The interaction and pharmacological modulation of the cardiorespiratory responses to primary thoracic blast injury, haemorrhage resuscitation

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Blast injuries represent a problem for civilian and military populations. The response to thoracic blast injury involves a reflex bradycardia, hypotension and apnoea. Casualties who have suffered a blast injury are likely to receive morphine as an early treatment, and may go on to suffer a haemorrhage, thus requiring fluid resuscitation. Aims of this thesis included determination of the effect of blast injury on the response to haemorrhage and whether these responses or their interaction are modified by morphine, and to compare the cardiovascular effects of early and late resuscitation with different solutions following blast injury and haemorrhage. Early cessation of the blast-induced apnoea is important if the patient is to adequately maintain arterial oxygen tensions and thus prevent the development of tissue hypoxia and a subsequent secondary inflammatory response. Therefore, the final aim of this thesis was to determine whether doxapram could shorten the duration of apnoea induced by thoracic blast.

Results confirmed that the response to thoracic blast injury involves a bradycardia, hypotension and apnoea, and also a vasodilation and a reduction in blood flow in the femoral vascular bed. New findings from this thesis show that thoracic blast augments the bradycardia and hypotension seen during haemorrhage and that morphine attenuates this effect. The hypovolaemic blast-injured patient may be resuscitated early or late after haemorrhage with blood, 0.9% saline, colloids (modified gelatin and hydroxyethyl starch) hypertonic saline or hypertonic/hydroxyethyl starch. These fluids restored blood pressure and femoral blood flow to pre-haemorrhage levels for at least 30 minutes. However, resuscitation with hypertonic saline/dextran was shown to be deleterious following blast injury and haemorrhage as blood pressure and femoral blood flow was not maintained for longer than 5 minutes following resuscitation with this fluid. The blast-induced apnoea and hypotension can be significantly attenuated by doxapram immediately following blast injury. This respiratory stimulant may also result in an improvement in ventilation/perfusion matching in the lungs and thus better tissue oxygenation, as administration of doxapram resulted in an improvement in the indices of metabolic acidosis. The new information gained from the work covered by this thesis could potentially lead to better treatment of the blast-injured victim.

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1. Introduction

1.1 The History of Blast Injuries

Blast injuries were first described in 1768 in an account by Jars (M. Jars) relating to a miner who died in an explosion (Clemedson, 1956; Maynard *et al.* 1997). Further detailed descriptions resulted from the First World War when men were frequently found dead in areas of an explosion with no external signs of injury, often with bloodstained fluid coming from the nose or mouth (see Zuckerman *et al.* 1940). Interest in the subject of blast injuries increased following similar reports during The Spanish Civil War (Clemedson, 1956; Zuckerman *et al.* 1940; Williams, 1942) and the Second World War (Clemedson, 1956). Unfortunately almost no *post mortem* examinations were carried out on these casualties. However, experimental work investigating the effects of explosions in animals revealed rupture of the alveoli as well as haemorrhages and bruising in the lungs upon *post mortem* examination of these animals (e.g., Hooker, 1924; and 2 reports from Crile 1917 and Hill 1918, commented upon in Williams, 1942).

The risk of blast injuries is still a threat today with increasing acts of terrorism. In 1995 a terrorist bombing in Oklahoma City, USA killed or injured 869 people (Mallonee *et al.* 1996). Thirteen of the 168 that died (Mallonee *et al.* 1996) had massive internal pathological chest conditions with no lethal external signs of injuries (reported in Irwin *et al.* 1997). Of the survivors, 13 admitted to hospitals had pulmonary contusion, pneumothorax, or went on to develop Adult Respiratory Distress Syndrome (Mallonee *et al.* 1996). Other survivors were reported to be hypotensive following the blast, again with no external signs of injury (reported in Irwin *et al.* 1997).

Blast injuries from explosions can be classified into 3 main categories (Cooper, 1996; Ripple & Phillips, 1997):

1. Primary blast injury occurs when the blast wave interacts with the body causing a small but rapid displacement of the body wall. The blast wave travels through the body causing damage particularly at air:water interfaces such as the lung, ear, and bowel. Often there are no external signs of this type of injury.
2. Secondary blast injury occurs when fragments or debris caused by the explosion collide with the body, often causing penetrating injuries.

3. Tertiary blast injuries result from gross body displacement or limb avulsions following fracture by a shock wave.

This thesis will focus on the physiological effects of primary blast injuries to the thorax, pharmacological modulation of the response, and how the response to the injury modifies reflex responses to other insults such as haemorrhage.

1.2 Physics of Blast

A blast wave originates from a detonation or explosion. These explosions may occur naturally for instance when a volcano erupts or from lightening, or may be accidental (e.g., gas or dust cloud explosions or from an explosion from pressurised gas containers) or from intentional physical, chemical or nuclear explosions (Iremonger *et al.* 1997). Energy is released upon explosion and an intense shock wave is generated in the air surrounding the charge. Atmospheric pressure rises to its peak blast pressure almost instantaneously, between 0.5 and 0.1 milliseconds after detonation. This positive pressure phase with a duration of approximately 2-3 milliseconds (Irwin *et al.* 1997) is then followed by a negative, sub-atmospheric pressure phase of longer duration (Williams *et al.* 1942. See Figure 1.1). It is the positive pressure phase that is thought to be more injurious (Clemedson, 1956). Although the reason for this is not clearly defined it is thought to be due to rapid acceleration of the thorax as the blast wave interacts with the body (Cooper *et al.* 1991). The shock wave travels with the velocity of sound (Cooper *et al.* 1983; Clemedson *et al.* 1956), with its peak pressure and impulse (time in positive phase) decreasing exponentially therefore rapidly losing its injurious power (Clemedson *et al.* 1956; see section 1.3).

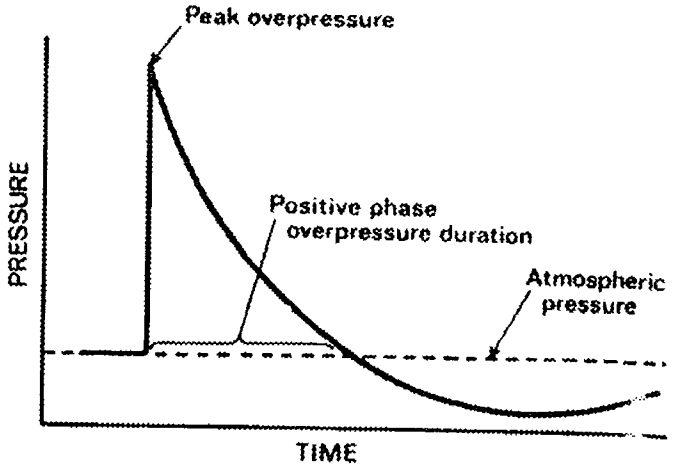


Figure 1.1 Schematic diagram of an idealised shock front resulting from the detonation of an explosive under free-field conditions (reproduced with permission from Cooper *et al.* 1983).

1.3 Mechanism of Injury from a Blast Wave

Three possible theories to explain the mechanism whereby a blast wave causes injury (particularly lesions in the lung) were put forward by Zuckerman in 1940:

1. Reduction in intra-alveolar pressure due to the sub-atmospheric pressure phase of the blast wave. This could cause sudden expansion and rupture of the pulmonary capillaries.
2. The positive pressure phase forcing air down the airways causing distension of the lungs (J. Barcroft, 1941 unpublished. Reported in Maynard *et al.* 1997).
3. Direct impact of the blast wave with the chest wall leading to sudden compression of the lung (Hooker, 1924).

The first and second theory were discounted when blast experiments on animals showed that protection of the chest with a steel cylinder, whilst leaving the head exposed with an open airway, produced no lung damage, when in unprotected animals blast led to severe damage or death (Zuckerman, 1940). However, it has been suggested that the negative pressure component of the blast wave could increase the severity of the lung damage produced by the positive pressure phase (Latner, 1942), but the extent of this is not thought to be significant (Maynard *et al.* 1997).

Further experimental studies have now shown that the mechanism of injury by a short-duration blast wave is as a consequence of a coupling of the blast wave with the body wall. The pressure wave is propagated through the body, reportedly at about 650

metres.sec^{-1} (Clemedson, 1956), and produces pressure differentials at air:water interfaces (such as in the lungs, ear and bowel) which lead to shear and stress waves which disrupt air:tissue interfaces and can also displace organs (Cooper *et al.* 1991; Cooper *et al.* 1997). The probability of developing primary blast injury depends upon the magnitude of the peak overpressure and the duration of this positive pressure phase (Clemedson, 1956; Cooper *et al.* 1983; Cooper *et al.* 1997).

1.4 Organ Damage due to Blast Exposure

Exposure to overpressure due to air blast can result in a condition known as blast lung (Cooper *et al.* 1983; Maynard *et al.* 1997). The features of blast lung are pulmonary oedema due to disruption of capillary fluid movement in the pulmonary circulation (see Chapter 4a, section 4a.1) as the alveolar-capillary diffusion barrier is disrupted, and pulmonary contusions due to haemorrhaging into the alveolar space, again as the alveolar-capillary diffusion barrier is disrupted. The result is insufficient gas exchange and a low arterial oxygen tension (Cooper *et al.* 1983). Before looking in more detail at the histological damage to the lungs following a primary blast injury, the following will briefly describe possible damage that could occur to other organs (both air and non-air containing organs) as a result of direct exposure to a blast wave in air.

1.4.1 *Damage to non-air-containing organs*

Damage to solid organs due to blast injury are not common and injuries to the pancreas, kidney and liver for example, are more consequential due to the haemorrhage and haemoperitoneum that can result from damage to these organs following blast exposure (Gordon-Taylor, 1942).

Studies of blast exposure to rabbits found no changes in the brain (Hunter, 1941) until higher pressures were achieved. Pial haemorrhages could then be seen, in addition to haemorrhaging into the ventricles. Lesions in the grey and white matter were not found (Hunter, 1941) but in monkeys and rabbits zones of oedema could be seen around the central canal of the spinal cord particularly in the thoracic region (Hunter, 1941).

Although a bradycardia is almost always present after thoracic blast injury this may not be due to a direct effect on the heart. The threshold for producing cardiac contusion has been shown to be higher than that for producing pulmonary contusion (Clemedson, 1956). In studies where blast pressure was sufficient to cause cardiac damage pericardial haemorrhages were seen along the coronary vessels towards the apex of the heart anteriorly and posteriorly and no leakage of blood was seen in the pericardial sac (Cameron *et al.* 1942). However, these experiments were carried out under water and animals showing cardiac damage were within 40yards of the charge where mortality rates were 80-100%. Other studies also showed direct lesions to the heart in severe blast injury (see Clemedson, 1956). These lesions consisted mainly of myocardial haemorrhages, ruptured muscle bundles and myocardial infarction thought to be due to air emboli originating in damaged pulmonary vessels. Clemedson reported (1956) some of the most frequent changes seen on the electrocardiogram are a flattening of the QRS complex, indicative of ventricular conduction problems, and prolonged P-R interval, which is consistent with an increase in vagal activity to the atrioventricular node, leading to a slowing down of conduction through the node. A recent study by Harban and colleagues (2001) investigated the effects of thoracic blast injury on cardiac function in the anaesthetised pig. It was concluded that blast injury caused "an immediate and sustained reduction in myocardial function", however, the first recordings were not carried out until 30 minutes after the blast had occurred. In contrast to all this, Hooker found no evidence of a reduction in heart function after air blast in experimental animals, however, it is pointed out that "no particular attention was paid to cardiac function" (Hooker, 1924).

1.4.2 *Damage to air-containing organs*

Organs containing gas or air are particularly susceptible to damage due to air blast as the blast wave passes through the higher density fluid-containing tissues and into the air or gas cavity which has a lower density and as such, a differing acoustic impedance. The stress wave is then reflected as a tensile wave and leads to damage and disruption at the air:tissue interface (Maynard *et al.* 1997). It has been said that the lungs are more vulnerable to blast-induced lesions than the abdomen (Gordon-Taylor *et al.* 1942; Hunter, 1941) and perhaps this is due to the many air:water interfaces within the lung parenchyma. However, abdominal lesions do occur and the sections of gastrointestinal

tract that suffer the most lacerations and perforations are the large intestine (Hunter, 1941), particularly the transverse and sigmoid colon, with the ascending colon, rectum and caecum also being affected (Huller *et al.* 1970; Guy *et al.* 1998). Trapped gas pockets within the GI tract may become compressed as the blast wave travels through the abdomen. Upon re-expansion or implosion of the gas "bubble" gut perforations may ensue. The small intestine and stomach can also be subjected to blast-induced damage (Gordon-Taylor *et al.* 1942) and subserosal haemorrhages are commonly found (Huller *et al.* 1970).

Another susceptible air-containing organ is the ear. The fragile tympanic membrane is very frequently ruptured (Hooker, 1924; Williams, 1942), sometimes blood may be found in the auditory canal (Hooker, 1924) and the eardrum may also become damaged by the blast wave (Williams, 1942).

The principle pathological finding following air blast injury is damage to the pulmonary tissue (Clemedson, 1956).

1.4.3 *Macroscopic damage to the lungs*

Pulmonary haemorrhages are one of the prominent features of blast lung (Williams, 1942; Clemedson, 1956; Zuckerman, 1940; Cooper *et al.* 1983; Maynard *et al.* 1997). The distribution pattern of the haemorrhagic lesions seem to be more concentrated in areas adjacent to bone such as at the costo-phrenic angles (Maynard *et al.* 1997; Williams, 1942), around the mediastinum where the stress waves can be reflected (Maynard *et al.* 1997; Cooper *et al.* 1991) and following the lines of the ribs (Williams, 1942). However, rib fractures are rare and are often associated with death (Maynard *et al.* 1997) possibly as a consequence of a severe blast injury. The haemorrhagic lesions can vary from slight spotting on the surface of the lung tissue, to continuous lesions often involving whole lobes (Zuckerman, 1940; Maynard *et al.* 1997). It is interesting to note that often the right lungs show more damage than the left (Cameron, 1942; Williams, 1942). Other reported pathological findings include air emboli from the damaged pulmonary vessels (Cooper *et al.* 1983; Clemedson, 1956), pneumothoraces and haemopneumothoraces (Maynard *et al.* 1997) as well as pulmonary oedema

(Cooper *et al.* 1983; Clemedson, 1956; Guy *et al.* 1998; Brown *et al.* 1993; Zuckerman, 1940).

1.4.4 *Microscopic damage to the lungs*

Rupture of the alveolar walls can be seen upon microscopic examination of the lung parenchyma (Williams, 1942; Clemedson, 1956; Zuckerman, 1940; Cooper *et al.* 1983; Maynard *et al.* 1997). Due to their close proximity, this is often associated with haemorrhaging from disrupted pulmonary capillaries (Maynard *et al.* 1997) although the actual capillary damage is more difficult to see in light microscopy (Cooper *et al.* 1983). Haemorrhage may be seen in the terminal bronchioles and in severe blast injury may also be seen in the walls of the bronchi or the larger blood vessels (Cooper *et al.* 1983; Maynard *et al.* 1997). The epithelial lining of the bronchioles can become damaged or even stripped, and a loss of its ciliated surface has also been observed (Maynard *et al.* 1997). The pulmonary lymphatic vessels have also been noted to be dilated with fluid and blood after blast injury (Maynard *et al.* 1997). The origin of the blood is likely to be from disrupted pulmonary capillaries and the fluid possibly as a result of an attempt to rectify the ensuing pulmonary oedema (see Chapter 4a, section 4a.1).

1.4.5 *Ultrastructural damage to the lungs*

Following blast injury in anaesthetised rats in 1993, Brown and colleagues noted marked pinocytosis in the endothelial and epithelial cells of the pulmonary tissue under electron microscopy. It was postulated that this was an early compensatory mechanism for the increase in capillary permeability following blast injury. No damage was reported in the cellular organelles (Brown *et al.* 1993).

1.4.6 *Scoring lung damage*

The extent and type of lung damage following blast injury was classified and a scoring system was devised to allow pathological evaluation of the severity of primary blast injury (Dodd *et al.* 1997):

0 = no lesions

1 = 0-10% of the lung shows traces of petechial haemorrhagic lesions

2 = 11-30% of the lung is damaged and may show slight ecchymoses. This is considered a moderate blast injury

3 = 31-60% of the lung is damaged showing confluent ecchymoses. This is considered a severe blast injury

4 = 61-100% of the lung is involved, with diffuse ecchymoses being evident.

In addition, *post mortem* lung weight can be used as a tool to evaluate the severity of lung damage. Lung weight has been shown to be increased following primary blast injury as a result of pulmonary oedema and haemorrhage in the lung tissue (Maynard *et al.* 1997; Guy *et al.* 1998), and correlates well to the severity of blast injury (Elsayed *et al.* 1997).

1.5 The Mechanism of the Response to Primary Blast Injury

The response to primary thoracic blast injury involves a triad of bradycardia, hypotension and apnoea followed by rapid shallow breathing (Clemedson, 1949; Jaffin *et al.* 1987; Irwin *et al.* 1997; Krohn *et al.* 1942; Guy *et al.* 1998). This pattern of response has been shown to be present after thoracic but not abdominal blast exposure (Guy *et al.* 1998). It has been suggested that the response is a reflex involving the vagus nerve as bilateral vagotomy in rabbits reduced the rapid shallow breathing after thoracic blast injury (Krohn *et al.* 1942), and combined cervical vagosympathectomy and atropine abolished the bradycardia and hypotension (Irwin *et al.* 1999). However, this study was not designed to allow distinction between afferent and efferent pathways. A more recent study confirmed that the response to thoracic blast injury was indeed due to a reflex with the afferent and/or efferent pathways carried in the vagus nerve as a cervical vagotomy abolished the bradycardia and apnoea, and attenuated the hypotension (Ohnishi *et al.* 2001). In addition, the bradycardia was markedly attenuated

by the muscarinic acetylcholine receptor antagonist atropine (Ohnishi *et al.* 2001), again, showing that the efferent pathway mediating the bradycardia is carried in the vagus nerve. Further evidence to suggest this was a reflex and not just due to cardiac damage is the latency of onset of the cardiorespiratory response. The bradycardia was evident after approximately 4 seconds, while the hypotension had a latency of onset of approximately 2 seconds after blast (Ohnishi *et al.* 2001). Had the response been due to direct cardiac damage from the blast the response would be instantaneous.

The only currently known reflex that could be responsible for eliciting the **full** triad of the response to primary thoracic blast injury is the pulmonary 'J' reflex. The response elicited by activation of the pulmonary 'J' reflex involves a bradycardia, hypotension, apnoea followed by rapid shallow breathing and vasodilation in the femoral vascular bed in the anaesthetised cat (Daly & Kirkman, 1988). In this respect the pattern of response is identical to that following thoracic blast injury. The pulmonary 'J' reflex can be activated by stimulation of the juxtapulmonary capillary receptors within the lung parenchyma. These so-called 'J' receptors are pulmonary afferent C-fibre nerve endings which can be stimulated either pharmacologically using a 5-Hydroxytryptamine (5HT) agonist such as phenylbiguanide (PBG; Daly *et al.* 1988) or mechanically by pulmonary oedema (Paintal, 1969). It is known that pulmonary oedema is a feature of thoracic blast injury (Guy *et al.* 1998).

The afferent limb of the Pulmonary 'J' Reflex (the pulmonary C-fibres), is carried in the vagus nerve (Coleridge *et al.* 1984) and although the afferent pathway of the response to thoracic blast injury is currently unknown, the efferent limb to the heart mediating the bradycardia, and that mediating the apnoea in both reflexes, is also carried in the vagus nerve (Daly *et al.* 1988; Ohnishi *et al.* 2001). The efferent limb to the vasculature mediating the vasodilation and hypotension in the pulmonary 'J' reflex is carried in the sympathetic nerves and involves an inhibition of sympathetic outflow (Daly *et al.* 1988). However, it is currently unknown whether this is also true for the response to thoracic blast injury. In fact evidence against the possibility of these two reflexes being part of the same reflex arc comes from the work of Dr. M. Ohnishi as part of his research towards a Ph.D. thesis (currently unpublished).

One of the studies carried out by Dr. Ohnishi and his colleagues aimed to determine whether thoracic blast leads to a reduction in peripheral vascular resistance via

sympathoinhibition. This was done by administration of a noradrenergic blocking agent guanethidine. Guanethidine prevents reflex changes in sympathetic nerve activity whilst maintaining sufficient vascular tone to allow the expression of any non-sympathetic noradrenergic vasodilation. Thus if the blast-induced vasodilation was due to sympathoinhibition, as is the vasodilation due to the pulmonary 'J' reflex, then it would be abolished by guanethidine. However, the results of this study showed that guanethidine failed to block the vasodilation in the skeletal muscle vascular bed after blast, and thus it was concluded that the efferent pathway mediating the vasodilator response to thoracic blast injury was not carried in the sympathetic nerves to the vasculature. Nevertheless, guanethidine was reported to attenuate the recovery of arterial blood pressure following blast injury in this study, possibly indicating that following thoracic blast injury there is a sympathetically-mediated vasoconstriction in other vascular beds such as the vital organs and/or an increase in sympathetic outflow to the heart contributing to the recovery of blood pressure. A haemodynamic redistribution of blood flow away from vital/metabolically active organs has important implications for the development of organ ischaemia, but this will be discussed in Chapter 3, section 3.4.

Further evidence against the theory that the reflex response to pulmonary C-fibre activation and the response to thoracic blast injury share the same reflex arc again came from studies carried out by Ohnishi and colleagues (unpublished Ph.D. thesis). Ondansetron is a $5HT_3$ receptor antagonist and the pulmonary 'J' reflex can be elicited by $5HT_3$ agonists such as PBG (Daly & Kirkman, 1988). Ondansetron will block the response elicited by PBG (M. Ohnishi, unpublished Ph.D. thesis). Ohnishi and colleagues therefore postulated that if the two reflexes (pulmonary 'J' reflex and the reflex response to thoracic blast injury) share the same afferent pathway then systemic administration of ondansetron should block the response to thoracic blast injury. The results, however, showed no significant difference in the response to thoracic blast following administration of ondansetron from the control group (M. Ohnishi, unpublished Ph.D. thesis).

Administration of a centrally-acting 5HT receptor antagonist, methiothepin, also failed to block the triad response to thoracic blast. Indeed it was shown to potentiate the bradycardia and apnoea (M. Ohnishi, unpublished thesis), in contrast to its blockade of the bradycardia elicited by a PBG-induced pulmonary 'J' reflex (Bogle *et al.* 1990).

The combined results of the work carried out by Ohnishi *et al.* suggest that the reflex response to thoracic blast and that to pulmonary C-fibre activation do not share the same afferent or central nervous pathway and thus it is unlikely that they are the same reflex. There are, however, still a number of other reflexes which could be possible candidates for at least part of the response, and in combination, could be responsible for the whole response to thoracic blast injury:

- the cardiac afferent C-fibre reflex
- the arterial baroreceptor reflex
- and activation of the pulmonary stretch receptors (the Hering-Breuer reflex)

The role of these reflexes in the response to thoracic blast warrants further investigation, and are considered further in sections 3.1.1.2, 3.1.1.1 and 6.1.1.

1.6 Clinically Relevant Questions and Aims of the Thesis

Blast casualties may often sustain blood loss as a consequence of their injuries (Cooper *et al.* 1983). The pattern of response to blood loss in the absence of a blast injury is biphasic (Barcroft *et al.* 1944; see Chapter 3, section 3.1), with phase I maintaining blood pressure by the action of the baroreflex (Secher & Bie, 1985; Little *et al.* 1989). It seems that after a blast injury baroreceptor reflex sensitivity may be modulated since the blast induced hypotension is associated with a bradycardia rather than a tachycardia which would be expected were the baroreflex functioning normally. This has potential clinical implications as patients with a reduced baroreflex sensitivity may experience greater falls in blood pressure for a given blood loss. If baroreflex sensitivity were altered following a blast injury then this may alter the response to a subsequent haemorrhage, in particular phase I as this is mediated by the baroreflex. This could potentially lead to a clinician failing to diagnose an internal haemorrhage in a blast victim as the usual pattern of response to haemorrhage may be altered. This could have potentially fatal consequences and so one of the aims of this thesis (addressed in Chapter 3) will be to determine the pattern of response to blood loss following primary thoracic blast injury.

As part of the treatment of a blast casualty morphine may be administered as an analgesic since this is standard military practice were service personnel are issued with morphine auto-injecters for use in the case of injury. Morphine is known to attenuate the

vagally-mediated reflex bradycardia seen in the second phase of the response to haemorrhage alone (Ohnishi *et al.* 1997), and the vagally-mediated bradycardia associated with activation of the pulmonary/cardiac afferent C-fibres (Ohnishi *et al.* 1999a). However, morphine has *no effect* on the vagally-mediated bradycardia induced by blast, but augments the apnoea and delays the recovery of blood pressure (Ohnishi *et al.* 1999b). Therefore a further aim addressed in Chapter 3 is to look at the effects of administering morphine to a blast injured victim whom will also go on to suffer blood loss.

The next step in the treatment of a hypovolaemic blast victim would be to restore plasma volume by fluid resuscitation. There are several reasons why the response to fluid resuscitation after blood loss may be different in blast-injured victims compared to those suffering haemorrhage in the absence of blast. The direct mechanical damage caused by the blast can increase capillary permeability and it is known that blast injuries lead to the development of pulmonary oedema and a reduction in PaO_2 (Guy *et al.* 1998). This hypoxaemia will result in tissue hypoxia and may trigger a secondary inflammatory response (see Chapter 4a, section 4a.4) and augment an already established pulmonary oedema. Some fluids e.g. crystalloids have been shown to increase the degree of pulmonary oedema (Fulton *et al.* 1973; Richardson *et al.* 1974; Tranbaugh *et al.* 1982). However, others such as hypertonic solutions have demonstrated anti-inflammatory properties (Corso *et al.* 1999; Nolte *et al.* 1992) and so have the potential to reduce pulmonary oedema. The optimum time to administer fluids may be later rather than sooner as resuscitation too soon after haemorrhage has been reported to increase the rate, volume and duration of haemorrhage (Sakles *et al.* 1997; Bickell *et al.* 1992; Krausz *et al.* 1992; Marshall *et al.* 1997), as well as increasing mortality (Bickell *et al.* 1992; Krausz *et al.* 1992; Marshall *et al.* 1997). Therefore one of the aims of this thesis is to address the issue of fluid resuscitation following blast injury and haemorrhage. Along with determining the response to fluid resuscitation in these subjects, the type of fluid as well as the timing of the administration of the fluid will be assessed (see Chapters 4a, 4b and 5).

Finally, the bradycardia and apnoea can be prevented and the hypotension attenuated by vagotomy (Ohnishi *et al.* 2001). In addition, the bradycardia can be blocked pharmacologically using atropine. However, no pharmacological means of attenuating the hypotension and apnoea is currently known. It is important clinically to attenuate the

hypotension in order to maintain adequate tissue perfusion, and attenuation of the apnoea would prevent the hypoxaemia, tissue hypoxia and thus secondary inflammation escalating the whole response. Doxapram is an analeptic (O'Connor *et al.* 1996), that is it stimulates respiration (Uehara *et al.* 2000; De Villiers *et al.* 1998; Bairam *et al.* 1993; Peers *et al.* 1991) reducing the frequency and duration of apnoea in humans (Yamazaki *et al.* 2001; Poets *et al.* 1999; Huon et al 1998) and animals (Bairam *et al.* 1992). Doxapram is also reported to have some pressor actions (Huon *et al.* 1998; Cote *et al.* 1992). Therefore the effects of doxapram on the cardiorespiratory response to thoracic blast injury will be assessed in Chapter 6.

2 Methods

This chapter describes the techniques common to all the studies throughout this thesis. Any additional techniques, and the protocol for the studies, can be found in the relevant chapters.

The studies were conducted on male Wistar rats of the Harlan Olac strain, kept on a 12 hour light/dark cycle and fed on Beekay standard rat and mouse diet (B & K Universal Ltd., UK) and allowed access to water *ad libitum*.

Anaesthesia was induced in all animals with inhalation isoflurane (Abbott Laboratories Ltd. UK; 3.5% in O_2/N_2O, FIO_2 = 0.5) in an anaesthetising chamber (Fluorovac, International Market Supplies, UK). Once the criteria indicating a surgical level of anaesthesia were reached, i.e., when locomotor activity ceased, postural muscle tone and the righting reflex was lost, anaesthesia was then maintained by delivery of 2.5% isoflurane in 0.4L $O_2.min^{-1}$, via a co-axial anaesthetic delivery/scavenging system and a nose cone (Fluovac^{TM}, International Market Supplies, UK.). The concentration of isoflurane was adjusted to maintain a surgical level of anaesthesia whereby there was no spontaneous movement, righting reflex or withdrawal to noxious foot pinch.

2.1 Surgical Preparation and Physiological Measurements

In each rat a cannula (2FG, Portex Ltd., UK) was inserted into the ventral tail artery and advanced until its tip lay in the abdominal aorta. Arterial blood pressure was monitored via this cannula using a strain gauge manometer (Sensonor 840^{TM}, SensoNor a.s., Norway). Both lateral tail veins were cannulated (2FG, Portex Ltd., UK) for drug administration. All cannulae were initially filled with heparinised saline (20 $iu.mL^{-1}$ heparin, Monoparin ^{TM}, CP Pharmaceuticals, UK, in 0.9% saline).

The isoflurane was then discontinued and anaesthesia maintained with alphadolone/alphaxolone (Saffan^{TM}, Pitman-Moore, UK) administered by continuous

intravenous infusion (19-24 $mg.kg^{-1}.h^{-1}$) using an infusion pump (Harvard 22™, Harvard Apparatus Ltd., UK) while the animals breathed air.

For the blast and doxapram study only (Chapter 6), the trachea was exposed via a midline incision and a tracheal tube inserted via a tracheostomy (see Chapter 6, section 6.2).

For measurements of blood flow in the femoral vascular bed the left femoral artery was carefully exposed so as not to cause any damage to the femoral nerve or vein, and a 0.5mm transit time ultrasonic flow probe (Transonic Systems Inc., USA) was positioned around the left femoral artery. K-Y lubricating jelly (Johnson & Johnson, UK) was used as an acoustic coupler. Femoral vascular resistance was calculated as mean arterial blood pressure divided by femoral blood flow. The electrocardiogram was recorded using needle electrodes placed in the skin of the ventrum, and heart period measured from the electrocardiogram. All physiological variables were amplified and recorded using a computerised Data Acquisition System (MacLab 8s™, ADInstruments, UK) Body temperature was monitored using a thermocouple (Medical precision thermometer; Ellab Copenhagen DM 852) inserted via the anus and advanced until its tip lay 6-8 cm beyond the anal sphincter, and maintained at approximately 38°C throughout the study using a thermally-insulated operating mat and a heating lamp. Samples of arterial blood were withdrawn anaerobically from the ventral tail artery for blood gas/pH determination (ABL5™, Radiometer Ltd. Denmark). The volumes of blood removed for blood gas analyses were replaced by equal volumes of isotonic colloid solution (Haemaccel).

2.2 Blast Wave Generator

The blast wave generator used has been described previously (Jaffin *et al.* 1987). This blast wave generator is a relatively small tabletop device that requires no explosives and permits the blast wave to be focused on a specific area of the body. Briefly, compressed air was used to generate a pressure of approximately 1500psi behind a solenoid-controlled valve (see Figure 2.1). When the solenoid-controlled valve is released the stored compressed air is discharged into a 20-mm internal diameter blast nozzle. This pressure ruptures a 0.55mm-thick aluminium bursting disc mounted within the nozzle,

releasing the high pressure air. This results in a short duration blast wave (lasting less than 1ms; Jaffin *et al.* 1987), that leaves the device through the base of the nozzle and is directed at the animal lying below. Altering the distance between the movable blast nozzle and the animal lying underneath regulates the force of the blast wave administered to the subject. The animals were positioned supine under the blast apparatus. Those subjected to blast were positioned directly under the blast nozzle (which delivers the blast wave to the animal), with the blast nozzle 3.5 cm above the ventral surface of the thorax, midway between the sternal notch and the xiphoid, while those subjected to sham blast were not. Thus all animals could be subjected to the sound of the blast, but only those positioned directly under the nozzle were subjected to the physical effect of the blast wave.

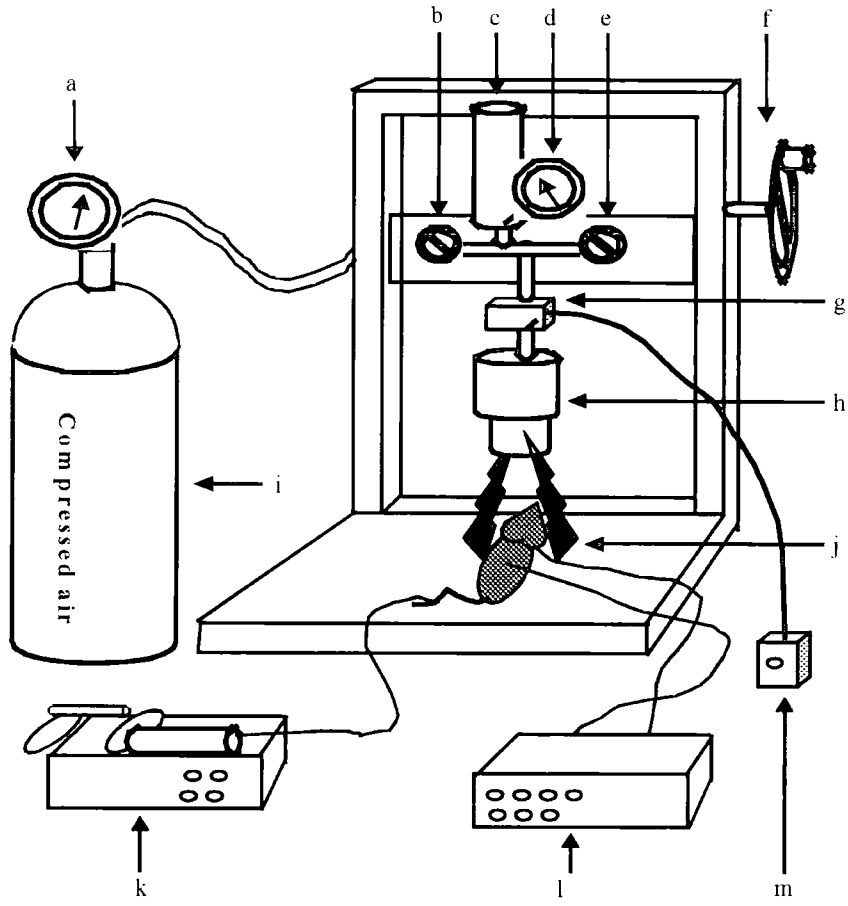


Figure 2.1 Schematic diagram of the blast wave generator used in this thesis. a) air cylinder pressure dial (inlet pressure), b) isolation valve, c) pressure reservoir 150mL, d) working pressure dial 1500psi, e) vent valve, f) rack to change distance between blast nozzle and the animal below, g) solenoid valve, h) blast nozzle, i) compressed air cylinder, j) blast wave, k) anaesthetic pump (Harvard 22, Harvard Apparatus Ltd., UK), l) computerised Data Acquisition System (MacLab 8s^{TM}, ADInstruments, UK), m) solenoid release switch. For full explanation see text.

At the end of the study, all animals were killed with an overdose of 0.5mL of 60mg.mL^{-1} sodium pentobarbitone (Sagatal, Rhône Mérieux (Ireland) Tallaght, Dublin) administered intravenously. A *post mortem* was performed to assess whether any internal damage had occurred from the insertion of the intra-arterial (i.a.) cannula or the thermometer, and to macroscopically assess the state of the lungs and the fullness of the bladder.

2.3 Statistical Analysis

Mean arterial blood pressure was calculated as diastolic pressure plus one third of the pulse pressure. Data are presented as mean±standard error of the mean (SEM) unless indicated otherwise. Statistical comparisons were made using a 2-way analysis of variance for repeated measures (time) (SPSS/PC+ v4.01) unless indicated otherwise, and the degrees of freedom adjusted using the Greenhouse-Geisser correction to minimise the risk of type 1 error (Ludbrook, 1994). Comparisons of non-repeated measurements between groups (baseline values, body weights) were made using one-way analyses of variance followed, where appropriate, by a Tukey post-hoc test (SPSS/PC+ v4.01). In all cases $P<0.05$ was considered statistically significant.

Each study was conducted in accordance with the Animals (Scientific Procedures) Act, 1986.

3 The Effects of Haemorrhage and Morphine on the Cardiorespiratory Responses to Primary Thoracic Blast Injury in the Anaesthetised Rat

3.1 Introduction

Blast weapons are a threat to both military and civilian populations (Cooper *et al.* 1983) and they produce a spectrum of injuries, ranging from direct effects of the blast wave, seen predominantly at gas-containing organs, to the displacement of casualties with subsequent injury to all body systems (Cooper *et al.* 1983).

Primary blast injury to the thorax produces a bradycardia, hypotension and apnoea (Krohn *et al.* 1942; Guy *et al.* 1998). This is a reflex response involving the vagus nerve (Irwin *et al.* 1999; Ohnishi *et al.* 2001). The afferent pathway mediating the apnoea and part of the hypotension is vagal since vagotomy abolishes the apnoea and attenuates the hypotension, and the efferent pathway mediating the bradycardia is also vagal since the fall in heart rate can be blocked with atropine (Ohnishi *et al.* 2001). It is unknown whether the afferent pathway mediating the bradycardia is also vagal. The reflex pathway involved in this response has not been fully characterised, although the response has many similarities to those induced by activation of the pulmonary afferent C-fibres (Daly & Kirkman, 1988; see Chapter 1, section 1.5). In addition, the persistence of hypotension combined with bradycardia after blast indicates that there may also be a modulation of the arterial baroreceptor reflex following blast injury (Ohnishi *et al.* 2001).

Blast injured casualties will often sustain haemorrhage as a consequence of their primary, secondary or tertiary blast injuries (Cooper *et al.* 1983). The pattern of response to a progressive simple haemorrhage (blood loss in the absence of tissue damage and nociception) is biphasic (Barcroft *et al.* 1944), with an initial tachycardia and maintenance of blood pressure via the arterial baroreceptor reflex (Secher & Bie, 1985; Little *et al.* 1989; see section 3.1.1.1). As haemorrhage progresses, and blood loss exceeds about 30% of total blood volume, a second phase becomes apparent (see section 3.1.1.2). This involves a vagally-mediated bradycardia, (which can be blocked by administration of atropine; Little *et al.* 1989) and a significant fall in blood pressure due

to a reduction in peripheral vascular resistance (Barcroft *et al.* 1944; Evans & Ludbrook, 1990). This phase is due to the recruitment of one or more reflexes (Little, *et al.* 1989). Although the identity of their afferent limb is currently uncertain (Scherrer *et al.* 1990; Shen *et al.* 1990; Kirkman & Little, 1994; see section 3.1.1.2) some studies suggest the possible involvement, in the rabbit at least, of cardiac vagal afferents as well as input from several brain pathways and circulating or neuronally released hormones (Evans *et al.* 2001; see section 3.1.1.2). However, what is known is that phase II of the response to haemorrhage is not due to a failure of the baroreflex as sensitivity is still high at the onset of this second phase (Little *et al.* 1984).

3.1.1 *Mechanisms underlying the cardiovascular response to a progressive simple haemorrhage.*

3.1.1.1 Phase I; The arterial baroreceptor reflex

The baroreflex is a negative feedback mechanism minimising moment to moment changes in arterial blood pressure (Cowley *et al.* 1973). The baroreceptors are slowly adapting mechanoreceptors which respond to stretch of the arterial wall produced by the absolute level of blood pressure, and to the rate of change of pressure, i.e., pulse pressure. They are therefore rate sensitive (Angell-James & Daly, 1970). During a progressive simple haemorrhage (blood loss in the absence of any significant tissue damage) there is a reduction in venous return, a reduction in cardiac filling (end-diastolic volume) and hence, by Starling's law of the heart, a reduction in stroke volume. This will lead to a reduction in arterial pulse pressure (see Little, Kirkman & Ohnishi, 1998), and thus an unloading of the baroreceptors (Angell-James & Daly, 1970) resulting in a reflex tachycardia (due to sympathoexcitation and vagal inhibition) and a sympathetically-mediated increase in total peripheral resistance.

The baroreceptors are found in the aortic arch and the carotid sinuses (Kirchheim, 1976). The afferent information is carried to the brain via myelinated and unmyelinated fibres in the sinus nerve (a branch of the glossopharyngeal nerve) from the carotid sinus and via the vagus nerve from the aortic arch (Kircheim, 1976). The first synapse of the baroreflex can be found in the nucleus tractus solitarius (NTS) in the medulla of the brainstem (Spyer, 1984). The efferent limb of the baroreflex is carried in the sympathetic

nerves to the heart and vasculature, and the vagus nerve to the heart (Kircheim, 1976). The cell bodies of the parasympathetic neurones are found in the nucleus ambiguus (NA), which receives an input from the NTS, and the dorsal vagal motor nucleus in the medulla of the brainstem (McAllen & Spyer, 1976; Jordan *et al.* 1982). This is the origin of the vagus nerve and it is cells in this area of the brain that would need to be influenced to change baroreceptor efferent activity. The efferent limbs of many vagally-mediated reflexes originate in the NA, as well as the dorsal vagal motor nucleus. The origin of the sympathetic efferent limb of the baroreflex can be found in the intermediolateral cell column of the spinal cord. The sympathetic motor neurones receive a tonic descending excitatory drive from a group of neurons in the rostral ventrolateral medulla (RVLM). Indeed, studies involving c-fos, a gene coding for the protein Fos expressed in cells such as neurons in response to external physiological stimuli (see McAllen *et al.* 1992), have shown increased c-fos immunoreactivity in spinally projecting neurons originating in an area within the rostral ventrolateral medulla (an area of the brain stem known to be a major source of sympathetic neurons supplying the heart and vasculature; see McAllen *et al.* 1992) following a 25% blood volume haemorrhage in conscious cats (McAllen *et al.* 1992). The RVLM receives an inhibitory input from a group of neurones in the caudal ventrolateral medulla (CVLM), which receives input from the NTS.

The sympatho-activation is not uniform to all vascular beds; those with the greatest dependence on oxygen are generally subject to the least vasoconstriction, while areas less dependent on oxygen, or more tolerant to transient reductions in oxygen delivery, have the greatest constriction. Thus the arterial baroreceptor reflex aims to maintain arterial blood pressure and thus preserve blood flow to areas which need it most.

3.1.1.2 Phase II; The 'depressor' reflex

Phase II is characterised by a fall in heart rate to below pre-haemorrhage levels and a profound fall in arterial blood pressure (Barcroft *et al.* 1944; Secher & Bie, 1985). This is thought to be a protective mechanism to prevent high levels of cardiac work with insufficient coronary perfusion (Öberg & Thorén, 1972). The reflex bradycardia is mediated by activation of the vagus nerve, as this can be blocked with atropine in humans (Lewis, 1932. Barcroft *et al.* 1944) and experimental animals (Little *et al.* 1989). Inhibition of the sympathetic efferent nerves to the vasculature mediates the fall in total

peripheral resistance (TPR; Öberg & Thorén, 1972). Recently there has been increased interest in the central nervous pathways mediating this response. Some studies have shown increased Fos protein expression (which is taken as a marker of increased neuronal activity) following haemorrhage in both the ventrolateral periaqueductal grey (vlPAG) and the caudal midline medulla (Badoer *et al.* 1993; Henderson *et al.* 2000). Both of these areas are known to cause inhibition of sympathetic efferent activity and, in the case of the vlPAG, increased vagal efferent activity (see Evans *et al.* 2001). The result of activation of the cardiac vagus nerve and inhibition of sympathetic efferent nerves to the vasculature is a profound fall in blood pressure. Phase II of the response to a simple haemorrhage is not due to a failure of the baroreflex as sensitivity is still high (Little *et al.* 1984), but due to a second 'depressor' reflex.

For many years it was thought that the depressor reflex was due to activation of cardiac vagal afferent C-fibres. The mechanosensitive receptor endings of these fibres are found in the ventricular myocardium and are stimulated by the abnormal deformations of the ventricular walls as the heart beats forcefully around an almost empty chamber during haemorrhage (Öberg and Thorén, 1972). The role of the cardiac C-fibres in phase II of the response to haemorrhage was supported by a body of circumstantial evidence:

- It is known that when these receptors are stimulated they lead to a reflex bradycardia due to vagal activation, and a reduction in vascular resistance due to sympatho-inhibition, i.e., similar to the response to a severe haemorrhage (Daly, Kirkman & Wood, 1988).
- The instillation of procaine into the pericardial sac, which would block the supposed afferent pathway of the cardiac C-fibre reflex, blocks the depressor response to severe haemorrhage (Burke & Dorward, 1988; Evans *et al.* 1989).
- The depressor response to severe haemorrhage was absent in animals treated neonatally with capsaicin, the capsaicin causing them to become deficient in afferent C-fibres (Little *et al.*1989). However, this was not conclusive proof of the involvement of the *cardiac* C-fibres as capsaicin destroys *all* C-fibres (Fizgerald, 1983).

Evidence against the involvement of cardiac C-fibres in the depressor response has been brought to light by more recent studies. Firstly, procaine has been shown to have more widespread effects than simply just blocking cardiac afferent C-fibres (Evans *et al.* 1993;

Gentry & Lukas, 2001). Secondly, cardiac transplant subjects with no functional innervation of the ventricles were reported to display the depressor response to severe haemorrhage in animals (Shen *et al.* 1990), and in response to an increasing dose of glycerol trinitrate (GTN) in man (Scherrer *et al.* 1990). This could not have been due to activation of the cardiac C-fibres as this afferent pathway was absent. Thirdly, this depressor response does not share the same central nervous pathway as the cardiac C-fibre reflex, as methiothepin (a 5-HT_{1A} receptor antagonist) administered centrally, will block the reflex bradycardia associated with activation of cardiac C-fibres in the rat (Bogle *et al.* 1990), but it did not block the bradycardia and hypotension seen during a severe haemorrhage (Kirkman *et al.* 1994). Therefore if the cardiac C-fibres are involved in phase II of the response to haemorrhage, they cannot be the only mediator in the initiation of this reflex, and the major contributor to the activation of the depressor phase of the response to haemorrhage may be species dependent (Evans *et al.* 2001).

The reflex bradycardic response to both blast (Irwin *et al.* 1999; Ohnishi *et al.* 2001) and haemorrhage (Lewis, 1932; Barcroft *et al.* 1944; Little *et al.* 1989) are mediated by activation of the vagus nerve. As they are both vagal reflexes it is pertinent to determine whether they interact. The outcome cannot be predicted from the published literature as the only situation were an interaction between haemorrhage and injury has been assessed is in the context of musculo-skeletal injury. Because the response to musculo-skeletal injury is fundamentally different to that induced by thoracic blast (the former yielding tachycardia and hypertension while the latter gives bradycardia and hypotension) it is impossible to predict the effects of the response to thoracic blast on that to haemorrhage. Since a blast casualty may go on to haemorrhage it is important to determine whether the response to thoracic blast may modify that to a subsequent haemorrhage.

In assessing the interaction between the response to blast and haemorrhage it would be important to look at the haemodynamic changes following blast and haemorrhage since these may be important for long term survival (see section 3.1.1.3 and 3.1.2.1).

3.1.1.3 Haemodynamic changes following a progressive haemorrhage

During a simple haemorrhage blood flow is diverted towards organs with a low ischaemic tolerance (e.g., the intestines) at the expense of those with a high ischaemic

tolerance (e.g., skeletal muscle), thereby maximally utilising the available oxygen supply (Mackway-Jones *et al.* 1999). However, when a haemorrhage occurs together with a model of musculo-skeletal tissue injury, a reversal of this haemodynamic situation is seen, i.e. blood is diverted towards the relatively quiescent skeletal muscle at the expense of the metabolically active vital organs such as the gut (Mackway-Jones *et al.* 1999). As a consequence of this, the gut may become ischaemic and the gut barrier may become compromised possibly resulting in bacterial translocation and septicaemia (Mackway-Jones *et al.* 1999). However, it is not known whether these haemodynamic changes will also occur with another type of injury, namely primary blast injury.

The response to blast may also give a reflex alteration in vascular resistance in addition to the bradycardia as abolishing the reflex by vagotomy attenuates the hypotension, whereas blocking the bradycardia with atropine does not modify the change in blood pressure (Ohnishi *et al.* 2001).

3.1.2 *The effects of morphine on the individual responses to primary thoracic blast injury and that to haemorrhage*

Morphine has been shown to attenuate the reflex bradycardia associated with the response to haemorrhage (Ohnishi *et al.* 1997) and those induced by activation of the pulmonary/cardiac afferent C-fibres with phenylbiguanidine (Ohnishi *et al.* 1999a). By contrast, morphine had no effect on the bradycardia induced by primary thoracic blast, but augmented the apnoea and delayed the recovery of blood pressure (Ohnishi *et al.* 1999b). Both the haemorrhage-induced bradycardia (Lewis, 1932; Barcroft *et al.* 1944; Little *et al.* 1989) and the blast-induced bradycardia (Ohnishi *et al.* 2001) are mediated by the vagus nerve, however, it appears that morphine is able to modify one vagally mediated bradycardia but not the other. Thus, it would be interesting to determine the effects of morphine on the haemorrhage-induced bradycardia when it is on a background of thoracic blast injury. This is especially relevant since battlefield casualties are very likely to receive morphine as an early treatment before attempts at resuscitation can be made.

3.1.2.1 Haemodynamic changes following morphine administered during blood loss

There are many similarities between the effects of morphine and those of musculo-skeletal injury on the response to haemorrhage, i.e. both morphine and musculo-skeletal injury block the bradycardia and delay the onset of the hypotension seen in phase II of the response to a simple haemorrhage (Ohnishi *et al.* 1997; Little *et al.* 1989). Although Ohnishi *et al.* (1997) did not determine the haemodynamic pattern of response, the hypotension seen during a severe haemorrhage is mediated by sympathoinhibition (McAllen *et al.*1992) and morphine can prevent sympathoinhibition by activation of μ opioid receptors (Evans & Ludbrook, 1990). Thus it is possible that morphine may have reversed the haemorrhage-induced hypotension by causing an increase in total peripheral resistance. Although it could not be speculated as to where those increases in resistance may be greatest, other studies have shown that administration of morphine after simple haemorrhage reduces survival, despite better maintenance of blood pressure (Marshall *et al.* 1998). Thus, this may be as a result of an increase in vascular resistance in the vital organs. Blood flow to these metabolically active organs would then fall resulting in ischaemic damage. As morphine delays the recovery of blood pressure after a blast injury (Ohnishi *et al.* 1999b) it is important that the haemodynamic changes after a blast injury and a subsequent haemorrhage are investigated.

Thus the aims of this study are to determine the effect of thoracic blast injury on the cardiorespiratory response to haemorrhage and whether these responses, or their interaction, are modified by morphine. Mackway-Jones *et al.* (1999) have already determined that changes in blood flow to the skeletal muscle occur during a progressive haemorrhage. To allow comparison, a preliminary study was conducted to determine whether any haemodynamic changes occur in the same vascular bed following blast injury and subsequent haemorrhage. Examination of blood flow to skeletal muscle using an ultrasonic flow probe will allow us to determine the haemodynamic pattern of response to blast and haemorrhage in the femoral vascular bed. This will give an insight to the timepoints at which significant changes in femoral blood flow occur. Future studies will then permit the elucidation of blood flow to other organs, such as the gut, at these specific time points (i.e., where significant changes occurred in skeletal muscle). Due to the nature of the blast injury, this will be carried out using fluorescent microspheres (Schimmel *et al.* 2001).

3.2 Methods

The study was conducted on male Wistar rats (Harlan Olac; body weight range 265-337g) terminally anaesthetised and prepared for recording as described in Chapter 2.

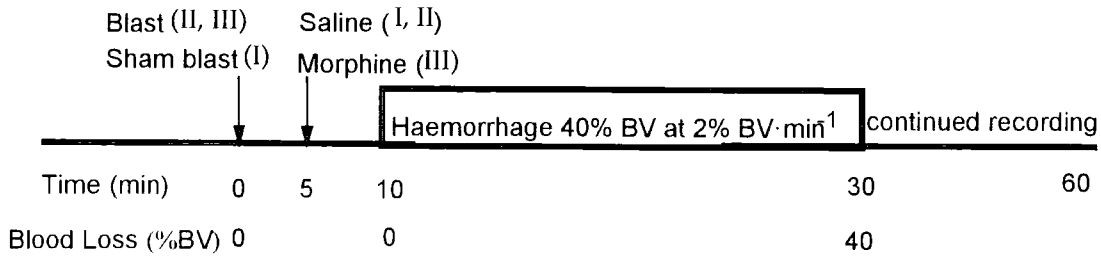
3.2.1 *Experimental protocol*

Following the surgical preparation, isoflurane (Abbott Laboratories Ltd., UK) was discontinued and anaesthesia maintained with alphadolone/alphaxalone (Saffan, Pitman-Moore, UK, 19-21mg.kg^{-1}.h^{-1} iv) using an infusion pump (Harvard 22^{TM}, Harvard Apparatus Ltd., UK) while the animals breathed air. The infusion rate of anaesthetic was adjusted to maintain a level of anaesthesia whereby a noxious pinch to the foot caused a mild withdrawal and a pressor response of approximately 10 mmHg. Rats were allowed to stabilise for 60 minutes positioned supine in the blast apparatus. Those subjected to blast were positioned with the ventral thorax 3.5cm below the blast nozzle (which delivers the blast wave to the animal, see Chapter 2, section 2.2) and baseline measurements of heart period, blood pressure and blood flow were made. The protocol shown in Figure 3.1 was then followed. A pressure of 1500psi was generated in the blast apparatus and animals in the blast groups received a single discharge from the apparatus to the ventral thorax. Animals were then randomly allocated to one of three groups:

- Group I (n=8) sham blast, haemorrhage and 0.9% saline (1mL.kg^{-1}) given intravenously 5 minutes after sham blast (5 minutes before the start of haemorrhage)
- Group II (n=8) blast, haemorrhage and 0.9% saline (1mL.kg^{-1}) given intravenously 5 minutes after blast (5 minutes before the start of haemorrhage)
- Group III (n=5) blast, haemorrhage and morphine (1mL.kg^{-1} 0.5mg.mL^{-1}) given intravenously 5 minutes after blast (5 minutes before the start of haemorrhage).

The cardiovascular measurements were made continuously from immediately prior to blast/sham blast, until 5 minutes after blast/sham blast whilst duration of apnoea was determined visually and recorded using a stopwatch.

Protocol



Group	Drug	Blast Injury	
I	Saline (1ml.kg^{-1}, i.v.)	-	n=8
II	Saline (1ml.kg^{-1}, i.v.)	+	n=8
III	Morphine (1ml.kg^{-1} 0.5mg.ml^{-1} saline, i.v.)	+	n=5

Figure 3.1 Diagrammatic representation of the protocol followed in this study (see section 3.1 for full explanation). Plus sign (+) indicates presence of haemorrhage and blast injury in that group.

Five minutes after blast (or sham blast) animals received either 0.9% saline (Groups I and II) or morphine (Group III) intravenously. Five minutes later cardiovascular measurements were repeated in all groups. Arterial blood was then withdrawn anaerobically into heparinised syringes from the ventral tail artery in all animals. The blood was withdrawn in 12 equal aliquots each over a 100 second cycle consisting of a 70 second withdrawal period and a 30 second recording period, giving an overall haemorrhage rate of 2% blood volume/minute which resulted in a loss of 40% of the total blood volume (BV; 6.06 mg.100g^{-1} body weight; Elebute & Little, 1978).

Cardiovascular measurements were repeated after the withdrawal of each aliquot of blood, and each blood sample was subjected to blood gas analysis (ABL5^{TM}, Radiometer, Denmark). Cardiovascular measurements were continued at 5 minute intervals for the 20 minutes following the end of haemorrhage. A final arterial blood sample was then withdrawn anaerobically for blood gas analysis and the animals were then killed with an overdose of 0.5mL of 60mg.mL^{-1} (89-113mg.kg^{-1}) sodium pentobarbitone (Sagatal, Rhône Mérieux (Ireland) Tallaght, Dublin) administered intravenously.

3.3 Results

There were no significant differences in the baseline (pre-blast or pre-sham blast) heart period or mean arterial blood pressure between any of the groups (See Table 3.1).

	Group I	Group II	Group III
n	8	8	5
Body wt (g)	306.9±2.9	316.9±8.6	319.2±12.3
HP (ms)	139.1±4.7	149.1±5.1	155.2±8.4
MBP (mmHg)	111.0±4.3	105.4±5.1	108.5±8.4
Hcrit (%)	40.7±0.6	38.7±0.8	40.0±0.5
Temp (oC)	37.9±0.1	37.5±0.2	37.6±0.2

Table 3.1 Baseline values for heart period (HP), mean blood pressure (MBP), haematocrit (Hcrit), body temperature (Temp) and body weight (Body wt). *n* denotes number of animals in group. Data presented are means±S.E.M.

3.3.1 *Effects of thoracic blast*

Sham blast produced no significant changes in heart period or mean arterial blood pressure in Group I (Figure 3.2). Thoracic blast (Group II) produced a significant increase of 317±28 ms in heart period from a pre-blast control of 149±5 ms and a significant fall in mean arterial blood pressure of 71.8±7.5 mmHg from a pre-blast level of 105.4±5.1 mmHg (Figure 3.2). Thereafter there was a partial recovery of heart period and mean arterial blood pressure although the animals were bradycardic and hypotensive for the subsequent 10 min after blast: in Group II heart period was significantly above and mean arterial blood pressure significantly below the corresponding levels seen in Group I for 10 min after blast. Blast in Group III produced effects on heart period and mean arterial blood pressure similar to those seen in Group II; there were no significant differences between these two groups for the first 10 min after blast (Figure 3.2).

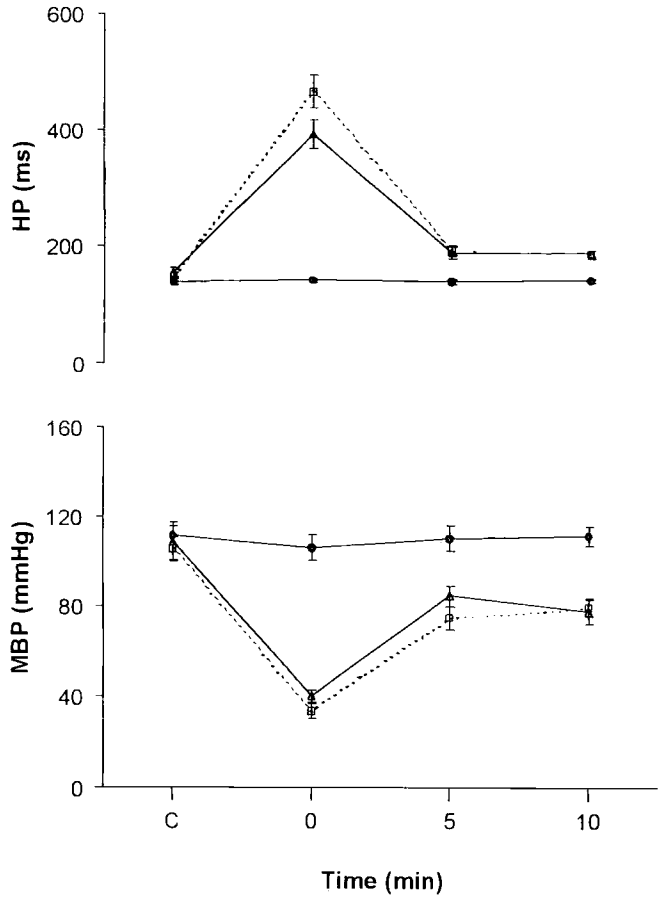


Figure 3.2 Effects of a thoracic blast injury/sham blast in anaesthetised rats on heart period (HP) and mean arterial blood pressure (MBP). Group I; saline, sham blast (●), Group II; saline and blast (□) and Group III; morphine and blast (Δ). Data recorded immediately before blast (C), and thereafter immediately (0) and at 5 and 10 minutes after blast. Values are means±S.E.M.

Sham blast (Group I) produced no change in femoral vascular resistance or blood flow (Figure 3.3 n=4). Immediately after thoracic blast (Groups II and III) there was a fall in vascular resistance, followed by a recovery. Consequently blood flow was maintained in the two groups despite the blast-induced hypotension (Figure 3.3; n=4 & 3 respectively, preliminary study conducted on a sub-set of animals in respective groups). No statistical analysis was performed on this data due to the small number of animals where it was possible to record femoral blood flow during blast.

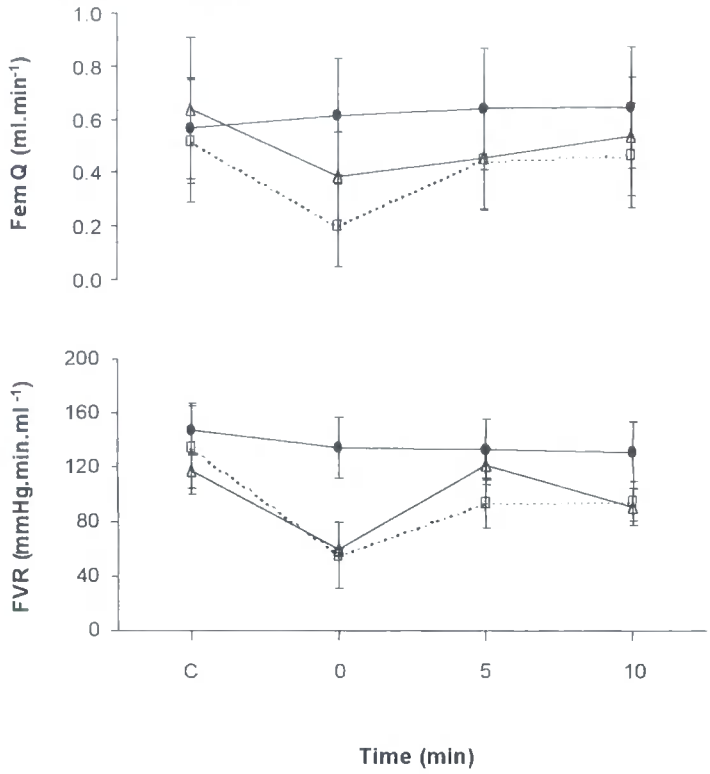


Figure 3.3 Effects of a thoracic blast injury in anaesthetised rats on femoral vascular resistance (FVR) and femoral arterial blood flow (Fem Q). Group I; saline, sham blast (●), Group II; saline and blast (□) and Group III; morphine and blast (Δ). Data recorded immediately before blast (C), and thereafter immediately (0) and at 5 and 10 minutes after blast. Values are means±S.E.M.

Thoracic blast produced an apnoea of duration 19.0±2.0 s and 16.5±1.7 s respectively in Groups II and III; there was no significant difference (Student's independent *t* test) in the duration of apnoea between these two groups (Figure 3.4), while sham blast did not produce apnoea.

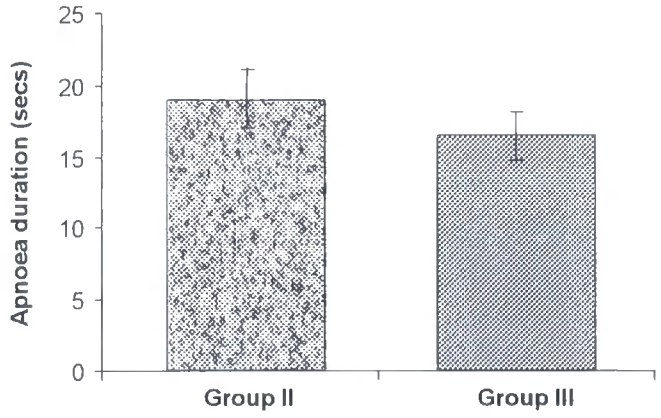


Figure 3.4 Apnoea duration following thoracic blast injury in anaesthetised rats in Group II; saline and blast, and group III; morphine and blast. Values are means±S.E.M.

3.3.2 *Effects of progressive haemorrhage*

Haemorrhage (all Groups) produced a significant change in heart period (Figure 3.5). Heart period increased significantly above pre-haemorrhage control (Groups I & II;

Figure 3.5). Different animals reached their peak tachycardia and bradycardia at different amounts of blood loss, consequently the graph shown in Figure 3.5 underestimates the peak tachycardia and peak bradycardia in each group. However, as the individual tachycardias in each animal are smaller than the individual bradycardias, the overall mean tachycardia is not so clear. The following section will therefore compare the peak changes in heart period corresponding to the tachycardia and bradycardia from each individual animal.

In Group I a progressive haemorrhage produced a biphasic response (Figure 3.5). There was an initial tachycardia: heart period initially decreased in all animals in this group with the maximum changes being seen after blood losses in the range 3.3-20.0% blood volume (BV) in different individuals, yielding a significant (Student's paired *t* test) reduction in heart period of 10±3 ms from a pre-haemorrhage level of 138±5 ms after the loss of 12.5±2.2 %BV. Thereafter there was a bradycardia in all animals, the peak increase being seen in the range 26.6-40.0 %BV loss giving a significant (Student's paired *t* test) maximum increase in heart period of 51±6 ms above pre-haemorrhage control after the loss of 32.1±1.6 %BV. Mean arterial blood pressure was initially maintained at a pre-haemorrhage level of 110.8±3.3 mmHg before falling progressively, the hypotension attaining statistical significance (compared to pre-haemorrhage control) after the loss of 13.3 %BV (Figure 3.5).

In Group II pre-haemorrhage heart period was significantly higher and mean arterial blood pressure significantly lower than the corresponding values in Group I because of the effects of blast in the former. The pattern of response to haemorrhage was significantly different in Group II compared to Group I (Figure 3.5; ANOVA). The first compensatory phase of the response to blood loss was absent in Group II. Examination of peak changes from each individual shows that there was no significant tachycardia in Group II while the bradycardia (significant peak increase in heart period of 30.6±8.1 ms; Student's paired *t* test) was seen after the loss of 25.5±0.8 %BV. Although there was no significant difference in the peak increase in heart period induced by severe haemorrhage between Groups I and II, the peak bradycardia in Group II was attained at a significantly lower (Student's independent *t* test) volume of blood loss when compared to Group I. Furthermore, in Group II mean arterial blood pressure was not maintained during the haemorrhage and started to fall after the first aliquot of blood had been removed (Figure

3.5), the hypotension achieving statistical significance (compared to pre-haemorrhage control) after the loss of 10% BV.

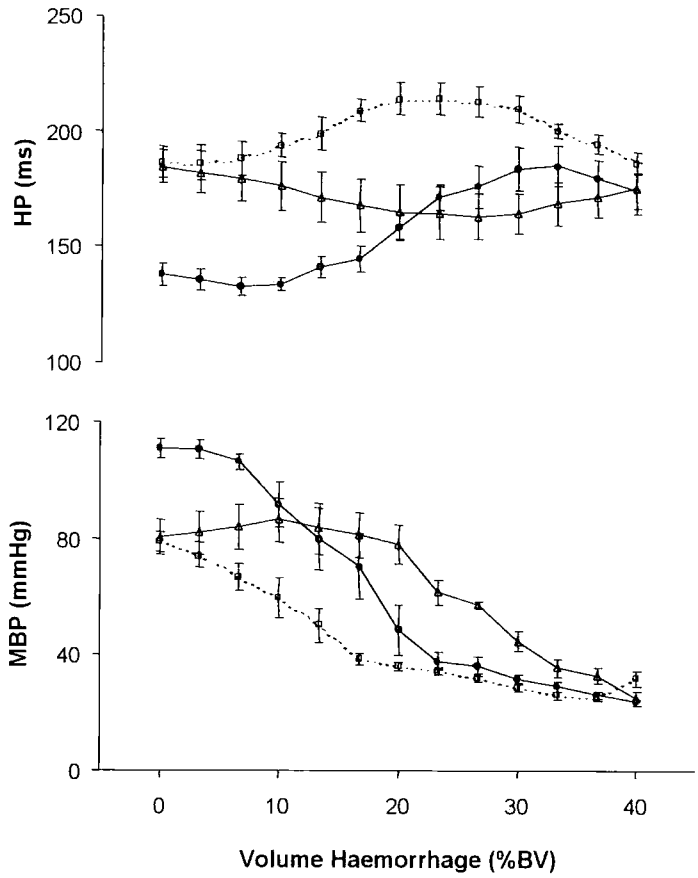


Figure 3.5 Effects of a progressive haemorrhage following thoracic blast injury (or sham blast) in anaesthetised rats on heart period (HP) and mean arterial blood pressure (MBP). Group I; saline, sham blast (●), Group II; saline and blast (□) and Group III; morphine and blast (Δ). Values are means±S.E.M.

There were no significant differences in pre-haemorrhage heart period or mean arterial blood pressure in the morphine-treated animals of Group III when compared to Group II (Figure 3.5). However, the pattern of response to haemorrhage was significantly different in Group III. Examination of the peak changes from each individual revealed that the animals of Group III exhibited a significant tachycardia with a maximum decrease in heart period of 24±6 ms (Student's paired *t* test) being seen after a loss of 25.9±2.6 %BV, while there was no significant bradycardia in this group. In addition mean arterial blood pressure was maintained until the loss of 20 %BV, thereafter falling with mean arterial blood pressure becoming significantly below pre-haemorrhage control after the loss of 33.0 %BV. Consequently mean arterial blood pressure was significantly higher in Group III compared to Group II between 13.3 and 35.8 %BV loss (Figure 3.5).

Thus, progressive haemorrhage in the absence of blast produced a biphasic response of tachycardia followed by bradycardia with mean arterial blood pressure being maintained

initially before falling. Following blast the initial compensatory phase of the response to blood loss was abolished: the tachycardia was absent while mean arterial blood pressure fell as soon as haemorrhage commenced. Although it is impossible to compare the absolute values between Groups I and II due to the different pre-haemorrhage baselines the bradycardic, hypotensive response to haemorrhage is seen after significantly smaller blood losses in Group II. Finally, pre-treatment with morphine before the haemorrhage abolished the bradycardia induced by haemorrhage and led to longer maintenance of mean arterial blood pressure.

Although the sample sizes are too small to apply statistical analysis to the haemodynamic data (n=3-4), preliminary results appear to show no difference in femoral blood flow between all groups with flow falling throughout the haemorrhage (Figure 3.6). Although there appears to be no difference in the pre-haemorrhage vascular resistance the subsequent response to haemorrhage appears to differ between groups. In Group I FVR appears to be maintained constant throughout the haemorrhage whilst after blast Group II appears to show a vasoconstriction after the loss of approximately 15%BV. However, after the loss of approximately 20% BV Group III animals treated with blast and morphine appear to show a vasodilation.

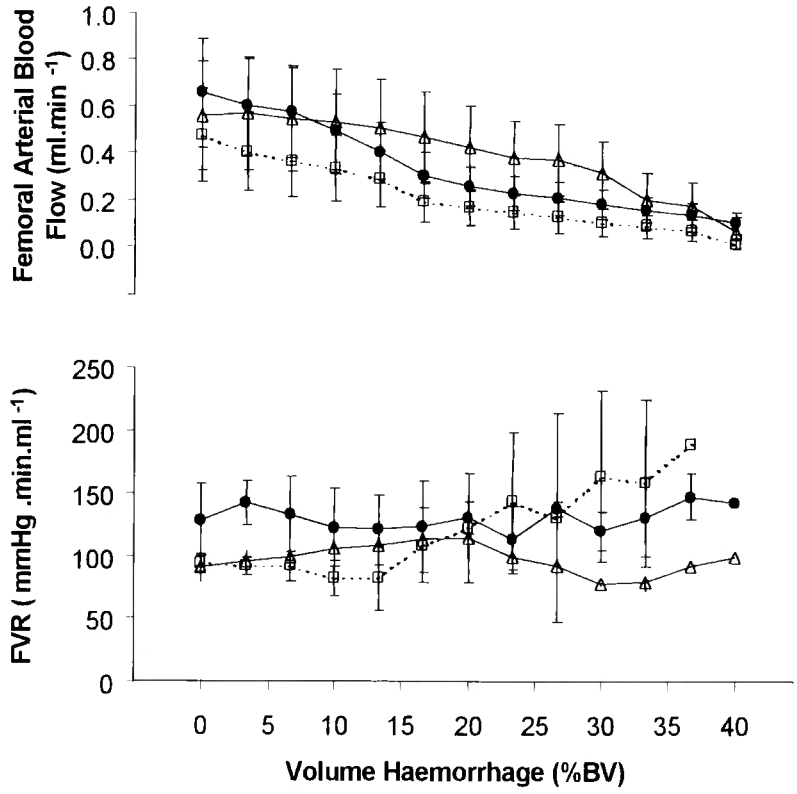


Figure 3.6 Effects of a progressive haemorrhage following thoracic blast injury (or sham blast) in anaesthetised rats on femoral arterial blood flow (Fem Q) and femoral arterial vascular resistance. Group I; saline, sham blast (●), Group II; saline and blast (□) and Group III; morphine and blast (Δ). Values are means±S.E.M.

3.3.3 *Effects of haemorrhage on arterial blood gases*

Ten minutes after thoracic blast (Group II) arterial oxygen tension (PaO_2) and arterial pH was significantly below those seen in the sham blast treated animals of Group I (Figure 3.7). Administration of morphine after blast (Group III) lead to a further significant decrease in PaO_2 and arterial pH, and a significant increase in arterial carbon dioxide tension ($PaCO_2$; Figure 3.7). Progressive haemorrhage led to a significant increase in PaO_2 and a fall in $PaCO_2$ and arterial pH in all groups (Figure 3.7). There was no significant difference in the pattern of response between the groups. However, there was a significant difference in the absolute levels of PaO_2, $PaCO_2$ and arterial pH between groups with Group I displaying the highest PaO_2 and arterial pH and the lowest $PaCO_2$ while Group III displayed the lowest PaO_2 and arterial pH and the highest $PaCO_2$ (Figure 3.7).

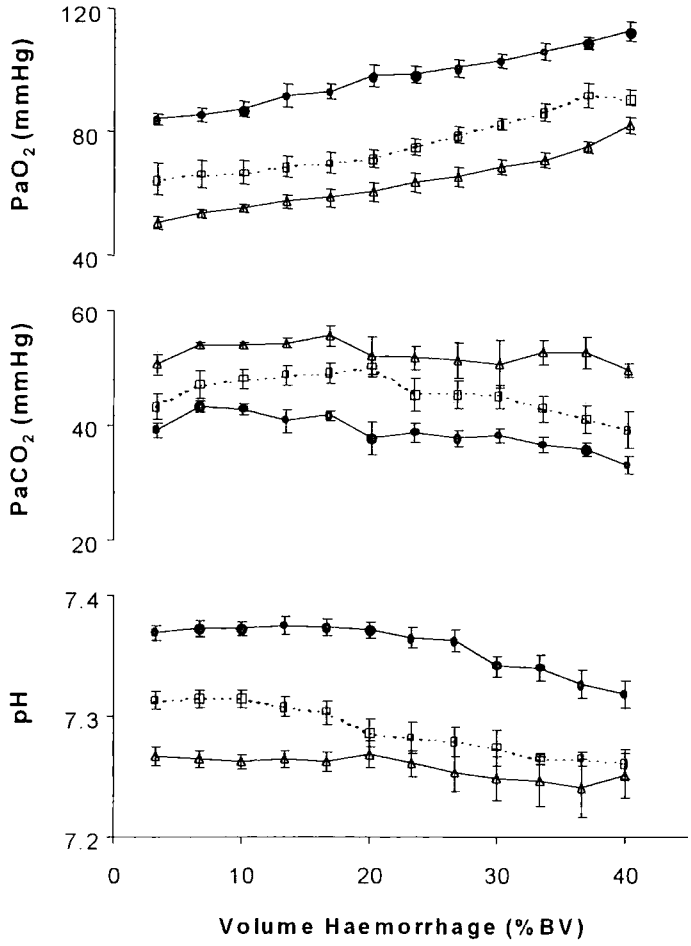


Figure 3.7 Effects of a progressive haemorrhage of 40% total blood volume following thoracic blast injury (or sham blast) in anaesthetised rats on arterial oxygen tension (PaO_2), arterial carbon dioxide tension ($PaCO_2$) and arterial pH. Group I; saline, sham blast (●), Group II; saline and blast (□) and Group III; morphine and blast (Δ). Values are means±S.E.M.

There was a significant fall in arterial base excess during haemorrhage in Groups I and II, with values in the animals given sham blast (Group I) being significantly higher during

the early stages of haemorrhage than those seen in animals given haemorrhage after blast (Group II, Figure 3.8). Values of arterial base excess in the morphine-treated Group III were significantly lower than Groups I and II until 20% BV loss. However in Group III there was no significant fall in base excess associated with blood loss (Figure 3.8).

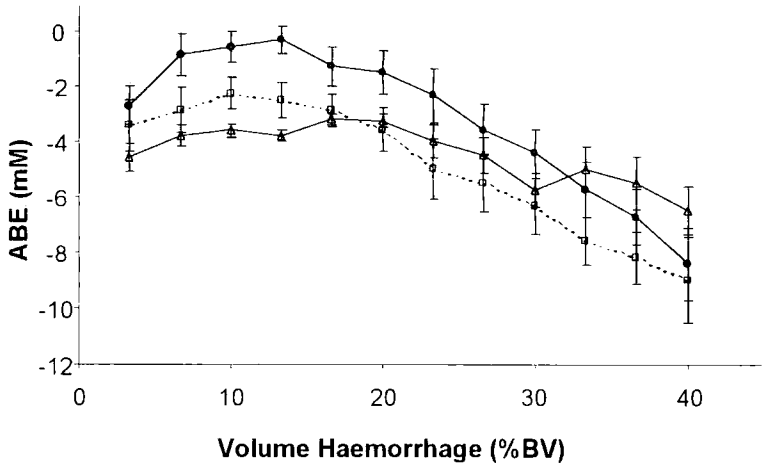


Figure 3.8 Effects of a progressive haemorrhage of 40% total blood volume following thoracic blast injury in anaesthetised rats on arterial base excess (ABE). Group I; saline, sham blast (●), Group II; saline and blast (□) and Group III; morphine and blast (Δ). Values are means±S.E.M.

3.3.4 *Post haemorrhage phase*

There were no differences in heart period or mean blood pressure between groups after haemorrhage. Furthermore, there were no significant changes in either parameter for the subsequent 20 minutes until the end of the study (Figure 3.9). Femoral arterial blood flow also appears to show no difference between groups or over time for the same period (Figure 3.10) although due to the small sample size no statistical analyses was performed on the haemodynamic results.

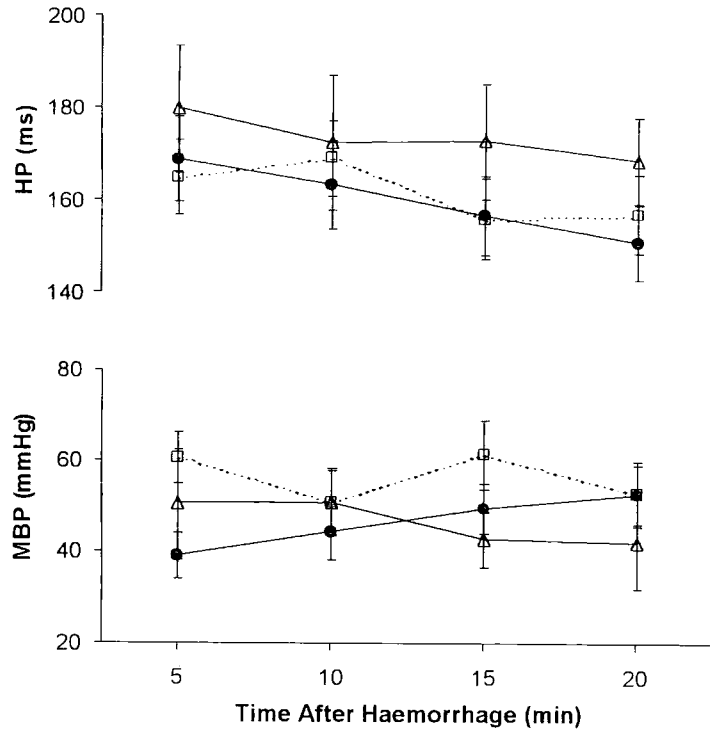


Figure 3.9 Heart period (HP) and mean arterial blood pressure (MBP) following thoracic blast (or sham blast) and a progressive haemorrhage of 40% total blood volume in anaesthetised rats. Group I; saline, sham blast (●), Group II; saline and blast (□) and Group III; morphine and blast. Data recorded at 5, 10, 15 and 20 minutes after haemorrhage. Values are means±S.E.M.

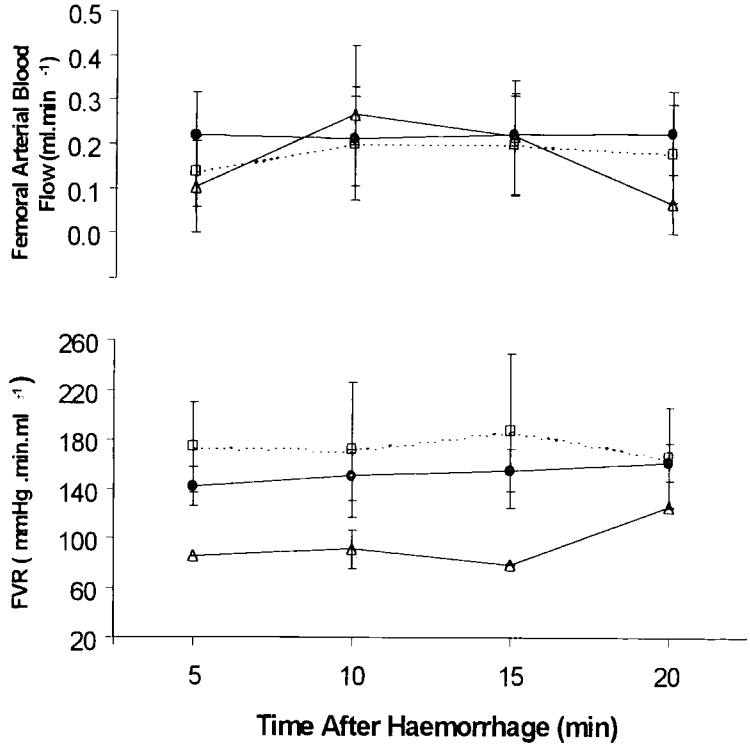


Figure 3.10 Femoral arterial blood flow and femoral arterial vascular resistance (FVR) following thoracic blast (or sham blast) and a progressive haemorrhage of 40% total blood volume in anaesthetised rats. Group I; saline, sham blast (●), Group II; saline and blast (□) and Group III; morphine and blast. Data recorded at 5, 10, 15 and 20 minutes after haemorrhage. Values are means±S.E.M.

There was no significant difference in PaO_2, $PaCO_2$, pH or ABE 20 minutes after the end of haemorrhage (Table 3.2).

Group	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH	ABE (mM)
I	99.3±1.4	32.6±0.6	7.26±0.05	-8.6±1.3
II	95.0±2.4	33.5±1.0	7.29±0.00	-10.0±0.6
III	93.3±6.2	39.3±3.8	7.25±0.02	-10.0±2.5

Table 3.2 Arterial blood gas results 20 minutes after the end of haemorrhage. Group I; sham blast, saline & haemorrhage, Group II; blast, saline & haemorrhage, Group III; blast, morphine & haemorrhage. PaO_2; arterial oxygen tension, $PaCO_2$; arterial carbon dioxide tension, pH; arterial pH, ABE; arterial base excess.

3.4 Discussion

Results from this study demonstrate that blast injury augments the bradycardic, hypotensive response to haemorrhage, and that morphine administered following blast injury, can attenuate this effect. Administration of morphine after blast does not affect the blast-induced bradycardia, consistent with earlier studies (Ohnishi *et al.* 1999b), or hypotension (which could not be predicted since pre-treatment with morphine delayed the recovery of blood pressure after thoracic blast; Ohnishi *et al.* 1999b). So it appears that morphine delays the recovery of the hypotension due to blast but once this response had been initiated, morphine had no effect.

The present study indicates that there is a facilitatory interaction between the reflexes responsible for the response to blast injury and severe haemorrhage. This is in marked contrast to the effects of musculo-skeletal injury on the response to blood loss where tissue injury attenuates the hypotension and bradycardia induced by severe haemorrhage (Little *et al.* 1989).

The results from this study suggest that prior exposure to thoracic blast augments the hypotensive, bradycardic second phase of the response to blood loss such that the tachycardic compensatory first phase is essentially absent and the peak bradycardia

occurs at significantly lower blood losses than those seen in the absence blast. There are a number of possible reasons for this effect of blast. Firstly, the response to blast may attenuate the baroreflex, which is responsible for the first phase of the response to haemorrhage, or secondly, the response to blast may augment the 'depressor' reflex responsible for the bradycardia and hypotension associated with the second phase of the response to haemorrhage. The first possibility would seem feasible since after a thoracic blast injury there is a bradycardia associated with hypotension, rather than a tachycardia, which would be expected were the sensitivity of the baroreflex normal. However, the effects of morphine in our study are not consistent with this explanation. In the present study morphine modified the response to haemorrhage after blast so that the compensatory phase was seen, including initial maintenance of arterial blood pressure and tachycardia, while the bradycardia associated with severe haemorrhage was abolished. It is unlikely that morphine achieved this effect by increasing the sensitivity of the baroreflex since it is known that μ opioid receptor agonists, in the absence of blast, themselves reduce baroreflex sensitivity (Gordon, 1990; Hamra *et al.* 1999; Eltraifi *et al.* 1988, 1989). The alternative explanation, namely that the response to blast augmented the 'depressor' reflex(es) initiated by severe haemorrhage, is consistent with the effects of morphine seen in the present study since it has previously been shown that morphine attenuates the depressor reflex associated with severe haemorrhage (Ohnishi *et al.* 1997, Evans & Ludbrook, 1990; Evans *et al.* 1989). Therefore, the most likely explanation based upon the current data, is that the response to blast augmented the depressor reflex associated with severe haemorrhage, which then overcame the baroreflex leading to an early fall in blood pressure and bradycardia. When this depressor reflex was blocked by morphine the baroreflex-mediated compensatory phase 1 was again uncovered. Another alternative may be that the "depressor" phase of the response to haemorrhage shares the same (unknown) afferent pathway of the response to thoracic blast injury and thus is potentiated by the effects of blast. Hooker reported in 1924 an active loss of venous tone with subsequent venous engorgement and pooling of blood within the abdominal veins after blast injury in experimental animals. Within 2 hours this engorgement was replaced by a decrease in size of the veins and this was thought to be as a consequence of transudation of blood plasma. It is possible therefore that venous return is reduced after blast due to increased venous capacitance and consequently a fall in effective circulating blood volume may cause the second phase of haemorrhage to be initiated sooner. If this were the case in this study, the reduction in venous return would far more likely be due

to venous pooling than a reduction in plasma volume due to the timescale.

The present study also raises an interesting question regarding the precise nature of the baroreflex modulation following blast. It is clear that the baroreflex is modulated after blast since blast induced hypotension is associated with bradycardia (or at least no change in heart rate) rather than the tachycardia which would be expected were the baroreflex functioning normally. This effect could be mediated by a change in set point as well as an alteration in sensitivity and so the effects of blast on the baroreflex need to be addressed specifically. There is clear clinical significance to this question since patients with reduced baroreflex sensitivity may display greater transient falls in blood pressure with any haemorrhage, a feature which is not addressed in the current study but may occur even in the presence of morphine.

However, baroreflex sensitivity may not be reduced after a blast injury, indeed after a severe haemorrhage when both blood pressure and heart rate have fallen it has been shown that baroreflex sensitivity is actually increased (Little *et al.* 1984). The blast itself may be initiating a reflex, which overrides the baroreceptor reflex. Ten minutes later at the start of haemorrhage this effect may still be ongoing and so the compensatory reflex response to haemorrhage is masked, i.e. the first phase is absent.

Further studies involving the phenylephrine pressor test (Jones *et al.* 1989) performed after thoracic blast exposure might aid in determining any alterations in baroreflex sensitivity.

Assuming baroreflex sensitivity were reduced by a blast injury, then a musculo-skeletal injury superimposed on blast may well reduce baroreflex sensitivity further, as it is known that baroreflex sensitivity is reduced by musculo-skeletal tissue injury (Redfern *et al.* 1984). Administration of morphine to a patient in this state may augment the effect further as morphine is also known to reduce baroreflex sensitivity after musculo-skeletal tissue injury (Wyatt *et al.* 1995). This may have serious clinical implications as anything a clinician may do to a patient with reduced baroreflex sensitivity that may result in any amount of blood loss, will result in greater falls in blood pressure in that patient.

The change in arterial blood gases after blast and subsequent haemorrhage are consistent

with previous studies. Animals subjected to thoracic blast displayed a lower PaO_2 and higher $PaCO_2$ when compared to those given sham blast, consistent with impaired pulmonary gas transport after blast (Guy *et al.* 1998). A further reduction in PaO_2 and elevation in $PaCO_2$ in morphine treated animals is consistent with the respiratory depressant effects of morphine (Houmes *et al.* 1992). Subsequent elevation of PaO_2 and falls in $PaCO_2$ during haemorrhage in each of the groups is consistent with a haemorrhage-induced increase in ventilation suggested to be due to arterial chemoreceptor reflex activation as a consequence of reduced blood flow to the chemoreceptors (Acker & O'Regan, 1981; Potter & McCloskey, 1987). However, these changes in respiratory activity and arterial blood gases are unlikely to account for the effects of morphine on the cardiovascular response to severe haemorrhage. Indeed, any respiratory depression would be predicted to enhance, rather than reverse, the bradycardia associated with severe haemorrhage (Daly & Kirkman, 1988; Daly *et al.* 1988; Blake *et al.* 1994). The fall in arterial pH and base excess in Groups I and II is consistent with the development of a metabolic acidosis, possibly due to a failure of oxygen delivery to metabolically-active tissues. However, they appear to be maintained in Group III. This may be due to a greater reduction in blood flow to the splanchnic bed severe enough that the metabolites simply aren't being washed out.

It is impossible from these studies to determine the site of action of morphine in attenuating the depressor response to severe haemorrhage. A number of central nervous loci are known to show increased activity during severe haemorrhage. These areas include the ventrolateral periaqueductal grey and the rostral ventrolateral medulla (see Evans *et al.* 2001). It is known that the endogenous opioid system participates in the depressor response to severe haemorrhage; in the rat this is predominantly via activation of δ_1 opioid receptors in the periaqueductal grey (Cavun *et al.* 2001) and δ_1 and μ opioid receptors in the spinal cord (Ang *et al.* 1999). The picture is complex because other studies have shown that activation of μ opioid receptors can also *attenuate* the depressor response to haemorrhage (Evans *et al.* 1989; Evans & Ludbrook, 1990, 1991; Ohnishi *et al.* 1997). However, it is unlikely that morphine is acting within the spinal cord to block the depressor response to severe haemorrhage since it is blockade, rather than activation, of μ receptors at this site which attenuates the depressor response to blood loss (Ang *et al.* 1999). Potential sites of action for morphine include the nucleus tractus solitarius, an afferent nucleus for a number of cardiovascular reflexes, the rostral ventrolateral medulla

and the nucleus ambiguus (Evans *et al.* 1989; Evans & Ludbrook, 1990, 1991). Since morphine attenuated both the bradycardia and hypotension during severe haemorrhage it is possible that it is acting early in the reflex pathway, before the sympathetic and vagal limbs diverge.

3.4.1 *Haemodynamic changes following thoracic blast injury and subsequent haemorrhage*

The preliminary results of the haemodynamic response to thoracic blast injury appear to be a decrease in vascular resistance in the femoral vascular bed. This may be the result of a decrease in sympathetic outflow to the vasculature. However, this finding is contrary to that reported in the literature. Irwin and colleagues and Dodd *et al.* reported in 1997 that during thoracic blast injury alone i.e., without the added insult of a haemorrhage, 'systemic vascular resistance index remained unchanged' (Irwin *et al.* 1997; Dodd *et al.* 1997). However, in the current study vascular resistance was calculated using blood flow measurements taken in the femoral bed, whereas Irwin and colleagues (1997) and Dodd *et al.* (1997) calculated vascular resistance using cardiac index. Perhaps then *total* peripheral resistance remains unchanged during the response to blast, thus there must be intense vasoconstriction in other vascular beds whilst there may be a vasodilatation in the femoral vascular bed.

A recent study reported an increase in total peripheral resistance at the first recording 30 minutes after a blast injury in the pig (Harban *et al.* 2001). If total peripheral resistance is *increased* after blast but vascular resistance is low in the skeletal muscle then, again, it follows that there must be an overall bigger increase in resistance in other vascular beds. Were this to occur in the vital organs it would result in a marked reduction in blood flow to these metabolically active organs. This may have serious clinical implications as a low blood flow to the gut may lead to a breakdown of the mucosal barrier and thus bacterial translocation (Mackway-Jones *et al.* 1999), this may lead to a systemic inflammatory response and possibly multiple organ failure.

At the start of haemorrhage there is no evidence showing a difference in femoral vascular resistance (FVR) between the blast, haemorrhage and saline group (Group II) and the blast, haemorrhage and morphine group (Group III). However, as the haemorrhage

becomes severe FVR in Group III falls below that in Group II (Figure 3.6), but there appears to be no difference in flow as pressure is initially higher in the morphine group. At the end of the 40% haemorrhage there is no difference in mean blood pressure and femoral arterial blood flow between any of the groups, but there appears to be a vasodilation in the femoral vascular bed in the morphine treated animals (Figure 3.6; however, this is preliminary data with a small sample size). Evans and colleagues showed in 1990 that no vasodilation occurred during phase 2 of the response to haemorrhage after administration of morphine. However, in Evans' experiments flow was measured in the ascending aorta and therefore TPR was calculated. So again, if there is a vasodilation in the femoral vascular bed after a morphine-treated blast and subsequent haemorrhage, and no change in total peripheral resistance (TPR; Evans & Ludbrook, 1990) then it follows that there must be a concomitant vasoconstriction elsewhere, e.g., the splanchnic organs.

In the 20 minutes following the end of haemorrhage there is no difference in femoral blood flow, mean blood pressure or heart period between any of the groups, and no difference in femoral vascular resistance between Groups I and II. However, preliminary data does appear to show a persistence of the vasodilation in Group III, the morphine treated group, for at least the first 15 minutes after the end of haemorrhage.

Further studies measuring flow to the gut or kidney as well to the femoral vascular bed might aid in determining the full haemodynamic response to blast and haemorrhage. Due to the manner in which the blast is delivered it would not be feasible to measure blood flow in the splanchnic organs using the ultrasonic flow probe used in this study. The blast wave may dislodge and damage the probe, and this may cause damage to the blood vessel in which blood flow is being measured. However, flow measurements could be carried out using fluorescent microsphere beads (Schimmel *et al.* 2001). The beads would be injected at the appropriate time when a blood flow measurement is required and this would provide a histological 'snap shot' of blood flow in several different organs at the same moment in time. A disadvantage to this technique is that it does not provide a continuous measurement. However, the use of different coloured beads at different times throughout the course of the experiment would provide a set of serial measurements.

In conclusion, this study indicates that thoracic blast modifies the response to progressive haemorrhage such that the compensatory first phase of the response to haemorrhage is lost and the hypotensive, bradycardic second phase is augmented and occurs after smaller blood losses. This effect is prevented by morphine. In addition, thoracic blast also appears to cause a fall in femoral vascular resistance which increases during a subsequent progressive haemorrhage whilst morphine attenuates this increase, an effect which appears to persist for at least 15 minutes post haemorrhage. It is therefore possible that blast may modify the clinical signs of blood loss in a patient. Pre-haemorrhage treatment with morphine prevents this effect but care must be exercised before viewing this effect of morphine as being protective since morphine in the absence of blast increases mortality after haemorrhage despite allowing longer maintenance of blood pressure (Marshall, *et al.* 1998).

4a Pulmonary and Cardiovascular Effects of Resuscitation after Thoracic Blast and Haemorrhage: Comparison of Whole Blood, Isotonic Saline and Colloid with Hypertonic Saline/Dextran (Early Resuscitation)

4a.1 Introduction

In a recent study (Chapter 3; Kirkman *et al.* 2000a; Sawdon *et al.* 2002) it was shown that primary thoracic blast injury modifies significantly the cardiovascular response to progressive haemorrhage. The tachycardic phase I of the response to haemorrhage, where blood pressure is maintained by the baroreflex, is absent when the blood loss is associated with blast. While the reflex hypotensive bradycardic phase II of the response to haemorrhage is significantly augmented following blast. Since casualties who have suffered blood loss are likely to be given fluid resuscitation it is now important to determine the response to resuscitation following haemorrhage in animals which have also been subjected to blast injury. Therefore the present study aims to compare resuscitation of the blast injured hypovolaemic patient with various solutions (see below). Initially this will need to be carried out in a pure haemorrhage/resuscitation model, i.e., before the possibility of any secondary damage occurring to remote organs due to tissue hypoxia resulting from prolonged hypotension, and so the subject will be resuscitated early, after a 5 minute hypovolaemic "shock" period.

There are several reasons why the response to fluid resuscitation after blood loss may be different in blast-injured animals compared to those suffering haemorrhage in the absence of blast. Potential differences may include not only the cardiovascular response but also the presence and degree of pulmonary oedema (Guy *et al.* 1998) and resulting change in arterial blood gases.

Thoracic blast injury leads to the development of pulmonary oedema (Zuckerman, 1940; Clemedson, 1956; Cooper *et al.* 1983; Brown *et al.* 1993; Guy *et al.* 1998; see Chapter 1, section 1.4.3). This may have unrelenting consequences for the outcome of the blast injured patient (see section 4a.4.1.1, Table 4a.3 & Figure 4a.14). Oedema occurs as a

result of an alteration in the parameters that govern fluid exchange across a capillary wall, and so it is important to understand the discipline of fluid exchange in order to attempt to treat or prevent tissue oedema.

4a.1.1 *Transcapillary fluid exchange*

The endothelium consists of a continuous monolayer of endothelial cells lining the entire lumen of all the blood vessels within the body. Overlying the endothelium is the vascular smooth muscle, which together with the endothelium regulates vascular tone (see Burnstock & Ralevic, 1994). The endothelium, together with its basement membrane, also functions as a barrier in the capillaries and is the site of exchange for solutes/solvent between the blood and interstitial fluid (see Holliday, 1999), as well as providing a smooth surface, which inhibits widespread blood clotting.

The entire plasma volume circulates between the intravascular space, the interstitial space and the lymphatic system at least once a day (see Levick, 1991). This fluid turnover helps to regulate plasma volume (see Holliday, 1999). The rate of filtration of the plasma can be altered by the balance of four forces (Starling, 1896), which collectively are referred to as Starling forces.

4a.1.2 *Starling forces*

In 1896 Ernest Starling first described the basic rules of fluid exchange between the interstitial and intravascular compartments across the capillary endothelium. The movement of fluid across a capillary wall is governed by the sum of four forces acting upon that vessel; the hydrostatic pressure inside the capillary due to capillary blood pressure; P_c, the hydrostatic pressure of the interstitial fluid; P_i, the oncotic (osmotic) pressure due to plasma proteins; π_c, and the oncotic pressure due to proteins in the interstitial space; π_i (Starling, 1896). Hence the Starling equation can be written as:

(Filtration rate per unit area) $J_v/A = K_c [(P_c - P_i)-(\pi_c-\pi_i)]$

Where K_c is the capillary filtration coefficient, a measure of the permeability of the vessel (Michel, 1997; Nolan, 1999; Holbeck & Grände, 2000; see Levick, 1991). Capillaries with a low K_c value have 'tight' intercellular junctions between their endothelial cells. These continuous capillaries can be found in the lungs, connective tissue, muscle, fat and skin (see Levick, 1991). The brain has particularly tight junctions. In tissues where fluid transfer is a particular feature of that tissue such as the kidney and gut mucosa, capillaries are fenestrated, that is they have 50nm pores within the endothelium. These pores are highly permeable to water and hence these vessels have a high K_c value (see Levick, 1991; see Kirkman & Sawdon, 2001).

The capillary wall behaves as a semi-permeable membrane with respect to the larger protein molecules as it restricts their movement whilst allowing the passage of water (Nolan, 1999). Consequently, plasma proteins, chiefly albumin and to a lesser extent globulins, exert a sustained osmotic force called oncotic pressure. However, the capillary wall is rarely a perfect semi-permeable membrane and does allow a small leakage of these large protein molecules, which therefore escape into the extravascular space to exert an oncotic pressure within the interstitial fluid compartment. Exactly how much protein escapes depends on another proportionality coefficient, the reflection coefficient; σ (Nolan, 1999; see Kirkman & Sawdon, 2001; see Levick, 1991; Michel, 1997). This can be thought of as a measure of how much a particular solute is reflected by the capillary wall or how freely a particular solute can pass through the pore in the endothelium, and hence how much osmotic pressure that solute can exert. For example, if the solute radius is much bigger than the pore radius and none of the solute can pass through then σ has a value of 1 (as in the cerebral vasculature; Nolan, 1999) and the solute is completely effective osmotically. On the other hand if the pore radius is much bigger than the solute radius and all of the solute can pass through freely then σ has a value of 0 and the solute exerts no osmotic pressure (see Holbeck & Grände, 2000; Levick, 1991; Michel, 1997). Generally though most capillaries have a reflection coefficient between these two extremes (typical values for σ are 0.8 – 0.95) and so the solute exerts some degree of osmotic force within the capillary (Michel, 1997; see Kirkman & Sawdon, 2001).

This can now be entered into the Starling equation to correct for the effective osmotic pressure at the capillary wall

$J_v/A = K_c [(P_c - P_i) - \sigma(\pi_c - \pi_i)]$

If the sum of this equation is positive filtration of fluid out of the capillary occurs, if it is negative absorption occurs (Michel, 1997; see Kirkman & Sawdon, 2001).

4a.1.2.1 The magnitude of the forces

Capillary hydrostatic pressure is around 40 mmHg at the arterial end, whereas plasma oncotic pressure is around 25 mmHg (see Levick, 1991; Michel, 1997) favouring filtration at the arterial end. Interstitial fluid pressure is subatmospheric at 0 to -2 mmHg in most tissues (see Levick, 1991) while interstitial oncotic pressure is generally larger than this at about 10 mmHg (see Levick, 1991).

As fluid is lost from the capillary down its length, P_c diminishes and hence net filtration petres off towards the venous end (figure 4a.1, panel a; see Levick, 1991) but net absorption in this steady state is not reached as was traditionally believed, due to the high oncotic pressure of the interstitial fluid. In the event that capillary pressure is lower than usual at the arterial end such as in hypovoleamia or after arteriolar vasoconstriction, then absorption can occur at the venous end (see Levick, 1991). As capillary pressure decreases along the length of the vessel due to filtration, it reaches a point at which the opposing forces are greater and absorption occurs (see Figure 4a.1 panel b). However, this effect is transient as interstitial fluid pressure is now diminishing due to dehydration and hence oncotic pressure of the interstitium rises thus opposing the absorption and reverting back to a net filtration of fluid from the capillary (Figure 4a.1 panel c; see Levick, 1991)

Fluid absorption is thus a self-limiting process (except in tissues specialised for fluid absorption), as absorption raises interstitial oncotic pressure resulting in filtration (see Levick, 1991).

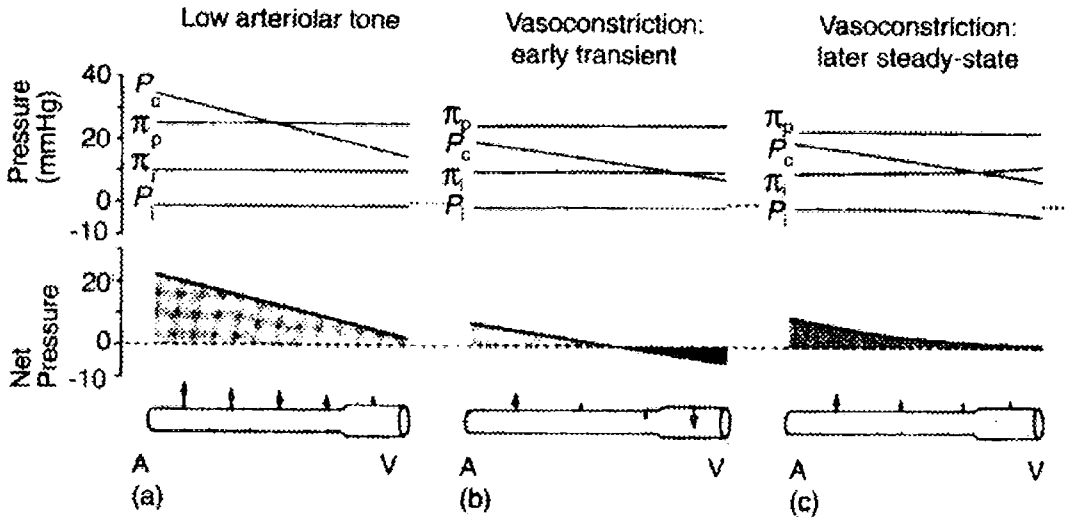


Figure 4a.1 *The effect of arteriolar constriction on fluid exchange.* From top: axial gradients of capillary hydrostatic pressure, P_c; plasma oncotic pressure, π_p (π_c); interstitial oncotic pressure, π_i; interstitial fluid pressure, P_i; and their sum, the net pressure (with correction of oncotic pressures for $\sigma = 0.9$). Sketch below shows direction of fluid exchange from arteriolar beginning of capillary (A) to its venular end (V). (a) Well perfused capillary. (b) Immediately after arteriolar vasoconstriction; transient absorptive state due to reduced capillary pressure. (c) Eventual steady state if vasoconstriction is maintained; downstream absorption abolished by rise in interstitial oncotic pressure and fall in interstitial hydraulic pressures. Source: Levick, 1991.

Despite mechanisms to maintain a steady state of fluid exchange across the capillary wall, (see Kirkman & Sawdon, 2001 and Levick, 1991 for review of Starling forces and buffering of filtration) there are certain pathological conditions (e.g., trauma) which can lead to circumstances of increased capillary permeability as a consequence of an inflammatory response. As a result intravascular proteins may leak into the interstitium causing a reduction in the forces opposing filtration of fluid out of the capillary and thus lead to tissue oedema, as the usual buffering mechanisms cannot cope. However, there are certain fluids available which reportedly attenuate the inflammatory response (Sun *et al.* 1999; Mazzoni, 1990; Akgur *et al.* 1999; Brown, 1990; Haljamae, 1985; Sheilds *et al.* 2000; Corso, 1999; Nolte, 1992) and so the type of fluid chosen for resuscitation may have important implications for the outcome of the patient.

4a.1.3 *Effects of blast injury*

Primary blast injury leads to shearing effects at air:water interfaces, e.g., in the lungs (see Chapter 1, section 1.4.2). It may be possible that this direct mechanical damage could lead to a rapid increase in capillary permeability, and it is known that blast injury leads to the development of pulmonary oedema, a reduction in PaO_2 and, if severe enough, an elevation in $PaCO_2$ (Guy *et al.* 1998). This hypoxaemia may result in tissue hypoxia, a known trigger for an inflammatory response (Combe *et al.* 1997; Michiels *et al.* 1996) with a longer duration of onset than the initial insult of the blast injury (Ohnishi *et al.* 2001; see also Table 4a.3). Within the lungs, a secondary inflammatory response could potentially augment an already established pulmonary oedema.

Other forms of blunt chest trauma can also lead to pulmonary oedema. Fluid resuscitation following blunt chest trauma, especially with crystalloid solutions, has been shown to increase the degree of pulmonary oedema and hypoxaemia (Fulton *et al.* 1973; Richardson *et al.* 1974; Tranbaugh *et al.* 1982). A recent study (Cohn *et al.* 1997) compared the effects of resuscitation with normal saline *vs* small volumes of hypertonic (7.5%) saline following haemorrhage and pulmonary contusion since resuscitation with hypertonic solutions can potentially reduce tissue oedema (see section 4a.4.2). However, in the case of localised pulmonary contusion no difference in the degree of oedema could be seen between normal and hypertonic saline-treated animals (Cohn *et al.* 1997).

As microvascular permeability may be increased, thus giving a low reflection coefficient, and an inflammatory response may be initiated after primary blast injury (see above), then this may imply that fluids may act differently to results obtained from fluid resuscitation studies in the absence of primary blast injury. Fluids reported to have good intravascular expansion properties may now leak out of the microvasculature.

The type of fluid chosen in the resuscitation of the trauma patient should reflect the needs of the patient at that time, and thus compounding factors need to be taken into account, such as the amount of fluid lost, underlying pre-existing medical conditions, and the extent and type of injuries to the patient. The crystalloid-colloid debate continues after many decades due to the lack of reliable evidence supporting one over the other (Nolan, 1999). Large volumes of crystalloids are required for adequate intravascular

volume expansion, however, this may be thought of as beneficial as some argue that the extravascular compartment also needs replenishing after hypovolaemia (Haljamäe *et al.* 1997). In contrast to crystalloids, it is with colloid solutions that anaphylactic reactions occur; though colloids have greater intravascular persistence. And so the debate continues. Resuscitation with whole blood may be the "Gold Standard" but the use of this fluid is not always practical, especially in military situations. A fluid that has been of increasing interest for pre-hospital resuscitation of the trauma victim is hypertonic saline/dextran (HSD, Vassar *et al.* 1993). This fluid is reported to be a potent volume expander and claims to markedly improve survival in the hypovolaemic patient. Indeed the more seriously injured the patient, the greater the reported benefit from HSD use (Wade *et al.* 1997a).

Below is an overview from the literature of each of the fluids examined in this study.

4a.1.4 *Whole blood*

Resuscitation with whole blood is preferable especially for haemorrhagic shock as it has significant oxygen carrying capacity (Nolan, 1999) and is reported to attenuate reperfusion injury, possibly by returning the increase in neutrophil activity seen after haemorrhage, back to baseline (Rhee *et al.* 1998) and attenuating apoptosis in the lungs (Subrato *et al.* 2000). In addition, no significant increase in the expression of the adhesion molecules that affect neutrophil-mediated reperfusion injury, E & P selectins, was reported after whole blood resuscitation (Alam *et al.* 2000). However, whole blood is expensive and in short supply, and is often not easily available especially in a military setting, as it requires special storage facilities and cross-matching (Nolan, 1999).

4a.1.5 *Crystalloid solutions*

Physiological isotonic saline (0.9%) is a commonly used inexpensive crystalloid solution. It contains no oncotic molecules and will therefore cross the vascular endothelium rapidly. Because of this property, large volumes are required for resuscitation of hypovolaemia and approximately 1.5 – 2 litres of crystalloid is needed for the

replacement of a 450mL blood loss (Nolan, 1999). Although this solution is well distributed across the extracellular compartment (interstitial space and intravascular space) and thus compensates for the disturbance in haemostasis due to the internal fluid shifts experienced after trauma (see Holliday, 1999; Haljamäe, 1985), the extra fluid accumulation in the interstitium contributes to tissue oedema, compromising capillary blood flow and hence oxygen delivery to the tissues (Schött *et al.* 1988). A recent study by Alam and colleagues in 2000 showed resuscitation with Lactated Ringers solution (LR), another commonly used isotonic crystalloid solution, resulted in the early up-regulation of adhesion molecules E & P selectins in the lung and spleen, leading to the development of pulmonary oedema (see section 4a.4.2). Other studies showed an increase in neutrophil activation after resuscitation with LR solution only, when compared to whole blood and hypertonic saline (Rhee *et al.* 1998) and an immediate up-regulation of Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1; Sun *et al.* 1999). This suggests that expression of pro-inflammatory mediators may be inhibited by some solutions (e.g., shed blood; Sun *et al.* 1999), whereas other solutions (e.g., crystalloids; Sun *et al.* 1999) confer no protection against their up-regulation.

4a.1.6 *Colloids*

The use of colloids in trauma resuscitation is advantageous given that they have a prolonged plasma volume support and are reported to improve microcirculatory blood flow (Kreimeier *et al.* 1995). These fluids maintain plasma oncotic pressure, and so the risk of tissue oedema is reduced because these relatively large molecules do not cross the endothelium easily. However, there is still a certain amount of debate as to which colloid to use in the resuscitation of the trauma victim. Gelatin solutions tend to be favoured clinically in the UK as an initial plasma volume expander (Nolan, 1999). This colloid has an average molecular weight of about 30 000 Daltons but contains a high proportion of lower molecular weight components (Nolan, 1999). Thus the plasma volume support of gelatins is limited as the low molecular weight components rapidly leak from the vascular compartment (Allison *et al.* 1999) and so within 1-2 hours of resuscitation the effect on plasma expansion is similar to that of crystalloid solutions (Lamke *et al.* 1976). This effect is reflected in a study comparing the effects of various fluids on capillary fluid

permeability in an isolated, autoperfused and denervated cat skeletal muscle. Gelatin was shown to increase the capillary filtration coefficient and thus induced trans-capillary filtration and tissue oedema during and after infusion (see Holbeck & Grände, 2000). However, this preparation is far from a more normal *in vivo* situation and so the results should be viewed with a certain degree of caution. The use of gelatins has been associated with a high incidence of anaphylactic reactions (Ring & Messmer, 1977). However, since this study the rate of occurrence of anaphylaxis has fallen due to a modification in the preparation of this colloid. Despite this, the use of gelatin solutions in the UK still remains high compared to the rest of the world (Nolan, 1999).

Another colloid used commonly in trauma resuscitation is hydroxyethyl starch solutions (HES). HES has been shown to reduce capillary leak (Allison *et al.* 1999) and reduce reperfusion injuries (Wisselink *et al.* 1998). This may be as a result of an inhibition of oxygen free radical formation (Nielsen *et al.* 1997) and a down regulation of pro-inflammatory mediators such as interleukin 6 (IL-6; Schmand *et al.* 1995). An increase in gas exchange 48 hours after resuscitation with HES in a human trauma study by Allison and colleagues in 1999 may be a reflection of a reduction in pulmonary oedema when compared to resuscitation with a gelatin solution.

HES is the least expensive colloid used for plasma volume expansion (Schmand *et al.* 1995) and is a bigger molecule than gelatin with an average molecular weight of 250 kDaltons (mean molecular weight of HES used in this study is 200kDa) and so does not pass through the plasma membrane easily, potentially giving better plasma volume support (Allison *et al.* 1999). HES is made of cross-linked glucose units, some of which are substituted with a hydroxyethyl subgroup. This allows for a longer half-life (and therefore better plasma volume support) in the body (Nolan, 1999). However, the more hydroxyethyl subunits that are substituted in the molecule, the less likely it is that the molecule will be broken down and this could lead to anaphylactic reactions in some patients (Nolan, 1999; degree of substitution of HES used in this study is 0.5).

4a.1.7 *Hypertonic saline/dextran*

Resuscitation with hypertonic solutions is advantageous particularly in a prehospital setting, as only small volumes are required. Indeed the infusion of 250mL of hypertonic (7.5%) saline in conjunction with the colloid dextran results in a similar plasma volume expansion to that achieved in resuscitation with 3 litres of a crystalloid solution (Hillman *et al.* 1997). Hypertonic saline/dextran (HSD) is reported to improve microcirculatory blood flow (Kreimeier *et al.* 1997) by reducing endothelial cell swelling (Mazzoni, 1990; Corso *et al.* 1998), and causing a vasodilation (Mazzoni *et al.* 1988), thus increasing the luminal diameter of the capillary. Other advantages from the use of HSD in trauma resuscitation include a reduction in reperfusion injuries by inhibiting leukocyte adhesion to the endothelium (Corso *et al.* 1999; Nolte, 1992). This would reduce free radical-induced injury to endothelial cells in both the systemic and pulmonary vasculature, which would otherwise result in endothelial cell contraction and colloid leak due to an increase in capillary permeability. Thus HSD has the potential to attenuate tissue oedema, which in the lungs could reduce the risk of developing Adult Respiratory Distress Syndrome (ARDS). Despite these beneficial reports of HSD the increase in blood pressure attained with HSD resuscitation after haemorrhagic shock, was transient, lasting only 10 minutes in one study with unanaesthetised rats (Chang & Varma, 1992) and, along with restoration of cardiac output in bled dogs, was poorly maintained in another study (Curtis & Cain, 1992).

It is now important to determine whether, and to what degree, resuscitation exacerbates pulmonary oedema after a combination of haemorrhage and the more generalised pulmonary injury induced by thoracic blast, and whether resuscitation with hypertonic saline/dextran may be beneficial in this model. In our study it will also be important to determine the cardiovascular and haemodynamic effects of resuscitation since there is evidence that resuscitation with normal saline after blast alone impairs cardiovascular performance (Wikoff *et al.* 1999). Additionally, some studies have suggested that resuscitation with hypertonic solutions may have a positive inotropic effect (Mouren *et al.* 1995) leading to a potentially beneficial effect of hypertonic resuscitation in blast casualties, which needs to be investigated. The study will assess the effects of resuscitation with various fluids in comparison to that with whole blood as whole blood is reported to reduce reperfusion injuries (Rhee *et al.* 1998; Subrato *et al.* 2000) whereas

resuscitation with crystalloids is reported to augment reperfusion injuries (Rhee *et al.* 1998; Sun *et al.* 1999).

The aim of the present study was to compare the cardiovascular effects of early resuscitation with different solutions, autologous blood, isotonic colloids (Haemaccel and hydroxyethyl starch), isotonic crystalloid (0.9% saline), and hypertonic saline/dextran following thoracic blast and a haemorrhage of 40% blood volume. In addition, the pulmonary effect was assessed by measuring *post mortem* lung weight ratios and arterial blood gases during the experiment.

4a.2 Methods

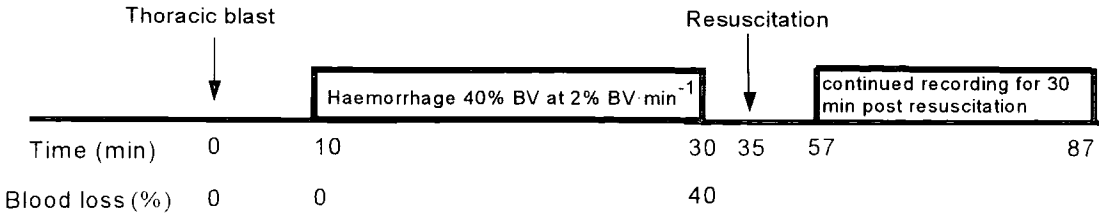
The study was conducted on male Wistar rats (Harlan Olac; body weight 231-283g) which were terminally anaesthetised and prepared for recording as described in Chapter 2.

4a.2.1 *Experimental protocol*

Following the surgical preparation the rats were positioned directly under the blast nozzle (which delivers the blast wave to the animal, see Figure 2.1), with the blast nozzle 3.5 cm above the ventral surface of the thorax. The isoflurane (Abbott Laboratories Ltd., UK) was discontinued and anaesthesia maintained with alphadolone/alphaxalone (Saffan ™, Pitman-Moore, UK) using an infusion pump (Harvard 22™, Harvard Apparatus Ltd., UK) while the animals breathed air. The anaesthesia was adjusted within the range 19-22 $mg.kg^{-1}.h^{-1}$ to maintain an experimental level of anaesthesia (mild withdrawal and a pressor response of approximately 10mmHg to a noxious pinch of the foot).

Following baseline cardiovascular, respiratory and blood gas measurements the protocol shown diagrammatically in Figure 4a.3 was then followed. All animals received a single discharge from the apparatus to the ventral thorax.

Protocol



Group	Haemorrhage	Blast	Resuscitation fluid	
I	+	+	Blood	n=6
II	+	+	7.5% Saline/Dextran	n=5
III	+	+	Modified Gelatin (Haemaccel)	n=6
IV	+	+	0.9% Saline	n=6
V	+	+	Hydroxyethyl Starch	n=6

Figure 4a.3 Diagrammatic representation of the protocol followed in this study (see section 4a.2.1 for full explanation). Plus sign (+) indicates presence of haemorrhage and blast injury in that group.

Five minutes later a control (pre-haemorrhage) recording was made in all groups. Ten minutes after administering the blast wave all animals were subjected to a controlled haemorrhage by anaerobic withdrawal of blood from the ventral tail artery in 12 equal aliquots at an overall rate of 2% estimated total blood volume per minute (6.06mL.100g^{-1} body weight, Elebute *et al.* 1978) until 40% of the total estimated blood volume had been withdrawn.

The animals were then allocated randomly to groups I-V and resuscitated 5 minutes after the end of haemorrhage with one of the following given intravenously via the tail vein at the standard clinical rate of 1.1mL.kg^{-1}.hr^{-1} with the exception of 0.9% saline which was administered at the standard clinical rate for crystalloid solutions: 3.3mL.kg^{-1}.hr^{-1} (ATLS guidelines);

- anti-coagulated (heparinised with Monoparin, CP Pharmaceuticals, UK) autologous blood (1:1 resuscitation volume:blood loss)
- hypertonic saline/dextran solution (RescueFlow®; 7.5% saline/6% dextran 70, 4mL.kg^{-1})
- isotonic colloid solution (modified gelatin, Haemaccel, Hoescht Marion

Roussel, Germany, 1:1 resuscitation volume:blood loss)

- isotonic crystalloid solution (0.9% saline, Fresenius Kabi Ltd. UK, 3:1 resuscitation volume:blood loss)
- isotonic hydroxyethyl starch (HAES-steril, Fresenius Ltd., UK, 1:1 resuscitation volume:blood loss)

See Table 4a.1 for a summary of treatments.

Cardiovascular measurements were made from 1 min before blast continuously until 5 min after blast, immediately before haemorrhage, after the removal of each aliquot of blood during haemorrhage, immediately before resuscitation, at 5 min intervals during resuscitation and thereafter immediately, 5, 10, 15, 20, 25 and 30 minutes after resuscitation. The duration of the blast-induced apnoea was determined visually and timed using a stopwatch. Blood gas analysis (ABL5^{TM}, Radiometer, Denmark) and haematocrit values (Hawksley micro-haematocrit reader) were obtained for samples taken immediately before blast, the first and last samples taken during haemorrhage, immediately after resuscitation and at 15 and 30 minutes after resuscitation.

All animals were killed 30 minutes after the end of resuscitation with an overdose of 0.5mL of 60mg.mL^{-1} (106-124 mg.kg^{-1}) sodium pentobarbitone (Sagatal, Rhône Mérieux (Ireland) Tallaght, Dublin) administered intravenously.

The lungs were removed and weighed to determine Lung Weight Index (lung weight/body weight).

Table 4a.1 *Summary of treatments:* All groups received thoracic blast followed 10 min later by a controlled haemorrhage of 40% estimated blood volume at 2% BV.min^{-1} then resuscitated with one of the following fluids;

Resuscitation Fluid	**Group** (resuscitated 5 min after haemorrhage)
Anti-coagulated autologous blood (1:1 resuscitation volume:blood loss)	I
Hypertonic saline/dextran (7.5% saline/6% dextran 70, 4mL.kg^{-1})	II
Isotonic colloid solution (modified gelatin, Haemaccel, 1:1 resuscitation volume:blood loss)	III
Isotonic crystalloid solution (0.9% saline, 3:1 resuscitation volume:blood loss)	IV
Isotonic hydroxyethyl starch (1:1 resuscitation volume:blood loss)	V

4a.3 Results

4a.3.1 *Baseline values*

Baseline (pre-blast) values for each group are presented in Table 4a.2. There were no significant differences between groups in the baseline cardiovascular or arterial blood gas variables, body weight or body temperature except for heart period between groups I and IV, femoral blood flow between groups II and III, and body temperature between groups III and V (see statistical analysis section, Chapter 2, section 2.3). However, these differences are so small they are generally of no physiological consequence. Body temperature did not change significantly during the course of the study in any group.

	Group I	Group II	Group III	Group IV	Group V
n	6	5	6	6	6
Body wt (g)	257.5±8.3	254.0±8.5	251.8±2.8	248.2±2.6	252.5±2.3
HP (ms)	165±3	153±3	148±6	146±2	148±6
MBP (mmHg)	102.1±2.3	104.0±2.2	106.5±4.4	100.9±2.9	96.9±6.1
Fem Q ($mL.min^{-1}$)	0.63±0.08	0.48±0.16	1.05±0.16	0.75±0.10	0.66±0.10
FVR ($mmHg.min.mL^{-1}$)	178±5	197±38	100±8	170±46	161±27
PaO_2 (mmHg)	89.3±1.6	83.4±2.8	84.7±2.4	88.2±1.0	87.8±5.1
$PaCO_2$ (mmHg)	33.8±1.3	32.2±1.2	37.3±1.1	36.0±1.8	32.7±0.9
a pH	7.35±0.02	7.37±0.01	7.36±0.01	7.38±0.01	7.37±0.01
ABE (mM)	-6.0±1.2	-5.4±0.7	-4.0±0.9	-3.2±1.1	-5.2±0.5
Hcrit (%)	34.2±1.3	33.4±1.2	32.5±2.3	32.7±1.2	33.0±0.7
Temp (oC)	37.5±0.2	37.7±0.2	37.9±0.1	37.7±0.0	37.2±0.1

Table 4a.2 Baseline (pre-blast) values in five groups of anaesthetised rats. Number of rats (*n*); body weight (body wt); Heart period (HP); mean arterial blood pressure (MBP); femoral arterial blood flow (Fem Q); femoral arterial vascular resistance (FVR); arterial oxygen tension (PaO_2), arterial carbon dioxide tension ($PaCO_2$) arterial pH (a pH) actual base excess (ABE), haematocrit (Hcrit) and body temperature (Tc). Values are mean ± SEM.

4a.3.2 *Effects of thoracic blast*

Thoracic blast in Group I, produced a significant increase of 307±60 ms in heart period from a pre-blast control of 165±3 ms (Figure 4a.4), and a significant fall in mean blood pressure of 64.8±5.1 mmHg from a pre-blast level of 102.1±2.3 mmHg (Figure 4a.4). Thereafter there was a rapid recovery in heart period and a partial recovery in mean arterial blood pressure (MBP). Ten minutes after blast MBP was still significantly below pre-blast levels.

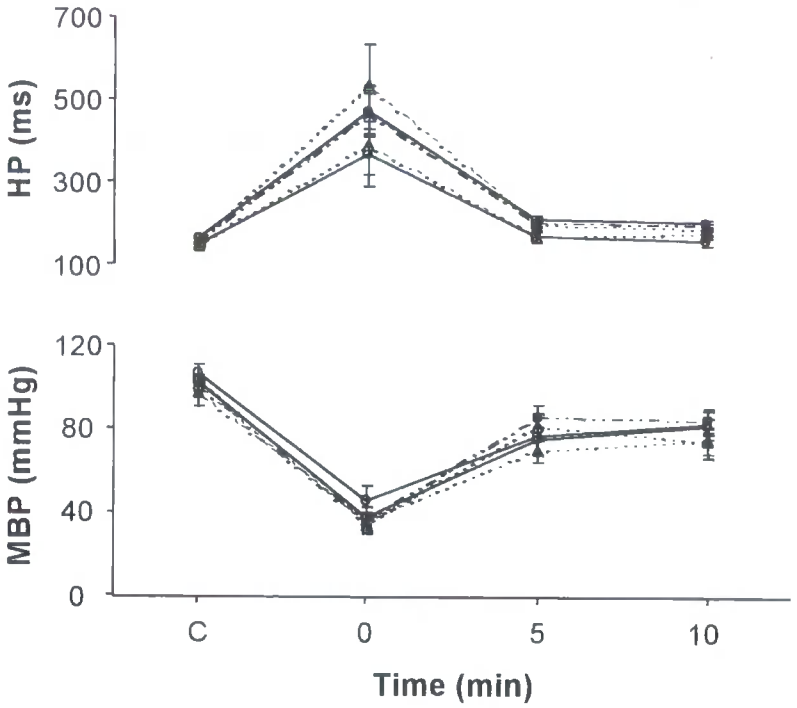


Figure 4a.4 Effects of a thoracic blast injury in anaesthetised rats on heart period (HP) and mean arterial blood pressure (MBP) in Group I; (●), Group II; (■), Group III; (o), Group IV; (Δ) and Group V; (▲). Data recorded immediately before (C) and thereafter immediately (0) and at 5 and 10 minutes after blast. Values are means±S.E.M.

In addition, thoracic blast (Group I) produced a significant (Student's independent *t* test) apnoea lasting 19.67±0.67 seconds (Figure 4a.5).

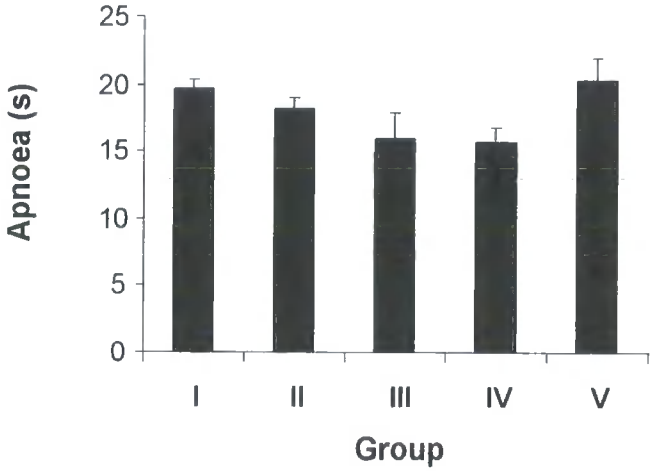


Figure 4a.5 Duration of apnoea in five groups of anaesthetised rats following thoracic blast injury. Values are means±S.E.M.

Furthermore, blast produced a significant, transient fall in femoral arterial blood flow of 0.51±0.08 mL.min^{-1} from a pre-blast control of 0.63±0.08 mL.min^{-1} (Figure 4a.6), and a significant, transient fall in femoral arterial vascular resistance of 97.3±5.0 mmHg.min.mL^{-1} from a pre-blast level of 177.9±5.0 mmHg.min.mL^{-1} (Figure 4a.6).

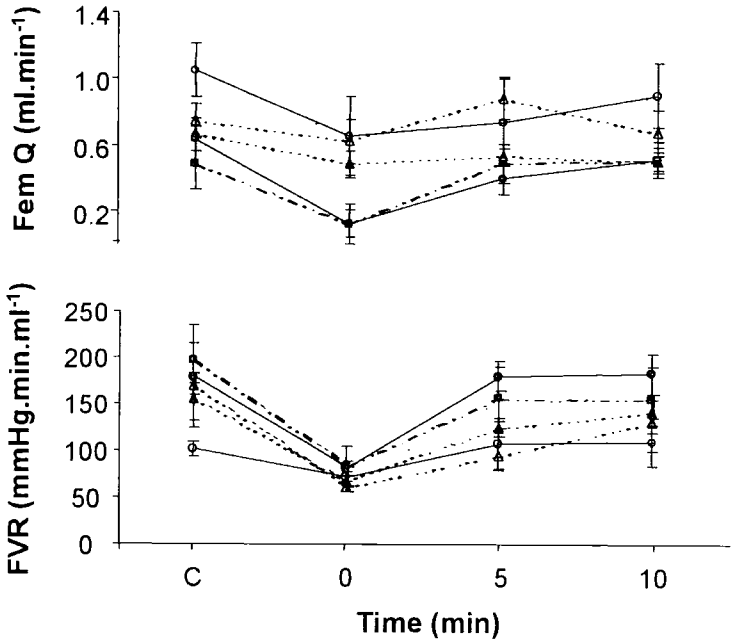


Figure 4a.6 Effects of a thoracic blast injury in five groups of anaesthetised rats on femoral arterial blood flow (Fem Q) and femoral arterial vascular resistance (FVR). Group I; (●), Group II; (■), Group III; (o), Group IV; (Δ) and Group V; (▲). Data recorded immediately before (C) and thereafter immediately (0) and at 5 and 10 minutes after blast. Values are means±S.E.M.

Following blast there was a significant fall in PaO_2 of 21.00±2.96 mmHg from a control pre-blast value of 89.33±1.65 mmHg, and arterial pH of 0.04±0.02 from a pre-blast control of 7.35±0.02 (Figures 4a.7). $PaCO_2$ and haematocrit both increased significantly following blast from a pre-blast level of 38.83±1.30 mmHg and 34.17±1.30 % to 41.00±1.44 mmHg and 38.80±0.78 % respectively (Figures 4a.7 & 4a.8).

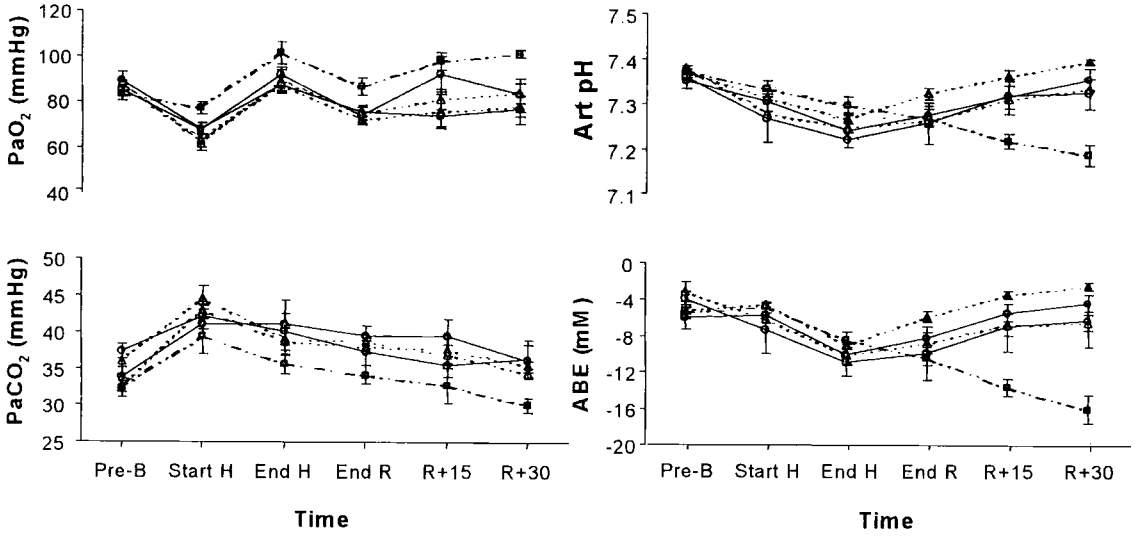


Figure 4a.7 Effects blast, haemorrhage and subsequent resuscitation in anaesthetised rats on arterial oxygen tension (PaO_2), arterial carbon dioxide tension ($PaCO_2$), arterial pH (art pH) and arterial base excess (ABE). Group I; blood (•), Group II; hypertonic saline/dextran (■), Group III; haemaccel (o) Group IV; 0.9% saline (Δ) and Group V; hydroxyethyl starch (▲). Data recorded immediately before blast (Pre-B), at the start of a haemorrhage of 40% total blood volume (Start H), at the end of haemorrhage (End H), at the end of fluid resuscitation (End R) and thereafter at 15 (R+15) and 30 (R+30) minutes after resuscitation. Values are means±S.E.M.

There was no significant change in ABE during this period. Thoracic blast in Groups II-V produced effects on the above parameters similar to those seen in Group I; there were no significant differences between groups for the first 10 minutes after blast except for femoral blood flow. This difference reflects the differences in the baseline values for femoral blood flow as there was no significant difference in the pattern of response of femoral blood flow in the first 10 minutes after blast.

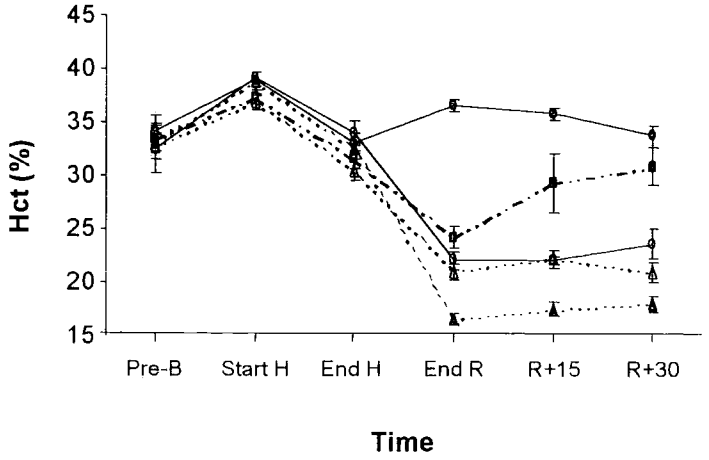


Figure 4a.8 Effects blast, haemorrhage and subsequent resuscitation in anaesthetised rats on haematocrit (Hct). Group I; blood (●), Group II; hypertonic saline/dextran (■), Group III; haemaccel (o) Group IV; 0.9% saline (Δ) and Group V; hydroxyethyl starch (▲). Data recorded immediately before blast (Pre-B), at the start of a haemorrhage of 40% total blood volume (Start H), at the end of haemorrhage (End H), at the end of fluid resuscitation (End R) and thereafter at 15 (R+15) and 30 (R+30) minutes after resuscitation. Values are means±S.E.M.

4a.3.3 *Effects of progressive haemorrhage*

Haemorrhage of 40% blood volume, initiated 10 minutes after thoracic blast, induced a significant change in heart period (Figure 4a.9) and mean blood pressure in all groups (Figure 4a.9). There was no evidence of the first, tachycardic, phase of the response to blood loss normally associated with haemorrhage in the absence of thoracic blast injury. The absolute mean blood pressure was significantly higher in Group III compared to Group V (ANOVA), however there were no significant differences in the pattern of response between groups. The following section will compare the peak change in heart period corresponding to the bradycardia from each individual animal.

Animals in Group I showed no significant tachycardia, while the bradycardia (significant peak increase in heart period of 26.52±7.06 ms; Student's paired *t* test) was seen after the loss of 20.56±4.08 % blood volume (Figure 4a.9). Furthermore, mean arterial blood pressure was not maintained in Group I during the haemorrhage and began to fall after the removal of the first aliquot of blood, the hypotension achieving statistical significance compared to pre-haemorrhage control after the loss of 6.7±0.0 % blood volume (Figure

4a.9). There was no significant difference in the peak increase in heart period, or in the hypotension induced by progressive haemorrhage between groups.

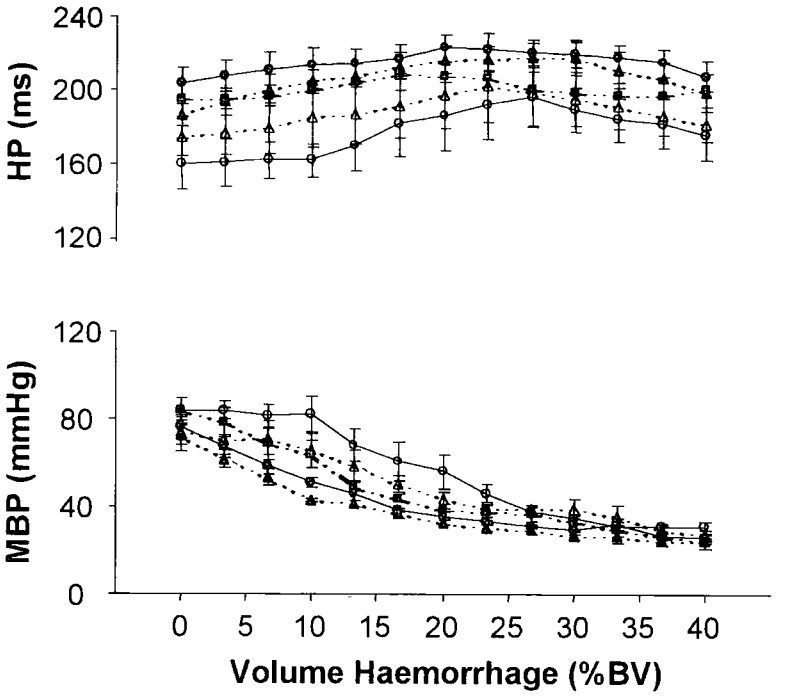


Figure 4a.9 Effects of a progressive haemorrhage following thoracic blast injury in five groups of anaesthetised rats on heart period (HP) and mean arterial blood pressure (MBP). Group I; (●), Group II; (■), Group III; (o), Group IV; (Δ) and Group V; (▲). Values are means±S.E.M.

Associated with the fall in arterial blood pressure there were significant reductions in femoral arterial flow in all groups. The fall in femoral blood flow became significant in Group I after the loss of 6.7±0.0 % blood volume (Figure 4a.10). However, there was no evidence of a change in femoral vascular resistance during the blood loss (Figure 4a.10).

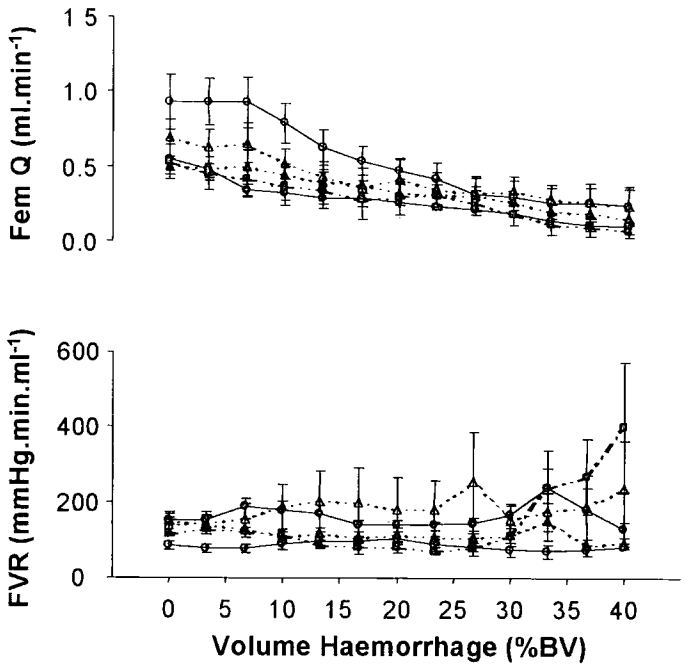


Figure 4a.10 Effects of a progressive haemorrhage following thoracic blast injury in five groups of anaesthetised rats on femoral arterial blood flow (Fem Q) and femoral arterial vascular resistance (FVR). Group I; (●), Group II; (■), Group III; (o), Group IV; (Δ) and Group V; (▲). Values are means±S.E.M.

By the end of the haemorrhage PaO_2 (Group I) had increased significantly by 19.2±2.94 mmHg from a pre-haemorrhage value of 68.3±2.96 mmHg (Figure 4a.7) while there was no significant change in $PaCO_2$ (Figure 4a.7). There was a fall in arterial pH and base excess of 0.07±0.04 and 4.33±0.97 mM from a pre-haemorrhage level of 7.31±0.02 and −5.67±0.92 mM respectively (Figure 4a.7). Haematocrit also fell by 5.8±0.9 % from a pre-haemorrhage control of 38.8±0.8 % (Figure 4a.8) in Group I. There was no significant difference in blood gas parameters between groups during the haemorrhage period.

4a.3.4 *Effects of resuscitation*

Five minutes after the end of haemorrhage animals in groups I-V were resuscitated with autologous blood (Group I), hypertonic saline/dextran (Group II), colloids; modified gelatin (Haemaccel, Group III) and isotonic hydroxyethyl starch (HES, Group V) or 0.9% saline (Group IV). Resuscitation with whole blood (Group I) induced a significant elevation in arterial blood pressure of 68.5±3.49 mmHg from a pre-resuscitation level of

37.4±3.48 mmHg (Figure 4a.11). There were no differences in the increase in mean arterial blood pressure associated with fluid resuscitation between groups. Thereafter MBP was maintained for the remainder of the study in groups I, III, IV and V (Figure 4a.11). However, MBP was not maintained after resuscitation with hypertonic saline/dextran (Group II). Within 5 minutes of resuscitation MBP in Group II had fallen significantly (compared to the other groups) by 11.0±8.3 mmHg from an end-resuscitation level of 75.0±5.8 mmHg, and continued to fall throughout the remainder of the study (Figure 4a.11). Additionally, one rat died within 25 minutes of resuscitation with hypertonic saline/dextran while all of the rats resuscitated with the other solutions survived until the end of the study, 30 minutes after resuscitation.

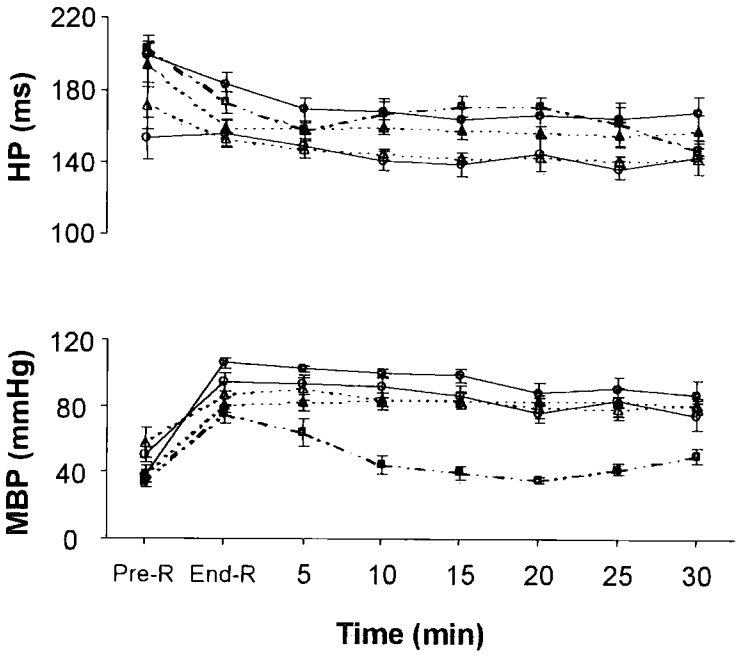


Figure 4a.11 Effects of resuscitation with various fluids following thoracic blast injury and subsequent haemorrhage of 40% total blood volume in anaesthetised rats, on heart period (HP) and mean arterial blood pressure (MBP) in Group I; blood (•), Group II; hypertonic saline-dextran (■), Group III; haemaccel (o), Group IV; 0.9% saline (Δ) and Group V; hydroxyethyl starch (▲). Data recorded immediately before resuscitation (Pre-R), and thereafter immediately (End-R) and at 5, 10, 15, 20, 25 and 30 minutes after resuscitation. Values are means±S.E.M.

Associated with the rise in arterial blood pressure immediately after resuscitation there was a fall in heart period (Group I) of 15.9± 6.5 ms from a pre-resuscitation value of 199.2± 10.8 ms, although this did not attain statistical significance (Figure 4a.11). Femoral vascular resistance fell, although not significantly, immediately following

resuscitation with whole blood (Group I) from a pre-resuscitation value of 276.26±86.22 mmHg.min.mL^{-1} to 196.17±25.6 mmHg.min.mL^{-1} (Figure 4a.9). However, there was a significant increase in femoral blood flow in Group I from a pre-resuscitation level of 0.18± 0.05 mL.min^{-1} to 0.57± 0.06 mL.min^{-1} (Figure 4a.12).

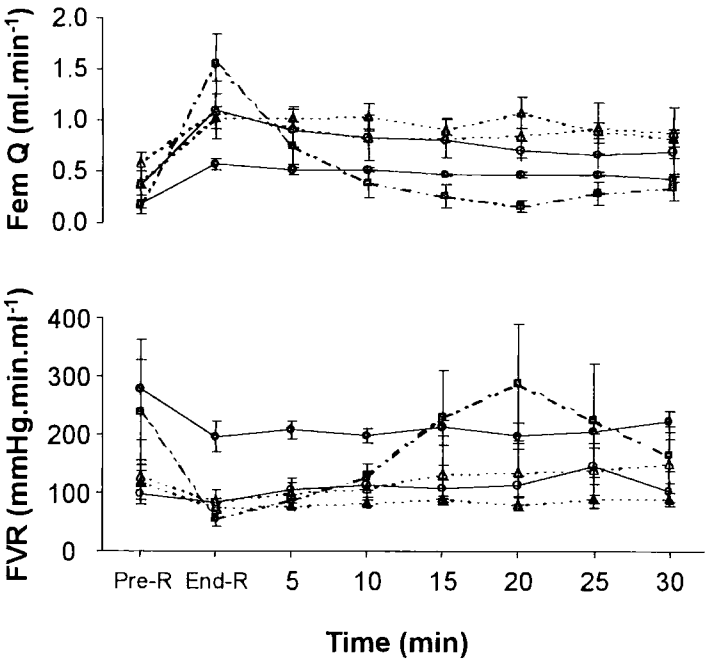


Figure 4a.12 Effects of resuscitation with various fluids following thoracic blast injury and subsequent haemorrhage of 40% total blood volume in anaesthetised rats, on femoral arterial blood flow (Fem Q) and femoral arterial vascular resistance (FVR) in Group I; blood (●), Group II; hypertonic saline-dextran (■), Group III; haemaccel (o), Group IV; 0.9% saline (Δ) and Group V; hydroxyethyl starch (▲). Data recorded immediately before resuscitation (Pre-R), and thereafter immediately (End-R) and at 5, 10, 15, 20, 25 and 30 minutes after resuscitation. Values are means±S.E.M.

There were no differences in the changes in heart period, or femoral vascular resistance and flow between groups I, III, IV and V immediately following resuscitation, although the absolute value of FVR in Group I is significantly higher, and femoral blood flow significantly lower than the other groups. The fall in femoral vascular resistance and increase in flow immediately after resuscitation was greatest in animals treated with hypertonic saline/dextran (Figure 4a.12), despite this group not showing the greatest increase in arterial blood pressure (Figure 4a.11). However, this effect rapidly waned with femoral vascular resistance rising and flow falling (Figure 4a.12) coincident with the fall in arterial blood pressure in this group. By contrast, in all the other groups femoral flow was well maintained with little further change in resistance (Figure 4a.12).

In Group I PaO_2 fell immediately after resuscitation by 11.7±3.2 mmHg from a pre-resuscitation value of 87.5±2.9 mmHg (Figure 4a.7). There was no difference in the fall in PaO_2 between groups immediately after resuscitation. Thereafter there was an elevation in PaO_2 in the groups treated with asanguinous fluids, this change being significant and most apparent in the group resuscitated with hypertonic saline/dextran which showed a significant rise of 14.5±3.8 mmHg by 30 minutes after resuscitation from 87.2±3.8 immediately following resuscitation. $PaCO_2$ did not change significantly following resuscitation (Figure 4a.7) except in the group given hypertonic saline/dextran where it had fallen significantly by 4.2±1 mmHg by 30 min after resuscitation, from 34.2±1.2 mmHg immediately after resuscitation. Directly after resuscitation there was a rise in arterial pH in Group I from a pre-resuscitation value of 7.24±0.04 to 7.28±0.02. This rise continued throughout the study and there was no difference between all groups in pH during or following resuscitation except that treated with hypertonic saline/dextran (Group II). In marked contrast arterial pH and base excess fell during resuscitation from pre-resuscitation values of 7.3±0.02 and –8.4±0.9 mM respectively, to 7.27±0.02 and –10.4±0.8 mM respectively, immediately post resuscitation. pH and ABE continued to fall significantly during the 30 minutes post-resuscitation in animals treated with hypertonic saline/dextran (Figure 4a.7). Arterial pH and base excess was significantly lower 15 and 30 minutes after resuscitation with hypertonic saline/dextran compared with the other groups, reaching levels of 7.19±0.02 (pH) and –16.0±1.5 mM (ABE) by 30 minutes post resuscitation with this fluid.

Haematocrit fell significantly following resuscitation in all groups given asanguinous fluids. The fall being greatest in Group V (HES). However, haematocrit rose following resuscitation in the animals treated with autologous blood (Group I; Figure 4a.8). Haematocrit was subsequently maintained at post-resuscitation levels in the groups treated with blood (Group I), both colloids (Groups III & V) and 0.9% saline (Group IV), with values seen in Group V being significantly lower than those seen in Groups I, III and IV, while values in Group I were significantly higher than Groups III-V. By contrast haematocrit increased significantly in the group treated with hypertonic saline/dextran to 30.8±1.8 %, so that by 30 minutes after resuscitation haematocrit was significantly higher in animals treated with hypertonic saline/dextran than in those given colloids or 0.9% saline (Figure 4a.8). Indeed, 30 minutes after resuscitation with

hypertonic saline/dextran haematocrit was not significantly different to that seen immediately *before* resuscitation (Figure 4a.8).

Post mortem Lung Weight Indices were not significantly different between groups (Figure 4a.13).

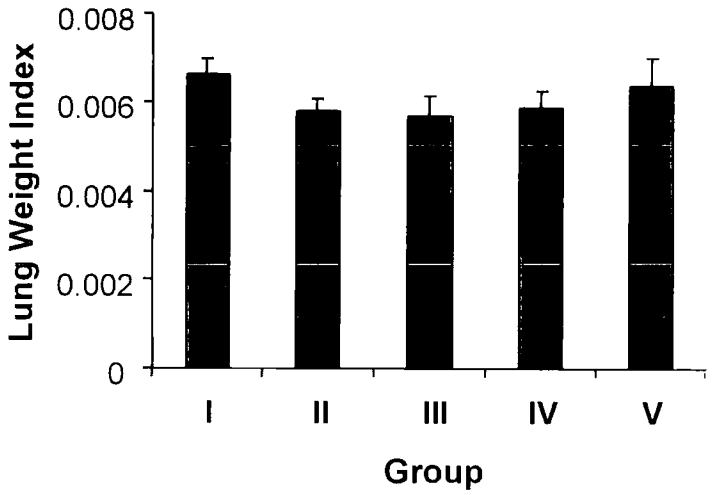


Figure 4a.13 Effects of various resuscitation fluids following thoracic blast injury and subsequent haemorrhage of 40% total blood volume, on *post mortem* lung weight index (post mortem lung weight/body weight) in anaesthetised rats. Group I; whole blood, Group II; hypertonic saline/dextran, Group III; haemaccel, Group IV; 0.9% saline, Group V; hydroxyethyl starch. Values are means±S.E.M.

4a.4 Discussion

Thoracic blast injury induced a bradycardia, transient apnoea and hypotension, the latter being rapidly but partially resolved. The response to subsequent controlled haemorrhage consisted of a progressive bradycardia and hypotension. The new findings from the study relate to the effects of subsequent resuscitation and a comparison of the efficacy of five resuscitation fluids: whole (autologous) blood, hypertonic saline/dextran, isotonic colloids; Haemaccel (modified gelatin) and hydroxyethyl starch, and 0.9% saline. Immediately upon completion of resuscitation the asanguinous fluids were able to restore arterial blood pressure and femoral blood flow to pre-haemorrhage levels while blood restored these parameters to higher (pre-blast) levels. The effects of all the fluids bar hypertonic saline/dextran was sustained for the following 30 minutes until the end of the study and resulted in a significant improvement (increase) in arterial pH and base excess.

By contrast the effects of hypertonic saline/dextran were very short-lived with arterial blood pressure and femoral flow falling within 5 minutes of resuscitation. Coincident with this fall in blood pressure and flow there was a marked fall in arterial base excess. The hypertonic saline/dextran solution did not attenuate any lung oedema since there were no differences in Lung Weight Indices between groups.

Hypertonic saline/dextran has been reported effective in resuscitation following simple haemorrhage (Barros *et al.* 1999; Corso *et al.* 1998) and to increase survival compared to normal saline after penetrating injuries (Wade *et al.* 1997, 1999). In addition, hypertonic saline/dextran has been reported effective in increasing cardiac output (Murphy *et al.* 1999) and improving tissue blood flow (Kien *et al.* 1996) following burn injuries. The deleterious effects of hypertonic saline/dextran reported from this study therefore contrast to its reported effectiveness for resuscitation following haemorrhage in the absence of blast injury. However, as mentioned earlier (see section 4a.1.9) one study of HSD resuscitation after haemorrhagic shock in unanaesthetised rats reported an increase in blood pressure, which was only sustained for 10 minutes (Chang & Varma, 1992). This is reminiscent of the present study in which mean arterial blood pressure was falling within 5 minutes of resuscitation with HSD.

The problems associated with hypertonic saline/dextran after blast and haemorrhage appears to be specific for this agent rather than being a general problem associated with resuscitation. Wikoff *et al.* (1999) had previously reported that resuscitation *per se* following blast alone was deleterious since it resulted in impaired cardiac performance. However, this is not the case following blast and haemorrhage since similar problems were not associated with resuscitation using 0.9% saline, colloids or whole blood in the present study.

The marked fall in blood pressure, without evidence of marked vasodilation, in the present study is consistent with a decline in cardiac output. It is possible that an impaired myocardium after blast (Wikoff *et al.* 1999) may be adversely affected by hypertonic saline/dextran. There have been varied reports concerning the cardiac effects of hypertonic saline with some suggesting a deleterious effect (Brown *et al.* 1990) while others suggest a positive inotropic effect (Mouren *et al.* 1995) in isolated hearts. However, others have failed to demonstrate any inotropic effect *in vivo* (Welte *et al.*

1995; Ogino *et al.* 1998). Further studies are now needed to assess cardiac changes following blast and haemorrhage and resuscitation with hypertonic saline/dextran, and to further investigate whether the deleterious effects of resuscitation with hypertonic saline/dextran are due to hypertonic resuscitation *per se*, or whether it is the combination of hypertonic saline with dextran.

An alternative or additional possibility to account for the reduced blood pressure after resuscitation is a rapid fall in venous return after the initial resuscitation with hypertonic saline/dextran. Again this is in contrast to the published effects of hypertonic solutions after non-blast injuries where the hypertonic solution produces a sustained increase in plasma volume by mobilising intracellular water, while the colloid augments plasma oncotic pressure (Tollofsurd *et al.* 1998; Moon *et al.* 1996; Saxe *et al.* 1996; Onarheim 1995) holding fluid in the vessels for longer, as even with hypertonic solutions some fluid may still move out the vessel by osmosis following the movement of sodium down its concentration gradient. This effect does not appear to be sustained after blast/haemorrhage since the initial fall in haematocrit seen after resuscitation with hypertonic saline/dextran was rapidly reversed. Again this appears to be a property of hypertonic saline/dextran since the reduction in haematocrit seen after resuscitation with 0.9% saline and colloids was sustained. It is possible that after blast capillary permeability is increased due to the direct lung damage caused by a coupling of the blast wave with the body (see Chapter 1, sections 1.3 & 1.4). As the pressure wave is propagated through the body the capillaries in the lungs may become disrupted leading to increased permeability and oedema in the lungs. Indeed haematocrit is increased 10 minutes after blast (at the start of the haemorrhage) in all groups (Figure 4a.8). Coupled with the possibility of an ensuing inflammatory response which may lead to secondary lung injury (see section 4a.4.1.2) the further insult of a haemorrhage could possibly potentiate this effect (Zunic, 2000). If permeability is increased enough to allow larger molecules to leak out after resuscitation with HSD, the dextran component may be leaking into the interstitial space, decreasing oncotic pressure in the vasculature and reducing the force which is holding the fluid within this compartment, possibly exacerbating any already existing oedema within the pulmonary circulation.

Anaphylactic reactions to dextran have been reported in both humans (Ljungstrom *et al.* 1988; Kreimeier *et al.* 1995) and animals (Hanahoe *et al.* 1983; Koller & Reed, 1992; de

Brito *et al.* 1982; de Brito & Hanahoe, 1983). Although the incidence in humans was reduced to 0.001% in 1985 due to the use of hapten prophylaxis; which consists of a preinjection of 20mL of dextran 1 (Ljungstrom *et al.* 1988; Kreimeier *et al.* 1995). This is in comparison to reports of allergic reactions to hydroxyethyl starch administration, which is thought to approach 30% (Spittal & Findlay, 1995). The adverse reaction to dextran has been shown to be due to naturally occurring specific antibodies predominantly of the IgG class, against the dextran molecule (see Miyamoto & Tashiro, 1996; Kreimeier *et al.* 1995). Dextran-induced anaphylactic reactions have been reported in patients who are already likely to have an ongoing inflammatory response such as in gastric ulcer or cancer patients (see Miyamoto & Tashiro, 1996). It is likely that the animals in our study are also experiencing an ongoing inflammatory reaction due to the blast injury and subsequent haemorrhage at the time of fluid resuscitation (see section 4a.1.5) and so may be more susceptible to suffer anaphylactic reaction to the dextran component of HSD. Reports in the literature of anaphylactic reactions to dextran only occur within certain colonies of Wistar rats (Hanahoe *et al.* 1983; Koller & Reed, 1992; de Brito *et al.* 1982; de Brito & Hanahoe, 1983). This would lead to an inflammatory response and an increase in capillary fluid filtration (Koller *et al.* 1997) and tissue oedema (de Brito & Hanahoe, 1983). Associated with this, dextran is also reported to induce a significant hypotension (Hoem *et al.* 1986). This may be the reason for the deleterious effect of HSD resuscitation in the present study. Blood pressure in the HSD treated animals began to fall within 5 minutes of resuscitation with this fluid. Systemic oedema due to dextran-induced anaphylaxis could explain the increase in arterial oxygen tension after HSD resuscitation. Systemic oedema would increase the diffusion barrier and decrease oxygen extraction from the blood. Further tests are now required to determine whether the adverse reaction to HSD resuscitation after blast and haemorrhage is due to dextran-induced anaphylaxis in this strain of rat. However, it must be stressed that in the literature it is reported to be rats from the Tuck colony which display dextran allergy (de Brito & Hanahoe, 1983) and the colony used in this study is the CFHB strain originating from Carworth, Europe.

Hypertonic saline/dextran did not reduce Lung Weight Index (LWI) in the present study. This is consistent with a report by Cohn *et al.* (1997) which indicated that hypertonic saline did not reduce the magnitude of lung injury or provide any physiological benefits over isotonic solutions following pulmonary contusions. However, HSD did not increase

LWI either. If an anaphylactic reaction to HSD had occurred, the increase in capillary fluid permeability may lead to tissue oedema in the systemic circulation rather than in the lungs. As mentioned in the previous paragraph, the increase in PaO_2 seen after resuscitation with hypertonic saline/dextran may not be indicative of improved pulmonary gas transfer, but rather may be due to poor oxygen extraction in the systemic circulation possibly due to an increase in the diffusion barrier between the capillaries and the tissue. Consistent with this it can be seen that the low arterial blood pressure and blood flow following resuscitation with hypertonic saline/dextran are associated with indices of severe metabolic disturbance: severe acideamia and negative base excess. A number of factors are likely to contribute to this. Firstly, low blood flow as a consequence of the reduced arterial blood pressure will reduce oxygen delivery. Secondly, any increase in systemic interstitial water is likely to increase the diffusion barrier for oxygen, and hence reduce oxygen extraction. In this context it is interesting to note that resuscitation with hypertonic saline/dextran is not reported to improve acid base balance (O'Bennar *et al.* 1998) and exacerbates base deficit (Cohn *et al.* 1997) even in situations where oxygen delivery is improved. Further studies are now needed to assess the microcirculatory effects of resuscitation with hypertonic saline/dextran after blast and its effects on tissue oxygen transport.

4a.4.1 *Possible mechanisms of ischaemia reperfusion injuries following a primary blast injury and subsequent haemorrhage.*

The potential for secondary damage following blast injury and its hypoxic consequences will form the basis for future studies in this field and so it is important to understand the basis of this secondary damage including the role of hypoxia and tissue mediators in this response.

The mechanism of the lung damage suffered by a blast victim may fall into two categories: direct lung injury and an inflammatory response initiated in the lungs or other organ systems by hypoxia which may, in turn, amplify the pulmonary insult and lead to an escalating whole body inflammatory response. Subsequent haemorrhage may augment this effect. Resuscitation with certain fluids may protect from this damage and some may not.

4a.4.1.1 Direct lung damage

Primary blast injury is known to cause lung injury (see Chapter 1, sections 1.3 & 1.4). The most notable and immediately life-threatening injury is blast lung (Cooper, 1996). The gross pulmonary changes associated with this include pulmonary contusion (Hunter, 1941), pulmonary oedema (Zuckerman 1940; Brown *et al.* 1993) and reduced gas transfer (Damon *et al.* 1971) resulting in a low PaO_2 and tissue hypoxia (see Figure 4a.14) a known trigger for an inflammatory response (Combe *et al.* 1997; Michiels *et al.* 1996). In addition to these changes, which may themselves lead to death over a period of hours, there are early cardiovascular and respiratory changes that can contribute to morbidity and mortality.

4a.4.1.2 Secondary lung damage

In addition to causing reduced oxygen levels in blood, the response to blast may cause cardiovascular changes which further reduce the delivery of oxygen to tissues, which in turn can initiate or amplify an inflammatory response contributing to secondary lung damage. The cardiovascular changes induced by blast include a transient apnoea and long-lasting bradycardia and hypotension (Guy *et al.* 1998; Ohnishi *et al.* 2001; Sawdon *et al.* In press; see also Chapter 3). Factors involved in the hypotension include a reduction in vascular resistance (see Figure 4a.6) and possibly impaired cardiac function (Harban *et al.* 2001), both of which can contribute to haemodynamic compromise and shock (failure of oxygen delivery to meet demand).

Blast victims may also suffer a haemorrhage, which is likely to exacerbate the hypoxia. During haemorrhage, microcirculatory blood flow and hence oxygen delivery to the microvasculature decreases. Insufficient oxygen to the endothelial cells results in cell swelling reducing the lumen of the capillaries and hindering blood flow further (see Menger *et al.* 1992; Mazzoni *et al.* 1989). The increased resistance in these vessels may lead to shunting of blood flow towards vessels with a lower resistance reducing functional capillary density, further augmenting the hypoxia and inflammatory response (see Figure 4a.14).

It is now widely accepted that shock can initiate a widespread inflammatory response (see Figure 4a.14) which leads to secondary injury. This secondary injury can become

manifest as damage and dysfunction in a number of organs including the lungs where Adult Respiratory Distress Syndrome (ARDS) may develop (Pepe *et al.* 1982; Hudson *et al.* 1995) and the gut where breakdown of the gut mucosal barrier and bacterial/endotoxin translocation may ensue (Deitch *et al.* 1988) possibly coupled with hepatic dysfunction (Rensing *et al.* 1999). Damage to these organs are particularly threatening since ARDS can lead to further failure of oxygen delivery while intestinal/hepatic damage can lead to sepsis () which in turn causes further inflammation and organ damage.

Thus, regardless of whether the hypoxia is the consequence of primary or secondary lung injury it is likely to initiate a widespread inflammatory response affecting other organs since the trigger for this is reduced oxygen delivery (possibly followed by re-oxygenation), which will occur regardless of whether the initial deficit is cardiovascular or pulmonary (see Figure 4a.14).

4a.4.2 *The inflammatory response*

The inflammatory response is orchestrated by the vascular endothelium. This involves a sequential expression of molecular mediators. A key element of the inflammatory response is the activation of leukocytes and transmigration from blood across the vascular endothelium of postcapillary venules (and capillaries in the lungs) into the tissue. This process is divided into stages of circulatory leukocyte margination and rolling along the endothelium, arrest of rolling and penetration into the tissues. The sequential appearance of molecular mediators is summarised in Table 4a.3.

Table 4a.3 Molecular markers and mediators of the inflammatory response

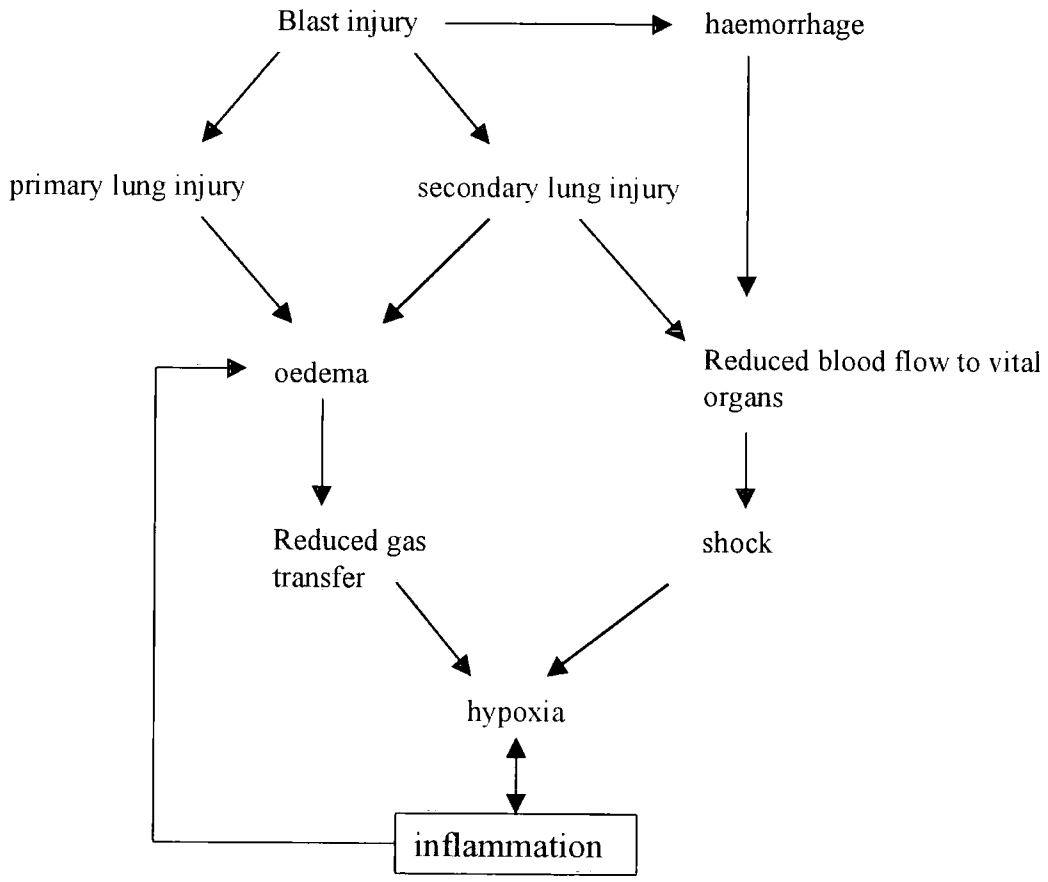
Stage	Molecular mediator/marker	Timing	Stimulus
Margination and rolling	Low affinity binding between endothelial P- and E-selectins and neutrophil PSGL-1 and L-selectin	P-selectin appears within 20 min of injury and E-selectin appears within 2 hours	Host of stimuli including IL-1β and TNFα released by lung macrophages in response to injurious stimuli. Other mediators include oxygen radicals, activated complement etc.
At this point the process may be aborted by anti-inflammatory mediators including glucocorticoids, IL-10 and NO. Otherwise the inflammatory process continues as follows:			
Arrest of rolling	A range of adhesion molecules on the endothelial surface including ICAM-1 and 2 and VCAM-1 forming high affinity bonds with leukocyte adhesion molecules including LFA-1 and MAC-1	6-9 h after injury	IL-1β and TNFα
Leukocyte activation	IL-8 secreted by endothelium	6-9 h after injury	IL-1β and TNFα
Leukocyte transmigration	Aided by endothelial PECAM-1		IL-8, Complement 5a
This leads to increased capillary permeability and tissue oedema. Endothelial cells further damaged by IL-1β and TNFα and by the secretion of elastase by activated neutrophils.			

ICAM-1 and 2, intercellular adhesion molecules 1 and 2; IL-10, IL-1β, IL-8, interleukins 1β, 8 and 10; LFA-1, leukocyte function associated antigen-1 or CD11a/CD18; MAC-1, macrophage-1 antigen or CD11b/CD18; NO, nitric oxide; PECAM-1, platelet-endothelial cell adhesion molecule 1; PSGL-1, P-selectin glycoprotein ligand 1; TNFα, tumour necrosis factor α; VCAM-1, vascular cell adhesion molecule 1.

As a consequence of the appearance or upregulation of a number of these mediators e.g. ICAM-1 and VCAM-1 in tissue, increased levels of activated neutrophils and IL-8 in pulmonary lavage fluid or evidence of increased microvascular permeability e.g. oedema, leakage of large molecules such as albumin from the vascular space are used as indices of an ongoing inflammatory response. The use of a number of these markers has now

become established in rat lung and other tissues. Thus, it has recently been shown that haemorrhagic shock leads to an upregulation of the levels of ICAM-1 and VCAM-1 in the rat lung, and that this can be modulated by the nature of fluid used for resuscitation (Sun *et al.* 1999). Thus fluid resuscitation aims to target several points within this vicious circle of inflammation. HSD was chosen in this particular study as it is reported in the literature to be particularly effective in improving microcirculatory blood flow by reducing vascular endothelial cell swelling (Mazzoni, 1990). It is reported to prevent P-selectin upregulation following haemorrhagic shock (Akgur *et al.* 1999) and also shows free-radical scavenging properties (Brown *et al.* 1990; Haljamäe, 1985), as well as reportedly inhibiting neutrophil activation (Sheilds *et al.* 2000) and post-ischaemic leukocyte adherence and transmigration through the vascular endothelium (Corso *et al.* 1999; Nolte, 1992), and thus is claimed to have the potential to limit the effects of ischaemia reperfusion injuries and attenuate end organ damage following a systemic inflammatory response. However, this appears to be contrary to the effects of HSD in this study. HSD failed to maintain blood pressure and femoral arterial blood flow following thoracic blast injury and haemorrhage and this was associated with severe acidaemia and negative base excess; indices of severe metabolic disturbance. An important future study should therefore aim to determine whether there is any inflammatory response after thoracic blast injury and haemorrhage by measuring inflammatory mediators such as ICAM-1 and VCAM-1, as well as markers of tissue damage to assess secondary organ injury such as α-glutathione-S-transferase (Redl *et al.* 1995; Rensing *et al.* 1999) a sensitive and specific marker of hepatocellular injury.

Figure 4a.14 Summary of escalating inflammatory response from an initial primary blast injury and subsequent haemorrhage



In conclusion the present study has shown that animals subjected to thoracic blast injury and severe haemorrhage can be resuscitated early with whole blood, isotonic saline and colloids (Haemaccel and hydroxyethyl starch), resulting in a restoration of arterial blood pressure, femoral blood flow and acid base status which is sustained for at least 30 minutes. The use of hypertonic saline/dextran is contraindicated following thoracic blast and haemorrhage since the effect is not sustained.

4b Pulmonary and Cardiovascular Effects of Delayed Resuscitation after Thoracic Blast and Blood Loss: Comparison of Whole Blood, Isotonic Saline and Colloid with Hypertonic Saline/Dextran

4b.1 Introduction

In the first section of this chapter (Chapter 4a) the effects of early resuscitation with autologous blood, 0.9% saline, 2 colloids (modified gelatin in the form of Haemaccel and hydroxyethyl starch) and hypertonic saline/dextran (HSD) following thoracic blast injury and haemorrhage were determined. The results showed that resuscitation with blood, normal saline and both colloids effectively increased mean arterial pressure and femoral arterial blood flow to pre-blast levels in the normal saline and colloid groups, and to levels higher than pre-blast in the whole blood group. These increases in blood pressure and femoral blood flow were sustained throughout the remainder of the study with no obvious detriment in the groups resuscitated with whole blood, normal saline or isotonic colloid. This was in contrast to the effects of resuscitation with HSD. Here the initial increase in mean arterial blood pressure and femoral blood flow was short-lived and both began to fall within 5 minutes of infusion of HSD. An ensuing metabolic acidosis was also apparent in the HSD group but was absent from the other groups.

This section of Chapter 4, and Chapter 5 aims to address the following questions; is the apparent detrimental effect of resuscitation with HSD following thoracic blast injury and blood loss simply due to its hypertonicity or were these subjects resuscitated too early after the insult? This latter question will be the focus of the remainder of this chapter with chapter 5 addressing the question of hypertonicity.

It is reported in the literature (Bickell *et al.* 1994; see Nolan, 1999) and often practised clinically (ATLS guidelines) to allow permissive hypotension in some hypovolaemic patients if immediate definitive treatment cannot be given. It is currently unknown however whether this will be beneficial or detrimental after a blast injury. The majority of casualties in a combat setting require early vigorous resuscitation with fluids (Weideman *et al.* 1999). However, recently animal studies have shown that resuscitation too soon after an uncontrolled haemorrhage will increase blood pressure, reverse

vasoconstriction, dislodge any thrombus formation and thus increase blood loss. The consequent decrease in oxygen delivery is reflected in the subsequent metabolic acidosis that develops (Bickell *et al.* 1989; Stern *et al.* 1993; see Nolan 1999). Indeed there are numerous reports in the literature of immediate resuscitation of haemorrhagic shock increasing the rate, volume and duration of haemorrhage (Sakles *et al.* 1997; Bickell *et al.* 1992; Krausz *et al.* 1992; Marshall *et al.* 1997; Stern *et al.* 2000), as well as increasing mortality (Bickell *et al.* 1992; Krausz *et al.* 1992; Marshall *et al.* 1997; Stern *et al.* 2000). One study showed significantly higher survival rates in those left unresuscitated when compared to those resuscitated with hetastarch (Craig *et al.* 1994), hypertonic saline (Solomonov *et al.* 2000) and in cases of penetrating torso injuries (Bickell *et al.* 1994; see Wade *et al.* 1997). Vassar and colleagues commented in 1993 that the exacerbated blood loss in animal models by hypertonic resuscitation is unlikely to be relevant in a clinical trauma setting as a clot would already have had chance to form, i.e., resuscitation is already delayed. Others claim that hypertonic resuscitation will dislodge an already formed early thrombus (Bickell *et al.* 1989; Stern *et al.* 1993, see Nolan 1999). However, Stern and colleagues' model of uncontrolled haemorrhage involves a single tear to a large vessel (1993), where early resuscitation might indeed be detrimental. However, if the haemorrhage were due to smaller amounts of damage to multiple smaller vessels then secondary organ damage resulting from prolonged hypotension may be the predominant risk and early fluid resuscitation to reperfuse vital organs may be the more beneficial option. It must be stressed however that most of the studies mentioned involve resuscitation of an uncontrolled haemorrhage (Craig *et al.* 1994; Solomonov *et al.* 2000; Bickell *et al.* 1989; Marshall *et al.* 1997; Stern *et al.* 1993 & 2000), whereas all the studies involving blood loss reported in this thesis are volume controlled.

The haemodynamic and cardiovascular effects of *early* resuscitation of primary thoracic blast injury and haemorrhage with whole (autologous) blood, normal (0.9%) saline, 2 colloids (modified gelatin in the form of Haemaccel, and hydroxyethyl starch; HES) and hypertonic saline/dextran have been investigated (Chapter 4a). Early resuscitation with HSD in this setting of blood loss on a background of thoracic blast injury was deleterious, with one subject not surviving the duration of the study (which was only 30 minutes post resuscitation). Therefore, this section will aim to reflect the more common clinical practice of delaying resuscitation.

The aim of the present study was to compare the cardiovascular and haemodynamic effects of *delayed* resuscitation with autologous whole blood, isotonic colloid (modified gelatin, Haemaccel), isotonic crystalloid (0.9% saline) and hypertonic saline/dextran solutions following thoracic blast and a haemorrhage of 40% blood volume (to reduce the number of animals used in this study only one colloid will be analysed). In addition, the pulmonary effect (as different fluids are reported to produce differing effects on pulmonary oedema; see section 4a.1.5 and 4a.4.2) was assessed by measuring *post-mortem* lung weight ratios and arterial blood gases during the experiment.

4b.2 Methods

The experiments were conducted on male Wistar rats (Harlan Olac, body weight 237-282g) which were terminally anaesthetised and prepared for recording as described in Chapter 2.

4b.2.1 *Experimental protocol*

Upon completion of the surgery the rats were positioned supine in the blast apparatus with the ventral thorax 3.5 cm below the blast nozzle (which delivers the blast wave to the animal; see Chapter 2, section 2.2). The animals were then allowed to stabilise for 1 h under alphadolone/alphaxolone (Saffan^{TM}, Pitman-Moore, UK) anaesthesia prior to exposure to blast. The infusion rate of anaesthetic was adjusted within the range 19-24 $mg.kg^{-1}.h^{-1}$ to maintain an experimental level of anaesthesia (mild withdrawal and a pressor response of approximately 10 mmHg to a noxious pinch of the foot).

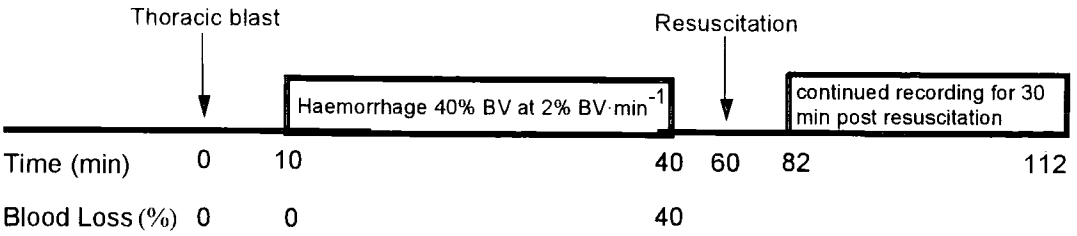
Following baseline cardiovascular, respiratory and blood gas measurements the protocol shown diagrammatically in Figure 4b.1 was then followed. A pressure of 1500 psi was generated in the blast apparatus and all animals received a single discharge from the apparatus to the ventral thorax. Ten minutes later the animals were subjected to a controlled haemorrhage. Blood was withdrawn anaerobically from the ventral tail artery in 12 equal aliquots at an overall rate of 2% estimated total blood volume per minute (6.06 $mL.100g^{-1}$ body weight, Elebute *et al.* 1978) until 40% of the total estimated blood volume had been withdrawn. The animals were then randomly allocated to groups

I-IV (see Table 4b.1) and resuscitated with one of the following given intravenously via the tail vein at the standard clinical rate of 1.1mL.kg^{-1}.min^{-1} (with the exception of 0.9% saline which was administered at the standard clinical rate for crystalloid solutions: 3.3mL.kg^{-1}.min^{-1}) and the standard clinical volume replacement (1:1 resuscitation volume:blood loss for whole blood and isotonic colloids, 3:1 resuscitation volume:blood loss for isotonic crystalloids, and 4mL.kg^{-1} for hypertonic solutions; ATLS guidelines)

- anti-coagulated (heparinised with Monoparin, CP Pharmaceuticals, UK) autologous blood (1:1 resuscitation volume:blood loss)
- isotonic colloid solution (modified gelatin, Haemaccel, Hoechst Marion Roussel, Germany, 1:1 resuscitation volume:blood loss)
- isotonic crystalloid solution (0.9% saline, Fresenius Kabi Ltd, UK, 3:1 resuscitation volume:blood loss)
- hypertonic saline/dextran (RescueFlow®; 7.5% saline/6% dextran 70, 4 mL.kg^{-1})

Each of the groups indicated above were resuscitated 20 min after the end of haemorrhage. The delay of 20 minutes between the end of haemorrhage and the onset of resuscitation was chosen empirically to reflect potential clinical practice.

Protocol



Group	Haemorrhage	Blast	Resuscitation Fluid	
I	+	+	blood	n=5
II	+	+	7.5% saline/ 6% dextran	n=5
III	+	+	modified gelatin (Haemaccel)	n=5
IV	+	+	0.9% saline	n=5

Figure 4b.1 Diagrammatic representation of the protocol used in this study (see section 4b.2.1 for full explanation). Plus sign (+) indicates presence of haemorrhage and blast injury in that group.

Table 4b.1 *Summary of treatments:* all groups were subjected to thoracic blast followed 10 min later by a progressive haemorrhage of 40% total estimated blood volume (BV) at 2% BV.min^{-1} then resuscitated with one of the following fluids;

Resuscitation fluid	Group (resuscitated 20 min after haemorrhage)
Anti-coagulated autologous blood (1:1 resuscitation volume:blood loss)	I
Hypertonic saline/dextran (7.5% saline/6% dextran 70, 4 mL.kg^{-1})	II
Isotonic colloid solution (modified gelatin, Haemaccel, 1:1 resuscitation volume:blood loss)	III
Isotonic crystalloid solution (0.9% saline, 3:1 resuscitation volume:blood loss)	IV

Cardiovascular measurements were made from 1 min before blast continuously until 5 min after blast, immediately before haemorrhage, after the removal of each aliquot of blood during haemorrhage, at 5 minute intervals following the end of haemorrhage for up to 20 minutes, immediately before resuscitation, at 5 min intervals during resuscitation and thereafter immediately, 5, 10, 15, 20, 25 and 30 minutes after resuscitation. Blood gas analysis (ABL5^{TM}, Radiometer, Denmark) and haematocrit values (Hawksley micro-haematocrit reader) were obtained for samples taken immediately before blast, the first and last samples taken during haemorrhage, immediately after resuscitation and at 15 and 30 minutes after resuscitation. Duration of apnoea was determined visually and timed using a stopwatch.

All animals were killed 30 minutes after the end of resuscitation with an overdose of 0.5mL of 60mg.mL^{-1} (106-127 mg.kg^{-1}) sodium pentobarbitone (Sagatal, Rhône

Mérieux (Ireland) Tallaght, Dublin) administered intravenously. The lungs were removed and weighed to determine Lung Weight Index (lung weight/body weight).

4b.3 Results

4b.3.1 *Baseline values*

Baseline (pre-blast) values for each group are presented in Table 4b.2. There were no significant differences between groups in the baseline cardiovascular or arterial blood gas variables, body weights or body temperature, with the following exceptions; ABE was significantly lower in Group II when compared to Groups I and IV, whereas arterial pH was significantly higher in Group I when compared to Group III. However, although these differences were statistically significant they were very small compared to the effects of blast and/or haemorrhage and were of little physiological significance. Femoral vascular resistance was significantly higher in Group III compared to Groups I and II. Body temperature did not change significantly during the course of the study in any group.

	Group I	**Group II**	**Group III**	**Group IV**
n	5	5	5	5
Body wt (g)	258±8	254±8	252±3	248±3
HP (ms)	154.7±4.4	147.2±2.2	149.5±4.6	146.6±3.4
MBP (mmHg)	106.2±4.2	101.4±2.2	105.8±4.2	106.8±1.9
Fem Q ($mL.min^{-1}$)	1.1±0.3	1.2±0.1	0.8±0.1	1.1±0.1
FVR ($mmHg.min.mL^{-1}$)	70.5±6.2	88.5±6.5	155.9±24.9	99.1±11.2
PaO_2 (mmHg)	91.0±1.2	87.2±1.3	87.0±1.3	88.8±2.9
$PaCO_2$ (mmHg)	34.6±1.2	32.4±0.8	36.2±0.9	37.8±2.2
a pH	7.37±0.01	7.35±0.00	7.35±0.00	7.37±0.01
ABE (mM)	-3.5±0.87	-6.2±0.4	-5.0±0.6	-3.2±0.7
Hcrit (%)	31.5±1.9	31.6±0.8	34.1±1.0	31.8±1.8
Temp (oC)	37.5±0.4	37.8±0.1	37.1±0.2	37.3±0.2

Table 4b.2 Baseline (pre-blast) values recorded in four groups of anaesthetised rats. Number of rats (*n*); body weight (body wt); Heart period (HP); mean arterial blood pressure (MBP); femoral arterial blood flow (Fem Q); femoral vascular resistance (FVR); arterial oxygen tension (PaO_2); arterial carbon dioxide tension ($PaCO_2$); arterial pH (a pH); actual base excess (ABE); haematocrit (Hcrit); and body temperature (Temp). Values are mean ± SEM.

The effects of blast, haemorrhage and subsequent resuscitation were qualitatively similar to those reported for early resuscitation (see Chapter 4a).

4b.3.2 *Effects of thoracic blast*

Thoracic blast (Group I) produced a significant increase in heart period of 388±64.4 ms from a pre-blast control of 154.7±4.4 ms and a significant fall in mean blood pressure of 71.1±2.9 mmHg from a pre-blast level of 106.2±4.2 mmHg (Figure 4b.2). Thereafter there was a rapid recovery in heart period and a partial recovery in mean arterial blood pressure (MBP).

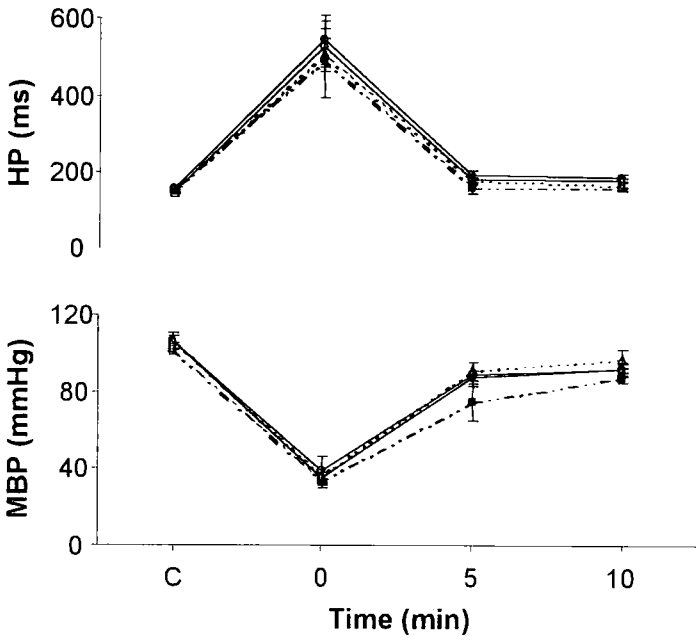


Figure 4b. 2 Effects of a thoracic blast injury in anaesthetised rats on heart period (HP) and mean arterial blood pressure (MBP) in Group I (●), Group II (■), Group III (o) and Group IV (Δ). Data recorded immediately before blast (C), and thereafter immediately after blast (0), and at 5 and 10 minutes after blast. Values are means±S.E.M.

In addition, thoracic blast produced a significant (Student's independent *t* test) apnoea lasting 21.4±2.7 seconds in Group I (Figure 4b.3).

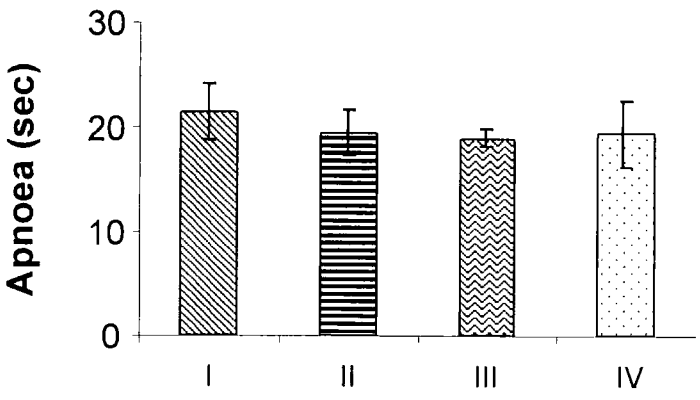


Figure 4b.3 Duration of apnoea following primary thoracic blast injury in four groups of anaesthetised rats. Values are means±S.E.M.

Furthermore, in Group I, blast produced a transient fall in femoral arterial blood flow of 0.81±0.28 mL.min^{-1} from a pre-blast control of 1.1±0.25 mL.min^{-1} (Figure 4b.4), and a transient fall in femoral arterial vascular resistance of 16.0±3.5 mmHg.min.mL^{-1} from a pre-blast level of 70.5±6.2 mmHg.min.mL^{-1} (Figure 4b.4).

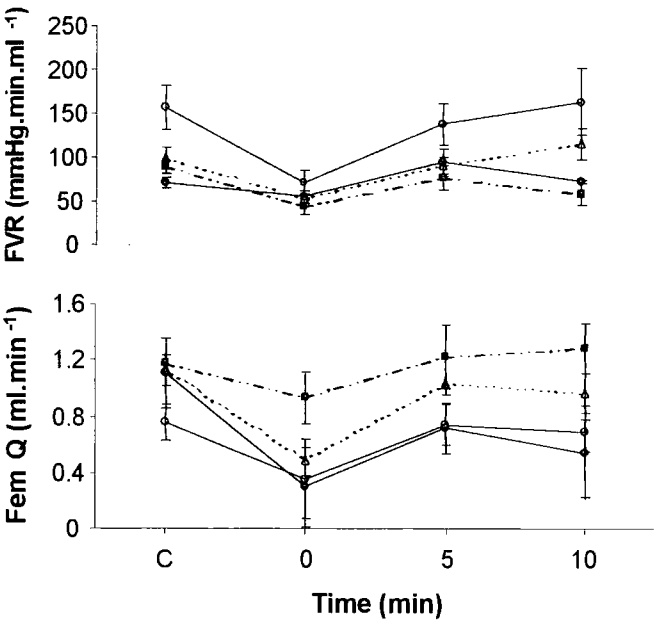


Figure 4b.4 Effects of a thoracic blast injury in anaesthetised rats on femoral arterial vascular resistance (FVR) and femoral arterial blood flow (Fem Q) in Group I (●), Group II (■), Group III (o) and Group IV (Δ). Data recorded immediately before blast (C), and thereafter immediately after blast (0), and at 5 and 10 minutes after blast. Values are means±S.E.M.

Ten minutes after blast heart period was still elevated and MBP was still significantly below pre-blast levels in Group I. Following blast there was a significant fall in PaO_2 of 23.40±1.17 mmHg in Group I, from a control pre-blast value of 91.00±1.22 mmHg, and a decrease in arterial pH of 0.05±0.01 from a pre-blast control of 7.37±0.01 (Figure 4b.5).

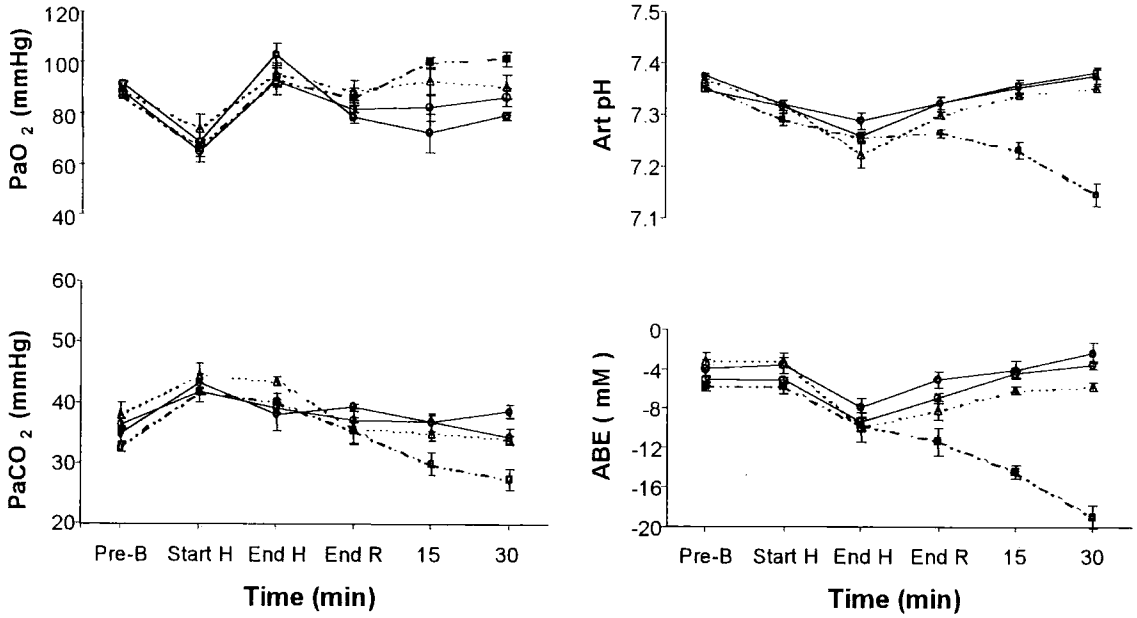


Figure 4b.5 Effects of blast, haemorrhage and subsequent resuscitation in anaesthetised rats on arterial oxygen tension (PaO_2), arterial carbon dioxide tension ($PaCO_2$), arterial pH (art pH) and arterial base excess (ABE). Group I; blood (•), Group II; hypertonic saline/dextran (■), Group III; haemaccel (o) and Group IV; 0.9% saline (Δ). Data recorded immediately before blast (Pre-B), at the start of a haemorrhage of 40% total blood volume (Start H), at the end of haemorrhage (End H), at the end of fluid resuscitation (End R) and thereafter at 15 and 30 minutes after resuscitation. Values are means±S.E.M.

Following thoracic blast there was a significant rise in $PaCO_2$ in Group I, of 8.6±1.5 mmHg from a pre-blast control level of 34.6±1.2 mmHg (Figure 4b.5) and haematocrit increased significantly following blast from a pre-blast level of 31.5±1.9 % to 38.1±0.49 % (Figures 4b.6). There was no significant change in ABE during this period (Figure 4b.5). Thoracic blast in Groups II-IV produced effects on the above parameters similar to those seen in Group I; there were no significant differences between groups for the first 10 minutes after blast.

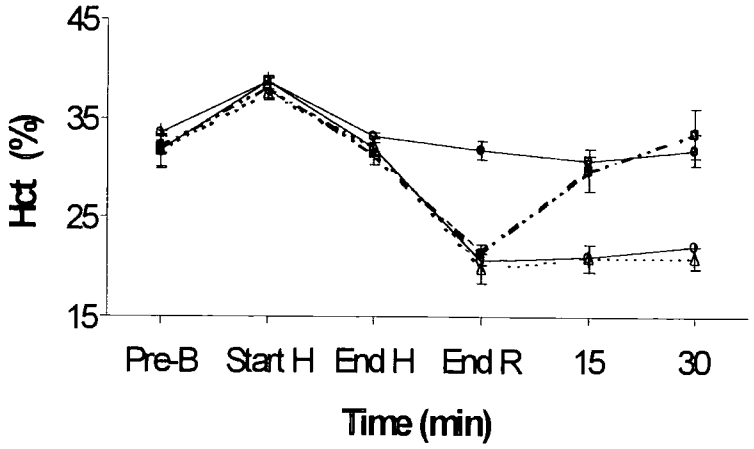


Figure 4b.6 Effects of blast, haemorrhage and subsequent resuscitation in anaesthetised rats on haematocrit (Hct). Group I; blood (•), Group II; hypertonic saline/dextran (■), Group III; haemaccel (o) and Group IV; 0.9% saline (Δ). Data recorded immediately before blast (Pre-B), at the start of a haemorrhage of 40% total blood volume (Start H), at the end of haemorrhage (End H), at the end of fluid resuscitation (End R) and thereafter at 15 and 30 minutes after resuscitation. Values are means±S.E.M.

4b.3.3 *Effects of progressive haemorrhage*

Haemorrhage of 40% blood volume, initiated 10 minutes after thoracic blast, induced a significant change in heart period (Figure 4b.7) and mean blood pressure in all groups (Figure 4b.7). There was no evidence of the first, tachycardic, phase of the response to blood loss normally associated with haemorrhage in the absence of thoracic blast injury. The following section will compare the peak changes in heart period from each individual animal.

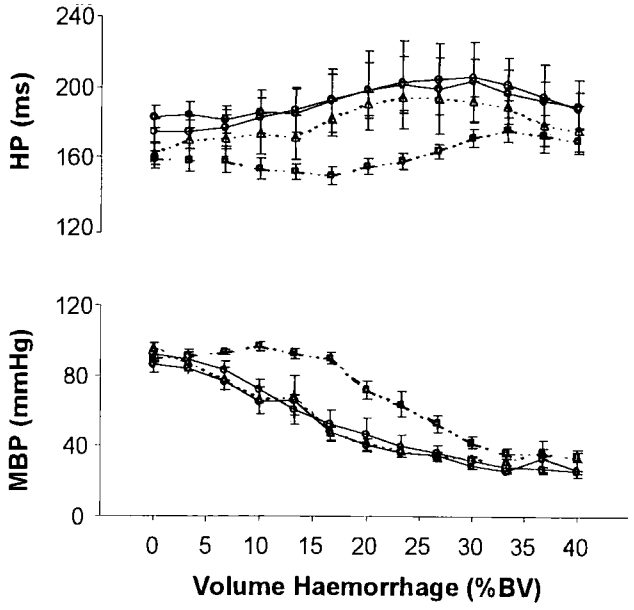


Figure 4b.7 Effects of a progressive haemorrhage of 40% total blood volume following a thoracic blast injury in anaesthetised rats on heart period (HP) and mean arterial blood pressure (MBP) in Group I (•), Group II (■), Group III (o) and Group IV (Δ). Values are means±S.E.M.

Animals in Group I showed no significant tachycardia, while the bradycardia (significant peak increase in heart period of 13.4±2.1 ms; Student's paired *t* test, geometric mean) was seen after the loss of 20.0±7.0 % blood volume (Figure 4b.7). Furthermore, mean arterial blood pressure was not maintained in Group I during the haemorrhage and began to fall after the removal of the first aliquot of blood, the hypotension being significant after the loss of 16.7 %BV (Figure 4b.7). There was no significant difference between groups in the peak increase in heart period seen during blood loss. There was no difference in the pattern of change in blood pressure induced by haemorrhage between groups I, III and IV, however the pattern was significantly different in group II where blood pressure was initially maintained before falling as blood loss exceeded 23.3%BV. Associated with the fall in arterial blood pressure there were significant reductions in femoral arterial flow in all groups (Figure 4b.8). However, there was no evidence of a change in femoral vascular resistance during the blood loss (Figure 4b.8).

By the end of the haemorrhage PaO_2 (Group I) had increased significantly by 34.4±5.41 mmHg from a pre-haemorrhage value of 67.6±1.17 mmHg (Figure 4b.5) while there was no significant change in $PaCO_2$ (Figure 4b.5). There was a significant fall in arterial pH and base excess (Group I) of 0.03±0.02 (pH) and 4.0±1.1 mM from a pre-haemorrhage level of 7.32±0.01 (pH) and –4.0±0.9 mM respectively (Figure 4b.5). Haematocrit (Group I) also fell significantly by 5.2±0.5 % from a pre-haemorrhage control of 38.1±0.5 % (Figure 4b.6). There were no significant differences between groups in the pattern of blood gas and haematocrit changes during haemorrhage.

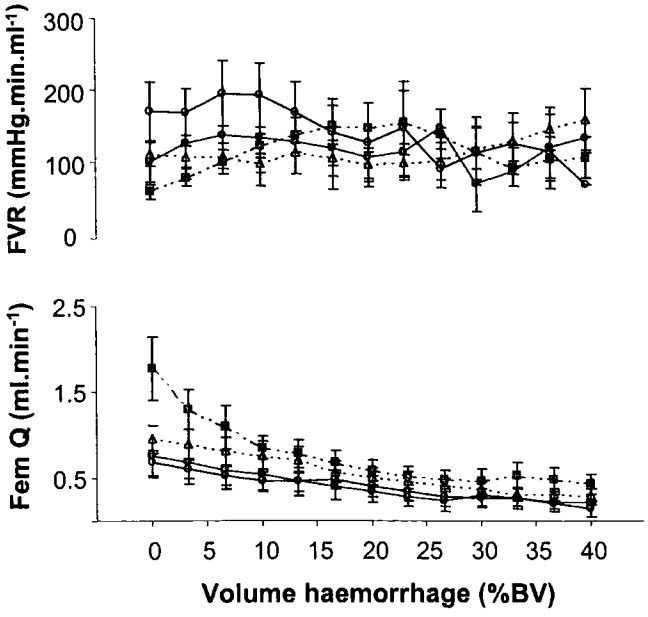


Figure 4b. 8 Effects of a progressive haemorrhage of 40% total blood volume following a thoracic blast injury in anaesthetised rats on femoral arterial vascular resistance (FVR) and femoral arterial blood flow (Fem Q) in Group I (●), Group II (■), Group III (o) and Group IV (Δ). Values are means!S.E.M.

4b.3.4 *Effects of 20 minute post-haemorrhage hypotensive period*

Following a 40% haemorrhage MBP (Group I) increased significantly over a 20 minute period by 29.7±5.0 mmHg from an end-haemorrhage level of 26.1±3.5 mmHg (Figure 4b.9). There was also a fall in heart period over this time, but this did not attain statistical significance (Group I; Figure 4b.9).

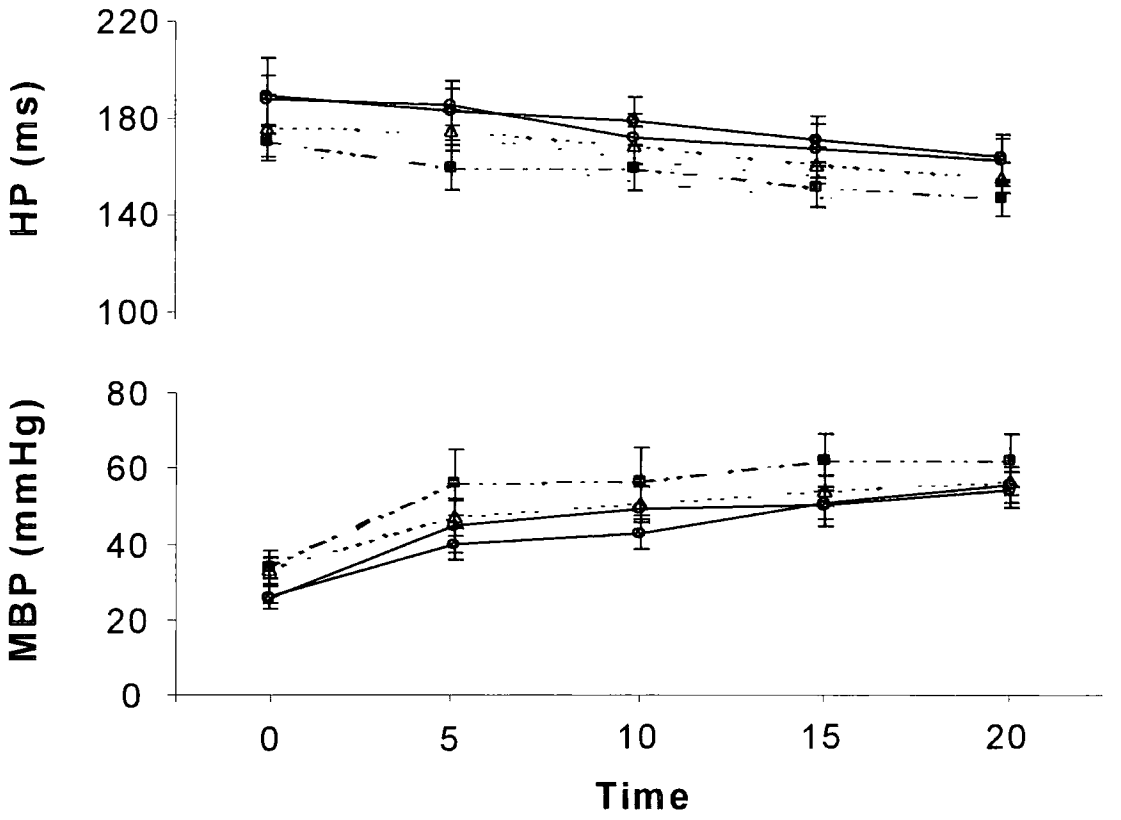


Figure 4b.9 Heart period (HP) and mean arterial blood pressure (MBP) following thoracic blast injury and a progressive haemorrhage of 40% total blood volume in anaesthetised rats. Group I (•), Group II (■), Group III (o) and Group IV (Δ). Data recorded immediately at the end of haemorrhage (0), 5, 10, 15 and 20 minutes after haemorrhage. Values are means±S.E.M.

Concomitant with the rise in blood pressure, there was a significant increase in femoral blood flow during the 20 min after blood loss, but there was no significant change in femoral vascular resistance (Figure 4b.10). There were no significant differences between groups in heart period, mean blood pressure, femoral blood flow or femoral vascular resistance during this 20 minute post-haemorrhage period.

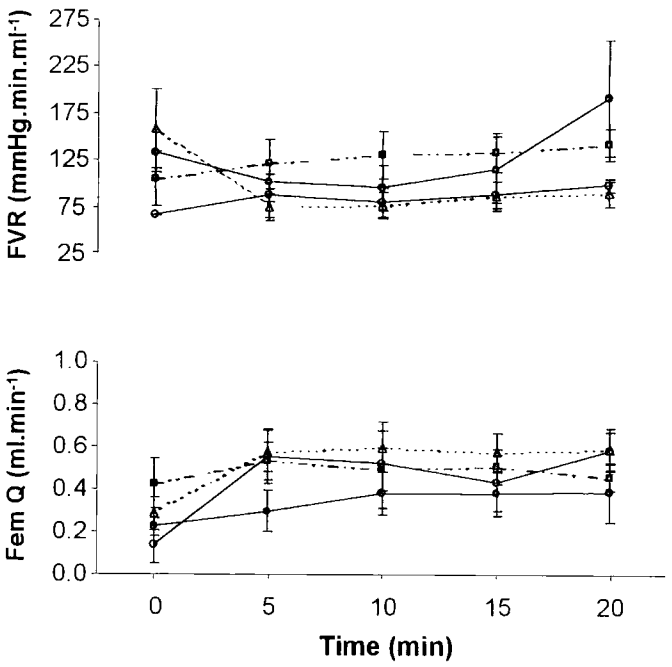


Figure 4b.10 Femoral arterial vascular resistance (FVR) and femoral arterial blood flow (Fem Q) following thoracic blast injury and a progressive haemorrhage of 40% total blood volume in anaesthetised rats. Group I (●), Group II (■), Group III (o) and Group IV (Δ). Data recorded immediately at the end of haemorrhage (0), 5, 10, 15 and 20 minutes after haemorrhage. Values are means±S.E.M.

4b.3.5 *Effects of resuscitation 20 minutes after haemorrhage*

Twenty minutes after the end of haemorrhage animals in groups I-IV were resuscitated with autologous blood (Group I), hypertonic saline/dextran (Group II), colloid (Haemaccel, Group III) or 0.9% saline (Group IV). Resuscitation with whole blood (Group I) induced a significant elevation in mean arterial blood pressure of 57.35±6.8 mmHg from a pre-resuscitation level of 55.84±4.9mmHg (Figure 4b.11). Although each fluid produced a significant increase in blood pressure, resuscitation with whole blood produced a significantly greater increase in blood pressure than was seen after resuscitation with HSD. There were no other differences between groups in the *initial* increase in blood pressure produced by resuscitation. Thereafter MBP was maintained for the remainder of the study in groups I, III, and IV (Figure 4b.11). However, MBP was not maintained after resuscitation with hypertonic saline/dextran (Group II). Within 5 minutes of resuscitation MBP in Group II had fallen significantly (compared to the other groups) by 23.2±5.2 mmHg from an end-resuscitation level of 92.1±5.5 mmHg, and continued to fall over the subsequent 5 minutes, thereafter remaining low for the remainder of the study (Figure 4b.11).

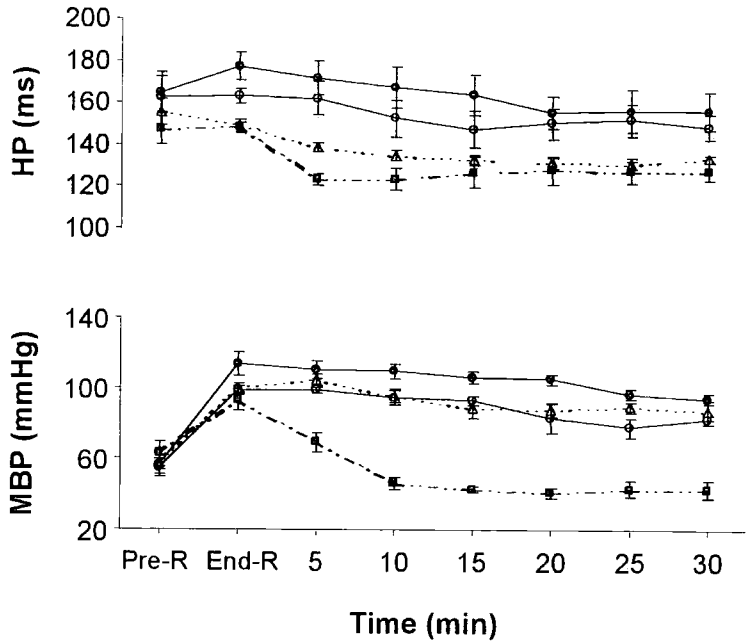


Figure 4b.11 Effects of resuscitation with various fluids following thoracic blast injury and subsequent haemorrhage of 40% total blood volume in anaesthetised rats, on heart period (HP) and mean arterial blood pressure (MBP) in Group I; blood (●), Group II; hypertonic saline/dextran (■), Group III; haemaccel (o) and Group IV; 0.9% saline (Δ). Data recorded immediately before resuscitation (Pre-R) and thereafter immediately after resuscitation (End-R) and at 5, 10, 15, 20, 25 and 30 minutes after resuscitation. Values are means±S.E.M.

There was no significant change in heart period immediately after resuscitation in any group (Figure 4b.11). However, within 10 minutes of resuscitation with HSD (Group II) heart period had fallen significantly by 24.9±2.6 ms from the end-resuscitation level of 147.9±2.4 ms (Figure 4b.11). Thereafter there was no significant change in heart period in any group for the remainder of the study (Figure 4b.11).

Concomitant with the increase in blood pressure, there was a significant increase in femoral blood flow in Group I from a pre-resuscitation level of 0.39± 0.14 $mL.min^{-1}$ to 1.05± 0.32 $mL.min^{-1}$ (Figure 4b.12). The increase in blood flow was significantly greater in the saline-treated group (Group IV) compared to the Haemaccel-treated group (Group III), there being no other differences between groups up to this time point (Figure 4b. 12). Thereafter blood flow was not sustained in Group II (HSD) and showed a persistent fall until the end of the study (Figure 4b. 12).

There was a small fall in femoral vascular resistance immediately following resuscitation in Groups I, II and IV, although this change was not statistically significant in any group (Figure 4b.12). Overall, there was a significant increase in vascular

resistance during the 30 min post-resuscitation, this effect being most pronounced in the HSD and Haemaccel-resuscitated groups (Groups II and III respectively, Figure 4b.12).

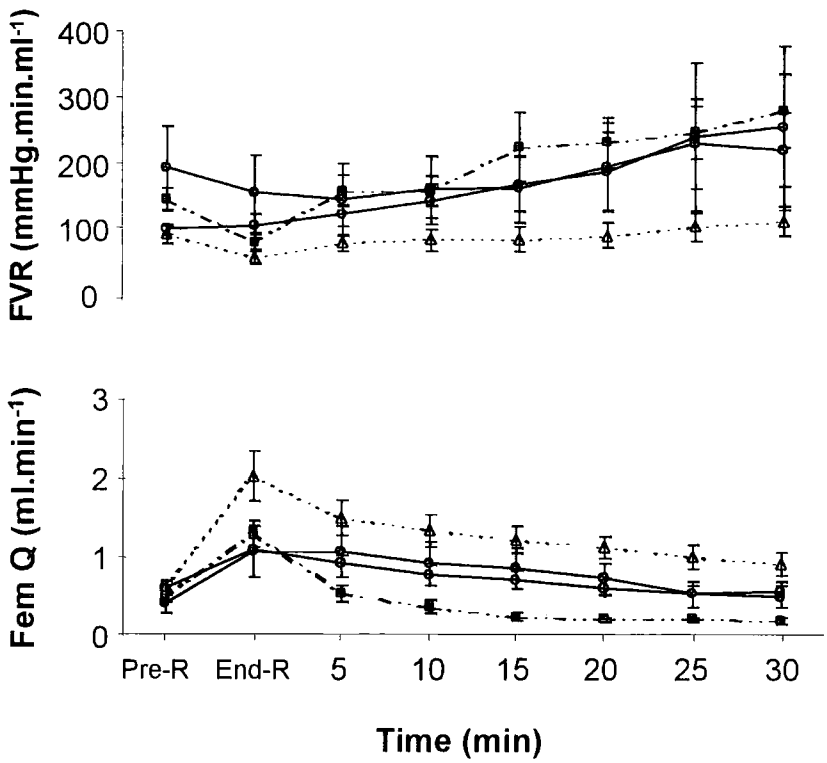


Figure 4b.12 Effects of resuscitation with various fluids following thoracic blast injury and subsequent haemorrhage of 40% total blood volume in anaesthetised rats, on femoral arterial vascular resistance (FVR) and femoral arterial blood flow (Fem Q) in Group I; blood (●), Group II; hypertonic saline/dextran (■), Group III; haemaccel (o) and Group IV; 0.9% saline (Δ). Data recorded immediately before resuscitation (Pre-R) and thereafter immediately (End-R) and at 5, 10, 15, 20, 25 and 30 minutes after resuscitation. Values are means±S.E.M.

Immediately after resuscitation there were significant falls in PaO_2: in Group I PaO_2 fell by 23.2±2.5 mmHg from a pre-resuscitation value of 102.0±5.4 mmHg (Figure 4b.5). There were no differences in the fall in PaO_2 between groups immediately after resuscitation. Thereafter PaO_2 was maintained without any further significant change in groups I, III and IV for the remainder of the study. However, PaO_2 rose significantly by 16.8±3.0 mmHg over the subsequent 30 min in the group resuscitated with hypertonic saline/dextran from 86.0±4.2 mmHg immediately following resuscitation. $PaCO_2$ did not change significantly following resuscitation (Figure 4b.5) except in the group given hypertonic saline/dextran where it fell significantly by 8.1±1.7 mmHg 30 min after resuscitation, from 35.3±2.1 mmHg immediately after resuscitation. After resuscitation there was a significant rise in arterial pH in groups I, III and IV. Arterial base excess (ABE) also increased significantly following resuscitation in Groups I, III and IV: in

Group I this increase amounted to 2.6±0.9 mM from a pre-resuscitation value of –8.0±1.1 mM, and continued to rise over the next 30 minutes. There was no difference in the rise of ABE between groups I, III and IV immediately following resuscitation and throughout the remainder of the study. Arterial pH did not change during resuscitation in Group II, however, base excess in Group II (HSD) fell significantly from a pre-resuscitation value of –9.2±0.4 mM to –10.3±1.0 mM immediately post resuscitation. Arterial pH did fall in Group II in the post resuscitation phase and both arterial pH and ABE continued to fall significantly during the 30 minutes post-resuscitation in these animals treated with hypertonic saline/dextran (Figure 4b.5). Arterial pH and base excess were significantly lower 15 and 30 minutes after resuscitation with hypertonic saline/dextran compared with the other groups, reaching levels of 7.15±0.02 (pH) and –19.0±1.4 mM (ABE) by 30 minutes post resuscitation with this fluid.

In Group II haematocrit fell significantly immediately following resuscitation by 11.6±1.2 % from a pre-resuscitation level of 32.4±0.4 %. There was no significant difference between any groups given asanguinous fluids (Figure 4b.6). However, haematocrit did not change following resuscitation in the animals treated with autologous blood (Group I; Figure 4b.6). Haematocrit was subsequently maintained at post-resuscitation levels in the groups treated with blood, haemaccel and 0.9% saline. By contrast haematocrit increased significantly in the group treated with hypertonic saline/dextran to 33.8±3.1 % by 30 minutes after resuscitation. Haematocrit was significantly higher at this point in animals treated with hypertonic saline/dextran than in those given haemaccel or 0.9% saline but not significantly different from those resuscitated with blood (Figure 4b.6). Indeed, 30 minutes after resuscitation with hypertonic saline/dextran haematocrit was higher than that seen immediately *before* resuscitation (Figure 4b.6).

Post mortem Lung Weight Indices were not significantly different between groups (Figure 4b.13).

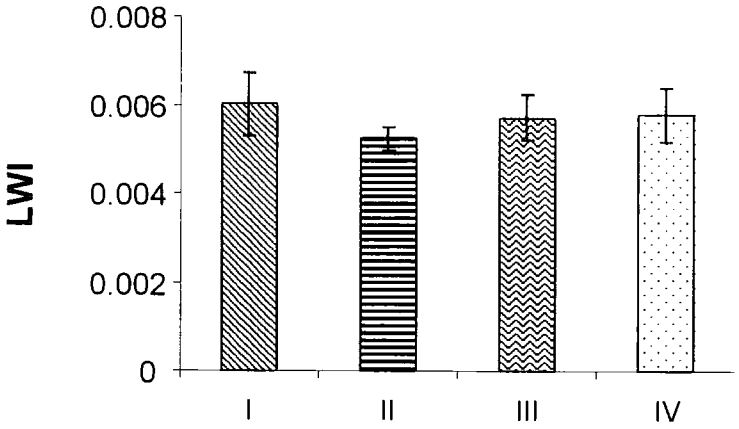


Figure 4b.13 Lung weight index (LWI), calculated as *post mortem* lung weight/body weight, following thoracic blast injury, subsequent haemorrhage of 40% total blood volume and resuscitation with autologous whole blood (I), hypertonic saline/dextran (II), colloid (III) and 0.9% saline (IV) in anaesthetised rats. Values are means±S.E.M.

4b.4 Discussion

Thoracic blast injury induced the expected bradycardia, transient apnoea and hypotension, which was rapidly but partially resolved. The response to subsequent controlled haemorrhage consisted of a progressive bradycardia and hypotension in Groups I, III and IV as has been reported previously (Chapters 3 & 4a). However, this was not apparent in Group II where blood pressure was maintained during the first 23% of blood loss (Figure 4b.7). It is not immediately obvious why the pattern of response to haemorrhage is different in Group II compared to the other three groups. One possible reason could be that the magnitude of the blast injury was less in Group II than in the other three groups. However, the physiological response to blast itself, duration of apnoea, was not different between any of the groups (Figure 4b.3).

The new findings from the study relate to the effects of subsequent *delayed* resuscitation and a comparison of the efficacy of four resuscitation fluids: whole (autologous) blood, hypertonic saline/dextran, isotonic colloid (modified gelatin, Haemaccel) and 0.9% saline. The outcome of delayed resuscitation with these four fluids was not significantly different to that seen with early resuscitation: resuscitation with the asanguinous fluids restored arterial blood pressure and femoral blood flow to pre-haemorrhage levels whilst blood restored these parameters to higher (pre-blast) levels. The effects of blood, colloid and 0.9% saline were sustained for the following 30 minutes until the end of the study and resulted in a significant improvement (increase) in arterial pH and base

excess. By contrast the effects of hypertonic saline/dextran were very short-lived with arterial blood pressure and femoral flow falling within five minutes of resuscitation. By comparing data recorded 15 minutes after resuscitation with hypertonic saline/dextran in the 'early' resuscitation series in Chapter 4a (Figures 4a.11 and 4a.12; approximately 20 minutes after blood loss) with that recorded 20 minutes after haemorrhage (Figures 4b.9 and 4b.10; immediately before resuscitation) in the present series, it can be seen that the blood pressure and flow were lower in animals resuscitated with hypertonic saline/dextran compared to no resuscitation at approximately equal times after blood loss. Thus, within this timescale, resuscitation with hypertonic saline/dextran appears to be deleterious compared to no resuscitation. This is in agreement with a study where survival rates were significantly higher in those left unresuscitated when compared to those resuscitated with hypertonic saline (Solomanov *et al.* 2000). Perhaps then it is the hypertonic component of the HSD that is producing the deleterious effects following thoracic blast injury and haemorrhage in this study. Accompanying the fall in blood pressure and flow was a marked fall in arterial pH and base excess after resuscitation with hypertonic saline/dextran, indicating a developing metabolic acidosis. This effect was not apparent in the other groups which is in contrast to a study in 1999 by Baron and co-workers in which delayed resuscitation of a pressure controlled haemorrhage in pigs, with blood and normal saline resulted in an increase in serum lactate levels and an increase in base deficit. However, that study did not evaluate the effect of delayed resuscitation with HSD. As with early resuscitation, hypertonic saline/dextran did not attenuate any lung oedema since there were no differences in Lung Weight Indices between groups (Figure 4b.13).

The results of Chapter 4 indicate that early and late resuscitation were equally effective as judged by gross haemodynamic and acid-base status after thoracic blast injury and blood loss. This is in contrast to many studies reported in the literature where resuscitation too early after an uncontrolled haemorrhage in an animal model resulted in an increased mortality (Bickell *et al.* 1992; Krausz *et al.* 1992; Marshall *et al.* 1997; Stern *et al.* 2000). This effect was apparent regardless of the type of fluid used, e.g., early resuscitation of haemorrhage with HSD or Ringers Lactate solution (RL) showed a significant increase in mortality (Bickell *et al.* 1992). Hypertonic saline resuscitation within 15 minutes of bleeding led to an increased blood loss and early death (Krausz *et al.* 1992). In a study of uncontrolled haemorrhage in rats, early resuscitation with RL led to the deaths of all animals in this group (Marshall *et al.* 1997). Finally, Stern and

colleagues (2000) showed that following an uncontrolled haemorrhage early increases in blood pressure and flow resulting from bolus infusions of HSD, resulted in a greater haemorrhage volume and mortality compared to slow infusion of HSD which allowed comparable increases in blood pressure and flow late in resuscitation. As mentioned earlier, some studies reported higher survival rates in subjects left unresuscitated. This was reported in both rats (Solomonov *et al.* 2000) and humans (Bickell *et al.* 1994) suggesting that resuscitation should be minimised or even withheld until control of bleeding is gained. However, Burris *et al.* showed in 1999 that controlled resuscitation with hypertonic hydroxyethyl starch or RL, to maintain a hypotensive state following an uncontrolled haemorrhage, increases survival when compared to no fluid resuscitation. This suggests that the deleterious nature of fluid resuscitation is due to the re-establishment of 'normal' blood pressure which may lead to an increase in blood loss. However, it must be stressed that the haemorrhage model used in this study is not uncontrolled but volume controlled.

As the deleterious outcome of resuscitation of thoracic blast injury and blood loss with HSD does not seem to be due to the timing of the resuscitation, as both early (section 4a) and late (section 4b) resuscitation periods were equally effective after thoracic blast injury and haemorrhage, the next chapter will aim to determine if hypertonicity plays a part in this response. Studies comparing isotonic fluids with hypertonic fluid resuscitation have yielded conflicting results. Kreimeier and co-workers (1997) concluded that survival rates were higher when severely injured patients were resuscitated with hypertonic saline when compared to isotonic saline. However, a meta-analysis of controlled clinical studies concluded no additional benefit with hypertonic saline resuscitation over isotonic solutions, but did however conclude that resuscitation of traumatic hypotension with HSD is likely to be more effective (Wade *et al.* 1997).

In conclusion Chapter 4 has shown that resuscitation of animals following primary thoracic blast injury and severe haemorrhage after an early (5 minute), and delayed (20 minute) resuscitation period with whole blood, isotonic saline or colloids (Haemaccel, early and delayed resuscitation; hydroxyethyl starch, early resuscitation), will result in a restoration of arterial blood pressure, femoral arterial blood flow and acid base status which will be sustained for at least 30 minutes. The use of hypertonic saline/dextran is contraindicated following primary thoracic blast and haemorrhage since the effect is not sustained, and indeed there is some evidence that within 15 minutes of resuscitation

with hypertonic saline/dextran animals have a lower arterial blood pressure and femoral arterial blood flow compared to those left unresuscitated.

5 Further Studies on Resuscitation Following Blast Injury and Haemorrhage: Comparison of Hypertonic Saline/Hydroxyethyl Starch, Hypertonic Saline and Isotonic Hydroxyethyl Starch with Hypertonic Saline/Dextran

5.1 Introduction

The previous chapter (chapter 4) compared the effectiveness of resuscitation with five different fluids, autologous blood, modified gelatin solution, 0.9% saline, hypertonic saline/dextran (HSD) and isotonic hydroxyethyl starch. These studies investigated the effect of resuscitation after blast and 40% haemorrhage following a short (5 minutes, section 4a), for all fluids, and long (20 minutes, section 4b) shock phase for all fluids except isotonic hydroxyethyl starch.

The results demonstrated that all types of asanguinous fluids evaluated were able to restore mean arterial pressure and femoral blood flow following their administration, whether this was 5 or 20 minutes after the haemorrhage. Autologous blood restored these parameters to values higher than the pre-blast level. The cardiovascular improvements with colloid, crystalloid and autologous blood were sustained following infusion and there were no apparent detrimental side effects (as has previously been reported with infusion of crystalloid alone in animals receiving blast; Wikoff *et al.* 1999). By comparison, HSD had a short-lived effect, causing an initial improvement in mean arterial pressure, but within 5 minutes of infusion in both studies (short and delayed resuscitation periods, chapters 4a and 4b respectively) there was a significant reduction in both mean arterial blood pressure and femoral arterial blood flow. At the same time, there was rapid development of a metabolic acidosis. Indeed, by comparing data recorded 15 minutes after resuscitation with hypertonic saline/dextran in the 'early' resuscitation series in chapter 4a (Figures 4a.11 and 4a.12; approximately 20 minutes after blood loss) with that recorded 20 minutes after haemorrhage (Figures 4b.9 and 4b.10; immediately before resuscitation) in chapter 4b, it can be seen that mean arterial blood pressure and femoral arterial blood flow were lower in animals resuscitated with hypertonic saline/dextran compared to no resuscitation at approximately equal times after blood loss. Thus, within this timescale, resuscitation with hypertonic saline/dextran

appears to be deleterious compared to no resuscitation. *Post mortem* examination showed no qualitative difference in gross lung pathology, as determined by the lung weight/body weight ratio, between any of the treatment groups indicating that non of the fluids tested either attenuated or augmented any pulmonary oedema that may be present as a result of the blast injury (Guy *et al.* 1998).

It is generally accepted that resuscitation with hypertonic saline confers greater benefits than resuscitation with isotonic crystalloids and some studies suggest that the addition of dextran confers additional benefit. This benefit seems to include a reduced ischaemia/reperfusion induced inflammatory response (Ciesla *et al.* 2001; Rizoli *et al.* 1998) and a reduction in tissue oedema. However, these conclusions are not universally accepted and the controversy may reflect different types of insult in various studies as will be discussed below.

The use of hypertonic saline (HS) for resuscitation after haemorrhagic shock is reported to improve tissue perfusion especially to the splenic and hepatic vascular bed when compared to Lactate Ringers solution (Kien *et al.* 1991) and limits the inflammatory response (Ciesla *et al.* 2001; Rizoli *et al.* 1998) by decreasing CD18 expression thereby limiting neutrophil adhesion (Rhee *et al.* 2000. See chapter 4a, section 4a.4.4 and Table 4a.3). Hypertonic saline resuscitation will also return neutrophil activation back to baseline following an increase due to resuscitation with Lactate Ringers (LR) solution (Rhee *et al.* 1998). Ischaemia/reperfusion-induced mRNA expression of the intercellular adhesion molecule ICAM-1 was suppressed in the hepatic circulation following resuscitation with hypertonic saline, leading to a decrease in leukocyte adhesion to the endothelial cells (Oreopoulos *et al.* 2000; Mazzoni, 1990; Sheilds *et al.* 2000). This is also reported to occur in the pulmonary circulation (Rizoli *et al.* 1998). HS resuscitation was shown to decrease pulmonary oedema following acid aspiration-induced lung injury (Rabinovici *et al.* 1996) and following a systemic inflammatory response to acute pancreatitis (Sheilds *et al.* 2000). However, this is not reported to be the case after pulmonary contusion (Cohn *et al.* 1997). Several studies looking at survival rates, comparing resuscitation fluids administered after haemorrhagic shock and in severely injured patients (Vassar *et al.* 1993) concluded that an increase in survival with hypertonic saline resuscitation was apparent when compared to isotonic saline (Kreimeier *et al.* 1997; Solomonov *et al.* 2000). However, a meta-analysis of controlled clinical studies concluded that HS was not more effective than standard isotonic

solutions for treating traumatic hypotension and that HSD was likely to be more effective (Wade *et al.* 1997b). This was not the case following severe injuries where the addition of dextran was reported to be of no benefit (Vassar *et al.* 1993). Waagstein and colleagues showed in 1997 that HS reversed ischaemia-induced haemodynamic and tissue metabolic disturbances with or without the addition of dextran. However, O'Benar reported in 1998 that HSD did not correct severe haemorrhage-induced metabolic disturbance, as ABE remained low.

Bigger molecules such as dextran or starch are added to HS to increase the oncotic pressure and prolong its volume expansion properties as fluid will still leak out of the vessel as sodium moves down its concentration gradient, taking fluid with it by osmosis. The addition of dextran in particular is reportedly beneficial due to, for example, its free radical scavenging properties amongst other reported benefits (see chapter 4a, section 4a.4.4). Consistent with this argument others have reported a significant attenuation of leukocyte adhesion in the hepatic microcirculation with HSD after haemorrhagic shock when compared to hypertonic hydroxyethyl starch (HHES; Bauer *et al.* 1993), but Vollmar and colleagues (1994) found HHES resuscitation restored the hepatic microcirculation and prevented the reperfusion-induced leukocyte stasis and adherence following haemorrhagic shock. In addition, a positive inotropic effect of HHES was shown in healthy volunteers, though it is thought this is unlikely to be clinically relevant (Goetz *et al.* 1995).

The picture becomes more complicated with studies of resuscitation following uncontrolled haemorrhage from a discreet lesion such as a large artery. Riddez reported in 1998 that 5 out of 8 pigs did not survive after HSD resuscitation of an uncontrolled haemorrhage, even if only two thirds of the recommended dose ($2.65 mL.kg^{-1}$ compared to the recommended $4 mL.kg^{-1}$) was administered (Riddez *et al.* 1998). However, this may reflect problems with rapid elevation of blood pressure causing further haemorrhage rather than a particular problem with hypertonic solutions.

The results from Chapter 4, have indicated a significant potential problem with using HSD in the resuscitation of the blast casualty. It is unclear whether this represents a generalised problem with hypertonic solutions when used after primary thoracic blast injury and blood loss or whether it is the dextran component of the HSD fluid. Therefore the present study aims to compare the effects of hypertonic saline alone (HS)

and together with hydroxyethyl starch (HHES), and isotonic hydroxyethyl starch (HES), with hypertonic saline dextran (HSD) administered 5 minutes after the end of a haemorrhage following primary thoracic blast injury.

5.2 Methods

The experiments were conducted on male Wistar rats (Harlan Orlac, body weight 232-283g), which were terminally anaesthetised and prepared for recording as described in Chapter 2.

5.2.1 *Experimental protocol*

Upon completion of the surgery the rats were positioned supine in the blast apparatus with the ventral thorax 3.5 cm below the blast nozzle (which delivers the blast wave to the animal). The animals were then allowed to stabilise for 1 h under alphadolone/alphaxolone (Saffan^{TM}, Pitman-Moore, UK) anaesthesia prior to exposure to blast.

The infusion rate of anaesthetic was adjusted within the range 19-22 $mg.kg^{-1}.h^{-1}$ to maintain an experimental level of anaesthesia (mild withdrawal and a pressor response of approximately 10 mmHg to a noxious pinch of the foot).

All animals were then treated in an identical manner to that reported in Chapter 4 (see section 4b.2.1) with regards to the administration of the blast injury and 40% haemorrhage.

Following baseline cardiovascular, respiratory and blood gas measurements the protocol shown diagrammatically in Figure 5.1 was then followed. The animals were allocated randomly to groups (see Table 5.1) and resuscitated with one of the following fluids given intravenously via the tail at the standard clinical rate of $1.1mL.kg^{-1}.min^{-1}$ (ATLS guidelines) 5 minutes after the end of haemorrhage:

- hypertonic saline/hydroxyethyl starch (7.5% saline/7% hydroxyethyl starch, HyperHes, Monoflac, Germany; $4\ mL.kg^{-1}$)

- hypertonic saline (7.5% saline; 4 $mL.kg^{-1}$)
- isotonic hydroxyethyl starch solution (HAES-Steril, Fresenius, UK; 1:1 resuscitation volume: blood loss)

In addition, data from animals treated with hypertonic saline/dextran (RescueFlow®; 7.5% saline/6% dextran 70, 4 $mL.kg^{-1}$) are presented in this chapter. Animals were not randomised into this group in the present study. The data are derived from a study reported previously in Chapter 4a and are included here to facilitate comparison.

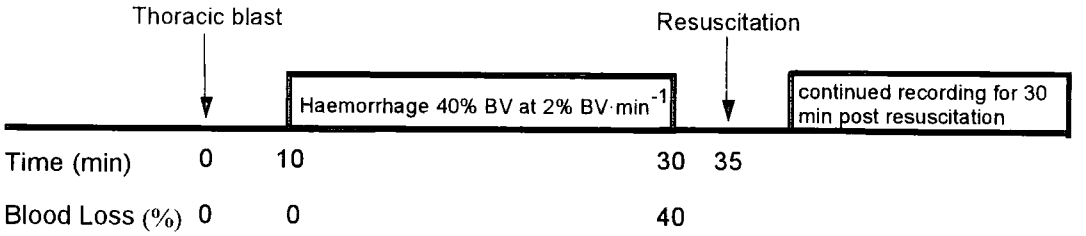
Table 5.1 *Summary of treatments:* all groups subjected to thoracic blast followed 10 minutes later by a progressive haemorrhage of 40% total estimated blood volume at 2% $BV.min^{-1}$.

Resuscitation fluid	**Group** (Resuscitated 5 minutes after haemorrhage)
Hypertonic saline/hydroxyethyl starch (7.5% saline/7% hydroxyethyl starch; 4 $mL.kg^{-1}$)	I
Hypertonic saline/dextran (7.5% saline/6% dextran 70, 4 $mL.kg^{-1}$)	II
Hypertonic saline (7.5% saline; 4 $mL.kg^{-1}$)	III
Isotonic hydroxyethyl starch solution (1:1 resuscitation volume: blood loss)	IV

Cardiovascular measurements were made from 1 minute before blast continuously until 5 minutes after blast, immediately before haemorrhage, after the removal of each aliquot of blood during haemorrhage, immediately before resuscitation and thereafter immediately, 5, 10, 15, 20, 25 and 30 minutes after resuscitation. Blood gas analyses were performed on samples taken immediately before blast, the first and last samples taken during haemorrhage, immediately before and after resuscitation and at 15 and 30 minutes after resuscitation. Duration of apnoea was determined visually and timed using a stopwatch.

All animals were killed 30 minutes after the end of resuscitation with an overdose of 0.5mL of 60mg.mL^{-1} (106-129mg.kg^{-1}) sodium pentobarbitone (Sagatal, Rhône Mérieux (Ireland) Tallaght, Dublin) administered intravenously. The lungs were removed and weighed to determine Lung Weight Index (lung weight/body weight).

Protocol



Group	Haemorrhage	Blast	Resuscitation Fluid	
I	+	+	7.5% saline / 7% hydroxyethyl starch	n=6
II	+	+	7.5% saline / 6% dextran	n=5
III	+	+	7.5% saline	n=6
IV	+	+	isotonic hydroxyethyl starch	n=6

Figure 5.1 Diagrammatic representation of the protocol followed in this study (see section 5b.2.1 for full explanation). Plus sign (+) indicates presence of haemorrhage and blast injury in that group.

5.3 Results

5.3.1 *Baseline values*

Baseline (pre-blast) values for each group are presented in Table 5.2. There were no significant differences between groups in the baseline cardiovascular or arterial blood gas variables, body weights or body temperature. Body temperature did not change significantly during the course of the study in any group.

	Group I	Group II	Group III	Group IV
n	6	5	6	6
Body wt (g)	253.5±1.8	254.0±8.5	251.7±3.2	252.5±2.3
HP (ms)	153.8±2.1	153.2±2.9	142.3±5.6	148.2±5.9
MBP (mmHg)	97.5±3.4	104.0±2.2	102.4±2.3	96.9±6.2
Fem Q ($mL.min^{-1}$)	0.93±0.12	0.48±0.16	0.70±0.07	0.67±0.10
FVR ($mmHg.min.mL^{-1}$)	115.0±15.5	197.5±37.9	158.2±23.7	155.02±22.7
PaO_2 (mmHg)	79.5±3.0	83.4±2.8	86.2±4.0	87.8±5.1
$PaCO_2$ (mmHg)	36.5±1.4	32.2±1.2	34.7±1.1	32.7±0.9
a pH	7.37±0.01	7.37±0.01	7.37±0.01	7.37±0.01
ABE (mM)	-3.5±0.4	-5.4±0.7	-4.5±0.4	-5.2±0.5
Hcrit (%)	34.6±1.6	33.4±1.2	35.6±0.6	33.0±0.7
Temp (oC)	37.7±0.2	37.7±0.2	37.8±0.1	37.2±0.1

Table 5.2 Baseline (pre-blast) values recorded in four groups of anaesthetised rats. Number of rats (*n*); body weight (body wt); heart period (HP); mean arterial blood pressure (MBP); femoral arterial blood flow (Fem Q); femoral vascular resistance (FVR); arterial oxygen tension (PaO_2); arterial carbon dioxide tension ($PaCO_2$); arterial pH (a pH); actual base excess (ABE); haematocrit (Hcrit); and body temperature (Temp). Values are mean ± SEM.

5.3.2 *Effects of thoracic blast*

Thoracic blast (Group I) produced a significant increase of 372.0±95.2 ms in heart period from a pre-blast control of 153.8±2.1 ms and a significant fall in mean blood pressure of 67.2±2.6 mmHg from a pre-blast level of 97.5±3.4 mmHg (Figure 5.2). Thereafter there was a rapid recovery in heart period and a partial recovery in mean arterial blood pressure (MBP).

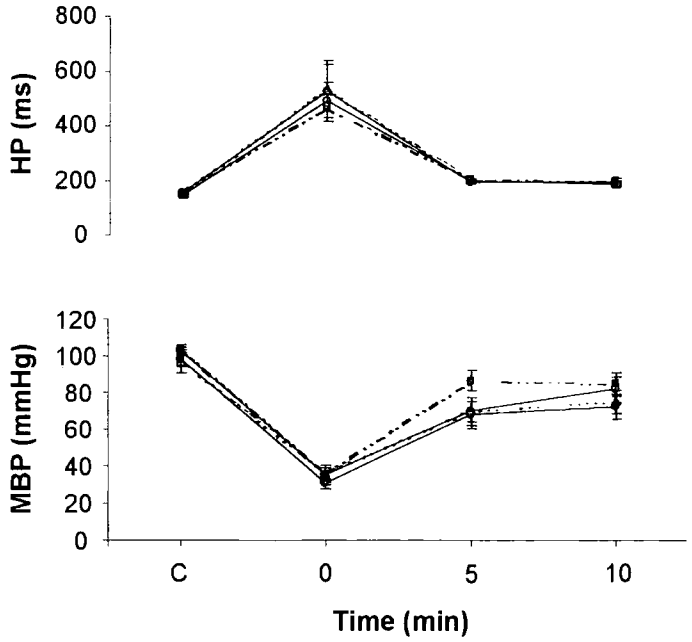


Figure 5.2 Effects of a thoracic blast injury on heart period (HP) and mean arterial blood pressure (MBP) in 4 groups of anaesthetised rats. Group I; (⊖), Group II; (■), Group III; (o) and Group IV; (Δ). Data recorded immediately before blast (C), and thereafter immediately after blast (0), and at 5 and 10 minutes after blast. Values are means±S.E.M.

In addition, in Group I, thoracic blast produced a significant (Student's independent *t* test) apnoea lasting 22.8±0.6 seconds (Figure 5.3).

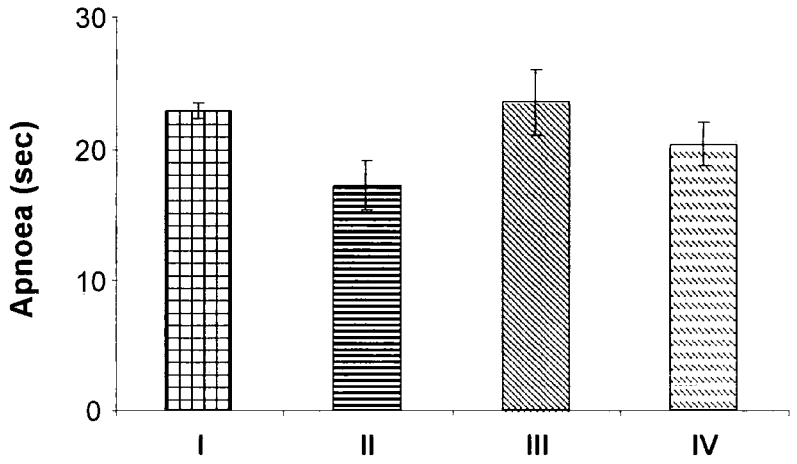


Figure 5.3 Duration of apnoea following thoracic blast injury in 4 groups of anaesthetised rats. Values are means±S.E.M.

Furthermore, blast (Group I) produced a transient fall in femoral arterial blood flow of 0.84±0.06 mL.min^{-1} from a pre-blast control of 0.93±0.12 mL.min^{-1} (Figure 5.4), and a transient fall in femoral arterial vascular resistance of 87.0±15.5 mmHg.min.mL^{-1} from a pre-blast level of 115.0 mmHg.min.mL^{-1} (Figure 5.4). Ten minutes after blast heart period was still elevated and MBP was still significantly below pre-blast levels.

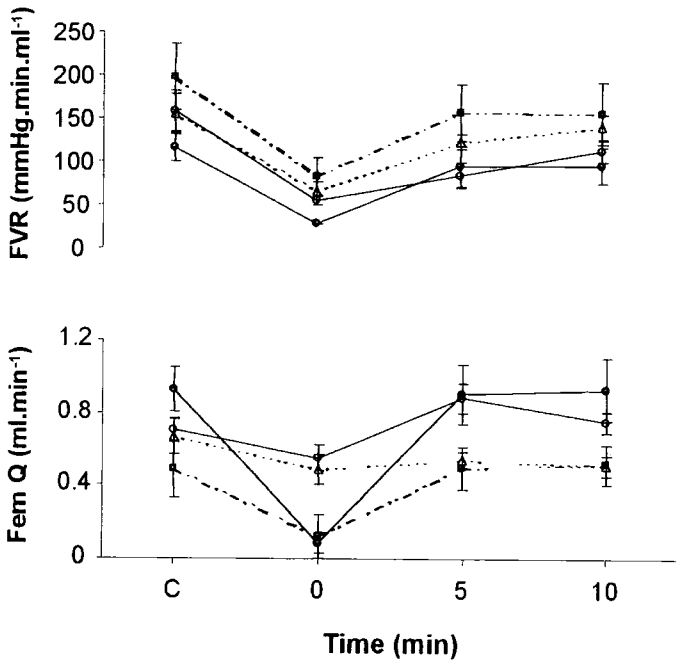


Figure 5.4 Effects of a thoracic blast injury on femoral arterial vascular resistance (FVR) and femoral arterial blood flow (Fem Q) in 4 groups of anaesthetised rats. Group I; (●), Group II; (■), Group III; (o) and Group IV; (Δ). Data recorded immediately before blast (C), and thereafter immediately after blast (0), and at 5 and 10 minutes after blast. Values are means±S.E.M.

Following blast there was a significant fall in PaO_2 of 19.3±5.6 mmHg (Group I) from a control pre-blast value of 79.5±3.0 mmHg and arterial pH of 0.06±0.01 from a pre-blast control of 7.37±0.01 (Figures 5.5).

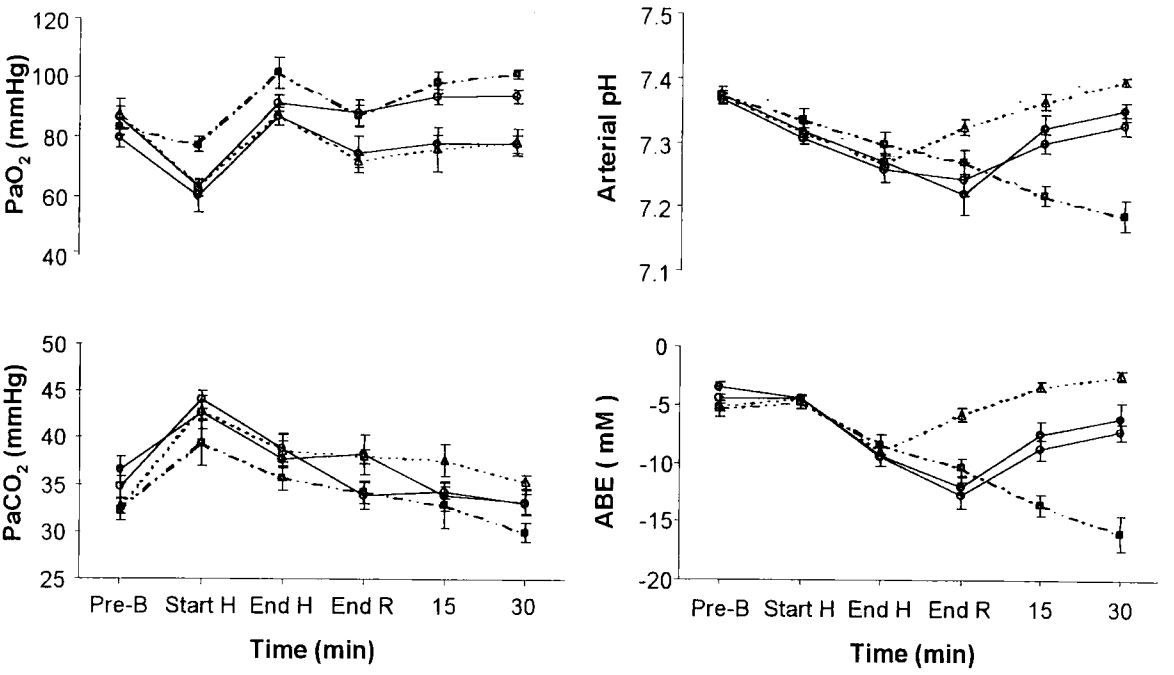


Figure 5.5 Effects blast, haemorrhage and subsequent resuscitation in anaesthetised rats on arterial oxygen tension (PaO_2), arterial carbon dioxide tension ($PaCO_2$), arterial pH and arterial base excess (ABE). Group I; hypertonic saline/hydroxyethyl starch (●), Group II; hypertonic saline/dextran (■), Group III; hypertonic saline (o) and Group IV; isotonic hydroxyethyl starch (Δ). Data recorded immediately before blast (Pre-B), at the start of a haemorrhage of 40% total blood volume (Start H), at the end of haemorrhage (End H), at the end of fluid resuscitation (End R) and thereafter at 15 and 30 minutes after resuscitation. Values are means±S.E.M.

Following thoracic blast there was a significant rise in $PaCO_2$ of 6.2±1.4 mmHg (Group I) from a pre-blast control level of 36.5±1.4 mmHg (Figure 5.5) and haematocrit increased significantly following blast from a pre-blast level of 34.6±1.6 % to 38.6±0.6 % (Figures 5.6). There was no change in ABE during this period (Figure 5.5). Thoracic blast in Groups II-IV produced effects on the above parameters similar to those seen in Group I; there were no significant differences between groups for the first 10 minutes after blast.

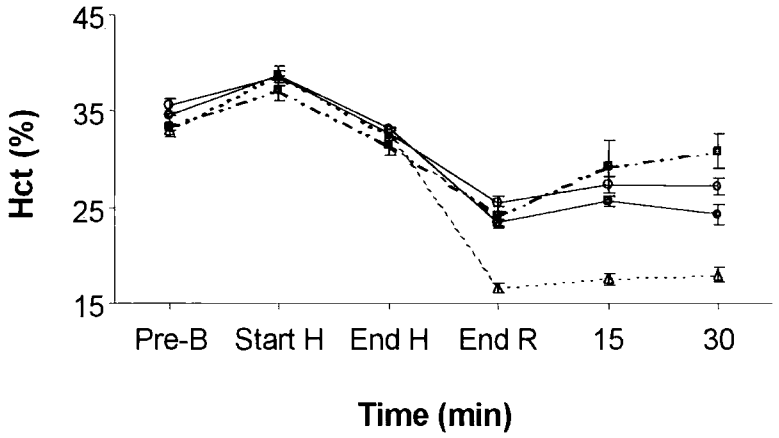


Figure 5.6 Effects of blast, haemorrhage and subsequent resuscitation in anaesthetised rats on haematocrit (Hct). Group I; hypertonic saline/hydroxyethyl starch (●), Group II; hypertonic saline/dextran (■), Group III; hypertonic saline (o) and Group IV; isotonic hydroxyethyl starch (Δ). Data recorded immediately before blast (Pre-B), at the start of a haemorrhage of 40% total blood volume (Start H), at the end of haemorrhage (End H), at the end of fluid resuscitation (End R) and thereafter at 15 and 30 minutes after resuscitation. Values are means±S.E.M.

5.3.3 *Effects of progressive haemorrhage*

Haemorrhage of 40% blood volume, initiated 10 minutes after thoracic blast, induced a significant change in heart period (Figure 5.7) and mean blood pressure in all groups (Figure 5.7). There was no evidence of the first, tachycardic, phase of the response to blood loss normally associated with haemorrhage in the absence of thoracic blast injury. The following section will compare the peak change in heart period corresponding to the bradycardia from each individual animal.

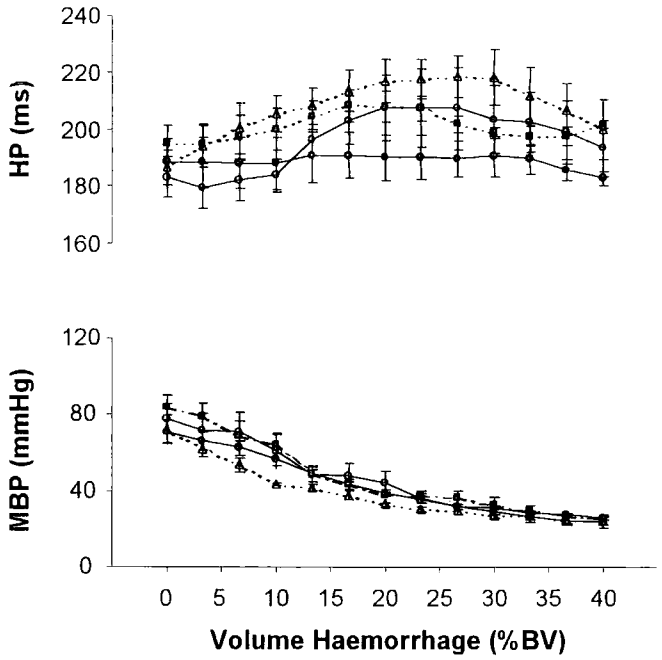


Figure 5.7 Effects of a progressive haemorrhage of 40% total blood volume following thoracic blast injury in 4 groups of anaesthetised rats on heart period (HP) and mean arterial blood pressure (MBP). Group I; (●), Group II; (■), Group III; (o) and Group IV; (Δ). Values are means±S.E.M.

Animals in Group I showed no significant tachycardia, while the bradycardia (significant peak increase in heart period of 8.62±2.75 ms; Student's paired *t* test) was seen after the loss of 27.2±4.6 % blood volume (Figure 5.7). Furthermore, mean arterial blood pressure was not maintained in Group I during the haemorrhage and began to fall after the removal of the first aliquot of blood, the hypotension becoming significant after the loss of 13.3%BV and reached a nadir of 22.56±1.44 mmHg after the loss of 39.44±0.56 %BV (Figure 5.7). There was no significant difference in the peak increase in heart period, or in the hypotension induced by progressive haemorrhage between groups. Associated with the fall in arterial blood pressure there were reductions in femoral arterial flow in all groups (Figure 5.8). However, there was no evidence of a change in femoral vascular resistance during the blood loss (Figure 5.8).

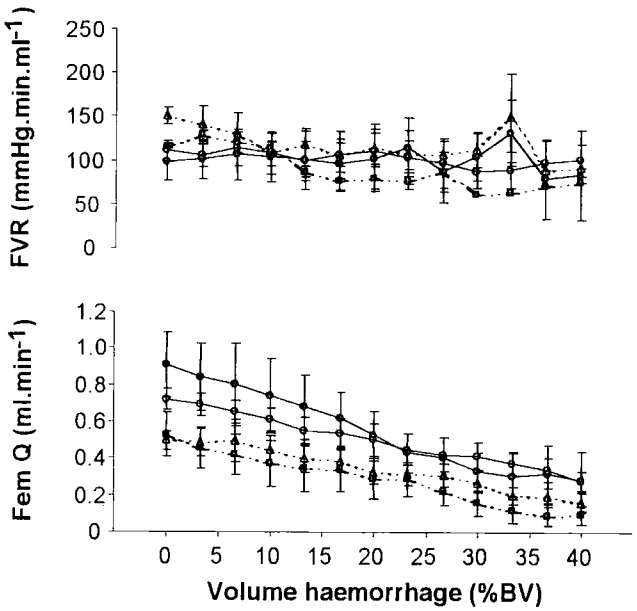


Figure 5.8 Effects of a progressive haemorrhage of 40% total blood volume following thoracic blast injury in 4 groups of anaesthetised rats on femoral arterial vascular resistance (FVR) and femoral arterial blood flow (Fem Q). Group I; (●), Group II; (■), Group III; (o) and Group IV; (Δ). Values are means±S.E.M.

By the end of the haemorrhage PaO_2 (Group I) had increased significantly by 26.8±5.6 mmHg from a pre-haemorrhage value of 60.2±3.1 mmHg (Figure 5.5) while $PaCO_2$ decreased significantly by 5.0±2.0 (Figure 5.5). There was a fall in arterial pH and base excess of 0.05±0.01 (pH) and 4.8±0.8 mM from a pre-haemorrhage level of 7.31±0.01 and –4.5±0.2 mM respectively (Figure 5.5). Haematocrit also fell by 5.4±0.2 % from a pre-haemorrhage control of 38.6±0.6 % (Figure 5.6) in Group I. There was no significant difference in blood gas parameters between groups during the haemorrhage period.

5.3.4 *Effects of resuscitation*

Five minutes after the end of haemorrhage animals in Groups I-IV were resuscitated with hypertonic saline/hydroxyethyl starch (HHES, 4 $mL.kg^{-1}$), hypertonic saline/dextran (HSD, 4 $mL.kg^{-1}$), hypertonic saline (HS, 4 $mL.kg^{-1}$) or isotonic hydroxyethyl starch (HES, 1:1 resuscitation:haemorrhage volume) respectively. Resuscitation induced a similar, significant, elevation in arterial blood pressure to pre-haemorrhage levels in all four groups (Figure 5.9). In Group I there was a further increase in MBP of 17±5 mmHg over the following 10 minutes which was maintained for the remaining 30 minutes of the study. In Group IV MBP was maintained for the remaining 30 minutes of the study without further increase (Figure 5.9). In Group III MBP was maintained for 15 minutes after resuscitation, thereafter falling significantly (Figure 5.9). However, in Group II the effects of hypertonic saline/dextran were not

sustained: MBP began to fall within 5 minutes of resuscitation, and by 10 minutes after resuscitation had fallen significantly by 30±5 mmHg and remained at the low level for the remainder of the study (Figure 5.9). One rat treated with hypertonic saline/dextran died 25 minutes after resuscitation, while all of the rats in the other groups survived until the end of the study (as reported in Chapter 4a.3.4).

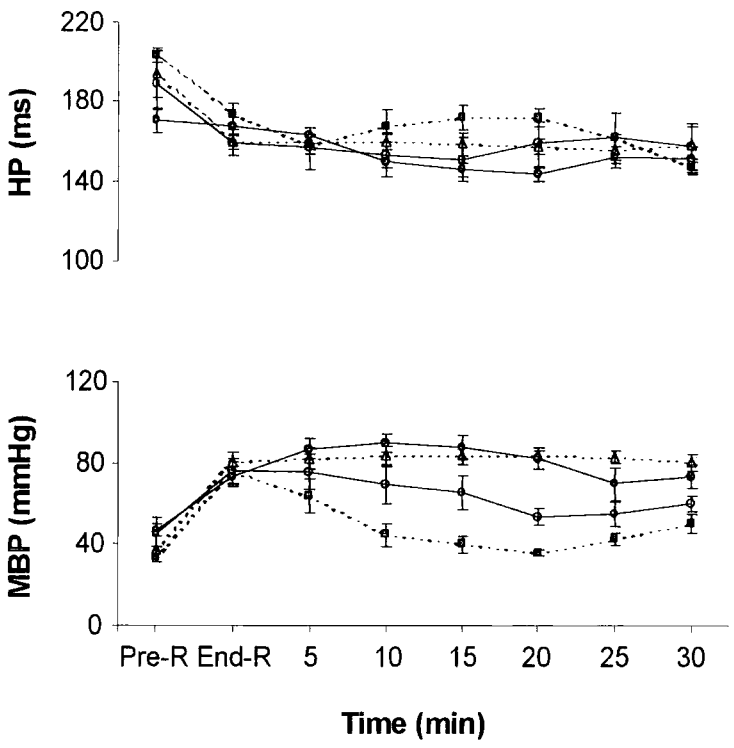


Figure 5.9 Effects of resuscitation with various fluids following thoracic blast injury and subsequent haemorrhage of 40% total blood volume in anaesthetised rats, on heart period (HP) and mean arterial blood pressure (MBP) in Group I; hypertonic hydroxyethyl starch (●), Group II; hypertonic saline/dextran (■), Group III; hypertonic saline (o) and Group IV; isotonic hydroxyethyl starch (Δ). Data recorded immediately before resuscitation (Pre-R), and thereafter immediately after resuscitation (End-R) and at 5, 10, 15, 20, 25 and 30 minutes after resuscitation. Values are means±S.E.M.

Associated with the rise in arterial blood pressure immediately after resuscitation there was a fall in heart period (Figure 5.9) and femoral vascular resistance (Figure 5.10), and a significant increase in femoral blood flow (Figure 5.10). This effect was sustained for the remainder of the study only in the animals resuscitated with isotonic hydroxyethyl starch solutions. In the remaining three groups femoral blood flow fell and vascular resistance increased as the study progressed, this waning of the effects of resuscitation being most pronounced in Group II (Figure 5.10).

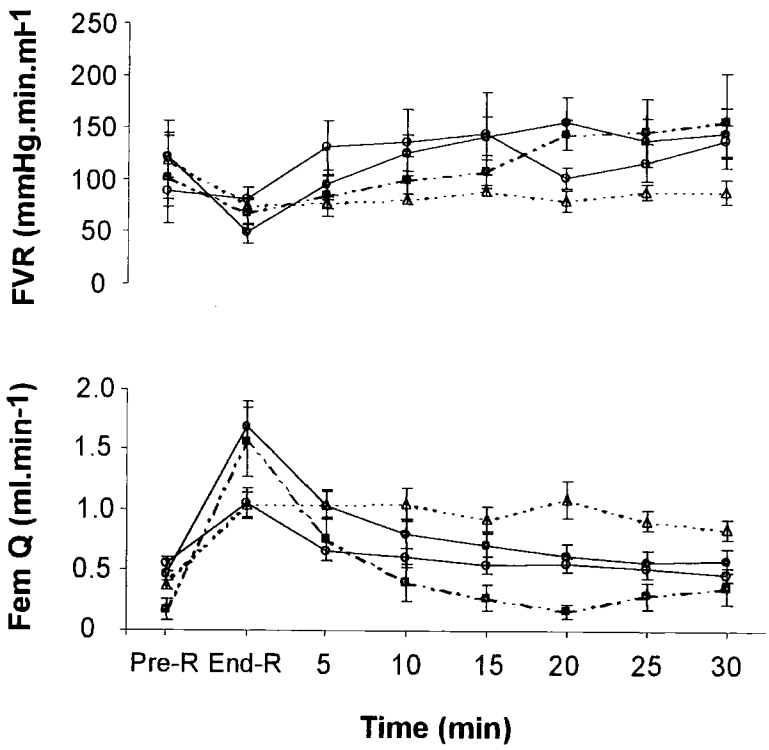


Figure 5.10 Effects of resuscitation with various fluids following thoracic blast injury and subsequent haemorrhage of 40% total blood volume in anaesthetised rats, on femoral vascular resistance (FVR) and femoral arterial blood flow (Fem Q) in Group I; hypertonic saline/hydroxyethyl starch (●), Group II; hypertonic saline/dextran (■), Group III; hypertonic saline (o) and Group IV; isotonic hydroxyethyl starch (Δ). Data recorded immediately before resuscitation (Pre-R), and thereafter immediately after resuscitation (End-R) and at 5, 10, 15, 20, 25 and 30 minutes after resuscitation. Values are means±S.E.M.

PaO_2 fell immediately after resuscitation in groups I, II and IV (Figure 5.5). Thereafter there was an elevation in PaO_2 in the group treated with hypertonic saline/dextran. $PaCO_2$ did not change significantly following resuscitation (Figure 5.5) except in the group given hypertonic saline/dextran where it had fallen significantly by 30 minutes after resuscitation. Immediately after resuscitation there was a rise in arterial pH and base excess in Group IV which continued for the remainder of the study. In Groups I and III both arterial pH and base excess increased within 15 minutes of resuscitation, and continued to do so for the remainder of the study. In contrast, animals treated with hypertonic saline/dextran (Group II) showed a significant continued fall in arterial pH and base excess during the 30 minutes post-resuscitation (Figure 5.5). At 15 and 30 minutes after resuscitation there were clear, significant, differences in arterial pH and base excess between groups, these parameters being highest in the animals given isotonic hydroxyethyl starch solution, intermediate (and equal) in those given hypertonic saline and hypertonic saline/hydroxyethyl starch and lowest in those given

hypertonic saline/dextran (Figure 5.5).

Haematocrit fell significantly following resuscitation in all groups (Figure 5.6), this effect being greatest in Group IV. Haematocrit was subsequently maintained at post-resuscitation levels in the groups treated with hypertonic saline/hydroxyethyl starch, hypertonic saline and isotonic hydroxyethyl starch. By contrast haematocrit increased significantly in the group treated with hypertonic saline/dextran (Figure 5.6). Indeed, 30 minutes after resuscitation with hypertonic saline/dextran haematocrit was not significantly different to that seen immediately *before* resuscitation (Figure 5.6). *Post mortem* Lung Weight Indices were not significantly different between groups (Figure 5.11).

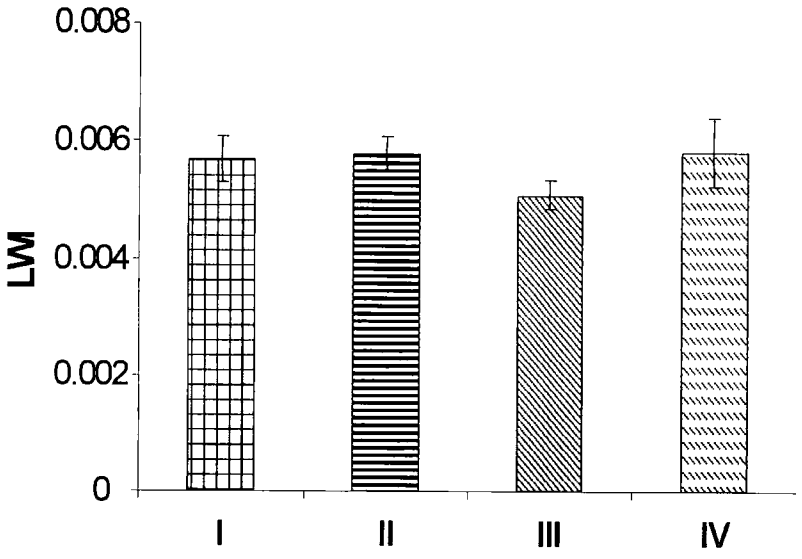


Figure 5.11 Lung weight index (LWI) calculated as *post mortem* lung weight/body weight, following thoracic blast injury and haemorrhage of 40% total blood volume and subsequent resuscitation in anaesthetised rats. Group I; hypertonic saline/hydroxyethyl starch, Group II; hypertonic saline/dextran, Group III; hypertonic saline and Group IV; isotonic hydroxyethyl starch. Values are means±S.E.M.

5.4 Discussion

We have previously shown that administration of whole blood or asanguinous isotonic solutions of either colloid (Haemaccel and isotonic hydroxyethyl starch) or crystalloid (0.9% saline) produce a sustained resuscitation after thoracic blast and haemorrhage (Chapter 4a). By contrast, it was shown in the same study that hypertonic saline/dextran was ineffective: although it initially restored arterial blood pressure the effects began to

wane within five minutes of the end of resuscitation and was associated with a development of a marked metabolic acidosis. The results were the same whether resuscitation was carried out 5 minutes or 20 minutes after the end of the haemorrhage (Chapters 4a & 4b respectively). The new finding from the present study suggests that this problem is specific for hypertonic saline/dextran rather than being a general problem with hypertonic saline solutions.

In the present study a comparison was made of the efficacy of several resuscitation fluids: hypertonic saline/hydroxyethyl starch, hypertonic saline alone and isotonic hydroxyethyl starch. In addition, data from animals treated with hypertonic saline/dextran are presented in this chapter. Animals from this group were not randomised into the present study. The data was derived from a study reported previously in Chapter 4a and was included here to facilitate comparison. Immediately upon completion of resuscitation the asanguinous fluids were all able to restore arterial blood pressure and femoral blood flow to pre-haemorrhage levels. The effects of isotonic hydroxyethyl starch were sustained for the following 30 minutes until the end of the study and resulted in a significant improvement (increase) in arterial pH and base excess.

Hypertonic saline/hydroxyethyl starch produced a further increase in mean arterial blood pressure over the 10 minutes subsequent to resuscitation. Interestingly, this was not associated with a further fall in haematocrit, suggesting that this latter effect was not due to further mobilisation of extravascular water and consequent increase in venous return. There was some evidence of a waning of the effects of hypertonic saline/hydroxyethyl starch and hypertonic saline since there was a late increase in femoral vascular resistance and a fall in flow. In addition the improvement of acid base status was not as marked with these solutions as it was with isotonic hydroxyethyl starch. It is now important to determine the extent of the reduced blood flow by comparing flow in a number of tissues e.g. heart, liver, kidney at selected times after resuscitation using fluorescent microspheres (Schimmel *et al.* 2001). In addition, potential ischaemic tissue damage should be assessed and a comparison made between selected resuscitation fluids. This could be achieved by assessing the expression of adhesion molecules, ICAM-1 and VCAM-1, which have previously been shown to be up-regulated after haemorrhagic shock, and to be affected by resuscitation fluids (Sun *et al.* 1999). Further assessment of liver damage could be effected by determining plasma

levels of α-glutathione-S-transferase, which is a sensitive and specific marker of hepatocellular injury (Redl *et al.* 1995; Rensing *et al.* 1999). In the context of blast injury the liver is of particular interest because of its role in defending the victim from the effects of bacteria and toxins which may gain access to the circulation as a consequence of either the primary blast injury (Guy *et al.* 1998) or secondary injury to the gut because of ischaemia and reperfusion. In addition to this, an assay to detect microalbuminuria, arising from damage in the kidneys, could be utilised as a measure of general inflammation (Kreuzfelder *et al.* 1988; Smith *et al.* 1994; Gosling *et al.* 1991).

It is difficult to explain the deleterious effects of hypertonic saline/dextran. This is clearly not a generalised problem associated with hypertonic solutions, but rather appears to be associated with the dextran component since it is absent with the same tonicity of saline when used alone or in combination with hydroxyethyl starch. One possibility is that the hypertonic solutions do increase vascular permeability by shrinking vascular endothelial cells as has previously been suggested (Corso *et al.* 1998; Kreimeier *et al.* 1997). Normally this action is associated with an improvement in microvascular perfusion (Corso *et al.* 1998). However, this has not been assessed after blast, and it is possible that under this circumstance there is a sufficient increase in permeability to allow leakage of the relatively small (70kDa) dextran molecule into the interstitium, with a resultant loss of plasma water and hence an increased haematocrit. The use of microalbuminurea as a measure of capillary leak may aid in assessing this (Kreuzfelder *et al.* 1988; Smith *et al.* 1994; Gosling *et al.* 1991) as dextran 70 and albumin are approximately the same size (70kDa vs 67kDa). However, it is possible that the strain of rat used in these studies is experiencing an anaphylactic reaction to the dextran molecule (see Chapter 4a, section 4a.4.4) and that it is this allergy that is leading to an increase in vascular permeability, although there is no evidence in the literature of a dextran allergy in this strain. Both these potential explanations require further investigation, in particular resuscitation with dextran alone should be looked at to assess any allergy to this molecule. If this is the case then it may reveal an augmented anaphylactic response than that seen with dextran in conjunction with hypertonic saline as hypertonic saline is reported to reduce the inflammatory response (Ciesla *et al.* 2001; Rizoli *et al.* 1998).

In conclusion, the present study has shown that after primary thoracic blast injury and haemorrhage hypertonic saline alone and in combination with hydroxyethyl starch were

able to restore blood pressure to pre-haemorrhage levels. This effect was sustained for at least 30 minutes after resuscitation and was associated with an improvement in acid base status. However, resuscitation with isotonic hydroxyethyl starch produced greater improvement in acid base status. This is in contrast to the effects of hypertonic saline/dextran, which were not sustained and were associated with severe metabolic acidosis. Finally, there was some indication that the initial improvement in blood flow induced by hypertonic saline and hypertonic saline/hydroxyethyl starch waned during the 30 minutes after resuscitation and further studies are needed to assess whether this is likely to cause tissue damage, especially if it is followed by further resuscitation.

6 The Effect of Doxapram on the Response to Primary Blast Injury: with Particular Reference to the Reflex Apnoea

6.1 Introduction

Primary blast injury to the thorax results in bradycardia, hypotension and apnoea (Krohn *et al.* 1942; Guy *et al.* 1998). Recent studies confirmed and extended these findings (see chapters 3-5). The bradycardia and apnoea have been shown to be reflex in nature, with afferent and/or efferent vagal pathways. The hypotension is also partially due to this reflex. The bradycardia is mediated via increased vagal efferent activity to the heart (Ohnishi *et al.* 2001), and although it has proven possible to pharmacologically block the bradycardia associated with thoracic blast injury with atropine (Ohnishi *et al.* 2001), currently there is no pharmacological treatment for the apnoea. It would be interesting, therefore to determine whether doxapram, a respiratory stimulant (Uehara *et al.* 2000; De Villiers *et al.* 1998; Bairam *et al.* 1993; Peers *et al.* 1991), could shorten the duration of apnoea if administered immediately after blast.

Doxapram is an analeptic (O'Connor *et al.* 1996), i.e., it stimulates respiration (Uehara *et al.* 2000; De Villiers *et al.* 1998; Bairam *et al.* 1993; Peers *et al.* 1991), reducing the frequency and duration of apnoea of prematurity in humans (Yamazaki *et al.* 2001; Poets *et al.* 1999; Huon *et al.* 1998) and in newborn animals (Bairam *et al.* 1992). Doxapram is also reported to have some pressor actions (Huon *et al.* 1998; Cote *et al.* 1992; Bamford *et al.* 1986). The mechanism of action of doxapram on respiration is thought to be similar to that of hypoxia, i.e., it stimulates chemoreceptors (Decanniere *et al.* 1992; Leeman *et al.* 1992a; Peers *et al.* 1991; Bairam *et al.* 1993), stimulating the carotid bodies in a dose-dependent manner (Bairam *et al.* 1991, 1993). Decreasing levels of PO_2 appear to be detected by an oxygen sensor in the plasma membrane of chemoreceptor cells. This sensor seems to be a hemoprotein (see Gonzalez *et al.* 1995) which becomes desaturated leading to an inhibition of O_2 sensitive potassium (K^+) channels in type I cells of the carotid bodies. This then triggers the depolarisation and opening of voltage-dependent calcium channels resulting in an influx of calcium into the type I cells and resulting in the release of catecholamine (see Gonzalez *et al.* 1995; Andersonbeck *et al.*

1995; Peers *et al.* 1991). However, the two latter studies on the mechanism of action of doxapram were carried out *in vitro*, and in 1993 Bairam and colleagues concluded that in newborn kittens doxapram acts in a manner independent of dopaminergic mechanisms on the carotid body. Doxapram is also reported to have actions on the central chemoreceptors ([1]Romeo *et al.* 1995; Scott *et al.* 1977; see Bamford *et al.* 1986) as it increases the ventilatory response to CO_2 (Calverly *et al.* 1983).

6.1.1 *The chemoreceptor reflex*

The peripheral arterial chemoreceptors are located at the bifurcation of the common carotid arteries in the neck (called the carotid bodies) and on the aortic arch in the thorax (called the aortic bodies; see Dampney *et al.* 2002; Gonzalez *et al.* 1995). The afferent pathway is carried in the sinus nerve and the vagus nerve respectively (see Dampney *et al.* 2002; Gonzalez *et al.* 1995). The peripheral chemoreceptors respond primarily to low arterial oxygen tension (PaO_2; see Dampney *et al.* 2002 & Gonzalez *et al.* 1995), but also to increased hydrogen ion concentration (arterial pH) and thus increased arterial carbon dioxide tension (essentially via increased hydrogen ion concentrations; Gonzalez *et al.* 1995). In the absence of the activation of any other reflex, stimulation of the peripheral chemoreceptors results in an increase in ventilation, in order to correct the fall in PaO_2, as well as varying effects on heart rate and peripheral vascular resistance. The cardiovascular effects of the reflex are variable because the respiratory part of the reflex is able to modify the cardiovascular part (Daly *et al.* 1988; Daly & Kirkman, 1988; see Figure 6.1). When the respiratory component of the reflex is absent, e.g., during a reflex apnoea, the primary cardiovascular effect of the peripheral chemoreceptor reflex becomes apparent. This involves a bradycardia due to an increase in vagal efferent activity to the heart, and vasoconstriction in most vascular beds particularly skeletal muscle, due to an increase in activity of sympathetic vasoconstrictor fibres (Daly *et al.* 1988; Daly & Kirkman, 1988). When respiration can increase, the increased activity of inspiratory neurones in the medulla of the brainstem and the activation of the lung stretch afferent fibres by lung inflation (the Hering-Breuer reflex), will inhibit the cardiac vagal motorneurones leading to an increase in heart rate (see Figure 6.1), and also cause a peripheral vasodilation (Daly *et al.* 1988; Daly & Kirkman, 1988).

[1] Article in Italian, information gained from English abstract.

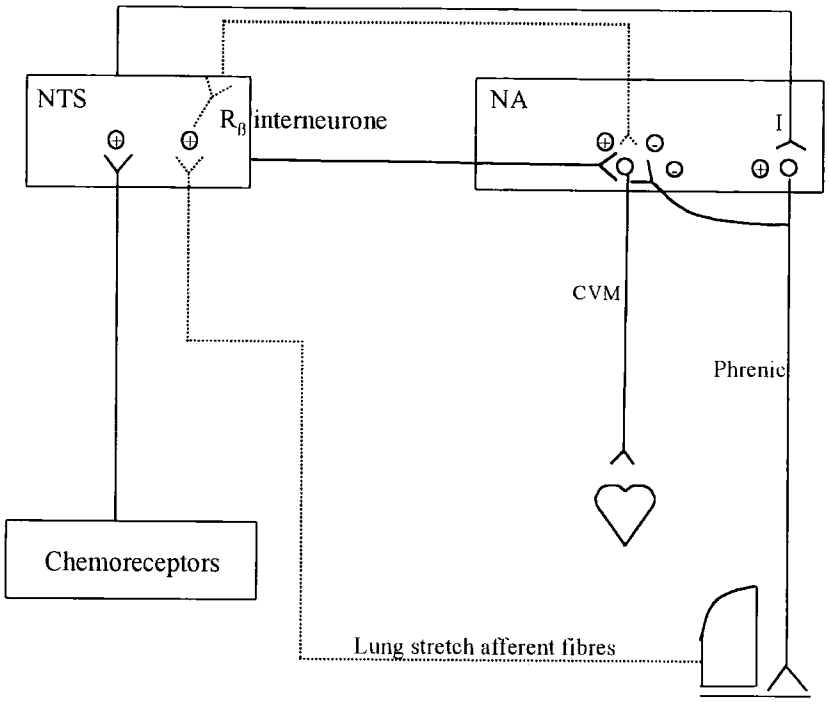


Figure 6.1 Schematic diagram of the neural pathway of the peripheral chemoreceptor reflex. Stimulation of the chemoreceptors results in an increase in respiration which leads to inhibition of the vagus nerve and hence a tachycardia (dashed line). However, when no increase in respiration is possible (e.g. during apnoea), the primary cardiovascular effect of the reflex (e.g. the bradycardia) becomes apparent (see section 6.1.1 for full explanation).⊕ denotes excitatory transmission, ⊖ denotes inhibitory transmission. NTS, nucleus tractus solitarius; NA, nucleus ambiguus; CVM, cardiac vagal motorneurone; I, inspiratory neurone.

6.1.2 *Matching ventilation to perfusion of the lung*

In order for oxygen and carbon dioxide exchange to occur in the lungs ventilation must adequately match perfusion. However, ventilation and perfusion are not uniformly distributed in the lung even in healthy individuals primarily due to the effects of gravity. This generally does not have a major effect on blood gases however, as there are mechanisms in place to compensate. For a given change in intrapleural pressure (i.e., when the thorax expands) there is a larger change in volume within the alveoli at the bottom of the lung as the alveoli here are smaller (due to weight of the lung tissue lying above), and thus more compliant, than at the apex of the lung. Thus the lung is better ventilated at the bottom of the lung compared to the top. However, not all of this

increased ventilation is wasted. Transmural pressure across the blood vessels increases towards the bottom of the lung, due to 'pooling' of blood because of the effects of gravity, causing the blood vessels to distend, and hence blood flow also increases toward the bottom of the lung. The opposite effect can be seen at the top of the lung.

The following will describe the two extremes of ventilation/perfusion mismatch. In reality the situation lies somewhere in between the two.

6.1.2.1 Wasted ventilation

Clinically, wasted ventilation may occur due to pulmonary embolism i.e., a blood clot blocking part of the pulmonary circulation. Ventilation to this part of the lung is wasted, as it cannot take part in gas exchange due to lack of blood flow to this area. The result is a low arterial oxygen tension and high arterial carbon dioxide tension (West, 1977).

6.1.2.2 Wasted perfusion (shunt)

When a proportion of blood flows through the pulmonary circulation without taking part in gas exchange this is termed right to left shunt or venous admixture. A small amount of venous admixture (approximately 1 to 2% of cardiac output) is normal. However, this proportion rises in certain pathological conditions and can result in hypoxaemia if compensatory mechanisms prove inadequate (West, 1977).

The regulation of pulmonary blood flow can be passive or active. Passive regulation occurs because the blood vessels within the pulmonary circulation are distensible and so increases in blood flow lead to decreases in pulmonary vascular resistance without affecting overall pulmonary arterial pressure (Ppa). Active regulation occurs in response to alveolar oxygen tension. Under normal conditions the blood vessels surrounding the alveoli are exposed to high oxygen tensions. The oxygen diffuses through the alveolar walls into the vascular smooth muscle cells. When this oxygen tension falls, for example when the fraction of inspired oxygen (FiO_2) is reduced or due to certain pathological diseases, the nearby arterioles constrict. The mechanism behind this hypoxia-induced arteriolar constriction is not fully understood (see below). This is in contrast to the effects of low oxygen tensions in the systemic circulation where low PaO_2 will relax the

resistance vessels by causing the release of vasoactive substances from endothelial cells. These vasodilatory mediators subsequently act in an autocrine manner to cause the release of nitric oxide, which will directly relax the vascular smooth muscle cells (Burnstock & Ralevic, 1994). In the lungs the vasoconstrictor response to low alveolar oxygen tensions, termed the hypoxic pulmonary vasoconstrictor (HPV) response, decreases blood flow in the region of lung suffering low oxygen tension and diverts it to better ventilated areas, hence matching ventilation to perfusion. This occurs with no overall effect on pulmonary vascular resistance (PVR) provided that less than 20% of the lung is involved. In cases of acute alveolar hypoxia where the entire lung is affected PVR may be twice normal values and systemic arterial oxygen tensions can drop (Berne & Levy, 1993). It is not fully understood how low oxygen tensions lead to pulmonary vasoconstriction but it is likely that it is via a direct action on the vascular smooth muscle cells. This may result in the local release of vasoconstrictor mediators such as thromboxane A_2, α-adrenergic catecholamines and endothelin possibly from the endothelium (Liu *et al.* 2001; Sato *et al.* 2000; Jones *et al.* 1999). Other studies have shown that in isolated pulmonary vascular smooth muscle cells acute hypoxia inhibits potassium channel activity and leads to depolarization of the smooth muscle cell (Post *et al.* 1995). A role for calcium channels within the pulmonary artery smooth muscle cells has also been implicated in the mechanism behind hypoxic pulmonary vasoconstriction. Hypoxia has been shown to enhance calcium channel currents in pulmonary resistance vessels (Franco-Obregon & Lopez-Barneo, 1996b). In experimental models of ARDS the HPV response is inhibited (Leeman *et al.* 1992b). Blood flow cannot then be diverted to better-ventilated areas of lung and systemic arterial oxygen tension falls.

6.1.3 *Doxapram and the HPV response*

In a normal lung administration of doxapram has been shown to increase the HPV response in anaesthetised animals (Leeman *et al.* 1992a; Decanniere *et al.* 1992) by displacing the FiO_2/Ppa stimulus response curve (Figure 6.2) to higher levels of Ppa for each given level of FiO_2. This means that for a given FiO_2 a higher pulmonary arterial pressure is achieved most likely via vasoconstriction of the pulmonary arterioles, and hence pulmonary gas exchange is improved (Leeman *et al.* 1992a). In contrast, Bjork and colleagues in 1996 concluded from their study that doxapram did not augment the

HPV response in normal piglets. However, they do suggest that interpretation of their data was difficult due to pronounced differences in metabolic and circulatory values between groups in the study. Therefore, under the influence of doxapram, pulmonary arterial pressure is likely to be higher at any FiO_2, potentially due to an increase in pulmonary arteriolar vasoconstriction.

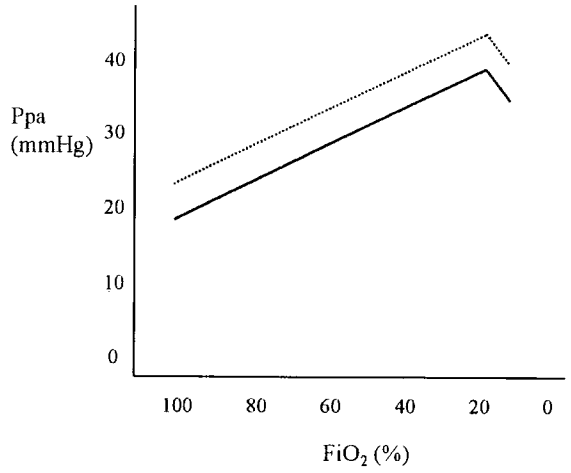


Figure 6.2 Schematic representation of a stimulus response curve showing the relationship between the fraction of oxygen inspired (FiO_2) and pulmonary arterial pressure (Ppa). As the fraction of inspired oxygen decreases, pulmonary arterial pressure increases in a biphasic manner (approximate values taken from Decanniere *et al.* 1992). Doxapram displaces this curve so that at any given level of FiO_2 pulmonary arterial pressure is higher. Dashed line, doxapram; solid line, saline.

What is more relevant for this study is the effect of doxapram on the HPV response when the lung is not normal. In man, doxapram has been shown to attenuate the impairment of pulmonary function post-operatively, again, mainly via effects on ventilation/perfusion (V/Q) ratios (Bjork *et al.* 1993). However, in a model of acute lung injury caused by oleic acid (an experimental model of ARDS; Leeman *et al.* 1992b), which increases intrapulmonary shunt in anaesthetised dogs, doxapram increased the HPV response as pulmonary arterial pressure (Ppa) was enhanced at all levels of perfusion. Though because there was no effect on arterial blood gases it was concluded that blood flow was not diverted to better-ventilated areas of lung (Leeman *et al.* 1992a). Another chemoreceptor agonist almitrine also increased the HPV response without affecting arterial blood gases (Leeman *et al.* 1992a). A possible reason for this may be that this particular model of lung injury involved more than 20% of the total lung volume. In anaesthetised rats with bleomycin-induced lung injury, doxapram did not

seem to improve V/Q mismatch as there was no change in PaO_2, however, there was a decrease in $PaCO_2$ (Horiuchi *et al.* 1995). However, almitrine did seem to improve ventilation/perfusion inequality as arterial blood gases were improved (Horiuchi *et al.* 1995)

Irwin and colleagues (1997) showed an increase in the calculated physiological shunt ratio (calculated as the difference between capillary oxygen content and arterial oxygen content divided by the difference between capillary oxygen content and venous oxygen content) after primary thoracic blast injury in anaesthetised rats. Macroscopic post mortem examination of the lungs after mild to moderate primary blast injury (as in this study) shows patchy pulmonary contusions (disruption of the pulmonary vasculature) involving approximately one third of the lung (Dodd *et al.* 1997), resulting in wasted ventilation. Analysis of blood gas data from blast injured anaesthetised rats also supports the theory of a V/Q mismatch as PaO_2 is always reduced and often $PaCO_2$ is also increased (see figures 4a.10, 4b.10 & 5.8). Doxapram administered after primary thoracic blast injury may improve arterial blood gases perhaps by augmenting the HPV response and shunting blood flow to uninjured, and hence better ventilated areas of the lungs.

The aim of the present study is to test the hypothesis that doxapram can shorten the duration of apnoea induced by thoracic blast, and to determine the effects of this agent on arterial blood gases after this type of lung injury. This is addressed by determining the effects of immediate treatment with either doxapram or a similar volume of vehicle (0.9% saline) on the cardio-respiratory response to thoracic blast.

6.2 Methods

The experiments were conducted on terminally anaesthetised male Wistar rats (Harlan Olac, 243-314g body weight), which were instrumented and prepared for recording as described in Chapter 2, except for the following procedure involving a tracheostomy which was performed on all the animals in this study under alphadolone/alphaxalone anaesthesia (Saffan, Pitman-Moore, UK, 0.5 $mL.h^{-1}$ i.v.)

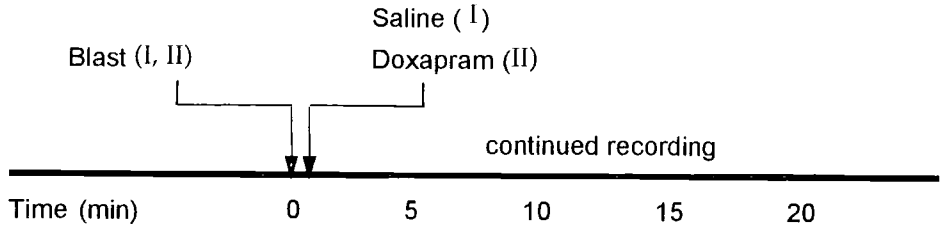
Tracheal cannulation; A 2.5 cm ventral skin incision was made over the trachea. The submaxillary gland was separated at the mid-ventral line and reflected bilaterally. The sternohyoideus was then exposed and also separated bilaterally at the mid-ventral line, allowing the trachea to be exposed. Thread was passed around the trachea, the distal thread aided the handling of the trachea and the proximal thread was used to fix the cannula to the trachea to minimise movement of the cannula. A small incision using scissors was made in the trachea, approximately 10 mm proximal to the larynx, to allow insertion of the cannula into the trachea. Once the tracheal cannula was inserted, the proximal loose loop of thread was tied firmly to fix the cannula in place. The cannula was then attached to a respiratory flow head (MacLab 8s^{TM}, ADInstruments, UK) during the experiment for measuring tidal volume.

All of the incisions produced by surgical preparation were covered with saline-soaked tissue to prevent from drying.

6.2.1 *Experimental protocol*

Upon completion of the surgery the rats were positioned supine in the blast apparatus (see Chapter 2, section 2.2). All animals were positioned with the ventral thorax 3.5 cm below the blast nozzle (which delivers the blast wave to the animal). The animals were then allowed to stabilise for 1 h under alphadolone/alphaxolone anaesthesia prior to exposure to blast. The infusion rate of anaesthetic was adjusted within the range 19-22 $mg.kg^{-1}.h^{-1}$ to maintain an experimental level of anaesthesia (mild withdrawal and a pressor response of approximately 10 mmHg to a noxious pinch of the foot). The protocol shown diagrammatically in Figure 6.3 was then followed.

Protocol



Group	Drug	Blast Injury	
I	Saline (1ml.kg^{-1}, i.v.)	+	n=8
II	Doxapram (1ml.kg^{-1}, 10mg.ml^{-1} saline i.v.)	+	n=8

Figure 6.3 Diagrammatic representation of protocol followed in this study (see section 6.2.1 for full explanation). Plus sign (+) indicates presence of blast injury in that group.

Following baseline cardiovascular, respiratory and blood gas measurements a pressure of 1500 psi was generated in the blast apparatus and all animals received a single discharge from the apparatus. The cardiovascular and respiratory variables were recorded continuously from 2 min prior to 5 min after blast then at 10, 15 and 20 min after exposure to blast. Samples of arterial blood for gas/pH analysis were taken anaerobically immediately before blast, then at 5, 10, 15 and 20 min after blast and were replaced with equal volumes of heparinised saline (20 iu.mL^{-1} heparin, Monoparin, CP Pharmaceuticals, UK, in 0.9% saline).

The animals were allocated to one of 2 groups as shown in Figure 6.3. Saline (Group I) or doxapram (Group II) were injected as soon as possible, within 1-4 s, after blast.

All animals were killed 20 minutes after the blast injury with an overdose of 0.5mL of 60mg.mL^{-1} (96-124mg.kg^{-1}) sodium pentobarbitone (Sagatal, Rhône Mérieux (Ireland) Tallaght, Dublin) administered intravenously. The lungs were removed and weighed to determine Lung Weight Index (lung weight/body weight).

6.3 Results

There were no significant differences in the baseline (pre-blast) parameters between any of the groups studied (Table 6.1).

	Group I	Group II
n	8	8
Body wt (g)	256.3±7.9	270.0±7.4
HP (ms)	140.1±4.7	143.8±3.7
MBP (mmHg)	119.9±3.9	115.8±4.8
Fem Q ($mL.min^{-1}$)	1.1±0.1	1.0±0.1
FVR ($mmHg.min.mL^{-1}$)	108.8±9.5	121.7±7.9
Vt (mL)	1.3±0.1	1.5±0.1
RR (bpm)	109.3±6.8	114.0±4.9
RMV ($mL.min^{-1}$)	138.1±16.2	176.0±15.8
PaO_2 (mmHg)	89.3±2.6	87.9±2.2
$PaCO_2$ (mmHg)	33.7±0.5	32.3±1.5
a pH	7.39±0.01	7.40±0.01
ABE (mM)	-3.0±0.5	-4.1±0.7
Hcrit (%)	35.0±1.3	34.3±1.5
Temp (oC)	37.6±0.2	37.8±0.1

Table 6.1 Baseline values for Group I (saline) and Group II (doxapram). *n* denotes number of animals in group, Body wt; body weight, HP; heart period, MBP; mean arterial blood pressure, Fem Q; femoral arterial blood flow, FVR; femoral arterial vascular resistance, Vt; tidal volume, RR; respiratory rate, RMV; respiratory minute volume, PaO_2; arterial oxygen tension, $PaCO_2$; arterial carbon dioxide tension, a pH; arterial pH, ABE; arterial base excess, Hcrit; haematocrit, Temp; body temperature. Data are presented as means±standard error of the means.

6.3.1 *The effects of saline on the cardio-respiratory response to thoracic blast*

In saline-treated animals (Group I) thoracic blast lead to a significant bradycardia, with heart period increasing by 289.14±27.44 ms from a pre-blast level of 140.11±4.73 ms (Figure 6.4), and a significant decrease in mean arterial blood pressure of 86.21±1.50 mmHg from a pre-blast value of 119.85±3.94 mmHg (Figure 6.4). Thereafter there was a rapid recovery in heart period such that by 2 minutes after blast heart period was not significantly different to pre-blast control. The recovery in mean blood pressure was

much slower with MBP remaining significantly below pre-blast control until 15 minutes after blast.

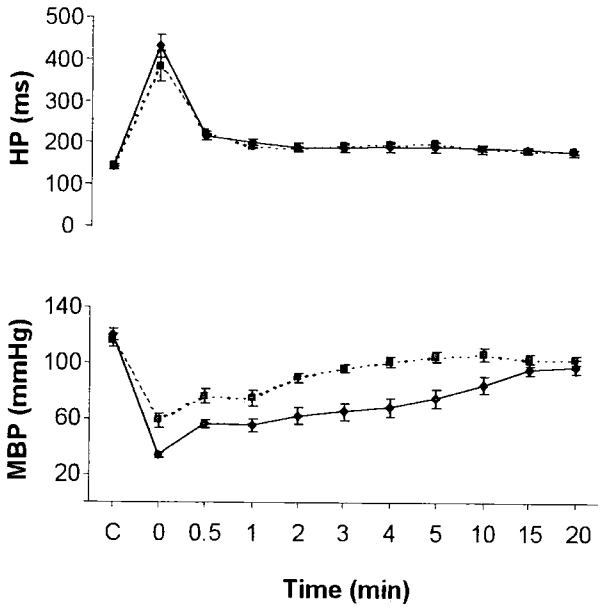


Figure 6.4 Effects of thoracic blast on heart period (HP) and mean arterial blood pressure (MBP) in anaesthetised rats treated with 0.9% saline (Group I; 1mL.kg^{-1}) (♦) or doxapram (Group II; 10mg.mL^{-1} in 1mL.kg^{-1}) (■) administered within 1-4sec of blast. All animals were subjected to blast at time 0 min. Data recorded immediately before blast (C), and thereafter immediately (0) and at 30 seconds, 1-5, 10, 15 and 20 min after blast. Values are mean±S.E.M.

In addition, blast produced a transient fall in femoral arterial blood flow of 0.67±0.07 mL.min^{-1} from a pre-blast control of 1.12±0.13 mL.min^{-1} (Figure 6.5), and a significant, transient fall in femoral arterial vascular resistance of 56.64±26.25 mmHg.min.mL^{-1} from a pre-blast level of 108.76±9.49 mmHg.min.mL^{-1} (Figure 6.5).

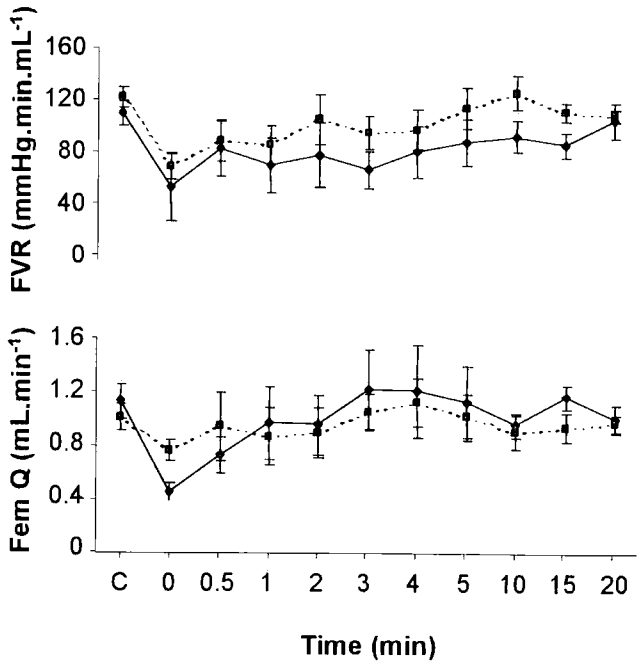


Figure 6.5 Effects of thoracic blast on femoral vascular resistance (FVR) and femoral arterial blood flow (Fem Q) in anaesthetised rats treated with 0.9% saline (Group I; 1mL.kg^{-1}) (♦) or doxapram (Group II; 10mg.mL^{-1} in 1mL.kg^{-1}) (■) administered within 1-4sec of blast. All animals were subjected to blast at time 0 min. Data recorded immediately before blast (C), and thereafter immediately (0) and at 30 seconds, 1-5, 10, 15 and 20 min after blast. Values are mean±S.E.M.

Thoracic blast (Group I) produced a significant (Student's independent *t* test) apnoea lasting 24.4±2.4 s (Figure 6.6).

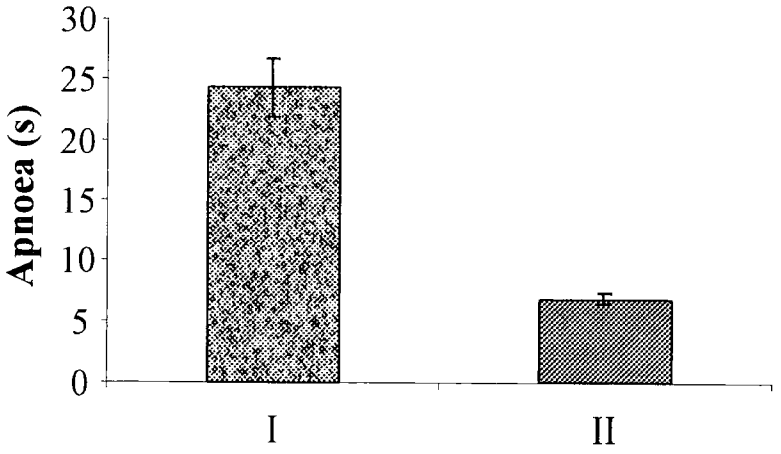


Figure 6.6 Duration of apnoea following thoracic blast in anaesthetised rats treated with 0.9% saline (Group I; 1mL.kg^{-1}) (♦) or doxapram (Group II; 10mg.mL^{-1} in 1mL.kg^{-1}) (■) administered within 1-4sec of blast. Values are mean±S.E.M.

Thirty seconds after blast tidal volume was not significantly different to pre-blast controls, however, respiratory rate had increased by 5.58±11.45 breaths.min^{-1} from a pre-blast level of 109.25±6.81 breaths.min^{-1}, hence respiratory minute volume also increased, by 5.2±19.0 mL.min^{-1} from a pre-blast control of 138.1±16.2 mL.min^{-1} (Figure 6.7). However, none of these values were significantly above pre-blast baseline.

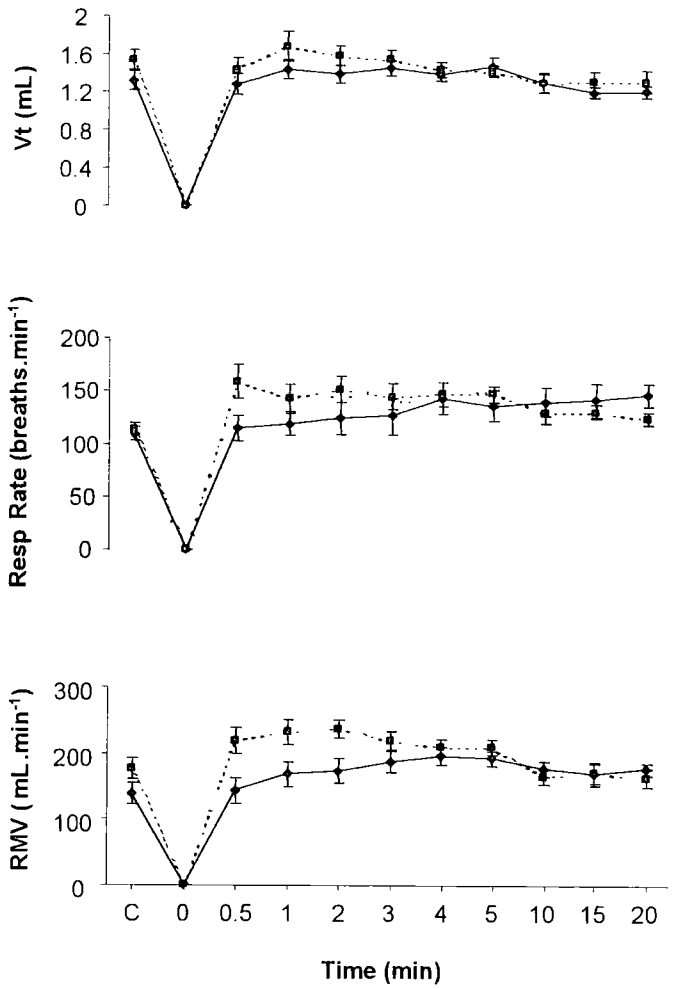


Figure 6.7 Effects of thoracic blast on respiratory tidal volume (Vt), respiratory rate (Resp Rate) and respiratory minute volume (RMV) in anaesthetised rats treated with 0.9% saline (Group I; 1mL.kg^{-1}) (♦) or doxapram (Group II; 10mg.mL^{-1} in 1mL.kg^{-1}) (■) administered within 1-4sec of blast. All animals were subjected to blast at time 0 min. Data recorded immediately before blast (C), and thereafter immediately (0) and at 30 seconds, 1-5, 10, 15 and 20 min after blast. Values are mean±S.E.M.

Five minutes after blast PaO_2 had fallen significantly by 26.17±2.40 mmHg from a control pre-blast value of 89.29±2.60 mmHg (Figure 6.8). $PaCO_2$ increased following blast from a pre-blast level of 33.71±0.47 mmHg to 35.75±3.25 mmHg (Figure 6.8). Arterial pH and ABE both fell following thoracic blast by 0.06±0.05 and 4.0±1.6 mM, from a pre-blast control of 7.39±0.01 and −3.0±0.5 mM respectively (Figures 6.8 and 6.9). Haematocrit also fell 5 minutes after thoracic blast by 1.0±1.1 % from a pre-blast control of 35.0±1.3 % (Figure 6.8).

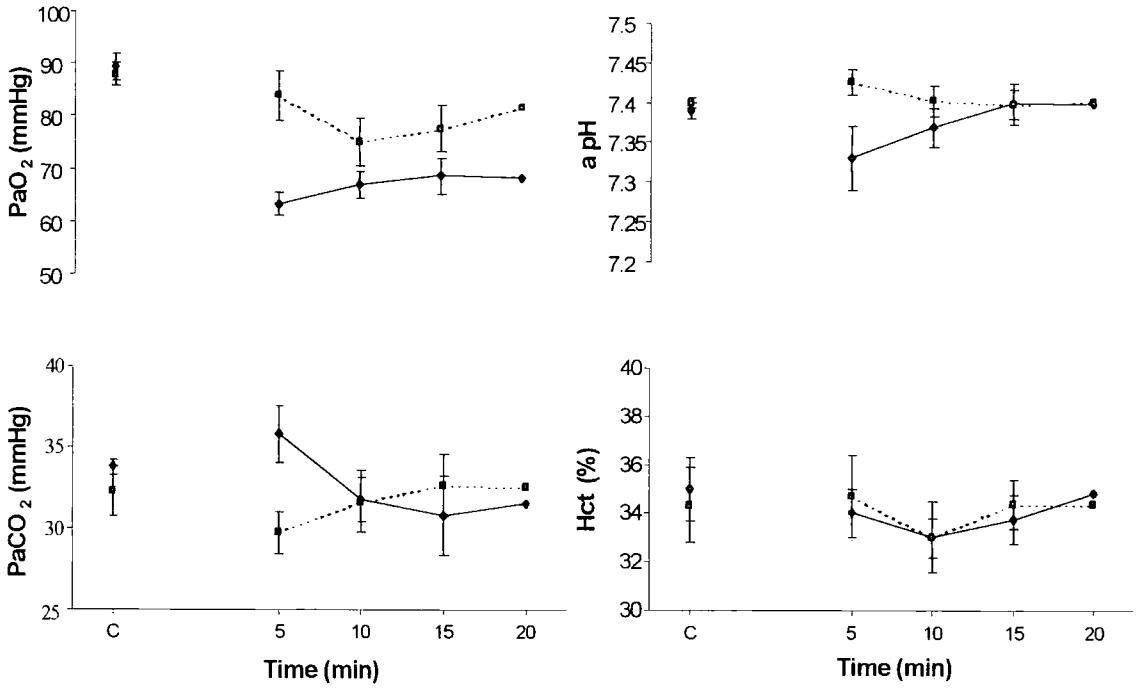


Figure 6.8 Effects of thoracic blast on arterial oxygen tension (PaO_2,) arterial carbon dioxide tension ($PaCO_2$), arterial pH (a pH) and haematocrit (Hct) in anaesthetised rats treated with 0.9% saline (Group I; 1mL.kg^{-1}) (♦) or doxapram (Group II; 10mg.mL^{-1} in 1mL.kg^{-1}) (■) administered within 1-4sec of blast. Data recorded immediately before blast (C), and 5, 10, 15 and 20 min after blast. Values are mean±S.E.M.

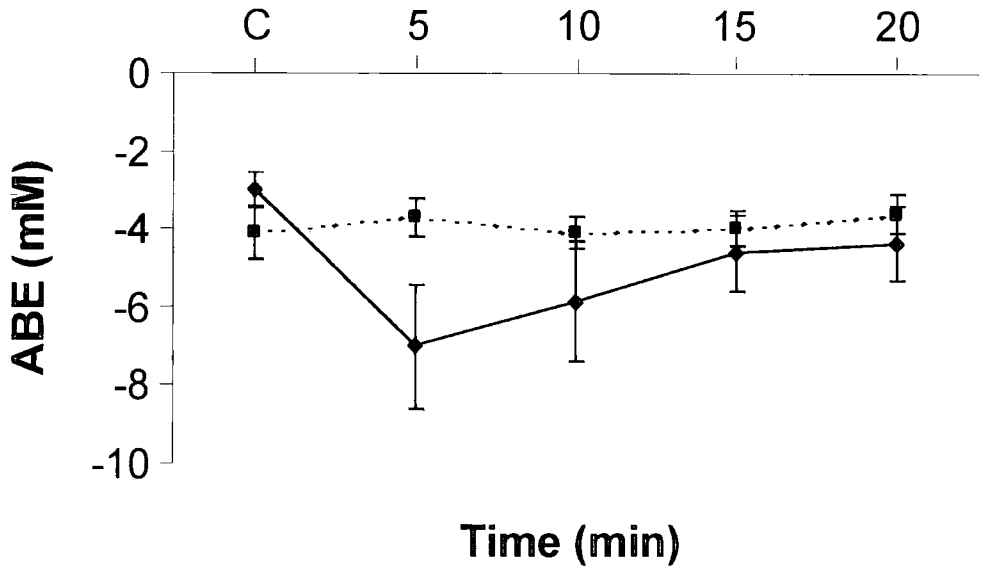


Figure 6.9 Effects of thoracic blast on arterial base excess (ABE) in anaesthetised rats treated with 0.9% saline (Group I; 1mL.kg^{-1}) (♦) or doxapram (Group II; 10mg.mL^{-1} in 1mL.kg^{-1}) (■) administered within 1-4sec of blast. Data recorded immediately before blast (C), and 5, 10, 15 and 20 min after blast. Values are mean±S.E.M.

6.3.2 *The effects of doxapram on the cardio-respiratory response to thoracic blast*

Administration of doxapram (10 $mg.kg^{-1}$) in Group II stimulated respiration within 0-3 s of injection, and significantly reduced the duration of apnoea to 6.8±1.5 s (Figure 6.6) compared to Group I. Thereafter a steady pattern of respiration was re-established, although in one animal there was a transient respiratory disturbance, i.e., during the first 30s after blast, rapid shallow breathing 7-21s after blast was followed by a second apnoea 21-25s after blast, then normal breathing resumed within a few breaths.

By 30s after blast there were no significant differences between respiratory tidal volume in doxapram and saline treated animals. However, respiratory rate and consequently respiratory minute volume were significantly higher in those treated with doxapram compared to saline (Figure 6.7). Respiratory rate (Group II) increased significantly 1 minute after blast by 29.11±13.15 $breaths.min^{-1}$ from a pre-blast control of 114.04±4.91 $breaths.min^{-1}$. Thus respiratory minute volume increased by 56.00±18.44 $mL.min^{-1}$ from a pre-blast level of 176.00±15.79 $mL.min^{-1}$ (Figure 6.7). Respiration rate was significantly higher in doxapram treated animals compared to those given saline for 1 minute after blast, while respiratory minute volume was significantly higher in the doxapram group for 2 minutes after blast.

PaO_2, (Figure 6.8), and ABE (Figure 6.9) were well maintained in the doxapram-treated animals compared to those given saline, the latter suggesting that doxapram may have beneficial cardiovascular as well as respiratory effects. $PaCO_2$ was significantly lower (29.7±0.9 mmHg) and arterial pH significantly higher (7.43±0.01) in the doxapram treated group compared to the saline-treated group, for the first 5 minutes after blast, thereafter returning to values not significantly different from the saline-treated group (Figure 6.8). Haematocrit was not significantly different from the saline-treated group throughout the study (Figure 6.8).

In addition to its respiratory effects doxapram markedly attenuated the hypotensive response to thoracic blast. Immediately after blast mean blood pressure fell significantly by 57.38±5.29 mmHg from a pre-blast control of 115.76±4.81 mmHg (Figure 6.4) and

remained significantly below pre-blast levels for only 2 minutes in this group, compared to 15 minutes in those given saline. In contrast, doxapram had no effect on the bradycardic response to thoracic blast (Figure 6.4) nor on haematocrit (Figure 6.8). Heart period increased by a similar amount to Group I immediately after blast, from 143.8±3.66 ms to 381.88±35.88 ms. In the doxapram-treated animals femoral vascular resistance was not significantly different from the saline-treated group but femoral arterial blood flow was significantly higher at 0.94±0.25 $mL.min^{-1}$ immediately after blast in the doxapram-treated group (compared to Group I which was 0.73±0.14 $mL.min^{-1}$), returning to values not significantly different to those in the saline group by 30 seconds after the blast (Figure 6.5). The higher blood flow in the doxapram-treated group immediately after blast is likely to be due to the pressor effect of this drug (Figures 6.4 and 6.5).

A comparison of *post mortem* lung weight indices (calculated as lung weight divided by body weight) showed lung weight index is significantly lower in the doxapram treated group compared to the saline treated group (Student's *t* test; see Figure 6.10).

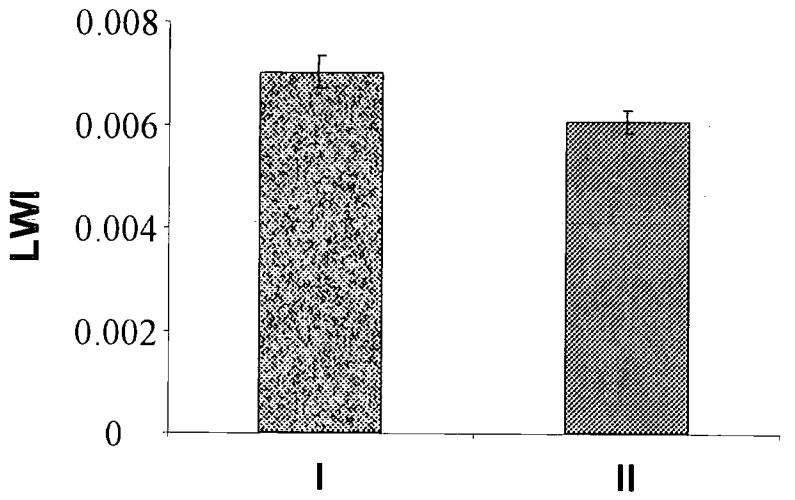


Figure 6.10 Effects of 0.9% saline (Group I; $1mL.kg^{-1}$) or doxapram (Group II; $10mg.mL^{-1}$ in $1mL.kg^{-1}$) on *post mortem* lung weight indices (LWI) following thoracic blast injury in anaesthetised rats. LWI calculated as *post mortem* lung weight/body weight. Values are mean±S.E.M.

6.4 Discussion

The results of this study suggest for the first time that it is possible to pharmacologically reverse the apnoea induced by thoracic blast. In addition doxapram significantly attenuated the hypotensive response for the first 2 min after thoracic blast and prevented the marked falls in PaO_2, arterial pH and base excess for at least 5 min after blast, but had no affect on the blast-induced bradycardia. This latter effect is in contrast to a study carried out in anaesthetised dogs (Hsu *et al.* 1985), where doxapram attenuated the bradycardia induced by xylazine (an α_2-adrenoreceptor agonist), and in contrast to this doxapram was shown to produce a reflex bradycardia in foetal lambs which could be abolished by vagotomy (Bamford *et al.* 1986). The attenuation of the blast-induced hypotension by doxapram is also consistent with earlier reports of its pressor action ([2]Bruckner *et al.* 1977; Huon *et al.* 1998; Cote *et al.* 1992) and with the report of doxapram potentiating the pressor effect of xylazine (Hsu *et al.* 1985). As xylazine is an α_2-adrenoreceptor agonist, then α_2-adrenoreceptors may be the site of action of doxapram's pressor effect as Bamford and colleagues reported (1986) a rise in arterial blood pressure following doxapram administration to a foetal lamb whose brain was destroyed above the cervical spinal cord.

The mechanism of the termination of the blast-induced apnoea by doxapram is likely to be stimulation of the carotid bodies thereby activating the chemoreceptor reflex and hence stimulating respiration. Doxapram has previously been shown to increase respiration by stimulating the chemoreceptors in a dose-dependent manner (Bairam *et al.* 1991, 1993). The potential benefits of early termination of apnoea are obvious, the present study further suggests that doxapram may also be exerting beneficial cardiovascular effects which warrant further investigation. That doxapram may exert a beneficial cardiovascular effect is suggested by a number of findings. Firstly, although the maintenance of PaO_2 after treatment with doxapram could be due to the increase in respiratory minute volume it is clear from previous studies that increasing ventilation alone is not sufficient to restore PaO_2 to normal values after blast. Thus, Ohnishi *et al.* (2001) showed that after blast PaO_2 fell significantly despite an increase in both respiratory tidal volume and rate (after the initial apnoea) and consequently a significant

increase in respiratory minute volume compared to pre blast control values (see Figure 6.11).

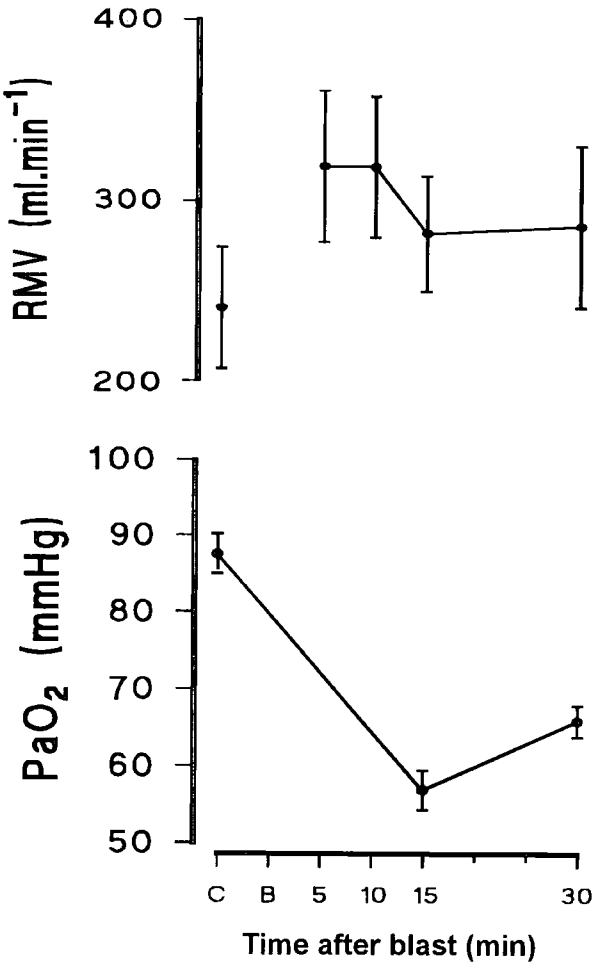


Figure 6.11 The effects of thoracic blast injury on respiratory minute volume (RMV) and arterial oxygen tension (PaO_2) in anaesthetised rats. Despite a significant increase in RMV, arterial oxygen tension stills remains low following a thoracic blast injury. Adjacent data points joined for clarity (E. Kirkman, unpublished data abstracted from Ohnishi *et al.* 2001).

This fall in arterial oxygen tension despite increased respiratory minute volume may be due to impaired gas exchange due to pulmonary oedema following blast injury. Thus doxapram may be exerting an additional effect on pulmonary haemodynamics, perhaps reducing shunt. This would be in contrast to reports in the literature where doxapram was shown to increase pulmonary artery pressure (i.e. cause pulmonary vasoconstriction) after lung injury in dogs but did *not* divert blood to better ventilated areas of the lung (Leeman *et al.* 1992a). However, in that particular study the injury may have involved more than 20% of lung volume and hence any increase in pulmonary arterial pressure would increase overall pulmonary vascular resistance and possibly lead to pulmonary oedema, further hindering gas exchange and counteracting any improvement in shunt.

[2] Article in German, information gained from English article.

Indeed, another study showed doxapram had no effect on gas exchange in piglets with atelectasis involving 50-75% of the lungs (Eyal *et al.* 1996). It seems a generalised increase in pulmonary arterial pressure (Ppa) and hence pulmonary vascular resistance (PVR) is deleterious when most of the lung is poorly ventilated. This may be as a result of an initiation or exacerbation of pulmonary oedema. As pulmonary oedema may also be seen after a more severe blast injury (Guy *et al.* 1998) administration of doxapram may be best avoided in that situation. However, after a milder blast injury, where lung damage is patchy *post mortem* macroscopic examination of the lungs after mild to moderate blast shows patchy contusions; Dodd *et al.* 1997), an increase in Ppa (and thus PVR) in response to low PO_2 may divert blood to better-ventilated areas aiding V/Q matching. This could potentially be the mechanism whereby doxapram increases PaO_2 after mild to moderate blast injury in this study. To investigate this possibility further future studies need to be carried out whereby V/Q is monitored before and after doxapram administration following differing grades of primary blast injury to the thorax and coupling this with parameters such as arterial blood gases and base excess. Doxapram also significantly reduced lung weight index following thoracic blast. This implies an attenuation of a blast-induced pulmonary oedema (Zuckerman, 1940; Brown *et al.* 1993), possibly via an increase in the hypoxic pulmonary vasoconstrictor response. A vasoconstriction in the pulmonary arterioles would lead to a decrease in capillary hydrostatic pressure which, due to an alteration in Starling forces, would reduce capillary filtration (see Chapter 4a, section 4a.1.2.1) and hence any pulmonary oedema. Additionally, an improvement in intrapulmonary shunt and hence gas exchange, would reduce hypoxia and thus reduce the hypoxic trigger for an inflammatory response, again reducing any pulmonary oedema (see Chapter 4a, section 4a.4.1.1).

In addition to the cardiorespiratory effects of doxapram, this respiratory stimulant may be exerting a beneficial systemic haemodynamic effect in the present study, in contrast to other agents that improve blood pressure at the expense of regional blood flow. Although blood flow was only transiently improved in the femoral vascular bed, the maintenance of arterial base excess may be due to improved tissue oxygenation, not only because of higher PaO_2 levels but also possibly because of improved blood flow to metabolically active regions (e.g. the gut and kidney). This could be investigated further in the rat using a combination of regional measurements of blood flow and tissue

oxygenation. Finally the time course of the studies should be extended to see whether any beneficial effects persist over a longer time scale, this could be combined with measurements of markers of secondary tissue damage such as microalbuminuria (Gosling *et al.* 1991) investigated over a range of levels of thoracic blast injury.

6.4.1 *Possible changes in chemoreceptor activity after sham blast and doxapram, and mechanism of action of doxapram*

The cardiorespiratory effects of doxapram (and saline) in the absence of thoracic blast should be investigated to adequately assess the interaction between the response to doxapram and that to primary blast injury. To this end, a pilot study has begun to determine the effects of doxapram or saline in sham blast animals. Currently there is only one animal in each group (saline or doxapram following sham blast). The animals in this pilot study were only subjected to the sound of the blast and not to the physical effect of the blast wave. Following sham blast, administration of saline had no cardiovascular or respiratory effect. However, doxapram gave an immediate increase in respiration (as soon as it was injected) and a small, transient vasoconstriction in the femoral vascular bed, followed by a transient bradycardia and hypotension as the respiratory effect waned. During the hypotension (following the vasoconstriction) there was a vasodilation as femoral flow remained constant (Figure 6.12). There are varying reports in the literature as to the cardiovascular effects of doxapram, with reports ranging from a modest increase in blood pressure with a small bradycardia (Bamford *et al.* 1986), to an increase of 49% in blood pressure with a 40.5% increase in heart rate within the first few minutes of administration ([3]Bruckner *et al.* 1977). However, within the same time scale as the bradycardia and hypotension reported in this study Mileitch and colleagues (1976) also reported an abrupt bradycardia and hypotension in anaesthetised and unanaesthetised goats which returned to, or exceeded, pre-injection values within 30 seconds.

[3] Article in German, information gained from English article.

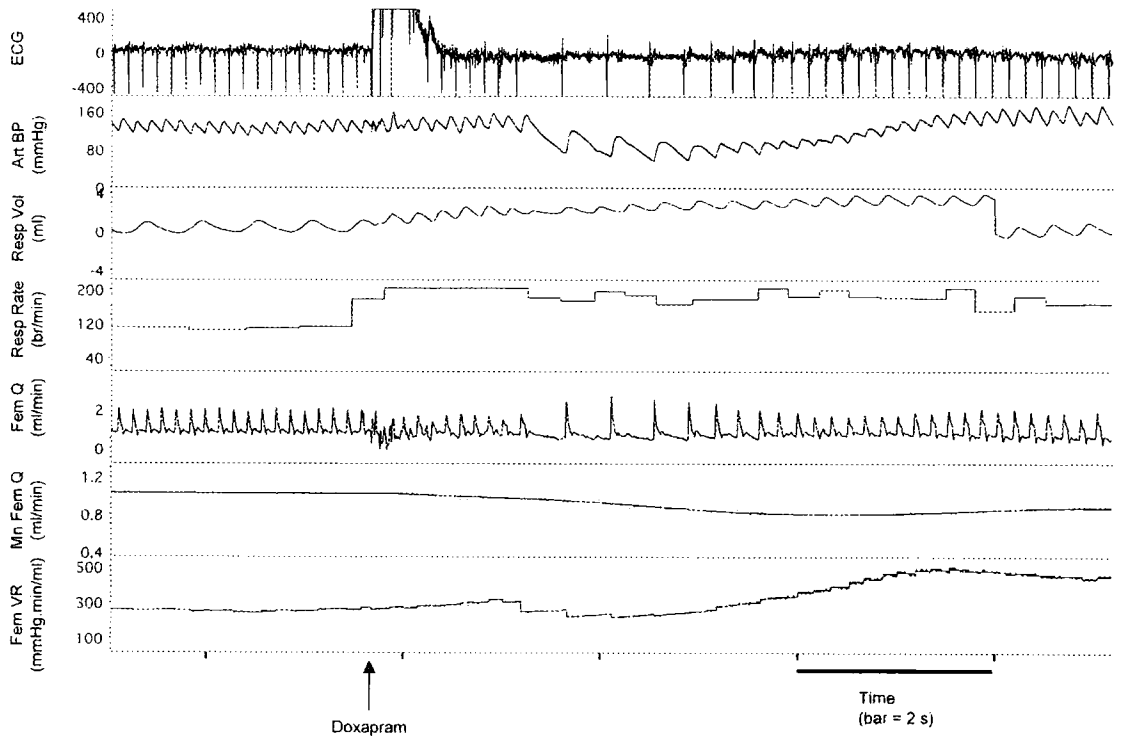


Figure 6.12 Original data trace of the electrocardiogram (ECG, top panel), arterial blood pressure (art BP), respiratory volume (Resp Vol; inspiration upwards), respiratory rate (Resp Rate), femoral blood flow (Fem Q), mean femoral blood flow (Mn Fem Q) and femoral vascular resistance (Fem VR) in one anaesthetised rat. The arrow denotes the point of sham blast administration and injection of doxapram ($1mL.kg^{-1}$, $10mg.mL^{-1}$).

6.4.2 *Implications for the chemoreceptor reflex and its interaction with the pulmonary 'J' reflex and the response to blast*

It was postulated in Chapter 1, section 1.5 that the pulmonary 'J' reflex may be responsible for the response to blast. Any reported studies on an interaction between the pulmonary 'J' reflex and the chemoreceptor reflex may allow comparisons with the response elicited in this study, the interaction between the response to blast and a possible doxapram-induced chemoreceptor reflex. One study showed an inhibition of the chemoreceptor reflex-induced increase in respiration by simultaneous pulmonary 'J' reflex-induced apnoea but did not comment on any cardiovascular changes (Paton, 1997a). Another study (Paton, 1998) looked at convergence of afferent fibres from both these reflexes onto neurones within the NTS in a working heart-brain stem preparation in the mouse. It was shown that there was a degree of convergence of the pulmonary C-fibres and chemoreceptor reflex afferent fibres within the NTS. Paton (1998) then

postulated that pulmonary oedema could induce a pulmonary 'J' reflex, thus reducing inspiratory drive and block the chemoreceptor reflex-induced increase in respiration. This could reveal the bradycardia of the primary cardiovascular response of the chemoreceptor reflex, which may result in a potentiation of the bradycardia from both reflexes.

However, after blast in this study, doxapram shortens the apnoea i.e. still gives the respiratory response, but no further bradycardia than that seen in the absence of doxapram. One interpretation of this would be that the peripheral chemoreceptors are no longer effective after a blast injury (and so the peripheral chemoreceptor induced-bradycardia is lost) and the respiratory drive comes from the central chemoreceptor effect of doxapram ([4]Romeo *et al.* 1995; Scott *et al.* 1977; Bamford *et al.* 1986).

This suggestion, however, seems unlikely since the response to blast can produce reduced PaO_2 *and* $PaCO_2$ (Ohnishi *et al.* 2001). This pattern of hypoxia and hypocarbia is typical of a situation where the peripheral chemoreceptors are stimulated by hypoxia and/or acidaemia with the resultant increased ventilation driving down $PaCO_2$. The central chemoreceptors limit, rather than cause, this effect since they usually respond to elevated $PaCO_2$, not to hypoxia or acidosis.

To examine whether doxapram is exerting its effects via actions on the peripheral chemoreceptor reflex a study could be carried out on the response to the administration of a drug that will only act on the peripheral chemoreceptors (e.g. cyanide; CN, see Daly, 1991), given before and after blast and sham blast, and determine whether the response will be similar to that of doxapram. If the response to such a drug (e.g., cyanide) was different to that of doxapram then perhaps the peripheral chemoreceptor reflex is not involved in the mechanism of action of doxapram after blast, or the peripheral chemoreceptor reflex doesn't respond after blast. Further, doxapram could be injected close-arterially to the carotid bodies in a very small dose and the response compared to that obtained when the same dose is injected intravenously.

[4] Article in Italian, information gained from English abstract.

The response to doxapram injection following denervation of the peripheral chemoreceptors would determine whether doxapram is acting via stimulation of this reflex (although no information could be gained about the bradycardia from these latter experiments as the denervation procedure for the aortic arch chemoreceptors is likely to destroy the vagal innervation to the heart). The above studies would aid in determining whether the peripheral chemoreceptors function after blast and the mechanism of doxapram's actions.

Finally, as the response to the pulmonary 'J' reflex is reminiscent of the response to thoracic blast (see Chapter 1, section 1.5), it would be interesting to determine whether the chemoreceptor reflex can generate a respiratory response during activation of the pulmonary 'J' reflex, and what the interaction would be on the bradycardia. The bradycardia due to the pulmonary 'J' reflex is likely to use a different population of cardiac vagal motorneurones than that due to the peripheral chemoreceptor reflex as the bradycardia induced by the pulmonary 'J' reflex cannot be modulated by respiration (Daly & Kirkman, 1988), however, the chemoreceptor-induced bradycardia is modulated by changes in respiration (Daly & Kirkman, 1988; Daly *et al.* 1988). Paton (1997) showed that the chemoreceptor-induced increase in respiration is inhibited by a simultaneous pulmonary 'J' reflex-induced apnoea, but no cardiovascular changes were commented on. Experiments involving simultaneous stimulation of the pulmonary 'J' reflex and peripheral chemoreceptor reflex could be compared to the above experiments involving blast/sham blast as this could potentially give some indication of the mechanism of the response to blast.

To summarise, preliminary evidence seems to confirm previous studies that administration of doxapram (in the absence of blast injury) stimulates respiration, but in this study doxapram gave a depressor response (however, it must be stressed that n=1). This is contrast to its reported pressor actions in the literature (Huon *et al.* 1998; Cote *et al.* 1992; [5]Bruckner *et al.* 1977), and its attenuation of the blast-induced hypotension in this study.

[5] Article in German, information gained from English article.

The main results from this study show that administration of doxapram following thoracic blast can pharmacologically reverse the reflex apnoea that results from this injury, and maintain arterial blood gases possibly by an improvement in ventilation and perfusion matching in the lungs. Additional to this, doxapram appeared to attenuate the hypotensive response to primary blast injury and may have reduced a blast-induced pulmonary oedema (as doxapram reduced lung weight index), but had no effect on the bradycardia.

7 Discussion

This thesis aimed to address several questions which are clinically relevant to blast victims. The following paragraphs will re-cap on these aims before going on to summarise the findings of this thesis and outline any future experiments that need to be carried out. How this information may be used in the assessment and treatment of the blast-injured casualty will also be discussed in this chapter.

7.1 Summary of the Aims and Results of this Thesis

The initial aim of this thesis was to determine the effect of thoracic blast injury on the cardiorespiratory response to haemorrhage, and whether these responses, or their interaction, are modified by morphine. The results showed that thoracic blast injury augments the bradycardic, hypotensive second phase of the response to haemorrhage, while morphine attenuates this effect.

Subsequent chapters addressed the topic of fluid resuscitation of the hypovolaemic blast-injured casualty. The cardiovascular effects of various isotonic fluids were compared to the increasingly popular hypertonic saline/dextran for resuscitation early and late after a thoracic blast injury and haemorrhage. Results of these studies showed adequate restoration of cardiovascular parameters with isotonic solutions, regardless of the timing of the resuscitation. However, resuscitation with HSD proved ineffective both early and late after a blast injury and haemorrhage in anaesthetised rats. Therefore the succeeding chapter investigated various hypertonic solutions in comparison to HSD, however, the same conclusion was derived; HSD was the only solution which failed to maintain arterial blood pressure, heart rate or femoral blood flow for longer than 5 minutes.

The final aim in this thesis was to investigate a potential pharmacological means of modifying the reflex apnoea that occurs as part of the response to thoracic blast injury. The respiratory stimulant doxapram proved to significantly attenuate the blast-induced apnoea, as well as improving arterial blood gases, reducing lung weight index and attenuating the hypotensive response to thoracic blast injury.

7.2 Potential Future Studies

In addition to those potential future studies mentioned in the discussion sections of the experimental chapters (sections 3.4, 3.4.1, 4a.4, 4a.4.1, 5.4, 6.4, 6.4.1 and 6.4.2) further studies could be carried out, e.g., to determine whether opioids modify the doxapram-induced attenuation of the apnoeic and hypotensive response to blast. This would also allow us to determine whether the blast-induced bradycardia is modified by morphine together with doxapram as it is not affected by either alone.

A full haemodynamic assessment is needed after blast (see Chapter 3, section 3.4.1). Blood flow in several different organs at the same timepoints following a blast injury could be assessed using different coloured fluorescent microspheres (Schimmel *et al.* 2001). This technique could also be used to assess haemodynamics after blast and doxapram administration. A bolus of doxapram will attenuate the blast-induced hypotension but this is short lasting. Clinically a continuous infusion of doxapram may therefore maintain BP for longer, however, this may be at the expense of blood flow to vital organs. These organs could also be assayed for markers of tissue damage *post mortem*, such as liver proteases, which are known to be increased following injury (REF).

As mentioned earlier in this chapter, there is an obvious need for the assessment of the integrity of the baroreflex to be carried out following blast injury as the blast-induced hypotension is associated with a bradycardia rather than a tachycardia which would be expected were the baroreflex functioning normally. A preliminary study is currently underway. The baroreflex is assessed using the phenylephrine pressor test before and at several timepoints following a blast injury in the anaesthetised rat. Preliminary results show that rather than the baroreflex being inhibited by the response to blast as one might assume from looking at the results of the blast and haemorrhage experiments (Chapter 3, phase 1 of the response to haemorrhage, which is due to the actions of the baroreflex, is absent; Sawdon *et al.* In press) it looks as though baroreflex sensitivity may actually be *increased*. This is consistent with a report by Little and colleagues (1984) whereby baroreflex sensitivity is increased during phase 2 of the response to a progressive simple haemorrhage. During this phase there is also a bradycardia associated with a fall in blood pressure. However, the preliminary findings are in contrast to another type of injury, musculo-skeletal tissue injury, where baroreflex

sensitivity is *decreased* in man (Anderson, Little & Irving, 1990) and rat (Redfern *et al.* 1984).

During blast injury the vagally mediated bradycardia is short lasting (over in 5-15 minutes) but powerful. Therefore, blast injuries may lead to the development of hypoxia. The defence reaction can be activated by hypoxia (Marshall, 1987) but this would cause a tachycardia and hypertension and not the bradycardia and hypotension seen with blast. It may be possible that the hypoxia driven defence reaction is overcome by blast, i.e., a bradycardia and hypotension become apparent and not the tachycardia and hypertension. Some anaesthetics (chloralose, urethane and barbiturates) are known to inhibit activation of the defence reaction from carotid body stimulation. However, the animals used in this thesis are anaesthetised with Saffan (an anaesthetic shown not to interfere with the cardiovascular component of the defence reaction) and so this may be allowing some defence reaction to come through (Timms, 1981) and is actually dampening the blast-induced bradycardia, i.e., in conscious man there may be an even more powerful bradycardia, perhaps fatal. After a model of head injury, where a pressure of 300 psi is administered to the parietal cortex (G. McMahon & E. Kirkman, unpublished study), there is a longer apnoea and no lung oedema with sodium pentobarbitone (Sagatal) anaesthetised rats compared to those anaesthetised with Saffan were there is a shorter apnoea and oedema of the lung severe enough to kill the majority of the animals. Future experiments should therefore include blast work under differing anaesthetics, comparing the magnitude of the bradycardias.

In a recent study looking at a model of musculo-skeletal tissue injury (bilateral hindlimb ischaemia) and haemorrhage in rats, whilst measuring blood flow in the superior mesenteric artery (SMA), it became apparent that in the group subjected to haemorrhage alone, phase 1 of the response to simple progressive haemorrhage was absent (Sawdon *et al.* 2001). This is reminiscent of the response to haemorrhage on a background of thoracic blast injury. What may be occurring in that study is a haemorrhage response on top of another type of injury, i.e., to the viscera (the intestines are displaced laterally to allow access to the SMA) in the same way that blast may be altering the response to haemorrhage due to movement of the viscera by the blast wave. Determining the response to haemorrhage following an abdominal blast could assess this.

7.3 Potential Treatment of the Blast-Injured Victim

The new information gained from the work covered by this thesis could potentially lead to improved assessment and treatment of the blast-injured victim. It has been shown that the response to blast modifies that to a subsequent haemorrhage such that the first compensatory phase of the response to blood loss is absent (Chapter 3; Sawdon *et al.* 2002) and so the volume of blood lost in these patients may be overestimated and hence too much fluid may be administered upon resuscitation. Administration of morphine for analgesia to a blast victim will alter the response to blood loss yet again, potentially masking any internal bleeding.

If the patient requires fluid resuscitation at some point, the clinician (or military personnel treating the victim in the field) will now be aware of the possible hazards associated with resuscitation with hypertonic saline/dextran. Therefore, one of the other fluids may be chosen such as hydroxyethyl starch or whole blood if in a clinical setting, or a hypertonic solution other than HSD if treatment is being administered out in the field.

If the patient is seen whilst still in the apnoeic stage of the response to blast injury, this cessation of breathing can be halted pharmacologically by the administration of the respiratory stimulant doxapram. This will also attenuate the hypotension and improve arterial blood gases as well as, theoretically at least, aiding oxygen delivery to the tissues thereby reducing ischaemic damage to organs and attenuating any lung oedema, possibly reducing the risk of developing adult respiratory distress syndrome (ARDS).

The combined insults of a reduced cardiac output due to blood loss and lung damage from a blast injury is likely to exacerbate the inflammatory response (see Chapter 4a, section 4a.4.1 and 4a.4.2). However, if future studies show an up-regulation or early expression of markers of inflammation such as ICAM-1 and VCAM-1 or microalbuminuria following blast injury and haemorrhage, then with prompt detection of these markers, early intervention may be possible, thus reducing any further risk of other clinical sequale such as adult respiratory distress syndrome. .

The combined results of this thesis show potential for improvements in the assessment and treatment of the hypovolaemic blast-injured patient. However, it must be stressed

that the injuries investigated in this thesis are well-defined injuries in anaesthetised rats, and further clinical trials are required before the results can be utilised in man.

8 References

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8.1 Books

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8.2 Unpublished Thesis

1. Ohnishi, Mitsuo. 'The Reflex Nature of Thoracic Blast Injury' (University of Durham. Ph.D. thesis, 2002)

9 Publications arising from this Thesis

9.1 Abstracts (peer reviewed)

Kirkman, E., Sawdon, M. A., Ohnishi, M., Cooper G. J. & Watkins, P. (2000). Effects of Primary Thoracic Blast Injury on the Response to Haemorrhage in the Anaesthetised Rat. *Journal of Physiology*. **523,** 292P.

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9.2 Other Abstracts

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9.4 Full Papers

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11 Summary of Abbreviations

σ	Capillary reflection coefficient (sigma)
πc	Capillary oncotic pressure
πi	Interstitial oncotic pressure
5HT	5 Hydroxytryptamine
ABE	Arterial base excess
ANOVA	Analysis of Variance
ARDS	Adult Respiratory Distress Syndrome
ATLS	Advanced Trauma Life Support
BP	Blood pressure
BV	Blood volume
CN	Cyanide
CVM	Cardiac vagal motorneuron
Fem Q	Arterial femoral blood flow
FiO2	Fraction of inspired oxygen
FVR	Femoral vascular resistance
Hct	Haematocrit
HES	Hydroxyethyl starch
HHES	Hypertonic hydroxyethyl starch
HP	Heart period
HPV	Hypoxic pulmonary vasoconstriction
HS	Hypertonic saline
HSD	Hypertonic saline/dextran
i.a.	Intra-arterially
i.v.	Intra-venously
ICAM-1	Intracellular adhesion molecule-1
IL	Interleukin
Kc	Capillary filtration coefficient
LWI	Lung Weight Index
MBP	Mean blood pressure
MmHg	millimeters of mercury
n	Sample size

N_2O	Nitrous oxide
NA	Nucleus ambiguus
NTS	Nucleus tractus solitarius
$PaCO_2$	Arterial carbon dioxide tension
PaO_2	Arterial oxygen tension
PBG	Phenylbiguanide
Pc	Capillary hydrostatic pressure
pH	Arterial pH
Pi	Interstitial hydrostatic pressure
Ppa	Pulmonary arterial pressure
psi	Pounds per square inch
PVR	Pulmonary vascular resistance
RL	Ringers lactate
RMV	Respiratory minute volume
RR	Respiratory rate
RVLM	Rostral ventrolateral medulla
SEM	Standard error of the mean
Temp	Body temperature
TPR	Total peripheral resistance
V/Q	Ventilation to perfusion ratio
VCAM-1	Vascular cell adhesion molecule-1
Vt	Tidal volume