
HNS20	Mean	8	22	23	17	17	22	19	19	19	19	19	19	
	SD		4	6	4	4	6	5	4	3	4	4	4	
HNS30	Mean	11	21	24	15	15	26	21	21	20	20	20	20	
	SD		6	7	5	5	4	5	4	5	5	2	2	

One-way ANOVA: * = P<0.05 vs. Control, # = P<0.05 vs. H30, @ = P<0.05 vs.HNS20, $ = P<0.05 vs. HNS30.

Table 4: Central venous pressure data (mmHg). Means and standard deviations.

			Control		Shock (min)			Reinfusion (min)					
Group		**n**	**C**	**CNS**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**
Control	Mean	10	6	-	5	5	5	5	5	4	5	5	4
	SD		2	-	2	2	2	2	2	2	3	3	3
NS	Mean	10	6	7	7	6	7	6	6	6	6	6	6
	SD		3	3	3	3	3	3	3	3	3	3	3
H20	Mean	7	8	-	5	5	7	6	5	5	5	4	4
	SD		4	-	3	3	3	3	3	3	4	4	4
H30	Mean	10	6	-	3	3	7	6	5	4	4	4	4
	SD		3	-	1	2	3	2	2	2	2	2	2
HNS20	Mean	8	6	6	4	5	7	5	5	5	5	4	4
	SD		4	4	3	4	4	3	3	3	3	3	2
HNS30	Mean	11	6	8	3	2	6	5	4	4	4	4	4
	SD		4	4	3	4	3	3	3	3	3	3	3

RANOVA: no overall group difference identified.

Table 5: Pulmonary artery wedge pressure (mmHg). Means and standard deviations.

			Control		Shock (min)		Reinfusion (min)					
Group		**n**	**C**	**CNS**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**
Control	Mean	10	9	9	9	9	10	9	8	8	9	8	8
	SD		4	4	4	4	4	3	2	3	4	3	3
NS	Mean	10	12	12	11	11	11	10	11	10	11	10	10
	SD		4	4	3	4	4	3	3	3	3	3	3
H20	Mean	7	11	11	9	9	13	11	11	10	10	10	9
	SD		2	2	2	2	3	3	2	2	3	3	3
H30	Mean	10	9	9	6	6	10	8	8	8	8	7	7
	SD		3	3	3	2	3	3	3	3	3	2	2
HNS20	Mean	8	11	11	7	7	9	9	8	8	8	7	7
	SD		2	3	2	2	2	3	2	2	3	3	3
HNS30	Mean	11	10	12	8	7	10	9	8	8	8	8	8
	SD		5	6	5	3	4	2	2	2	3	2	3

RANOVA: no statistically significant overall group differences were identified.

Table 6: Cardiac index (ml/min/kg). Means and standard deviations.

			Control		Shock (min)		Reinfusion (min)							
Group		**n**	**C**	**CNS**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**	**RANOVA**
Control	Mean	11	129	-	122 #	113	113	117	109	110	104	101	99	
	SD		36	-	26	28	33	24	22	20	16	17	19	
NS	Mean	10	167	177	157 + # @ $	147 # $	142	136	134	129	127	124	119	*
	SD		38	39	28	34	31	35	28	32	34	35	41	
H20	Mean	7	136	-	104	107	142	131	130	132	116	117	124	
	SD		60	-	45	47	58	49	44	44	46	47	43	
H30	Mean	10	133	-	72	79	136	120	112	100	102	103	97	
	SD		34	-	19	22	29	24	25	19	22	21	23	
HNS20	Mean	8	174	178	103	97	164	141	124	121	120	112	112	
	SD		40	35	29	22	22	14	28	14	14	20	20	
HNS30	Mean	11	179	200 * #	88	88	190 * #	158	146	141	132	139	131	* #
	SD		51	36	32	27	24	26	23	29	31	42	36	

One-way ANOVA: * = P<0.05 vs. Control, + = P<0.05 vs. H20, # = P<0.05 vs. H30, @ = P<0.05 vs. HNS20, $ = P<0.05 vs. HNS30.

Table 7: Stroke volume index (ml/beat/kg). Means and standard deviations.

			Control		Shock (min)		Reinfusion (min)						
Group		**n**	**C**	**CNS**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**
Control	Mean	11	0.81	-	0.67 # $	0.64 $	0.66	0.68	0.64	0.63	0.61	0.60	0.59
	SD		0.31	-	0.31	0.31	0.32	0.30	0.28	0.27	0.27	0.26	0.27
NS	Mean	10	0.82	0.77	0.67 # $	0.65 $	0.65	0.64	0.63	0.61	0.59	0.57	0.55
	SD		0.19	0.19	0.15	0.13	0.10	0.12	0.11	0.12	0.11	0.11	0.13
H20	Mean	7	0.71	-	0.49	0.53	0.82	0.74	0.74	0.73	0.64	0.67	0.69
	SD		0.19	-	0.17	0.18	0.29	0.24	0.21	0.20	0.22	0.23	0.20
H30	Mean	10	0.88	-	0.39	0.42	0.91	0.79	0.75	0.70	0.70	0.71	0.67
	SD		0.21	-	0.12	0.15	0.18	0.21	0.19	0.19	0.18	0.16	0.17
HNS20	Mean	8	0.78	0.70	0.39	0.40	0.72	0.61	0.59	0.54	0.54	0.50	0.51
	SD		0.21	0.18	0.15	0.14	0.18	0.18	0.22	0.15	0.14	0.15	0.13
HNS30	Mean	11	0.89	0.81	0.31	0.33	0.81	0.68	0.63	0.62	0.59	0.62	0.57
	SD		0.14	0.13	0.09	0.07	0.11	0.12	0.07	0.10	0.10	0.14	0.11

One-way ANOVA: # = P<0.05 vs. H30, $ = P<0.05 vs. HNS30.

RANOVA: no statistically significant overall group differences were identified. There were significant group by time interaction differences, which are shown in the statistics table (Table 3.7).

Table 8: Right ventricular stroke work (g/min). Means and standard deviations.

			Control		Shock (min)		Reinfusion (min)							
Group		**n**	**C**	**CNS**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**	**RANOVA**
Control	Mean	11	1.2	-	1.2 @ $	1.2 $	1.2	1.2	1.3	1.3	1.3	1.3 £	1.4 £ $	@ £ $
	SD		0.5	-	0.6	0.5	0.5	0.5	0.7	0.5	0.4	0.5	0.5	
NS	Mean	10	1.1	1.1	0.9	0.9	1.0	1.0	1.0	1.0	1.0	0.9	1.0	
	SD		0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.2	
H20	Mean	7	1.2	-	1.1 $	1.3 $	1.8 £ $	1.5 @ $	1.5 @	1.5	1.5 @ $	1.5 @ £ $	1.5 @ £ $	@
	SD		0.4	-	0.3	0.4	0.7	0.5	0.5	0.5	0.5	0.5	0.5	
H30	Mean	10	1.2	-	0.9	0.9	1.7 @ £ $	1.4	1.5	1.3	1.3	1.3 £	1.4	$
	SD		0.3	-	0.3	0.2	0.4	0.4	0.4	0.4	0.5	0.3	0.2	
HNS20	Mean	8	1.0	0.9	0.7	0.7	1.0	0.9	1.0	0.9	0.9	0.9	0.9	
	SD		0.5	0.6	0.4	0.5	0.7	0.6	0.6	0.4	0.4	0.5	0.4	
HNS30	Mean	11	1.0	0.9	0.6	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
	SD		0.3	0.3	0.3	0.4	0.6	0.4	0.4	0.4	0.4	0.4	0.4	

One-way ANOVA: @ = P<0.05 vs. HNS20, $ = P<0.05 vs. HNS30, £ = P<0.05 vs. NS.

Table 9: Left ventricular stroke work (g/min). Means and standard deviations.

			Control		Shock (min)					Reinfusion (min)				
Group		**n**	**C**	**CNS**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**	**RANOVA**
Control	Mean	11	6.5	-	6.4 + # @ $	6.6 + # @ $	6.6	6.4	6.4	5.8	5.8	5.8	5.6	@ $
	SD		1.9	-	1.9	2.0	2.1	1.9	1.8	1.5	1.4	1.6	1.2	
NS	Mean	10	6.1	6.1	6.2 + # @ $	5.7 # $	5.8	5.6	5.5	5.5	5.7	5.4	4.9	
	SD		1.1	0.7	0.9	1.3	1.3	1.4	1.2	1.8	1.7	1.9	1.9	
H20	Mean	7	4.6	-	3.3	4.1	5.6	4.7	4.6	4.7	4.5	4.2	4.5	
	SD		1.1	-	1.4	1.6	1.1	1.1	1.1	0.9	1.0	1.3	1.1	
H30	Mean	10	6.2	-	2.8	3.4	7.2	6.3	5.8	5.6	5.5	5.4	5.4	
	SD		1.4	-	1.0	1.1	2.0	1.8	2.0	1.9	1.6	1.6	1.5	
HNS20	Mean	8	5.2	5.2	3.5	3.7	5.7	5.5	5.1	5.2	5.3	5.1	5.1	
	SD		1.2	0.7	0.8	1.1	0.9	0.5	1.4	0.9	0.9	0.9	1.0	
HNS30	Mean	11	6.2	5.5	3.6	3.4	5.9	4.9	5.1	5.2	5.2	4.9	4.7	
	SD		1.4	0.5	1.4	1.3	1.1	1.0	1.2	1.2	1.2	1.1	1.5	

One-way ANOVA: + = P<0.05 vs. H20, # = P<0.05 vs. H30, @ = P<0.05 vs. HNS20, $ = P<0.05 vs. HNS30.

RANOVA: significance of F for the groups was 0.071. There were some statistically significant differences in group by time interaction and two significant group differences from the contrast with the control group (as shown in the RANOVA column).

Table 10: Systemic vascular resistance index (dyne.sec.cm^{-5}.kg). Means and standard deviations.

			Control		Shock (min)		Reinfusion (min)						
Group		**n**	**C**	**CNS**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**
Control	Mean	11	55788	-	55667	61524	61353	54774	56381	55148	55674	56862	57695
	SD		29576	-	19163	26515	31408	12851	11048	13177	13469	13750	15731
NS	Mean	10	47945	51834	57414	54590	54493	55982	55920	58106	62560	61417	59984
	SD		11351	13592	11841	10927	11420	17783	16177	23777	22372	28023	30886
H20	Mean	7	41690	-	42450	49506	47795	42616	41238	42101	49047	42184	44228
	SD		10048	-	7914	7100	17809	7817	6561	6185	16286	9214	14035
H30	Mean	10	45146	-	45200	51571	47736	48957	48718	50333	49371	47936	50901
	SD		9737	-	8180	11336	8230	9578	10925	13477	10781	12589	13566
HNS20	Mean	8	42668	47629	59735	61309	49801	57414	55073	60755	61551	65391	61564
	SD		9541	10165	19260	22807	9702	12466	11575	11074	10813	14098	8998
HNS30	Mean	11	44051	42786	74441 + #	67220	45200	46153	51083	53808	57087	51552	52877
	SD		12015	6943	26828	26741	10157	12921	14466	16444	16809	14945	17472

One-way ANOVA: + = P<0.05 vs. H20, # = P<0.05 vs. H30.

RANOVA: no statistically significant overall group differences were identified. There were significant group by time interaction differences, which are shown in the statistics table (Table 3.10).

Table 11: Pulmonary vascular resistance index (dyne.sec.cm^{-5}.kg). Means and standard deviations.

			Control		Shock (min)			Reinfusion (min)					
Group		**n**	**C**	**CNS**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**
Control	Mean	11	7367	*7367*	7781	8117	8392	7608	7980	8450	9337	9203	10122
	SD		3511	*3511*	5168	4903	3255	2918	3081	3162	3433	3447	3624
NS	Mean	10	5409	5837	6195	6032	6588	7021	6856	7672	7597	6689	7949
	SD		1334	1447	2369	2303	3170	3349	2505	2411	2797	3632	3323
H20	Mean	7	9217	*9217*	10961	12120	10993	10233	8973	9557	11479	11255	10156
	SD		4659	*4659*	4561	5540	6640	5523	4121	4625	7109	6967	4795
H30	Mean	10	6541	*6541*	9621	10626	10073	9121	9792	8674	9660	9081	9922
	SD		2074	*2074*	2564	2605	4092	4216	4002	3108	1838	2461	2222
HNS20	Mean	8	5076	5393	7313	8133	6517	6037	7088	7380	7781	8889	8786
	SD		996	1477	2063	1720	2360	1288	1166	932	1099	1824	1941
HNS30	Mean	11	5132	5632	8028	9645	5823	7229	7136	7526	7970	7578	7958
	SD		2261	1667	3926	4928	3982	2461	3321	2265	2389	2513	2575

RANOVA: no statistically significant group differences identified

Table 12: Haematocrit. Means and standard deviations.

			Control	Shock (min)		Reinfusion (min)					
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**
Control	Mean	11	29.2	30.2	29.3	28.6	28.4	27.8	28.4	28.0	27.7	28.3
	SD		3.5	3.7	3.7	3.8	3.7	3.8	4.4	4.8	4.3	4.8
NS	Mean	10	31.0	30.8	29.4	27.7	27.8	27.6	27.5	27.4	27.3	26.8
	SD		4.8	3.3	3.7	4.8	4.6	4.8	5.2	5.3	6.1	6.3
H20	Mean	7	29.8	29.3	26.8	28.4	28.6	27.8	27.4	27.8	27.2	26.5
	SD		1.5	1.8	4.9	2.1	1.9	1.9	2.1	2.7	2.2	2.8
H30	Mean	10	28.2	27.0	26.3	27.5	26.9	26.4	25.5	24.9	24.8	24.6
	SD		2.5	3.0	2.8	2.6	3.2	3.7	3.5	3.5	3.6	3.5
HNS20	Mean	8	33.7	32.4	30.5	31.0	30.9	29.7	29.0	28.6	28.0	27.6
	SD		1.7	0.8	1.7	3.1	3.5	2.3	2.7	2.3	1.7	1.4
HNS30	Mean	11	29.1	28.3	26.6	29.2	27.8	27.7	26.5	26.5	26.2	26.2
	SD		2.5	3.2	2.9	3.9	4.2	3.7	3.6	4.0	3.8	4.3

RANOVA: no statistically significant overall group differences were identified.

Table 13: Body temperature (°C). Means and standard deviations.

			Control	Shock (min)				Reinfusion (min)				
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**
Control	Mean	11	37.0	37.2	37.4	37.4	37.5	37.6	37.7	37.8	37.9	37.9
	SD		0.61	0.65	0.78	0.81	0.83	0.89	0.95	1.02	1.12	1.20
NS	Mean	10	37.5	37.9	38.1	38.2	38.4	38.4	38.6	38.6	38.8	38.9
	SD		1.11	1.10	1.24	1.37	1.39	1.49	1.52	1.58	1.58	1.59
H20	Mean	7.0	37.3	37.4	37.7	37.6	37.9	38.1	38.2	38.4	38.5	38.5
	SD		1.12	0.87	1.24	1.31	1.27	1.30	1.35	1.36	1.30	1.20
H30	Mean	10	36.4	36.7	36.9	36.7	37.1	37.4	37.4	37.5	37.6	37.7
	SD		0.99	0.80	0.69	0.75	0.87	0.87	0.93	1.02	1.07	1.15
HNS20	Mean	8	37.5	38.0	38.2	37.9	38.1	38.2	38.3	38.2	38.4	38.3
	SD		2.12	2.18	2.09	1.95	1.87	1.72	1.57	1.58	1.24	1.09
HNS30	Mean	11	38.0	38.6	38.7	38.4	38.8	38.9	39.0	39.2	39.3	39.4
	SD		1.90	1.81	1.77	1.57	1.52	1.49	1.52	1.52	1.58	1.63

RANOVA: no overall group differences.

Table 14: Arterial oxygen saturation (%). Means and standard deviations.

			Control	Shock (min)		Reinfusion (min)					
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**
Control	Mean	11	99.7	99.5	99.6	99.6	99.7	99.6	99.5	99.6	99.6	99.6
	SD		0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.1
NS	Mean	10	99.3	99.3	99.3	99.4	99.3	99.4	99.4	99.4	99.2	99.3
	SD		0.2	0.3	0.2	0.3	0.3	0.4	0.4	0.2	0.3	0.3
H20	Mean	7	99.4	99.0	99.1	99.2	99.5	99.6	99.6	99.5	99.4	99.7
	SD		0.4	1.3	1.0	0.6	0.2	0.4	0.3	0.3	0.5	0.2
H30	Mean	10	99.6	99.5	99.6	99.5	99.5	99.6	99.5	99.5	99.5	99.5
	SD		0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3
HNS20	Mean	8	99.4	99.6	99.5	99.5	99.6	99.6	99.5	99.5	99.6	99.5
	SD		0.3	0.2	0.2	0.3	0.2	0.2	0.3	0.2	0.1	0.1
HNS30	Mean	10	99.5	99.5	99.3	99.3	99.4	99.5	99.5	99.5	99.5	99.5
	SD		0.1	0.1	0.2	0.4	0.5	0.3	0.2	0.1	0.2	0.2

RANOVA: No overall differences were detected.

Table 15: Mixed venous oxygen saturation (%). Means and standard deviations.

			Control	Shock (min)		Reinfusion (min)						
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**
Control	Mean	11	68.3	66.2 + # $	63.4 # @ $	62.1	61.1	58.4	56.8	54.7	52.7	48.5
	SD		6.8	7.4	8.2	10.0	9.8	9.8	9.2	9.1	9.7	11.4
NS	Mean	10	75.0	72.0 + # @ $	67.1 # @ $	62.9	61.5	59.9	57.5	55.7	55.1	49.3
	SD		7.9	7.9	12.6	13.7	14.7	13.2	14.6	16.6	17.6	18.1
H20	Mean	7	62.4	46.4	48.0	62.4	56.3	53.2	56.6	51.5	48.6	52.0
	SD		14.0	17.3	16.7	10.2	14.1	11.8	13.1	15.9	15.6	13.0
H30	Mean	10	71.4	38.3	39.4	67.6	62.5	58.0	54.2	51.3	51.7	48.3
	SD		9.0	11.9	10.8	8.7	10.6	10.8	9.8	10.5	10.3	11.1
HNS20	Mean	8	75.3	49.3	44.1	68.1	63.7	57.3	57.6	54.6	55.2	48.0
	SD		5.6	12.2	13.7	6.8	7.0	10.2	8.2	8.5	9.1	11.2
HNS30	Mean	10	75.9	38.2	36.0	72.6	65.3	62.5	58.8	55.6	54.1	53.6
	SD		8.9	12.1	9.2	4.7	7.7	8.0	9.7	9.6	8.6	8.9

RANOVA: No overall group differences detected. There were group by time interaction differences. Contrasts and one way ANOVAs were performed to investigate these differences.

One-way ANOVA: * = P<0.05 vs. Control, + = P<0.05 vs. H20, # = P<0.05 vs. H30, @ = P<0.05 vs. HNS20, $ = P<0.05 vs. HNS30, £ = P<0.05 vs. NS

Table 16: Oxygen delivery index data (ml/min/kg). Means and standard deviations.

			Control	Shock (min)		Reinfusion (min)							
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**	**RANOVA**
Control	Mean	11	16	14	12	12	12	12	12	11	10	10	
	SD		4	5	5	6	5	5	5	5	5	5	
NS	Mean	10	21	19+ # $	18 # $	16	15	15	14	14	14	13	*
	SD		7	5	5	5	5	4	5	5	5	6	
H20	Mean	7	17	12	12	16	14	12	15	13	12	13	
	SD		8	5	6	6	5	7	4	5	4	5	
H30	Mean	10	15	7	8	15	13	10	10	10	10	9	
	SD		4	2	2	3	3	4	2	3	3	4	
HNS20	Mean	8	24	13	12	20 *	17	15	14	14	11	12	
	SD		6	3	2	4	3	3	2	2	5	2	
HNS30	Mean	11	20	9	9	21 *	17	16	15	13	14	13	#
	SD		6	3	3	4	4	3	4	4	5	5	

One-way ANOVA: * = P<0.05 vs. Control, + = P<0.05 vs. H20, # = P<0.05 vs. H30, $ = P<0.05 vs. HNS30.

Table 17: Oxygen consumption index data (ml/min/kg). Means and standard deviations.

			Control	Shock (min)		Reinfusion (min)						
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**
Control	Mean	11	5	5	4	4	5	5	5	5	5	5
	SD		1	2	2	2	2	2	2	2	2	2
NS	Mean	10	5	5	5	5	5	5	5	5	5	5
	SD		2	1	2	1	1	2	1	1	1	1
H20	Mean	7	5	6	6	5	6	6	6	6	6	6
	SD		1	1	1	1	1	1	1	1	1	1
H30	Mean	10	4	5	5	5	5	4	5	5	5	5
	SD		1	1	1	1	1	2	1	1	1	2
HNS20	Mean	8	6	7	7	8	8	6	7	7	6	7
	SD		2	2	2	4	4	2	3	3	2	2
HNS30	Mean	10	5	5	5	5	5	5	5	5	7	6
	SD		2	2	2	2	2	2	2	2	7	2

RANOVA: no statistically significant overall group differences were identified.

Table 18: Oxygen extraction ratio. Means and standard deviations.

			Control	Shock (min)		Reinfusion (min)					
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**
Control	Mean	11	0.32	0.34	0.37	0.38	0.39	0.42	0.43	0.45	0.47	0.51
	SD		0.07	0.07	0.08	0.10	0.10	0.10	0.09	0.09	0.10	0.11
NS	Mean	10	0.25	0.28	0.33	0.37	0.38	0.40	0.42	0.44	0.45	0.51
	SD		0.08	0.08	0.13	0.14	0.15	0.13	0.15	0.17	0.18	0.18
H20	Mean	7	0.37	0.53 £	0.52	0.38	0.44	0.50	0.44	0.48	0.51	0.48
	SD		0.14	0.17	0.17	0.10	0.14	0.10	0.13	0.16	0.16	0.13
H30	Mean	10	0.29	0.65 * £	0.61 * £	0.32	0.37	0.47	0.46	0.49	0.48	0.51
	SD		0.09	0.12	0.11	0.09	0.11	0.16	0.10	0.11	0.10	0.12
HNS20	Mean	8	0.25	0.55 * £	0.60 * £	0.39	0.43	0.47	0.48	0.51	0.50	0.57
	SD		0.06	0.19	0.19	0.22	0.20	0.21	0.19	0.19	0.21	0.19
HNS30	Mean	10	0.27	0.61 * £	0.64 * £	0.27	0.34	0.38	0.41	0.45	0.46	0.46
	SD		0.03	0.12	0.09	0.05	0.08	0.08	0.10	0.10	0.08	0.09

One-way ANOVA: * = P<0.05 vs. Control, + = P<0.05 vs. H20, # = P<0.05 vs. H30, @ = P<0.05 vs. HNS20, $ = P<0.05 vs. HNS30, £ = P<0.05 vs. NS

RANOVA: no statistically significant overall group differences were identified, but there were significant differences in group by time interactions, which are shown in the statistics table (Table 3.18).

Table 19: Arterial partial pressure CO_2 (mmHg). Means and standard deviations.

			Control	Shock (min)		Reinfusion (min)					
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**
Control	Mean	11	36.1	35.5	33.7	35.4	36.2	36.1	36.1	36.3	36.1	36.9
	SD		5.0	6.2	4.5	5.1	4.8	4.9	4.8	3.2	3.0	3.4
NS	Mean	10	35.0	37.5	36.4	36.2	36.6	36.5	36.0	36.5	36.4	36.0
	SD		3.0	3.5	5.0	5.0	3.3	4.0	2.8	3.5	2.7	2.9
20% Haem	Mean	7	36.2	37.1	38.1	40.5	38.0	38.0	38.1	39.3	37.0	36.7
	SD		5.1	4.7	5.6	5.7	5.5	5.5	5.2	4.4	5.3	3.0
30% Haem	Mean	10	32.6	36.1	37.6	37.8	36.5	37.5	35.8	35.8	36.7	37.1
	SD		4.0	3.2	3.7	3.0	3.8	3.9	4.3	4.9	3.8	3.7
20% HNS	Mean	8	33.3	35.0	34.0	37.2	34.8	33.6	35.3	35.7	36.9	36.9
	SD		6.6	6.4	5.4	6.6	5.8	5.0	3.3	3.5	4.0	5.6
30% HNS	Mean	10	35.3	36.0	35.9	40.7	36.9	37.0	36.7	37.5	37.9	37.2
	SD		4.8	5.1	3.4	9.5	5.7	3.7	2.9	2.2	2.6	3.1

RANOVA: No overall differences were detected.

Table 20: Arterial pH. Means and standard deviations.

			Control	Shock (min)				Reinfusion (min)				
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**
Control	Mean	11	7.49	7.48	7.50	7.50	7.50	7.50	7.50	7.49	7.49	7.48
	SD		0.05	0.08	0.07	0.05	0.05	0.06	0.05	0.03	0.03	0.04
NS	Mean	10	7.49	7.45	7.46	7.46	7.47	7.47	7.47	7.47	7.46	7.47
	SD		0.04	0.03	0.04	0.04	0.02	0.03	0.03	0.03	0.03	0.03
H20	Mean	7	7.48	7.46	7.45	7.44	7.46	7.47	7.48	7.47	7.48	7.48
	SD		0.06	0.06	0.04	0.05	0.06	0.06	0.05	0.06	0.06	0.04
H30	Mean	10	7.49	7.46	7.44	7.44	7.46	7.47	7.49	7.48	7.47	7.47
	SD		0.06	0.04	0.04	0.06	0.05	0.05	0.06	0.07	0.06	0.07
HNS20	Mean	8	7.49	7.45	7.44	7.42	7.45	7.47	7.46	7.45	7.44	7.44
	SD		0.05	0.07	0.08	0.09	0.08	0.06	0.04	0.04	0.03	0.04
HNS30	Mean	10	7.49	7.45	7.41	7.36	7.43	7.45	7.46	7.46	7.45	7.46
	SD		0.04	0.05	0.06	0.13	0.12	0.09	0.06	0.04	0.04	0.03

RANOVA: No overall differences were detected.

Table 21: Arterial bicarbonate (mM/L). Means and standard deviations.

			Control	Shock (min)		Reinfusion (min)							
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**	**RANOVA**
Control	Mean	11	27.4 #	27.3	27.0 $	27.1 $	27.8 @ $	27.7 @	27.6 @	27.6	28.5	27.0	@ £ $
	SD		1.6	2.1	1.7	2.2	1.5	1.3	1.2	1.1	4.6	1.5	
NS	Mean	10	26.2	25.6	25.6	25.4	26.0	25.9	25.8	25.8	25.6	25.4	
	SD		1.8	2.2	2.8	3.3	2.9	2.1	2.1	2.4	2.2	2.5	
H20	Mean	7	26.5	26.1	26.0	26.6	26.8	27.3	27.5	28.1	26.7	26.6	
	SD		1.1	2.0	2.3	1.9	2.3	1.8	1.7	1.2	1.5	1.8	
H30	Mean	10	24.9	25.8	25.4	25.3	25.9	27.1	26.7	26.4	26.4	26.6	
	SD		2.1	1.3	2.1	1.6	1.6	1.8	1.6	1.9	2.3	2.7	
HNS20	Mean	8	24.9	23.7	22.5	23.2	23.7	23.9	24.4	24.6	24.7	24.6	
	SD		1.3	2.0	2.9	2.6	2.5	2.4	2.1	1.8	2.0	2.2	
HNS30	Mean	10	26.8	24.4	22.7	22.3	23.8	25.3	25.5	25.9	25.9	26.2	
	SD		1.6	3.8	3.9	3.3	3.5	3.0	2.6	2.4	2.0	2.2	

One-way ANOVA and RANOVA : £ = P<0.05 vs. NS, # = P<0.05 vs. H30, @ = P<0.05 vs. HNS20, $ = P<0.05 vs. HNS30.

Table 22: Arterial base excess (mM/L). Means and standard deviations.

Group		n	C	S0	S30	R0	R30	R60	R90	R120	R150	R180
Control	Mean	11	4.0	4.3	4.1	3.9	4.5	4.4	4.4	4.3	3.7	3.7
	SD		1.5	1.3	1.4	2.3	1.5	1.5	1.1	1.2	1.0	1.5
NS	Mean	10	3.0	1.9	2.2	2.0	2.7	2.6	2.6	2.5	2.4	2.2
	SD		1.8	2.0	2.4	3.2	2.7	1.9	2.0	2.2	2.0	2.3
H20	Mean	7	3.1	2.5	2.4	2.6	3.3	3.9	4.2	4.6	3.5	3.5
	SD		1.5	2.3	2.0	1.8	2.5	2.0	1.5	1.7	1.9	2.2
H30	Mean	10	1.6	2.0	1.5	1.2	2.3	3.4	3.3	3.0	2.8	3.0
	SD		2.7	1.5	2.2	2.3	2.0	2.3	2.0	2.5	2.8	3.2
HNS20	Mean	8	2.1	0.5	-0.8	-0.7	0.4	1.0	1.1	1.3	1.2	1.1
	SD		1.0	1.9	3.2	3.1	2.5	2.1	1.9	1.5	1.5	1.6
HNS30	Mean	10	6.0	3.3	1.4	0.1	2.3	4.2	4.6	4.9	4.9	5.3
	SD		7.9	9.4	9.7	10.4	9.6	9.0	8.7	8.5	8.6	8.4

RANOVA: no overall group differences were identified.

Table 23: Arterial lactate concentration (mM/L). Means and standard deviations.

			Control	Shock (min)		Reinfusion (min)					
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R120**	**R180**	**RANOVA**
Control	Mean	11	1.3	1.3	1.2	1.0	0.9	0.9	0.8	0.8	
	SD		0.9	1.1	0.8	0.5	0.3	0.2	0.2	0.3	
NS	Mean	10	1.6	2.3	1.9	1.8	1.5	1.4	1.2	1.2	
	SD		1.2	1.3	1.4	1.3	0.8	0.6	0.5	0.6	
H20	Mean	7	2.0	2.1	2.0	1.8	1.3	1.2	1.1	1.0	
	SD		0.9	1.0	1.0	0.8	0.6	0.5	0.5	0.4	
H30	Mean	10	1.8	2.3	2.4	2.3	1.7	1.4	1.3	1.4	
	SD		1.2	1.4	1.6	1.9	1.6	1.2	1.1	1.8	
HNS20	Mean	8	1.9	3.1	3.8	3.4	2.6	2.2	1.6	1.5	*
	SD		0.8	1.6	2.4	2.0	1.7	1.4	0.9	1.1	
HNS30	Mean	11	1.4	3.3	4.5	4.8 *	4.2 *	2.7	1.6	1.5	* #
	SD		0.5	2.9	4.2	3.8	3.8	2.9	1.4	1.2	

One-way ANOVA: * = P<0.05 vs Control, # = P<0.05 vs H30.

Table 24: Normalised femoral blood flow data. Data has been normalised as flows were measured in electrical units only to assess changes rather than absolute flows. Means and standard deviations.

			Control		Shock (min)		Reinfusion (min)						
Group		**n**	**C**	**CNS**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**
Control	Mean	11	100	-	94 # @ $	86 # @ $	83	88	80	80	78	72	69
	SD		0	-	18	16	23	19	21	18	22	24	24
NS	Mean	10	100	103	101 # @ $	87 # @ $	84	84	81	77	81	75	67
	SD		0	16	20	28	31	33	33	39	38	40	37
H20	Mean	7	100	-	68	75 @ $	113	91	87	87	79	69	81
	SD		0	-	23	18	24	19	24	26	26	19	26
H30	Mean	10	100	-	40	43	103	86	76	69	68	69	71
	SD		0	-	32	19	36	31	31	29	25	27	29
HNS20	Mean	8	100	102	44	30	106	93	71	69	65	76	63
	SD		0	33	33	32	21	23	25	30	25	25	34
HNS30	Mean	11	100	105	36	33	110	90	78	73	70	66	72
	SD		0	22	27	21	36	35	24	19	17	12	22

One-way ANOVA: * = P<0.05 vs. Control, + = P<0.05 vs. H20, # = P<0.05 vs. H30, @ = P<0.05 vs. HNS20, $ = P<0.05 vs. HNS30, £ = P<0.05 vs. NS

RANOVA: no statistically significant overall group differences were identified. There were statistically significant differences in group by time interactions. These can be found in the statistics table (Table 3.24).

Table 25: Normalised gut blood flow data. Data has been normalised as flows were measured in electrical units only to assess changes rather than absolute flows (the precise location of the flow probes could not always be duplicated in all the experiments). Means and standard deviations.

			Control		Shock (min)		Reinfusion (min)							
Group		**n**	**C**	**CNS**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**	**RANOVA**
Control	Mean	11	100	-	100 # $	90 $	95	91	90	88	87	84	89	
	SD		0	-	17	24	16	21	20	21	21	24	23	
NS	Mean	10	100	114	125 + # @ $	104 $	102	98	94	93	87	77	79	
	SD		0	30	27	22	25	26	25	24	27	19	32	
H20	Mean	7	100	-	72	91 $	120	121	115	121	104	96	108	
	SD		0	-	8	13	31	54	51	59	33	26	47	
H30	Mean	10	100	-	60	74	98	105	107	104	100	99	103	
	SD		0	-	14	18	23	19	22	14	15	20	25	
HNS20	Mean	8	100	120	77	73	114	116	109	112	99	105	114	$
	SD		0	51	24	31	27	33	32	33	24	10	20	
HNS30	Mean	11	100	94	45	46	92	91	82	84	84	82	70	
	SD		0	16	19	18	49	35	26	24	33	24	19	

One-way ANOVA: * = P<0.05 vs. Control, + = P<0.05 vs. H20, # = P<0.05 vs. H30, @ = P<0.05 vs. HNS20, $ = P<0.05 vs. HNS30, £ = P<0.05 vs. NS

Table 26: Femoral vascular resistance (arbitrary units). Means and standard deviations.

			Control		Shock (min)		Reinfusion (min)							
Group		**n**	**C**	**CNS**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**	**RANOVA**
Control	Mean	11	0.84	*0.84*	0.90	0.97	1.05	0.95	1.04	1.01	1.02	1.12	1.19	
	SD		0.12	*0.12*	0.15	0.11	0.33	0.25	0.25	0.30	0.26	0.46	0.49	
NS	Mean	10	1.01 +	1.11	1.21	1.28	1.23	1.19	1.25	1.34	1.33	1.39	1.54	
	SD		0.12	0.20	0.24	0.27	0.34	0.24	0.30	0.36	0.33	0.41	0.66	
H20	Mean	7	0.74	*0.79*	1.28	1.05	0.75	0.82	0.85	0.91	0.99	1.00	1.03	
	SD		0.19	*0.27*	1.20	0.45	0.17	0.31	0.35	0.39	0.40	0.41	0.50	
H30	Mean	10	0.80	*0.80*	1.43	1.39	0.96	1.00	1.05	1.08	1.05	1.08	1.05	
	SD		0.16	*0.16*	0.63	0.50	0.29	0.25	0.18	0.26	0.26	0.38	0.38	
HNS20	Mean	7	0.95	1.12	3.38	6.56 * £ + #	1.06	1.16	1.48	1.86	1.70	1.52	1.81	* +
	SD		0.09	0.43	4.03	7.34	0.29	0.35	0.74	1.66	0.83	0.83	1.18	
HNS30	Mean	10	0.97 +	1.09	2.65	2.55	1.13	1.14	1.28	1.40	1.46	1.44	1.30	*
	SD		0.12	0.23	1.19	0.95	0.30	0.29	0.27	0.38	0.34	0.28	0.34	

One-way ANOVA: * = P<0.05 vs. Control, £ = P<0.05 vs. NS, + = P<0.05 vs. H20, # = P<0.05 vs. H30.

Table 27: Gut vascular resistance (arbitrary units). Means and standard deviations.

			Control		Shock (min)				Reinfusion (min)					
Group		**n**	**C**	**CNS**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**	**RANOVA**
Control	Mean	11	0.84	*0.84*	0.85	0.98	0.88	0.91	0.92	0.92	0.89	0.94	0.88	
	SD		0.12	*0.12*	0.20	0.29	0.28	0.28	0.30	0.31	0.24	0.30	0.36	
NS	Mean	10	1.01 +	1.04	0.92	1.07	1.14	1.11	1.20	1.16	1.35	1.41	1.55	
	SD		0.12	0.29	0.34	0.38	0.49	0.49	0.62	0.65	0.64	0.81	1.42	
H20	Mean	7	0.74	*0.74*	0.87	0.73	0.75	0.65	0.67	0.67	0.74	0.72	0.75	
	SD		0.19	*0.19*	0.39	0.20	0.15	0.15	0.23	0.25	0.23	0.28	0.28	
H30	Mean	10	0.80	*0.80*	0.90	0.82	0.98	0.82	0.78	0.72	0.76	0.77	0.75	
	SD		0.16	*0.16*	0.39	0.25	0.38	0.26	0.30	0.27	0.28	0.31	0.31	
HNS20	Mean	7	0.95	1.07	1.08	1.32	0.98	1.00	0.90	0.92	1.04	0.87	0.77	
	SD		0.09	0.61	0.53	0.86	0.37	0.46	0.31	0.25	0.39	0.20	0.14	
HNS30	Mean	10	0.97 +	1.13	2.01 * £ + # @	2.05	1.33	1.05	1.18	1.20	1.17	1.06	1.21	* # @
	SD		0.12	0.47	0.99	1.01	0.55	0.43	0.44	0.59	0.47	0.34	0.51	

One-way ANOVA: * = P<0.05 vs. Control, £ = P<0.05 vs. NS, + = P<0.05 vs. H20, # = P<0.05 vs. H30, @ = P<0.05 vs. HNS20.

Table 28: Portal venous oxygen saturation (%). Means and standard deviations.

			Control	Shock (min)		Reinfusion (min)						
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**
Control	Mean	11	71.4	67.8 # @ $	64.6 @	64.6	65.1	63.4	58.0	61.2	61.4	58.0
	SD		8.0	9.2	10.5	9.7	9.6	8.7	19.8	8.1	12.0	10.2
NS	Mean	10	72.8	73.6 + # @ $	70.0 @ $	67.6	66.0	64.6	64.1	63.1	62.3	56.5
	SD		5.3	6.7	6.7	8.1	9.1	7.4	8.2	9.0	8.6	10.6
H20	Mean	7	70.9	50.4	51.8	68.5	66.1	65.6	64.6	57.6	56.2	59.6
	SD		5.8	12.5	15.7	9.5	10.5	11.1	15.7	13.1	10.0	7.8
H30	Mean	10	74.1	45.5	50.4	71.7	72.3	70.0	65.7	64.0	64.1	61.0
	SD		7.1	9.2	5.5	7.2	6.7	6.7	9.3	8.6	6.2	6.5
HNS20	Mean	7	67.1	41.6	43.1	68.2	62.2	57.7	61.1	56.9	57.2	52.2
	SD		10.5	19.3	21.6	11.2	14.3	15.6	11.4	13.2	16.0	20.7
HNS30	Mean	10	74.2	43.2	45.7	75.3	74.5	71.2	65.4	65.5	64.8	57.6
	SD		7.2	13.2	14.6	5.7	8.4	9.0	13.8	14.0	14.7	14.6

RANOVA: No overall group differences detected. There were group by time interaction differences. Contrasts and one way ANOVAs were performed to investigate these differences.

One-way ANOVA: * = P<0.05 vs. Control, + = P<0.05 vs. H20, # = P<0.05 vs. H30, @ = P<0.05 vs. HNS20, $ = P<0.05 vs. HNS30.

Table 29: Portal oxygen extraction ratio. Means and standard deviations.

			Control	Shock (min)				Reinfusion (min)				
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R90**	**R120**	**R150**	**R180**
Control	Mean	11	0.29	0.32	0.35	0.35	0.35	0.37	0.42	0.39	0.39	0.42
	SD		0.08	0.09	0.10	0.10	0.10	0.09	0.20	0.08	0.12	0.10
NS	Mean	10	0.27	0.26	0.30	0.32	0.34	0.35	0.36	0.37	0.37	0.44
	SD		0.05	0.07	0.07	0.08	0.09	0.07	0.08	0.09	0.09	0.11
H20	Mean	7	0.29	0.49 £	0.48	0.31	0.34	0.35	0.35	0.42	0.44	0.40
	SD		0.06	0.12	0.16	0.10	0.11	0.12	0.16	0.13	0.10	0.08
H30	Mean	10	0.26	0.54 * £	0.50 £	0.35	0.28	0.30	0.34	0.36	0.36	0.37
	SD		0.07	0.09	0.05	0.24	0.07	0.07	0.09	0.09	0.06	0.04
HNS20	Mean	8	0.33	0.58 * £	0.57 * £	0.32	0.38	0.42	0.39	0.43	0.44	0.48
	SD		0.10	0.19	0.21	0.11	0.14	0.15	0.11	0.13	0.17	0.21
HNS30	Mean	10	0.26	0.57 * £	0.54 £	0.24	0.25	0.29	0.35	0.34	0.35	0.42
	SD		0.07	0.13	0.15	0.06	0.08	0.09	0.14	0.14	0.15	0.15

One-way ANOVA: * = P<0.05 vs. Control, + = P<0.05 vs. H20, # = P<0.05 vs. H30, @ = P<0.05 vs. HNS20, $ = P<0.05 vs. HNS30, £ = P<0.05 vs. NS

RANOVA: no statistically significant overall group differences were identified, but there were significant differences in group by time interactions, which are shown in the statistics table (Table 3.29).

Table 30: Portal lactate concentration (mM/L). Means and standard deviations.

			Control	Shock (min)		Reinfusion (min)				
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R120**	**R180**
Control	Mean	10	1.4	1.4	1.2	1.0	1.0	0.9	0.8	0.9
	SD		1.0	1.2	0.9	0.6	0.4	0.3	0.2	0.3
NS	Mean	7	1.8	2.4	2.1	1.9	1.4	1.2	1.1	1.2
	SD		1.4	1.6	1.8	1.5	0.8	0.5	0.5	0.6
H20	Mean	7	2.0	2.2	2.0	1.8	1.3	1.2	1.1	1.0
	SD		0.9	1.0	1.0	0.8	0.6	0.6	0.5	0.5
H30	Mean	9	1.8	2.2	2.4	2.3	1.8	1.4	1.3	1.5
	SD		1.1	1.3	1.5	1.9	1.6	1.2	1.2	2.0
HNS20	Mean	7	1.7	3.2	4.1	3.7	2.8	2.4	1.7	1.4
	SD		0.5	1.7	2.8	2.4	1.8	1.4	1.0	1.0
HNS30	Mean	7	1.4	3.6	5.3	5.2 *	4.1	2.9	1.6	1.4
	SD		0.5	3.1	4.9	4.6	4.3	3.2	1.6	1.0

RANOVA: Differences between groups: F = 2.38, df 5, sig. of F = 0.055.

One-way ANOVA: * = P<0.05 vs Control.

Table 31: Portal - arterial lactate concentration (mM/L). Means and standard deviations.

			Control	Shock (min)		Reinfusion (min)				
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R120**	**R180**
Control	Mean	10	0.04	0.02	0.01	0.00	0.02	0.02	0.00	0.03
	SD		0.14	0.14	0.11	0.06	0.07	0.07	0.06	0.08
NS	Mean	7	-0.06	-0.01	0.04	-0.05	0.01	-0.03	-0.04	-0.02
	SD		0.09	0.22	0.21	0.15	0.10	0.08	0.07	0.06
H20	Mean	7	0.01	0.10	0.02	0.01	-0.04	0.01	0.00	0.02
	SD		0.09	0.11	0.29	0.20	0.12	0.15	0.14	0.10
H30	Mean	9	-0.03	-0.13	-0.05	-0.06	-0.01	-0.05	0.03	0.01
	SD		0.23	0.21	0.22	0.12	0.11	0.10	0.07	0.08
HNS20	Mean	7	-0.04	-0.05	0.06	0.18	-0.01	-0.30	0.02	-0.02
	SD		0.15	0.22	0.44	0.36	0.25	0.59	0.22	0.18
HNS30	Mean	7	-0.10	-0.21	-0.03	0.04	-0.01	-0.16	-0.21	-0.01
	SD		0.18	0.50	0.57	0.52	0.64	0.64	0.20	0.16

RANOVA: No overall group differences were identified, although there were group by time interaction differences.

Table 32: Portal endotoxin data. Means, standard deviations and Area Under the Curve.

			Control	Shock (min)		Reinfusion (min)						
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R120**	**R180**	**RANOVA**	**AUC**	
Control	Mean	11	12	12	21	16	22	29	35	45		6903	
	S.D.		2.61	3.37	9.24	7.62	8.66	7.09	10.69	19.55		1526.86	
NS	Mean	10	13	12	25	18	34	56	76	85	*	12405	*
	S.D.		3.54	3.50	6.24	6.77	15.64	19.78	31.13	41.37		4271.74	
H20	Mean	7	14	14	18	26	26	31	41	41		7714	
	S.D.		6.27	3.76	7.56	15.92	13.45	18.80	25.07	21.68		3284.75	
H30	Mean	10	15	13	21	20	30	42	68	69	*	10770	
	S.D.		6.24	3.50	8.76	15.17	14.91	19.32	30.75	32.39		4453.68	
HNS20	Mean	8	14	11	19	23	40	61	77	116	*+	13838	*+
	S.D.		3.54	1.77	5.82	6.51	15.35	23.26	24.34	47.49		4050.60	
HNS30	Mean	11	12	13	18	15	26	36	51	83	@	9539	
	S.D.		2.61	3.44	6.07	5.68	14.80	18.04	24.71	42.03		3714.56	

RANOVA: * = $P<0.05$ vs. Control, + = $P<0.05$ vs. H20, @ = $P<0.05$ vs. HNS20.

One-way ANOVA of AUC: * = $P<0.05$ vs. Control, + = $P<0.05$ vs. H20.

Table 33: Arterial endotoxin data. Means, standard deviations and Area Under the Curve.

			Control	Shock (min)		Reinfusion (min)						
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R120**	**R180**	**RANOVA**	**AUC**
Control	Mean	11	15	12	20	13	18	24	28	36		5767
	S.D.		8.61	2.36	5.77	2.82	6.82	12.89	16.99	23.02		2382.98
NS	Mean	10	13	11	26	17	19	41	54	65	*	9322.5
	S.D.		2.64	2.11	8.96	5.30	6.26	13.90	20.82	24.09		2265.52
H20	Mean	7	14	14	16	26	34	40	40	46	*	8175
	S.D.		4.76	3.76	4.50	28.68	24.40	22.58	19.79	20.50		3774.42
H30	Mean	10	15	17	22	22	27	40	49	61	*	9375
	S.D.		5.99	6.26	10.59	24.29	18.29	19.64	21.58	22.79		3515.68
HNS20	Mean	8	13	13	23	26	37	59	72	96	*	13003 *
	S.D.		2.67	3.72	11.65	21.12	17.31	24.70	32.18	48.09		4362.16
HNS30	Mean	11	11	11	21	12	19	38	53	79	* @	8543
	S.D.		1.58	2.02	7.78	4.83	7.75	22.26	23.83	47.26		4242.04

RANOVA: * = $P<0.05$ vs. Control, @ = $P<0.05$ vs. HNS20.

One-way ANOVA of AUC: * = $P<0.05$ vs. Control.

Table 34: Arterial-Portal endotoxin levels. Means, standard deviations and Area Under the Curve.

			Control	Shock (min)		Reinfusion (min)						
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R120**	**R180**	**RANOVA**	**AUC**
Control	Mean	11	3	1	-2	-3	-3	-6	-7	-9		-1000
	SD		9.77	3.71	10.29	7.28	9.88	17.93	16.74	20.11		2543.08
NS	Mean	10	0	-1	1	-1	-15	-15	-22	-21		-3082.5
	SD		4.71	4.59	10.66	6.15	19.72	17.23	27.51	35.39		3788.05
H20	Mean	7	0	0	-2	1	8	8	-1	5		621
	SD		2.89	5.48	9.51	28.78	17.04	8.16	21.49	21.79		1740.00
H30	Mean	10	-1	4	1	3	-3	-3	-19	-9		-1395
	SD		6.85	5.30	9.37	25.30	23.36	22.88	22.83	20.82		3961.41
HNS20	Mean	8	-1	3	4	3	-3	-2	-5	-20		-834
	SD		5.82	4.63	12.46	19.27	22.19	29.02	28.91	54.84		5183.68
HNS30	Mean	11	-2	-2	4	-4	-8	0	3	-5		-285
	SD		3.50	4.74	10.22	7.75	17.04	18.71	12.30	39.33		1932.87

RANOVA: No significant differences found between the groups.

One-way ANOVA of AUC: No significant differences found between the groups.

Table 35: Portal TNFα data. Means, standard deviations and Area Under the Curve.

			Control	Shock (min)		Reinfusion (min)						
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R120**	**R180**	**RANOVA**	**AUC**	
Control	Mean	9	0	2	5	0	0	9	18	21		2292	
	SD		0	4	12	0	0	11	22	21		2190	
NS	Mean	11	0	0	6	0	5	18	37	47	*	4711	
	SD		0	0	11	0	13	18	21	28		2906	
H30	Mean	10	0	0	10	2	4	28	34	35	*	4568	
	SD		0	0	11	6	7	18	9	15		1777	
HNS30	Mean	9	1	2	6	1	8	24	34	37	*	5183	*
	SD		3	6	10	3	11	14	16	20		1310	

RANOVA: * = $P<0.05$ vs. Control.

One-way ANOVA of AUC: * = $P<0.05$ vs. Control.

Table 36: Arterial TNFα data. Means, standard deviations and Area Under the Curve.

			Control	Shock (min)		Reinfusion (min)						
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R120**	**R180**	**RANOVA**	**AUC**	
Control	Mean	9	0	3	6	2	4	9	10	14		1783	
	SD		0	7	8	7	9	14	15	21		2389	
NS	Mean	11	0	0	2	0	2	7	15	27		2011	
	SD		0	0	7	0	6	12	20	16		2106	
H30	Mean	10	0	0	5	2	16	29	38	38	*	5288	* £ $
	SD		0	0	8	6	16	18	18	13		2401	
HNS30	Mean	9	0	0	7	0	0	7	23	21	#	2442	
	SD		0	0	10	0	0	11	15	13		1563	

RANOVA: * = $P<0.05$ vs. Control, # = $P<0.05$ vs. H30.

One-way ANOVA of AUC: * = $P<0.05$ vs. Control, £ = $P<0.05$ vs. NS, $ = $P<0.05$ vs. H30 + NS.

Table 37: Arterial-Portal TNFα levels. Means, standard deviations and Area Under the Curve.

			Control	Shock (min)		Reinfusion (min)						
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R120**	**R180**	**RANOVA**	**AUC**
Control	Mean	9	0	1	0	2	4	-1	-8	-7		-508
	SD		0	2	11	7	9	15	28	26		3299
NS	Mean	11	0	0	-3	0	-4	-11	-24	-20	*	-2591
	SD		0	0	14	0	15	18	26	20		2644
H30	Mean	10	0	0	-5	0	12	1	4	4		645 £
	SD		0	0	13	9	16	10	12	12		1249
HNS30	Mean	9	-1	-3	0	-1	-7	-17	-12	-18	* #	-2350
	SD		3	6	3	3	11	18	20	21		2257

RANOVA: * = $P<0.05$ vs. Control, # = $P<0.05$ vs. H30.

One-way ANOVA of AUC: £ = $P<0.05$ vs. NS.

Table 38: Portal IL-6 levels. Means, standard deviations and Area Under the Curve.

			Control	Shock (min)		Reinfusion (min)				
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R120**	**R180**	**AUC**
Control	Mean	9	0	2	3	0	3	10	24	47	3517
	SD		0	4	6	0	10	17	21	25	3038
NS	Mean	11	0	2	7	0	4	18	30	59	4718
	SD		0	5	11	0	8	19	27	37	3447
H30	Mean	10	0	3	0	1	4	16	35	58	4545
	SD		0	6	0	3	10	20	15	20	2088
HNS30	Mean	10	0	2	14	0	2	11	31	56	4035
	SD		0	6	15	0	6	20	21	30	3248

RANOVA: No significant differences found between the groups.

One-way ANOVA of AUC: No significant differences found between the groups.

Table 39: Arterial IL-6 levels. Means, standard deviations and Area Under the Curve.

			Control	Shock (min)		Reinfusion (min)					
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R120**	**R180**	**AUC**
Control	Mean	9	0	1	4	0	0	10	19	34	2733
	SD		0	4	6	0	0	12	20	23	2251
NS	Mean	11	0	0	5	0	8	13	35	54	4452
	SD		0	0	11	0	15	20	41	49	5203
H30	Mean	10	0	0	0	0	3	17	41	64	5190
	SD		0	0	0	0	9	18	23	22	2760
HNS30	Mean	9	0	0	12	0	2	13	36	65	4992
	SD		0	0	13	0	7	13	18	20	1507

RANOVA: No significant differences found between the groups.

One-way ANOVA of AUC: No significant differences found between the groups.

Table 40: Arterial-Portal IL-6 levels. Means, standard deviations and Area Under the Curve.

			Control	Shock (min)		Reinfusion (min)					
Group		**n**	**C**	**S0**	**S30**	**R0**	**R30**	**R60**	**R120**	**R180**	**AUC**
Control	Mean	9	0	0	2	0	-3	0	-6	-13	-783
	SD		0	0	8	0	10	18	25	28	2946
NS	Mean	11	0	-2	-2	0	5	-5	2	-5	-266
	SD		0	5	8	0	19	23	29	20	3413
H30	Mean	10	0	-3	0	-1	-1	1	6	5	435
	SD		0	6	0	3	14	18	18	20	2123
HNS30	Mean	9	0	-3	-1	0	0	4	6	10	675
	SD		0	6	19	0	10	25	37	40	4235

RANOVA: No significant differences found between the groups.

One-way ANOVA of AUC: No significant differences found between the groups.