An examination into the origins of microscopic gas emboli in blast

Joanne Elizabeth Harding

School of Medical Sciences

The University of Adelaide

Submitted for Degree of Doctor of Philosophy

7 January 2015

Contents

Appendices

Abstract

Injuries from blast are found in a variety of clinical settings following exposure to an explosion. An explosion results in a blast wave, formed as it releases (and expands) the gas produced, into the immediate surrounding environment. While blast trauma is well documented in the literature, the origins of microscopic gas emboli resulting from blast are not. As such the management of gas emboli for blast victims is currently not based upon research evidence.

The major objective of this research was to outline and test an alternative theory to microscopic gas emboli development in blast other than the popular and untested translocation theory.

This research has shown a rapid decompression effect liberates a dissolved gas (carbon dioxide) from blood to gas bubble, this was supported by a lowered carbon dioxide content in active samples and aligned acid-base chemistry using a blood gas analyser. These findings justified a further experiment using an explosives experiment, again using blood samples and blood gas analysis. Although the blast experiment did not provide clear evidence in support of the autologous theory for microscopic gas emboli formation (due to the effects of the positive pressure phase), it justifies the continuation of the search for mechanisms of emboli development other than translocation because the bubbling in blood could not possibly have resulted from translocation via damaged pulmonary architecture.

Statement

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

…......................................

Joanne Elizabeth Harding

Acknowledgements

Chapter 1

Introduction

1.1 Outline

Studying the pathological root of injury enables clinicians to provide to patients care that is derived from evidence. Finding that evidence is not always straightforward, theories are posed and evolve over time, and can become entrenched. As years pass, research methods develop, old theories can be reviewed and retested using new methods, paving the way for new research providing evidence for clinical practice and improved health outcomes for patients.

1.2 The blast wave and related trauma

An explosion - an extremely rapid release of energy, generally in the form of a compressed gas, into an environment of relative lower pressure, such as atmosphere - can occur through accidental (industrial or domestic), environmental (natural fires or volcanoes) and as well deliberate (military or criminal/terrorist) means. The blast force an explosion produces is proportional to the type and size of the base material (Arnold, Halpern & Tsai, 2004; Iremonger, 1997). Blast trauma is the injury caused by exposure to an explosion.

As a clinical category, blast trauma includes an assortment of injuries with varying physiological aetiologies; some victims suffer multiple injuries just as road trauma victims do, but the origins of those injuries can be quite different. The injuries received in blasts result from a multitude of factors, not least of all the direct impact of the blast wave front against the body. Kirkman et al. (2008, p. 105) described blast injury resulting from artificial explosives,

as '...the biochemical and pathophysiological changes and the clinical syndrome resulting from exposure of the living body to detonation of high explosive.'

A blast wave front evolves from the expansion of gas released when an explosion occurs. Upon release, the gaseous expansion compresses the immediate surrounding atmospheric environment, creating a high pressure blast wave front. The force of the wave front fades rapidly as it moves away from the detonation point. As this compression effect decays behind the wave front the resultant rarefaction leaves an inertia effect, producing a negative pressure in the surrounding environment (Iremonger, 1997; Sattin et al. 2008). A diagram outlining a blast wave pressure time line is provided as Figure 1.1, showing the difference in over-pressure (in red) and under-pressure (in yellow) phases over time. The positive pressure phase is above the ambient pressure line, *(x* axis), the under-pressure phase is sub-atmospheric and begins as the wave descends below the ambient pressure line (*x* axis), until it returns to ambient pressure level.
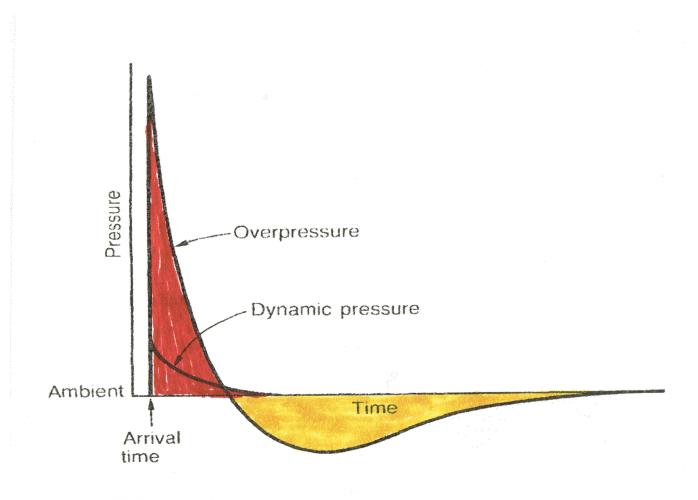
Figure 1.1 An open air blast wave, expressed as a pressure-time line. Adapted from Yelverton, 1997, p. 200.

The implications from both the over-pressure and under-pressure waves for the human body vary greatly. Various injuries from blast have been observed for many years; they include high impact trauma, penetrating injury from flying debris, deceleration injury, traumatic amputation and burns (Mellor, 1997; Sattin et al. 2008). The high pressure exerted on the body by the positive pressure wave results in a unique 'blunt barotrauma', an injury firmly linked to high morbidity (Axelsson & Yelverton, 1996; Bass, Rafaels & Salzar, 2008; Bowen, Fletcher & Richmond, 1968; Cooper et al. 1991; Jaffin et al. 1987; Phillips & Richmond, 1991). The over-pressure wave primarily affects air and fluid filled organs such as lungs, ears and bowel.

The effects of over-pressure have been researched in depth for many years (Benzinger, 1950; Bowen, Fletcher & Richmond, 1968; Cooper et al. 1991; Desaga, 1950; Hoge et al. 2008; Iremonger, 1997; Irwin et al. 1999; Long et al. 2009; Phillips & Richmond, 1991; Ramasamy et al. 2009; Teland, 2012), but the effects from under-pressure are not as well documented (Latner, 1942; Zhang et al. 1996). The research described in this thesis was designed in part to address this deficit in research into the effects of under-pressure.

1.3 Research history

Research into blast injuries peaked in the 1950s and 60s, when the majority of the fundamental work on explosives injury was done (Benzinger, 1950; Bowen, Fletcher & Richmond, 1968; Chiffelle, 1966; Desaga, 1950). There followed a slow progression in detailed work until the major terrorist attacks on the United States in 2001, which revived blast research.

Recent research, with modern experimental tools, has delved deeper into human physiology and injury potential from blast than ever before. Current research is exploring the inclusion of immune and inflammatory response to blast. This evolving area includes explorations in biochemical and gene activation responses to injury, with a focus on any link to blast injury specifically (Elsayad & Gorbunov, 2008; Gorbunov et al. 2005; Surbatovic et al. 2007). In addition, research is producing a growing awareness of blast specific brain injury (Ling et al. 2008; Rosenfeld & Ford, 2010).

While the clinical management of blast-specific brain injury is consistent with other types of brain injury, the blast brain injury (in particular mild traumatic blast brain injury) shares some symptomatology with post-traumatic stress disorder, making isolating the physical from the psychological injury difficult for researchers in both these fields (Ling et al. 2008; Reneer et al. 2011; Taber, Warden & Hurley, 2006). Blast injury research has also shown the benefits of applying specific resuscitation methods for blast victims (Garner et al. 2009; Kirkman et al. 2008).

The development of blast injury knowledge has been attained by bringing information from the biological and the engineering communities together through a multidisciplinary approach. Knowledge from this body of resources related to blast trauma research is explored through further chapters of this thesis providing a solid foundation for the experimental phase of the project.

1.4 Current blast injury management

Today, blast victims, even severe cases, recover much faster than those in our recent past; this is attributable to advanced resuscitative practices, surgical techniques and specialist units' technologies and practice regimes (Alfici, Ashkenazi & Kessel, 2006; Arnold, Halpern & Tsai, 2004; Hirshberg et al. 1999; Lavery & Lowry, 2004; Sattin et al. 2008; Shamir et al. 2006). These successes, however, come at a cost for individuals and health care systems. Individuals can suffer long term hospital stays with extended rehabilitation time, at high costs to the public purse. Having specialist retrieval, emergency care systems, alongside critical care, burns and rehabilitation units help avoid protracted burden through expedited specialist care staff and specifically focused regimes (Arnold, Halpern & Tsai, 2004; Peleg et al. 2003; Rosenfeld et al. 2005).

Also today, blast research is further enriched by clinical experiences, although it is unfortunate that blast injury seems more common these days, those documented experiences are assisting in the care of victims by providing Level III evidence for practice (Arnold, Halpern & Tsai, 2004; Bochicchio et al. 2008; Hoge et al. 2008; Pizov et al. 1999; Scherer et al. 2007; Shamir et al. 2006). In the four years between 2001 and 2005, blasts in civilian settings, outside intense terrorist/war zones such as Iraq and Afghanistan, left almost twice as many casualties compared with deaths (Rosenfeld et al. 2005).

Blast trauma victims present with a pattern of injury different from that of conventional trauma, a unique injury complexity with more body regions affected, more admissions to intensive care units, longer hospital stays, more surgical interventions and ultimately an

increased mortality rate (Kluger et al. 2004; Stuhmiller, 2008; Wade et al. 2008). These differences are shown in Table 1.1.

Table 1.1 Trauma types observed in terrorist bombings and conventional trauma events. Sattin et al. 2008, (p. 25).

VARIABLE	TERRORIST BOMBING	OTHER TRAUMA
ISS > 15	28.7	10.0
GCS < 6	9.5	2.9
Admission BP <90mmHg	6.2	2.5
ICU admission	26.0	7.1
Body regions injured >3	28.3	6.2
Surgical procedures	50.8	36.6
In hospital mortality	6.1	2.0

The relatively large number of casualties demanding high levels of specialist care following blast means expectation of research based evidence for that care is justified. It is the education of clinicians through evidence based training programs that provides knowledge translation from research findings to the bedside (Grimshaw et al. 2012).

Immediate management for blast victims requires simple expedient trauma management to manage what is commonly, but not always a mass casualty event, however it is reasonable to assume that multiple injuries will be discovered even in a single patient scenario. Training regimes such as Early Management of Severe Trauma and Advanced Trauma Life Support assist in guiding the clinician through the trauma patient scenario, and are well known in most

of the developed world where successful patient outcomes are proven (American College of Surgeons, 2008; Kilner, 2000).

With appropriate adjustment to a blast situation, be it mass casualty, and/or chemical or radiological contamination, an individual patient can be managed in much the same way as any other trauma casualty using the principles of resuscitation known as Danger, Response, Airway, Breathing, Circulation (DRABC) (Alfici, Ashkenazi & Kessel 2006; Rosenfeld et al. 2005). Yet, because injuries resulting from terrorist bombings are likely to produce more multiple trauma and very different injury patterns from other disaster scenes, appreciation of the fundamental mechanisms of blast is imperative for effective initial resuscitation and safe transportation from the scene (Arnold, Halpern & Tsai, 2004; Frykberg, 2002; Kluger et al. 2004).

If treatment begins rapidly and to the highest standards, it is not unreasonable to expect that a successful discharge and rehabilitation will result for most cases in the developed world. To achieve this, discrete injuries require individual attention within the context of their aetiology, just as the cervical spine fracture is considered when deceleration occurs in a vehicle accident.

Microscopic gas emboli formation is a discrete phenomenon arising from blast's many possible injuries and deserves the same level of attention as the cervical spine injury from a vehicle accident. An intellectual, well-versed approach to treating trauma requires prudent, focused laboratory and clinical research on the injuries; the possibility of blast emboli formation should not be discounted in a blast victim's presentation because it is integral to their injury history, which in turn is of paramount importance to prospective care planning (American College of Surgeons, 2008; Kilner, 2000).

1.5 Microscopic gas emboli, a blast phenomenon

A gas embolus is a bubble of gas identified in the blood vessel that is mobile; gas emboli are usually found as a result of a physiological insult and may be iatrogenic or not. As emboli move through the circulation they are threatening to the vessel endothelium, the peripheral circulation and end organs, where ultimately they may cause death (Francis & Mitchell, 2004; Muth & Shank, 2000; Suzuki, Armstead & Eckmann, 2004). Insight into blast gas emboli began with their discovery through experimental work in 1950, by Benzinger (1950). Three theories about how the emboli develop (by translocation, through spalling and by autologous formation) eventually arose, each embraced by different science disciplines with varying degrees of enthusiasm. Each discipline rationalises its argument succinctly, but not conclusively, because of a lack of research and their use of an inductive approach to the debate.

The first theory considers the possibility of airways gas 'translocating' as emboli from alveoli to capillaries, when the lung parenchyma is disrupted as the blast wave passes through the chest (Benzinger, 1950; Clemedson & Hultman, 1954). The lung is considered to be the most vulnerable to a blast wave impact and when it is affected by the pressure wave, it is known as a primary lung injury (Sharpnack, Johnson & Phillips, 1991). Given that blast emboli have only been found in central arterial and pulmonary circulation, the theory is superficially plausible. This theory relies directly on the origins of microscopic gas emboli arising from the effects of the blast overpressure and subsequent lung injury.

The 'translocation theory' dominates the medical literature to the point where treatment regimes have been designed around the concept in an effort to mitigate perceived iatrogenic

consequences (Ciraulo & Frykberg, 2006; DePalma et al. 2005; Riley, Clark & Wong, 2002; United States Department of Defense, 2005).

Another theory originally produced in 1950, by Schardin, describes 'spalling' as a cause for emboli development (Schardin, 1950). The spalling effect is the result of coupling, where the blast wave travels from a higher density medium to a lower density medium, whereupon it produces a tension wave as it passes through the chest wall (dense medium), then through internal lung parenchyma, including gas filled alveoli (the less dense medium).

Engineers believe the ensuing tension wave can throw off material at the interface (Phillips & Richmond, 1991). Today's engineers in this field consider the material thrown off at this time may be emboli, leaving 'spalling' as a possible way of producing microscopic emboli in blast victims (Ritzel, personal communications, 2004 & 2011). Unfortunately, there is no explanation as to what those emboli may constitute, by way of blood particles or gas.

In years gone by the content of an embolus appeared inconsequential by absence of any discussion because effects of emboli were more focused on regional effects with regard to organ damage from impaired blood supply (Benzinger, 1950; Clemedson & Hultman, 1954; Phillips & Richmond, 1991). However today, information on emboli contents is imperative in determining how emboli may react with the blood vessel's endothelium by way of biochemical responses affecting both anatomy and physiology of the endothelial lining when they are present (Drew, Helps & Smith, 1995; Eckmann & Armstead, 2006; Kapoor & Gutierrez, 2003; Suzuki, Armstead & Eckmann, 2004). Biochemical responses to blast injury is one of the latest foci in blast injury research, therefore learning more about emboli

development in blast may also provide information to this specific biochemical research arena.

While the first two theories of blast emboli development rely on the biophysical effects from the high over-pressure phase of blast and ensuing lung injury, the third theory holds that emboli develop in the sub-atmospheric or under-pressure phase of the blast wave. The theory proposes that the fall in the surrounding air pressure to sub-atmospheric levels at that time brings dissolved gas in blood out of solution as bubbles.

Any consideration of this third theory (autologous development during the sub-atmospheric phase) was dismissed in the 1950's during discussions in the literature between Benzinger (1950), Rossle (1950) and Clemedson & Hultman (1954). A thorough literature search revealed no evidence that any research underpinned or followed these discussions to test their outcome. The debate rested as there was no further exploration to determine the origins of microscopic blast gas emboli, but some research addressing changes in blood during lowered and sub-atmospheric pressures occurred as early as 1952 (Kemph & Hitchcock, 1952). Unfortunately, no link was made between this work and emboli development during the under-pressure phase of blast. Kemph & Hitchcock's (1952) work showed changes in blood exposed to explosive or rapid decompression by way of foaming, alongside a loss of measured carbon dioxide.

Kemph & Hitchcock (1952) were seemingly unaware of the close relationship their work had to the autologous theory and its partnered sub-atmospheric phase of blast, and as a result of that, the autologous theory has become obsolete in the literature over the years, while the

translocation theory appears to have become lore (Benzinger, 1950; Clemedson & Hultman, 1954; DePalma et al. 2005; Horrocks & Brett, 2000; Mayo & Kluger, 2006; Rosenfeld & Ford, 2010; Rossle, 1950; Weiler-Ravell, Adatto & Borman, 1975; Wightman & Gladish, 2001). This occurred due in part to experts promoting a focus toward the impact of over-pressure effects, as this was seen to be where the serious injuries lay, and in the minds of the researchers and experts of the time so perhaps did the question of emboli formation.

As previously alluded to, testing a theory is only possible if the necessary knowledge and testing capability exists at the time. With no tools available to test for sub-atmospheric causes of gas emboli formation, over-pressure became the focus of blast trauma research.

1.6 Finding a pathway for research

Our current knowledge of blast injuries comes from a multitude of sources, including engineers, scientists and clinicians. Much of this knowledge is established using deduction and scientific method to provide evidence to support a theory; clinicians then contribute evidence from clinical settings, further developing an overall understanding of blast effects on humans. Unfortunately, the causes of some phenomena remain speculative because of a lack of research evidence. This is particularly evident when speculative explanation of a phenomenon is made by respected experts and is ongoing over extended periods. The tendency for a theory to be granted greater credibility than it is due is natural but it remains unscientific. This is not an uncommon situation in clinical practice, hence the push for evidence-based practice and knowledge translation for the utilisation of research in clinical practice and policy development (Grimshaw et al. 2012). It is paramount that research

information provides evidence for clinical practice, where a theory has been tested, thus providing the evidence (Grimshaw et al. 2012).

The revolution in our understanding of the cause, treatment and prevention of gastric ulcers is a spectacular and recent example of how the perceived wisdom of eminent clinicians and researchers can persist in the face of compelling evidence. Despite little evidence in support and some evidence to the contrary, the theory that gastric ulcers were caused by patients' stress had become lore (Palmer, 1954), and vagus nerve surgery was a common treatment. It took Marshall and Warren, winners of the Nobel Prize for Medicine in 2005, considerable time and effort to convince their colleagues that stomach ulcers are caused by a bacterium (Warren & Marshall, 1984).

History is littered with similar stories of opinion being accepted as fact (or refuted) over time, often only because the original question had not been revisited using a modern approach. Modern techniques and tools such as biopsies, electron microscopes, culturing, and ultimately genomics enabled Marshall and Warren to make their discovery. They succeeded not by abandoning earlier work or accepting the status quo, but taking the information along a different pathway to previous researchers. The story of gastric ulcers teaches us the importance of taking small but very deliberate steps based on existing knowledge and using it to examine pathways to an evidence-rich end. The project described in this thesis took a similar exploratory approach.

As already noted, the occurrence of gas emboli in blast is under-researched, and theories of their origins have been promulgated as truisms over the decades since Benzinger published his discovery of gas emboli in 1950. Since their discovery, new scientific tools, tangential

13

research observations and a more developed knowledge base have paved the way to investigating this phenomenon more fully. This thesis reviews the current understanding of the three existing theories in the development of microscopic gas emboli in blast victims, and tested one of those theories experimentally.

1.7 Project outline

We know air emboli occur because they have been isolated post mortem in the pulmonary and central arterial circulation. Some think they develop through leakage across damaged alveoli and blood vessel walls, and whilst this is also probably their place of origin, we do not know for sure, and this might be because we do not know the mechanism by which they are formed.

The major objective of this thesis was to determine whether microscopic blast emboli form in any way other than the translocation theory. Accepting the premise that microscopic gas emboli form by autologous means does not preclude the concept that they may at times form by translocation in injury scenarios consistent with that possibility, such as penetrating chest and lung trauma.

To discover a theory alternative to translocation forges a path for future research that may assist in determining whether there is reasonable justification for an additional or alternative theory to the most popular theory of how microscopic gas emboli form in the blast trauma scenario. This thesis provides a brief overview of blast science, alongside the clinical implications of blast trauma. Areas explored include wave theory, blast wave formation and an explanation of the biophysical effects of blast using information derived from biology,

physics, chemistry and clinical medicine. The thesis then outlines all three theories surrounding blast emboli formation, where a literature examination and application of deductive reasoning using Henry's Law reveals justification for exploring the theory that emboli develop during the sub-atmospheric phase of blast.

The subsequent experimental work focused on determining if gas can be released from solution (blood) during a rapid decompressive event such as that observed in the sub-atmospheric phase of a blast. In addition, the work set out to determine the type of gas liberated from the blood solution.

The results of this experimentation provide a platform for further work integral to blast specific emboli and their effects on the human body.

Chapter 2

Blast science

2.1 Outline

The focus of this research was the development of microscopic gas emboli in blast victims. To comprehend the potential range and scale of the impact blast exposure has on human physiology, an overall understanding of how an explosive device works, the wave theory and wave formation is necessary. This chapter explains the science behind blast, using the link between the blast wave and blast injury by relating the process of blast wave transmission in a variety of typical forms to its impact on the human body.

2.2 Common explosive materials

An explosion is a release of compressed gas that forms a high pressure shock wave that begins at the detonation point and moves very rapidly in all directions. In general terms, the peak high pressure is reached quickly, the wave front interfaces with the surrounding medium, and the pressure following the front decays as it moves away from the detonation or release point (Iremonger, 1997; Schardin, 1950).

The most common type of artificial explosion causing injury is chemical. A chemical explosion is produced by the oxidation of reactive chemical compounds or agents, releasing the rapidly expanding gases. The magnitude of the explosion depends on whether low or high-grade explosives are used, which in turn is dependent upon the speed at which they oxidise; the greater the speed the more energy released and the higher the grade (Iremonger, 1997; Mellor et al. 1997). Low-grade gunpowder, used since the 9th century in China, is still

used today in small arms and munitions such as flares, blank rounds and fireworks (Stuhmiller, Phillips & Richmond, 1991). Nitro-glycerine is an example of a high-grade explosive, and used today in dynamite[1]. Dynamite compounds are used extensively in mining and demolition work today alongside ammonium nitrate and trinitrotoluene (TNT) (Schardin, 1950; Stuhmiller, Phillips & Richmond, 1991).

Modern military explosives achieve selective levels of explosions through the use of plastic compounds containing RDX (or cyclotrimethylene-trinitramine) and de sensitisers (Lieutenant Colonel W. Jolly MBE, Royal Australian Corps of Engineers, personal communication, June 2007). Plastic explosives such as RDX provide more stability in varying climates and will not explode erroneously, as they require a detonator (Iremonger, 1997; Stuhmiller, Phillips & Richmond, 1991).

Cocktails of conventional and unconventional materials are different from those found in conventional historical military operations and commonly used by terrorist organisations (Elsayad & Gorbunov, 2008). Chemicals containing chlorine and propane are often used, and increase the potential of an explosive event, raising the speed by which the blast wave moves from less than 300 - 400 m/s up to 1,000 - 9,000 m/s (Elsayad & Gorbunov, 2008). Such compounds produce a high-grade explosion and carry a high injury potential because they produce a more instantaneous peak pressure rise and a faster blast wind.

Explosions in the industrial or community settings vary in propensity and occur often worldwide at great human and economic cost to victims, businesses, communities and

_____
[1] Discovered and patented by Alfred Nobel 1867 (Encyclopaedia Britannica, 2013).

receiving hospitals (Chapman, 2010; Dixon & Millar, 2014; Dobnik, 2014; Pomara et al. 2013; Video World News, 2009; Video World News 2013; Zhang et al. 2011).

2.3 Wave theory and the blast wave

Appreciating the fundamentals of a blast wave is essential to providing informed patient care, because it is intrinsically linked to understanding the mechanisms of injury caused by blast. Trauma management relies on the clinician understanding the mechanism of injury and the history of the traumatic event; a clinician's capacity to provide appropriate care is compromised if there are no foundations beyond the obvious injury on which to apply practice or even still logistical support within a health care system (Frykberg, 2002; Rosenfeld et al. 2005).

Blast wave behaviour was studied in depth between the 1960's an 70's. The results arising from those eminent researchers remain valid and feature prominently in blast injury literature (Bowen, Fletcher & Richmond, 1968; Iremonger, 1997; Mellor, 1997; Mellor et al. 1997; Phillips & Richmond, 1991; Stuhmiller, Phillips & Richmond, 1991; Yelverton, 1997). That prominence has lead more recent authors to cite them in their written work, as a consequence these researchers and authors dominate the discussion through the remainder of this chapter.

As the blast wave travels, it transfers energy from one point to the next, always moving away from its point of origin in a spherical pattern. The amount of energy transferred through this process depends on the level and type of chemical used to produce the wave, along with the density of the medium through which energy must transit. A blast wave is thinner than a

normal sound wave which relates to it being so highly pressurised, and the higher the pressure the faster the wave. The blast wave travels at a speed faster than sound, hence the noise of the explosion arrives after the flash and the physical effect of the explosion itself (Canon, 2001; Iremonger, 1997; Mellor et al. 1997; Stuhmiller, Phillips & Richmond, 1991; Teland, 2012). A blast wave is produced as an explosion releases (and expands) the gas produced into the immediate surrounding environment (medium). The sound wave leaves from a stationary point and moves in all directions from this central point, and the resultant vibration can travel through a variety of media before it is exhausted. The high pressure shock front leads the blast wave; as the pressure pulse of the wave steepens it compresses the atmospheric air, forming the shock front. This shock front occurs instantaneously as the opposing atmospheric pressure ceases to compete with the rapidly moving high pressure gas wave front arising from the explosion. The blast wind follows instantaneously, (the net motion of the gas released) and is measured as dynamic pressure (Schardin, 1950; Stuhmiller, Phillips & Richmond, 1991; Teland, 2012).

As the speed of the gas produced in an explosion is greater than the speed of sound, it results in an extremely rapid rise in pressure (referred to as over-pressure), temperature and flow density. The density of the medium in which the explosion occurs will impact on its over-pressure, the wave speed and any impending reflection. A free field wave is one released into a open space where the medium is only atmospheric pressure and there are no additional obstacles such as buildings or vegetation (Mellor et al. 1997; Stuhmiller, Phillips & Richmond, 1991).

The free field waveform or Friedlander waveform can be further described by Friedlander's equation used to measure a blast's potential:

$$P(t) = P_s (1 - t / t_0) \exp(- bt / t_0)$$

'Where P(t) is pressure over time, P_s peak pressure, t_0 is positive time duration and b is the decay constant (rate of over-pressure fall after the peak is reached)' (Stuhmiller, Phillips & Richmond, 1991, p. 245).

The free field blast wave as described is a blast wave progression in the purest form and used for demonstration purposes. Pressure is measured using a pressure waveform and expressed as a pressure time line, as illustrated in Figure 2.1. As a measure of pressure through time, the waveform consists of five definable measured points. Each is significant to the overall blast impact on injury: arrival time, positive pressure phase, including peak positive pressure (commonly referred to as peak over-pressure or POP), negative (or under) pressure phase, and a point of return to atmospheric pressure (Cannon, 2001; Iremonger, 1997; Mellor et al. 1997; Wightman & Gladish, 2001).
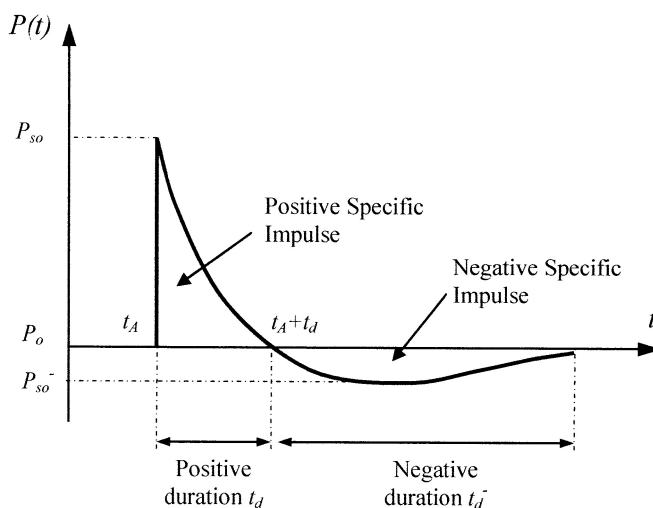
Figure 2.1 Positive and negative pressure specific impulses of a free field blast wave over respective duration times. tA = arrival time. (Ngo et al. 2007, p. 77).

The over-pressure time is the positive pressure time; from arrival time to the point where the wave falls below the atmospheric level marking the beginning of the under-pressure phase. The under-pressure time is the wave passage below the atmospheric level (or the *x* axis on the graph in Figure 2.1), until it returns to the *x* axis, or positive pressure juncture, once again.

The duration time of the wave is measured from arrival time to the point of return to atmospheric pressure following the under-pressure phase. The duration time equates to the time in which a victim is exposed to ambient pressure changes (Axelsson & Yelverton, 1996; Desaga, 1950; Mellor et al. 1997; Teland, 2012). Duration time, is a dynamic pressure measurement and influences injury because the impact of the pressure differentials throughout the time line on the human body is considered proportional to injury (Mellor 1997; Mellor et al. 1997; Stuhmiller, Phillips & Richmond, 1991; Teland, 2012; Wightman & Gladish, 2001). The pressure differential between positive and negative phases can be important for trauma (Cannon, 2001; Iremonger, 1997; Krauthammer & Altenberg, 2000), its place in the diagnosis of injury potential is discussed in detail later in this chapter.

2.4 Pressure changes and injury

Peak over-pressure (or static peak pressure) varies according to the size and type of explosive material and the medium into which it is released, concurrently the magnitude of that peak pressure with the duration time is directly proportional to injury; as such these measurements are important to clinicians. Peak pressures produced by military-type explosions can reach around 2,000 kPa (Iremonger, 1997). Such explosions are tactically significant, aimed at producing a mass casualty effect over a battlefield-sized area, whereas lethal peak

over-pressures for an individual human are much lower at 414 - 552 kPa (Iremonger, 1997; Mellor et al. 1997).

Exposure to the lower but still potentially lethal peak pressures is commonplace, and is observed in patients presenting to hospitals through industrial and domestic explosions. Such patients are victims of for example domestic gas hot water service incidents, industrial explosions or fireworks mishaps. Patients present with burns and other signs of exposure to blast overpressure such as electrocardiograph (ECG) changes and temporary hearing loss.

As the high pressure wave deteriorates exponentially, at the cube of the distance from detonation point, the dynamic pressure wave (or blast wind) continues as the pressure effect reverses, leaving a net movement of gas travelling back to the detonation point, ultimately producing a vacuum space within the blast zone space (Iremonger, 1997). This is known as the negative pressure phase. The complete wave, with its positive and negative pressure phases, is a cycle of compression and rarefaction, which attests for the powerful blast wind, it dies off over time, as shown as blast wave propagation in Figure 2.2.
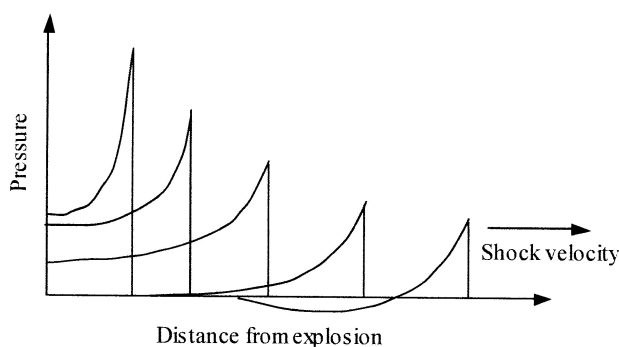
Distance from explosion
Figure 1: Blast wave propagation

Figure 2.2 Blast wave propagation. (Ngo et al. 2010, p. 76).

Injuries occurring in blast relate specifically to various external pressures the victim is exposed to at any given moment during the blast event. The impact of these pressure differentials on the human body is of greatest concern to clinicians (Mellor et al. 1997; Wade et al. 2008).

It is common for blast victims to present with multiple injuries in the clinical setting, particularly if the victim is close to the blast's detonation point and therefore exposed to all phases of the blast wave's pathway (as illustrated in Figure 2.1); the mortality rate, inevitably, is also higher (Alfici, Ashkenazi & Kessel 2006; Arnold, Halpern & Tsai, 2004; Desaga, 1950; Mellor et al. 1997).

The variety of trauma includes blast barotrauma injury, resulting from the coupling effect upon organs such as the lungs; shrapnel injuries, traumatic amputations; damage to large organs such as the liver; skeletal fractures; head injuries, and cervical spine injuries resulting from high speed-collisions and/or bodies being thrown by the blast wind (Mellor et al. 1997; Shamir et al. 2004). Such injuries reflect the powerful pressure differentials and also the wind that generates projectiles from the surrounding environment; the individuals within the space can become projectiles themselves. These multiple injuries complicate clinical management if the carer is naive to blast physics and thus unable to relate the progression and effect of the wave to the injury identified in the patient on presentation.

Blast injuries are classified according to the progression of the blast wave and its relationship with the type of injury inflicted at each stage of the wave progression. While blast injuries are examined in detail in the following chapter, it is important to appreciate that they relate directly to the pressure changes occurring in the blast zone, at the particular phase of the wave

to which the victim is exposed (Mellor et al. 1997; Sattin et al. 2008). Table 2.1, depicts the basic relationship between the pressure changes in the blast wave and the victims injuries.

Table 2.1 Relationship between injury and the blast wave. (Derived from: Frykberg, 2002, Mellor et al. 1997; Sattin et al. 2008; Wightman & Gladish, 2001).

Primary injury	Relates to the static overpressure.
Secondary injury	Relates to the dynamic pressure or blast wind.
Tertiary injury	Relates to the dynamic pressure or blast wind.
Thermal or quaternary injury	Relates to the thermal output.

2.5 The sub-atmospheric or negative pressure phase

The lack of research literature on the negative phase of blast demonstrates the lower importance placed on its role in blast injury over the years. This may be due to experts dismissing it because of its reduced speed when compared with the overpressure phase and the speed by which it occurs, as well as the fact it follows the more recognised and well versed higher pressure wave (Benzinger, 1950; Przekwas, 2008). In response to that lack of published evidence about the role of underpressure in blast, expert opinion is relied upon in the following section.

2.5.1 Explosive and rapid decompression

The negative pressure or sub-atmospheric phase of a blast wave time line, involves a rapid or explosive decompression event, in a time frame and depth of level met below atmosphere. This is similar to an event commonly expressed in the aviation industry where the event is considered an unplanned change in altitude, resulting in exposure to a fall below atmospheric pressure within a particular duration time. According to the Federal Aviation Administration in the United States of America (FAA), (2005), an explosive decompression time frame is less than or equal to 0.1 seconds, while a rapid decompression time frame is a maximum of 0.5 seconds, while the parameters for each vary, both are potentially problematic as explained below:

'An explosive decompression is a change in cabin pressure faster than the lungs can decompress. Most authorities consider any decompression which occurs in less than 0.5 seconds as explosive and potentially dangerous.'
'A rapid decompression is a change in cabin pressure where the lungs can decompress faster than the cabin. The risk of lung damage is significantly reduced in this decompression as compared with an explosive decompression.' (Federal Aviation Administration, 2005, p. 20).

Neither of these scenarios can be reasonably excluded in any one blast event, as any one individual's level of exposure will depend on distance from detonation point and the type/size of charge involved. Even though both rapid and explosive decompression are potentially injurious, the major differences between the events relate to the lungs' ability to equilibrate and the degree of lung injury and rapid decompression is more likely to be observed in survivors (Australian Transport Safety Bureau, 2009; Axelsson et al. 2000; Cooper et al. 1991; Federal Aviation Administration, 2005).

2.5.2 Negative pressure and injury

Zuckerman (1940), recognised the existence of a sub-atmospheric pressure wave in a blast event, but he treated it as a secondary element with respect to blast injury. He described the negative pressure wave as: '...much weaker than the pressure component, and in no case can it ever be greater than 15 pounds per square inch, since this corresponds to a perfect vacuum.' (Zuckerman, 1940, p. 6104). Exposure to a perfect vacuum has trauma implications that are directly related to explosive decompression. Understanding the negative pressure phase of blast begins with the victim's distance from the detonation point, and the amplitude and impulse of the preceding positive phase at that distance (Iremonger, 1997). The ensuing negative pressure phase is a product of the net movement of gas back to detonation point as the blast wind of the positive phase deteriorates.

As already noted the negative phase is not considered a major player in severe injury, but it is related mathematically to 'impulse time history' and 'the magnitude of the preceding positive wave' (Iremonger, 1997; Krauthammer & Altenberg, 2000). As the blast front and subsequent wind attenuates away from the detonation point a much lower air pressure develops behind, creating a suction effect at a possible -1 atmosphere, which reverses the direction of the blast wind causing a negative pull within the immediate environment (Iremonger, 1997). Despite Zuckerman's (1940) depiction of the sub-atmospheric pressure as weak, research in the modern age has assessed this, and found it can produce enough force to shift concrete structures (Krauthammer & Altenberg, 2000). Personal communication with D. Ritzel, (2004) confirmed research observations that this negative pressure phase is commonly accepted to be between three and four times longer than its preceding positive

pressure phase and can be in the magnitude of 15% of the preceding over-pressure phase (Iremonger, 1997).

D. Ritzel (personal communication, 2004), stated that the effect of the negative pressure phase is more profound further a-field than the positive phase effects, so it would then follow that people located further from the detonation point are more likely to suffer effects of the negative wave than the positive wave. This is logical given that, as Ritzel (2004) pointed out, as the positive wave deteriorates, it gives way to an 'N' wave in which the sub-atmospheric phase can be of equal pressure, in theory and logically this may stand for large explosions. However, in reality, like all blast waves, this may be countered by the environment at the time, the effects of which alter this absolute state to a relative state (D. Ritzel, email communication, 2004).

The blast waveform graph timeline, as illustrated in Figure 2.1, shows clearly how the over-pressure peaks rapidly, and falls abruptly, but the under-pressure phase continues three or four fold as long. Expert opinion is that the difference between the static pressure (the peak overpressure) and the dynamic pressure (the remainder of the timeline that is not the peak pressure and includes the sub-atmospheric pressure may, in addition to the distance from the detonation point, hold consequences that are not explored in research at this time (D. Ritzel, email communication, 2004; personal communication, 2011).

Possible injurious effects from the negative phase alone have been identified through laboratory research with animals involving rapid decompression in laboratory experiments (Chen, Wang & Ye, 2000; Latner, 1942; Zhang et al. 1996). As noted earlier, Kemph and

Hitchcock (1952) found alterations in blood resulting from rapid and explosive decompression, but failed to link this concept to blast.

2.5.3 The engineer's perspective

In the field of engineering there is a perception that a negative pressure wave produces a much weaker load on solid structures than a positive pressure load; however, Krauthammer and Altenberg, (2000) suggested that the peak positive load and peak negative load can reach similar magnitude over a scaled distance. Their experiment showed that: '...the inclusion of the negative phase of blast pulse caused the glass panel to exhibit large motions toward the incoming blast load (i.e. to be pulled toward the load).' (Krauthammer & Altenberg, 2000, p. 16). They explained that this may relate to a combined effect, of both positive and negative phases of the pressure pulse, on structures exposed to blast; this is consistent with Ritzel's (2004 & 2011) earlier opinion.

Figure 2.3 offers two different scales of the same pressure pulse wave, showing that the peak pressure values are similar in magnitude; the difference between positive and negative reduces as the distance extends. Krauthammer & Altenberg, (2000), surmised that the integrity of a structure is influenced by both the positive and negative pressure phase of the blast, because if the positive pressure alone does not cause a structure to fail, it may rebound and fail during the negative phase.
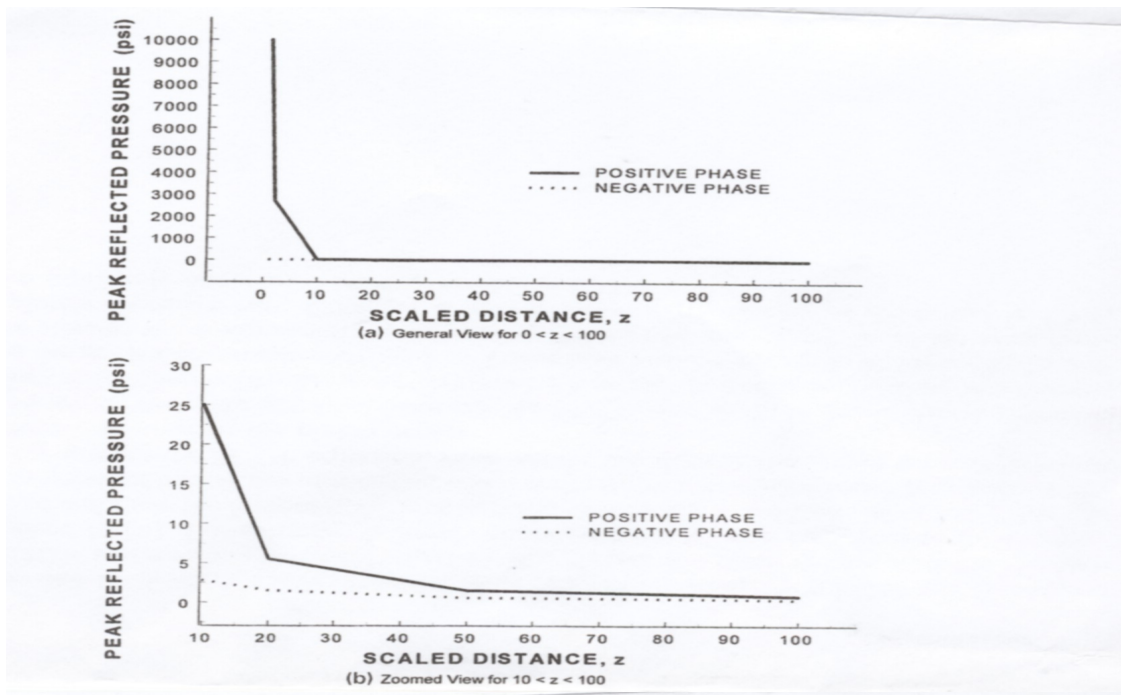
Figure 2.3 Positive and negative pressure pulse waves over distance. (Krauthammer & Altenberg, 2000, p. 6).

Teich and Gebbeken, (2010, p. 220) supported this with 'While the influence of the negative phase is minimal when analysing massive reinforced concrete structures, it dominates the structural response of flexible systems...'

To reiterate – if the positive phase has not overcome the structures' tensile strength and failed, the structure may still fail under the combined influence of the rebound over-pressure wave with the negative pressure wave, producing a structural failure in the opposite direction to that expected by the over-pressure wave. How this engineering concept translates to an animal model is unknown, except that the principle of the rebound wave and its relationship to ongoing waves and flexible objects (such as an animal or human) stands for all blast waves (Krauthammer & Altenberg, 2000; Teich & Gebbeken, 2010).

As mentioned previously, the blast loading effect resulting from the peak or static over-pressure in an animal varies according to the different tissue types it affects (Axelsson et al. 2000; Bowen, Fletcher & Richmond 1968; Mellor et al. 1997). Some tissue types are more vulnerable to the force exerted than others. Air and fluid interfaces are vulnerable because the wave passes from a high density medium (the chest wall) to a low density one (air or fluid filled spaces such as alveoli) at rapid speed (Mellor et al. 1997; Tsokos, 2008).

In simple terms, the effect of a shock wave on the human body is like an extremely intense, exceedingly rapid, (almost instantaneous), blunt injury. However, a blast trauma is complicated because a blast wave may travel at 300m/s or more, and the human body consists of varying densities, so the differential pressures affect different parts of the body differently at times, which is not the case with a blunt injury caused by for example a high speed motor vehicle (Mellor et al. 1997).

2.5.4 The relationship between negative and positive pressure phases

Several researchers have argued that the significant pressure differentials generated during a blast wave's progress throughout both positive and negative phases are largely responsible for injury (Krauthammer & Altenberg, 2000; Latner, 1942; Maynard, Coppel & Lowry, 1997; Ritzel, personal communications, 2004 & 2011). Some propose that injuries resulting from each phase are at times different, and yet at other times, similar (Latner, 1942; Tsokos et al. 2003).

Because little information has been published about the negative pressure phase of blast, a 'Blast Signature Database' was constructed as a preliminary step for this research. Shown as

Appendix I.   The database contains pressures and duration times measured from raw data acquired from live blast events conducted at the Defence Science Technology Organisation, Edinburgh, South Australia in 2006.   Fifteen events were obtained, with transducer output from eight different angles aligned to the detonation point.   Signatures with incomplete data were discarded leaving a complete group of 87 signatures.   The data were analysed as one group only, not identified by angles, for the purposes of realistically approximating blast wave. The following data were plotted along the blast timeline:

- peak over-pressure,

- over-pressure duration time,

- peak under-pressure,

- time to peak under-pressure,

- under-pressure duration time,

- the number of fluctuations above and below atmospheric pressure.

The database provided evidence of a number of issues including the relationship that the underpressure has with its respective overpressure, where most underpressure phase durations are at least three times greater than those of the corresponding overpressure phases.

The database shows that sub-atmospheric phases can reach quite extensive levels within duration times averaging 0.14 seconds.   This timing is proportional to the size of the explosion and the over-pressure generated from it.   Fluctuations of the signature above and below the 'atmospheric level' after the primary fall below 'atmospheric level' heralding the under-pressure phase, demonstrate the dynamic nature of the immediate post-positive pressure phase. These variations represent the difference between a stylised timeline, as described by the Friedlander Equation and reality.

A typical fluctuating waveform trace from the database is shown as Figure 2.4.
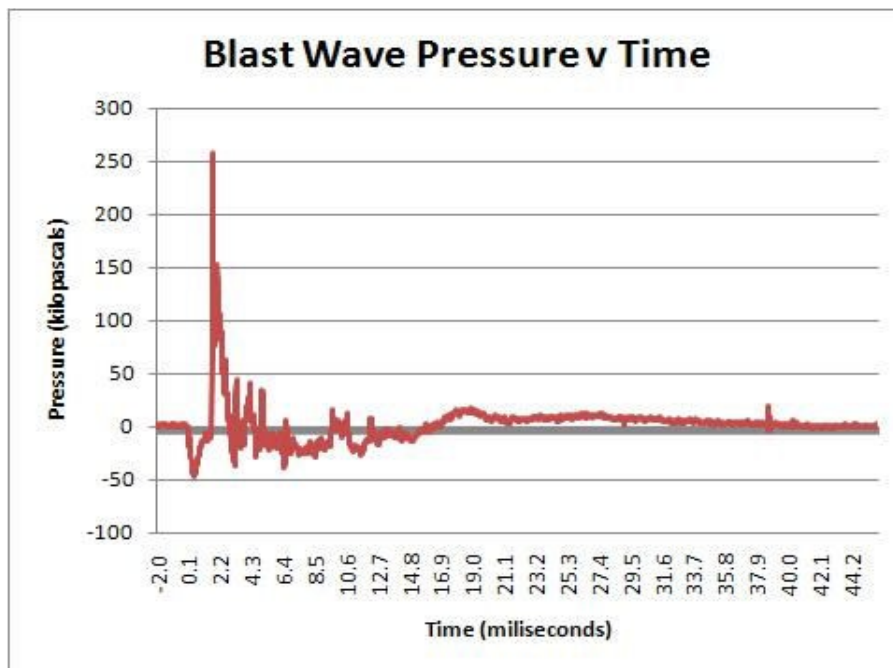
Figure 2.4 Graph of a blast wave signature (time compressed) showing the fluctuations above and below the *X* axis. Graph taken from Blast Signature Database, based on explosives testing event with DSTO, Adelaide, 2006.

These fluctuations above and below ambient pressure along the time line may be an area of research interest in the future, as the repeated fluctuations may increase the quantifiable area under the curve[2] in the over-pressure phase, and the area over the curve in the under-pressure phase; as such this phenomenon may increase injury potential because the areas are converging as a Riemann sum (Burton, 2005).

---

[2] The area under the curve refers to the mathematical calculation used to quantify a dynamic space on a graph. First described by Henri Lebesque (1871-1941), (Rudin, 1976). In blast the area under the curve denotes the area above atmospheric (ambient) pressure on the x axis and it is considered that injury potential is increased.

2.5.5 Studying the sub-atmospheric phase

Two of the three existing theories of the origins of microscopic gas emboli hold that blast-generated positive pressure causes primary lung injury. The third theory, relates to emboli developing at the sub-atmospheric or negative pressure phase. If the third theory is correct, then current injury prediction, injury treatment and education programs for clinicians are focused on a different injury demographic, and will neglect victims suffering from the negative phase effects of blast. If microscopic gas emboli do not arise from primary lung injury, as a consequence of over-pressure, patients suffering secondary and tertiary injuries are at greater risk of emboli than primary injured victims. This hypothesis accords with D. Ritzel's (email communication 2004) assertion that the negative wave has a more profound effect at a greater distance from the detonation point than the over-pressure wave, due to the N wave progression as the wave moves onward through the blast wind. This translates into the observation that victims at further distance from the detonation point, with mild or no apparent injuries would be more affected by the negative pressure wave than those in the primary injury zone, justifying research for a greater understanding of the negative pressure wave.

Research to date has failed to produce a successful simulation of the entire blast time line, whereas positive pressure research is well established since the development of the air driven shock tube, used in both blast research and non blast high impact blunt trauma research (Duff et al. 1966; Jaffin et al. 1987; Long et al. 2009; Reneer et al. 2011). Reneer et al. (2011), produced mild traumatic brain injury in small mammals using a new 'multi-mode shock tube'. The work showed brain injury, without primary lung injury, assessing the impact on the brain from varying non-lethal wave propagation.

Reneer et al. (2001) claimed their new shock tube produced a form of sub-atmospheric wave, a parameter previously unrecorded in shock tube research, but the authors unfortunately did not measure the negative pressure characteristics or parameters, stating: 'The duration of the negative phase was difficult to accurately estimate from the pressure-time histograms. The negative phase duration and impulse were therefore not calculated.' (Reneer et al. 2011, p. 100). This new shock tube may in time produce data for a simulated sub-atmospheric waveform, but at present alternative methods are necessary to generate more information on the negative blast wave and its effects on human physiology.

The extant blast injury literature generally accepts that the positive pressure phase, in particular the peak (static) over-pressure, is responsible for the more serious injuries resulting from blast as it represents the force exerted or mechanical blast loading on the body, and therefore the injuries inflicted produce the emboli. The evidence for the peak over-pressure producing injury is indisputable (Chiffelle, 1966; Iremonger, 1997; Mellor et al. 1997; Stuhmiller, Phillips & Richmond 1991); however, whether that injury results in microscopic gas emboli remains unknown.

2.6 Types of blast waves and their broad bio-mechanical effects

A blast wave in open air is simple, uncomplicated, notionally unimpeded, and comparable to that shown in Figure 2:1. The simple waveform of the free field example is the purest form, but it is not always the reality, and this fact has important clinical implications. Once a clinician has an appreciation of the basic tenets of a blast wave, understanding the type of

blast wave to which a victim has been exposed will assist in making health care timely and efficient.

2.6.1 Reflection waves

A ground level detonation produces a ground tremor as some of the energy is absorbed into the ground, but much of it is reflected, reinforcing the original wave (known also as the primary or incident wave), because this new reflection wave moves through a heated and compressed air medium. This situation raises the pressure, density and temperature within the zone, sometimes many times as the cyclic wave event continues, making ongoing reflection wave(s) more intense and lethal (Chiffelle, 1966; Iremonger, 1997; Mellor et al. 1997).

2.6.2 Mach stem

The higher the detonation point above the ground (if carried on a person or a vehicle) the less ground tremor, instead a reflection and reinforcement effect takes hold as the incident wave reflects off the ground below and extends outside the region of regular reflection. The region of regular reflection is the area closest to the detonation site (or ground zero) and is shown in Figure 2.5.
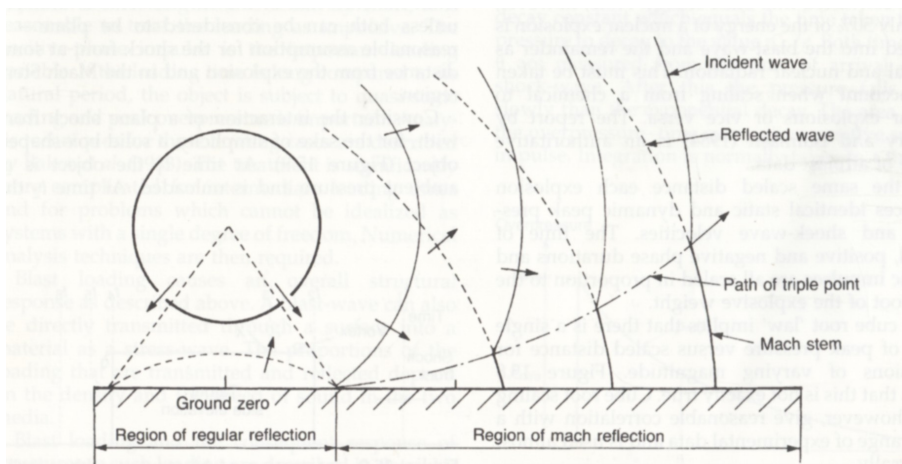
Figure 2.5 Mach stem reflection. (Iremonger, 1997, p.195).

This reflection wave moves through the altered blast affected medium and, like the normal reflection scenario, it is faster than the original wave, thereby increasing injury potential. The Mach stem is the point within a reflection wave at which the reflection effect is additional to the wave. As illustrated in Figure 2.5, the wave meets the outside of the regular reflection zone, the Mach stem system sets up the region of Mach reflection impacting on the course of reflection making the path of triple point reflection extend out from the region of regular reflection (Iremonger, 1997; Stuhmiller, Phillips & Richmond, 1991; Stuhmiller, 2008; Yelverton, 1997).

According to Schardin (1950), an increase in wave pressure occurs at the junction point shown by arrows in Figure 2.5 and the extent of pressure increase depends on the angle at which the points meet; a right angle produces a new Mach wave diverging from the primary wave where the front is at right angles to the reflecting plane as a Mach wave of its own. The Mach stem effect not only amplifies the injury potential by its reflective nature, it may alter the location of predicted injury because of the reflective nature of the complex waves. This means the position of the victim will impact on location and type of injury, because the victim

is vulnerable to reflection waves that are not in the direct line of the originating primary blast wave front (Chiffelle, 1966; Iremonger, 1997; Mellor, 1997; Yelverton, 1997).

2.6.3 Reflection and reinforcement phenomenon

The action of a reflection wave is more notable when the blast is released into a closed space. That closed space means that when the wave is released into the immediate environment it will hit a near-by hard surface such as a wall in the same manner it hits the ground and will reflect back into the immediate space. Reflection and reinforcement phenomena in a closed space (a room, bus or train) increase injury potential should the victim be standing in front of a wall or other solid surface (such as another person) because the victim directly in the wave path is hit by the oncoming wave and then again from behind by the reflection wave off the surrounding surfaces or people.

People subjected to a closed space blast are subjected to much higher, and more complex over-pressures due to reflection and reinforcement. It is well described that over-pressures are directly proportional to the degree of primary blast injury (Arnold, Halpern & Tsai, 2004; Lavery & Lowry, 2004; Mellor, 1997; Yelverton, 1997).

Corresponding patterns of severe injuries occurred as a result of 1991 terrorist bombing in

Birmingham, United Kingdom, illustrated in Figure 2.6.
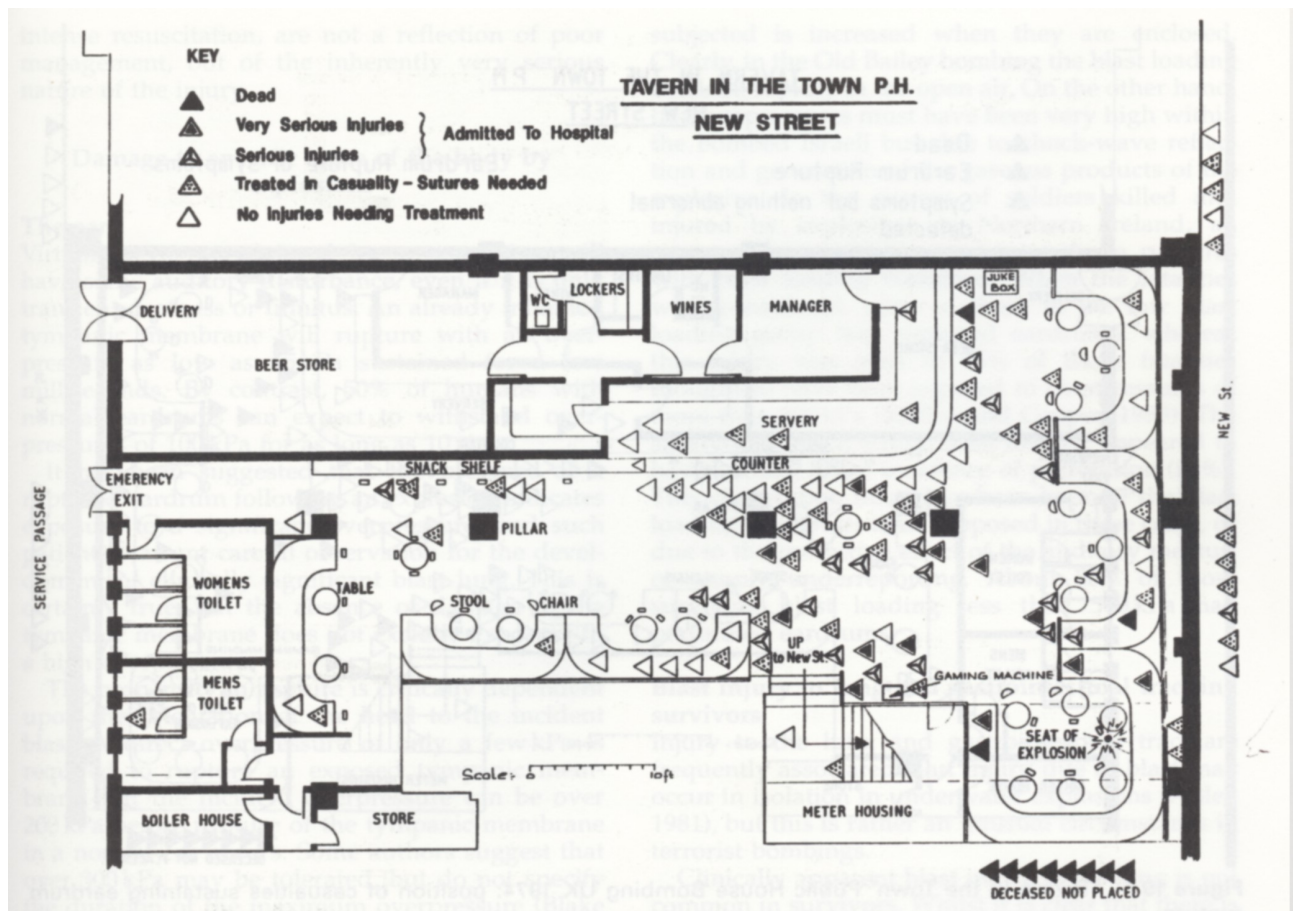
Figure 2.6 Tavern in the town. (Phillips & Richmond, 1991, p. 41).

The injuries received in the terrorist bombings in London (2005) and Madrid (2004) reflected

the victims being inside enclosed buses and trains (Katz et al. 1989). A database recording the

injuries from these events was established for ongoing education for emergency services, thus

educating them in the importance of the 'history of the event' element when planning care

(American College of Surgeons, 2008; Department of Human Services-Victoria, 2007; Kilner,

2000). Data from these terrorist events show that those injured inside (in trains or buses)

suffered more serious primary blast injury and/or concomitant injuries than those victims

identified further a field or outside on the street. Ultimately designing a complete database

from clinical observation data has assisted in training emergency and health care workers in Melbourne in 2007 for the emergency services response training exercise program. The database is owned by Department of Human Services-Victoria, and remains in place for future training. This author acted as database consultant for this exercise, (Department of Human Services-Victoria, 2007).

2.6.4 Blast waves exiting buried devices

Even though a pure open air blast wave distributes the pressure wave spherically, as described earlier, this may be countered by the placement of the explosive material. The direction of a wave is not always perfectly spherical because the direction of a wave is influenced by its immediate surroundings, particularly if it is buried or partially buried in soil (Ramasamy et al. 2009; Stuhmiller, 2008). When a buried device is detonated, the energy released as a shock wave, compresses the soil around it and the energy is transmitted therein as a reflection wave. This then creates a tension wave because only a small portion is released to air through the surface disruption at this stage. The tension wave then causes the soil to collapse and blast products are vented to air, in a Venturi effect. The end result is a crater. The depth at which the device is laid and the soil type affect the energy release and direction of the blast wave (Ramasamy et al. 2009; Stuhmiller, 2008). Determining the soil type in a crater can explain the 'spray' of the blast wave and ultimately provide information for clinicians on severity of injuries to expect, in addition to the obvious wound contamination from the soil.

2.6.5 Blast wave in water

While blast propagated through air produces a 'gradual' exponential decline in pressure, the blast wave through water is quite different, as demonstrated in Figure 2.7. The wave progression is truncated rapidly by the tension applied as the wave arrives at the water surface.
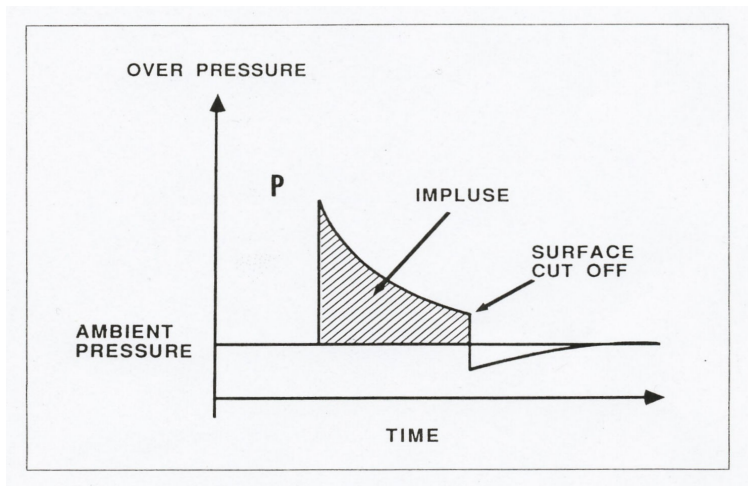
Figure 2.7 Underwater blast wave impulse. (Stuhmiller, Phillips & Richmond, 1991, p. 259).

An explosion in water produces a shorter and higher amplitude positive pressure waveform than a blast in soil or air (Ripple & Phillips, 1997; Stuhmiller, Phillips & Richmond, 1991). The abrupt cut off point in the wave front of the underwater blast changes the biophysical impact on the human body while increasing the overpressure time. In addition, the blast wave is intensified in deeper water because the air-water interface disrupts its progression (Ripple & Phillips, 1997). Figure 2.8 illustrates this phenomenon.
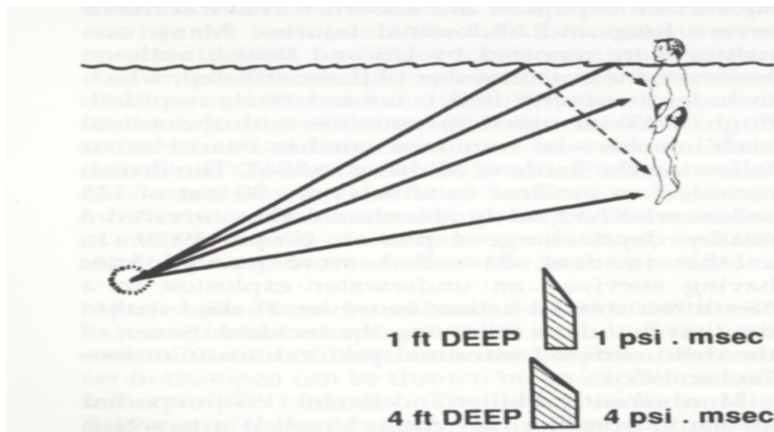
Figure 2.8 An underwater blasts intensity is magnified with increasing depth. (Ripple & Phillips, 1997, p. 252).

When detonation occurs underwater, the gas produces a large underwater bubble. This bubble expands in all directions when the resultant compressive shock wave arrives at the surface and produces small bubbles (spalling) from the tension produced at the air/water interface (Iremonger, 1997; Stuhmiller, Phillips & Richmond, 1991). The misconception that underwater blast injury produces more gastrointestinal injuries is common and lies in the assumption that the victim's head is above the surface, leaving the lower part of the body relatively exposed to the higher overpressure reflection tension wave (Phillips & Richmond, 1991). A victim in water will incur the same type of injuries as one in open air if the overpressure exposure is similar, because a smaller explosion under water can result in a higher overpressure than an open air blast using the same size charge, because of the waveform transition through the different media (Petri et al. 2001; Phillips & Richmond, 1991).

2.7 Summary

Clinicians likely to treat blast trauma victims should appreciate pressure wave behaviour and the effects arising from different media or obstacles. Since the medium and the surrounding environment alters the speed of the wave and influence the pattern of the waveform, the duration time and pressure magnitude are ultimately altered. These factors, are directly related to injury.

Chapter 3

Blast injury

3.1 Outline

By understanding blast wave behaviour as described in Chapter Two and knowing the relationship of the blast wave with the injury, clinicians can reasonably predict a pattern of injuries amongst casualties (Wade et al. 2008). This chapter builds on the earlier review of blast science from the engineering perspective, and explores the application of the science to the clinical world.

Clinicians refer to a blast injury classification scale, based directly on blast wave science, as a guide to predicting the clinical outcome of a blast injury (Mellor et al. 1997; Sattin et al. 2008). The classification system is well established, but it is important to continually reassess its relevance to current research and clinical practice to ensure it remains meaningful. For example, recent research into cytokine and inflammatory response to trauma, and blast specifically, may provide a basis for a new 'quinary injury' category as discussed later in this chapter. Similarly, it is important to consider whether microscopic gas emboli, currently allocated to the primary injury classification based on the assumption that this is where they originate, are correctly classified.

3.2 Determining the blast wave force

As previously outlined, understanding the type of blast to which a victim is exposed is helpful for clinicians, to identify the mechanism of injury or blast wave force. For example, knowing a victim has been exposed to an enclosed space blast enables clinicians to prepare resources for victims of reflection injuries, because the injuries are likely to stem from multiple

reinforced waves and, where there is a greater chance of a primary injury.

Understanding the mechanism and pattern of injury also assists in identifying the type and size of the blast itself and is helpful to forensic and military scientists alike as these teams work alongside the clinicians. Points of note include the victim's position or angle with respect to the detonation point, and whether debris was involved. For example, a large number of victims suffering penetrating injuries or traumatic amputations in the absence of primary injury would indicate they were exposed to blast wind and dynamic pressure rather than static overpressure, so were outside the lethal zone (Mellor et al. 1997; Sattin et al. 2008; Stuhmiller, 2008). Additionally, high rates of mortality, primary injury, and/or severe or multiple injury in a closed space would indicate intense reflection activity (Chaloner, 2005; Iremonger, 1997). The author observed the effects of reflection waves in an enclosed space on three adolescents killed in an explosives incident in East Timor in 2000:

'They were found in a small, close quartered, but partially internal walled concrete building near the West Timor border. We received the bodies in the late afternoon but the incident was already over 24 hours past. The odour emanating from the delivery was a small hint of what we could expect to see beneath the body bags. We were tasked by the United Nations coroner to perform post mortem XRay examinations as evidence for potential criminal charges to be laid. At first we tried to determine what lay beneath by palpable examination of the body bags, because of the smell. We could not identify the body planes properly. Once opened we saw multiple orthopaedic and visceral injuries consistent with their fatal outcomes: traumatic amputations, fractures and massive skeletal displacement; in one case a victim's arm was embedded in a fellow victim's body.....' (Personal diary extract: Harding J, 2000, p. 113, Volume II: East Timor).

To summarise, blast injuries depend on the size and type of explosion, the distance and angle of the victim from the detonation point, the environment into which the blast is released, and

finally the organs and tissues affected because tissue mechanics within the human body vary significantly. The mechanism of injury relates to each classification and they are listed accordingly: primary, secondary, tertiary and quaternary with a more recent citation of a possible fifth (quinary) category (Chaloner, 2005; Mayo & Kluger, 2006; Sattin et al. 2008).

3.3 Blast injury classification

Blast injuries were first classified in a rudimentary sense by Zuckerman (1940), as cited in Chaloner, (2005). Considerable refinement has taken place since, but overall the classifications are consistent with their original forms as the categories are derived from the physical effects of the blast wave.

Major recent modifications include Mayo & Kluger's (2006) suggestion of a quinary injury classification for hyper-inflammatory responses to toxins embedded in the explosive device. In addition, Kirkman and his colleagues advocated the inclusion of a miscellaneous class for burns and other effects such as drowning, poisoning from noxious gases and psychological disturbances (Kirkman et al. 2008).

The classification system as it is used today is shown as Table 3.1.

Table 3.1 Blast injury classification table. (Horrocks & Brett, 2000; Mellor et al. 1997; Stuhmiller, 2008).

Primary injury	Caused by the blast wave colliding with the body surface. Commonly affects air and fluid containing organs such as lungs, bowel and ear. Relates to static overpressure.
Secondary injury	Caused by projectiles released from the explosive device or local debris released into the zone. Penetrating and blunt injuries are common. Relates to dynamic pressure/blast wind.
Tertiary injury	Caused by the blast wind and turbulent environment. Includes displacement of the body (as a projectile), and traumatic amputations. [Some authors include crush injury caused by structural collapse due to the blast, others provide a separate classification] Relates to dynamic pressure/blast wind.
Thermal injury	Caused by the brief but intense heat. Flash burns, inhalation burns, varying degrees of burn injury. Burn injury is more profound if structures burn. Relates to thermal output and the environment.
Quinary injury**	Hyper inflammatory response. Caused by toxin embedded in the explosive device, producing an inflammatory response.

**Term c*oined by Mayo & Kluger, 2006*

Because the dominant theory holds that microscopic gas emboli develop in blast injury through gas translocation resulting from primary lung injury, the phenomenon is currently classified within primary injury.

3.4 Primary Injury

Primary injury is the most serious injury sustained from blast; it typically occurs in victims in close proximity to the detonation point. The blast wave moves spherically from the central detonation point and the blast front force dissipates in proportion to the cube distance. Primary injury generally occurs in victims from within the lethal zone or those in the inside rim of the injury zone (Ripple & Phillips, 1997). Here, there is a greater exposure to blast positive pressure loading because it is closer to the detonation point, as outlined in Figure 3.1.
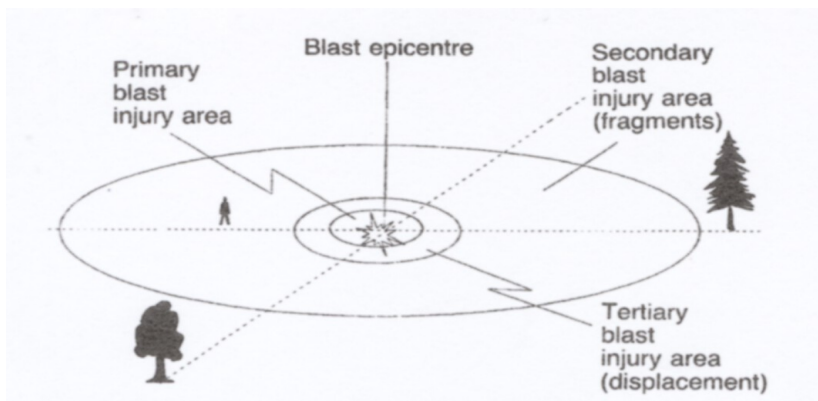
Figure 3.1 Blast wave injury potential zone. (Ripple & Phillips, 1997, p. 247).

3.4.1 Primary injury, the coupling effect and tensile strength

Blast loading is the force exerted on the body surface by the high pressure blast wave front, consisting of both shear and stress waves (Chiffelle, 1966; Maynard, Coppel & Lowry 1997; Mellor et al. 1997; Stuhmiller, Phillips & Richmond, 1991). The blast loading causes the body surface to move, setting up reverberations and stresses within the internal tissue framework, known as a 'coupling effect' (Iremonger, 1997; Mellor et al. 1997).

The coupling effect is profound when this energy is transferred through layers of tissue that are distinctly different in density from each other, such as the skin and internal organs containing air and fluid such as the lungs, ears, eyes and bowel. The waves move from the higher density (chest wall) to the lower density (the lung parenchyma), the effects of which are traumatising on fragile pulmonary architecture. Particularly vulnerable organs include the ear, lungs and bowel and to a lesser degree the spleen due to its spongy blood filled make up.

The structure of the lungs is particularly vulnerable to traumatic distortion from blast loading because blast over-pressure is so fast that the lungs cannot equilibrate as they would in a normal breathing cycle (Stuhmiller, Phillips & Richmond, 1991). It was long believed that lung injury was caused by the force of the blast tracking down the airways. When closed, the glottis should protect the lungs from damage within, so it was thought that if the glottis was open the lungs would incur the injury directly from the tracheal route (Benzinger, 1950). Benzinger tested the theory using a dog model and discovered that blast loading at the chest wall (and subsequent coupling) was responsible for the lung injury, and the force of the blast did not track down the airways.

The coupling effect causes rapid acceleration and deceleration that distorts and displaces internal structures from their connective tissue mantles caused by rapid acceleration and deceleration (Mellor et al. 1997). Engineering literature proposes that coupling produces spalling at the air/fluid interface, producing a tension wave; where material is thrown off the tissue interface by the tension wave as the wave moves through the different densities (Schardin, 1950). Schardin (1950) tested this theory using an azide charge in the centre of a glass disk. The process is visually obvious in underwater blasts as the blast wave hits the water surface and water disperses in a spray, but this has not been reconstructed in human or

animal models however, it is believed that in the lung this may produce vapour bubbles on the air filled membrane surfaces as it disrupts the surface, followed by implosion, resulting in collapse of the small gas containing alveoli (Phillips & Richmond, 1991; D. Ritzel, personal communication, 2004; Schardin, 1950).

In most people the threshold for injury to the tympanic membrane is 15-100 kPa, whereas lung damage is known to occur at overpressures of 175 kPa and 3 milliseconds duration. These overpressures are much less than the lethal limit, commonly regarded as 400-500 kPa (Iremonger, 1997; Stuhmiller, Phillips & Richmond, 1991). The lung parenchyma and other tissue interfaces have a limited amount of 'give', equating to tensile strength and threshold for injury (Stuhmiller, Phillips & Richmond, 1991). Table 3.2 shows the different tissue types and aligned tensile strengths.

Table 3.2 Tissue and non biological tensile strengths. (Stuhmiller, Phillips & Richmond, 1991, p. 263).

MATERIAL	TENSILE STRENGTH (MPa)
Common building materials	
Stainless steel	1,000
Silk	400
Oak	120
Marble	6
Biological fibres	
Resilin	3
Collagen	50 - 100
Biological tissues	
Tracheal membrane wall	0.4 – 2.2
Mixed arterial tissue	1.4 – 1.7
Elastic arterial tissue	0.8 – 1.0
Venous tissue	1.7 3.0
Large intestine	0.45 – 0.69

3.4.2 Primary lung injury, and microscopic gas emboli

Medical literature orthodoxy (for example: Horrocks & Brett 2000; Irwin et al. 1999; Katz et al. 1989; Mason et al. 1971; Riley, Clark & Wong, 2002; Rosenfeld & Ford, 2010; Weiller-Ravell, Adatto & Borman, 1975; Wightman & Gladish, 2001), has long accepted the translocation theory of microscopic gas emboli development and viewing the coupling event and subsequent disruption of lung architecture as the opportunity for air from damaged alveoli to 'shift' into capillaries, thereby causing the microscopic gas emboli (Benzinger, 1950; Clemedson & Hultman, 1954).

It is true that blast damages the alveoli and capillary network in a variety of ways, including vascular and interstitial congestion, alveolar ruptures and cuff-like peri-vascular haemorrhages in the interstitial spaces (Tsokos et al. 2003), but the question of whether air enters an intact and functioning vessel remains unanswered, particularly given the lack of an epidemiological link between blast lung injury and blast emboli (Ho & Ling, 1999; Tsokos et al. 2003). It is also known that air emboli occur in penetrating and blunt chest trauma, however, it is difficult to compare these injuries directly with those resulting from blast injury as the parameters of over-pressure, the coupling effect and the pressure differentials between over-pressure and under-pressure do not occur in other types of trauma (Ho & Ling, 1999).

3.4.3 Primary injury - prediction and prevention

Mathematical formulae that describe the blast wave curve have been used to quantify primary lung injury potential since the mid 20th century (Desaga, 1950). In 1968, Bowen, Fletcher and Richmond developed the injury risk curves for blast lung injury that are still in use today,

albeit with some refinements (Bass, Rafaels & Salzar, 2008; Bowen, Fletcher & Richmond 1968; Teland, 2012). The Bowen Curves, are based on the pressure changes that occur at coupling, with a wave speed greater than 400 mm/s on the lung parenchyma interfaces (the capillaries and alveoli) (Bass, Rafaels & Salzar, 1968). The pressure loadings Bowen, Fletcher and Richmond (1968) described produce irreversible damage to the lung micro-architecture; engineers know this as irreversible stress where the tensile stress of the material (capillaries and alveoli) is expended (Wightman & Gladish, 2001). Since that time, research has developed further. Bass, Rafaels and Salzar, (2008) argued that the measure (using Bowen Curves) for injury potential may not be sufficient with a wave duration time of less than 400 m/s. Both teams' models work for a defined blast wave amplitude and cannot be used to assess complex waves such as those expected with reflection activity.

Axelsson and Yelverton (1996) used both free field waves (Friedlander waves) and complex waves to predict injury. Their model has recently been found to have both numerical and experimental validity, but only with small charges (Teland, 2012). Elsayad and Gorbunov (2008) claimed that high-order explosives can reach 9,000 m/s. Bass, Rafaels and Salzar, (2008) echoed Elsayad and Gorbunov's (2008) claim suggesting that because most of the developers of military protective equipment use the Bowen curve measurements, soldiers are inadequately protected against blast injury. It is difficult to verify this claim as such protective clothing is generally made under high security and manufacturing procedures and specifications are not published.

In recent years research and development in protective clothing and equipment for occupational risk of blast injury has focused on mitigating the severity of primary injury because it carries the greatest risk to life. In particular, Western military equipment, clothing

and vehicles are designed to protect the individual from improvised explosive devices (IED). For example, the Australian Protected Mobility Vehicle (PMV) holds a proven record of withstanding IED attack; despite the loss of vehicles in current operations, no associated deaths or injuries have occurred in this vehicle as a result of exposure to a blast over-pressure wave. The PMV has a V-shaped hull design, that deflects ground laid blast and prevents a hull breach by the over-pressure wave and subsequent primary injury to the occupants (Australian Government, Defence, 2011).

Work today continues to improve injury prediction and prevention. The expectation that primary injury will be at the forefront of blast injury research is not unreasonable given its rates of morbidity and mortality.

3.4.4 Primary injury, the vulnerable organs

The high energy loads delivered to the body in blasts cause damage to delicate structures that destroy the anatomy or impair its function as well as related organs or systems. In primary blast lung injury, the blast load on the chest wall can distort the lung parenchyma to a point where the alveolar and capillary walls are damaged or destroyed, resulting in fluid leaving the capillaries for the interstitial spaces and in some instances into alveoli as well (Sharpnack, Johnson & Phillips, 1991; Wightman & Gladish, 2001). The resultant increase in alveolar and pulmonary interstitial pressure ultimately impairs gas exchange and patients present with a respiratory embarrassment or, in severe cases, early onset Acute Respiratory Distress Syndrome (ARDS) (Guy, Glover & Cripps, 1998; Lavery & Lowry, 2004; Stuhmiller, 2008; Tsokos, 2008). Alongside the respiratory effects, the increased interstitial pressure distorts nerve endings and stimulates pulmonary C-fibre receptor endings which reside in the alveolar

interstitial spaces near pulmonary capillaries (Guy, Glover & Cripps, 1998).   The signals travel via the vagus nerve to the vagal zones in the brain. This reflex increases cardiac and pulmonary cholinergic activity and a drop in alpha and beta adrenergic activities, resulting in a bradycardia, apnoea followed immediately by a responsive tachypnea, and a systemic non-cardiac, normovolaemic hypotension- a shock state that is claimed as unique to blast over-pressure trauma (Guy et al. 1998; Irwin et al. 1999).

The overall result of the lung injury is impaired gaseous exchange resulting from impaired micro-circulation, damaged alveoli, and additional interstitial fluid accumulation further compromising gas exchange. Microscopic examination of blast lung injury victims shows an assortment of structural damage ranging from enlargement of the alveolar spaces, thinning of walls, intra-alveolar haemorrhages to completely ruptured alveoli (Tsokos et al. 2003). It may be reasonable to predict that some of these injuries are recoverable with appropriate modern treatments and mechanical ventilation strategies, while other injuries may not. For those alveoli and capillaries that are ruptured, they cannot function, which means entrainment of air from damaged alveoli to damaged capillaries for transportation through the pulmonary circulation and onto central arterial circulation may be more limited than what is implied by the translocation theory.

Advanced mechanical ventilation strategies, for lung injury, aimed at alveolar recruitment for improving gas exchange, while minimising lung workload have generated some positive recovery reports (Lavery & Lowry, 2004; Moran, Bersten & Solomon, 2005; Pizov et al. 1999; Putensen et al. 2009; Shamir et al. 2006). Nevertheless recovery also depends on a host of other factors, such as surfactant production, the state of the pulmonary circulation, and the extent of the overall bleeding within the parenchyma, as well the iatrogenic complications

of positive pressure ventilation itself (Bellani et al. 2011; Lavery & Lowry, 2004; Moran, Bersten & Solomon, 2005).

Aside from the lung, organs at high risk of barotrauma from primary injury include the ear, the bowel, and to a lesser degree the spleen and the eye. The tympanic membrane of the ear responds similarly to the lung because it too is an air backed[3] organ. The membrane can rupture and victims may suffer temporary neuropraxia and dislocation of the ossicles. Tympanic membranes may heal without treatment in time, but severe cases may require surgery.

The bowel is vulnerable to primary injury because of the pockets of air that may be in the tract at any given time; the colon is the most vulnerable section of the bowel, suffering injuries ranging from small haemorrhages and perforations, to mesenteric tearing that may bleed and/or produce ischaemic bowel segments (Mellor et al. 1997; Stuhmiller, 2008). Bowel injury may not be obvious in the immediate resuscitative phase but insidious development of bowel ischaemia or slow leaking of bowel contents into the peritoneum from a small tear may develop over the short to medium time following a hospital admission. Once again a clear appreciation of the victims' position and distance from the blast and possible exposure to reflection mechanisms can assist with prospective and latent clinical assessment.

3.5 Other injuries

Other injuries include secondary, tertiary and thermal injuries. Secondary injuries are

_____

[3] Air backed" refers to an organ that has air inside it and against the organs perimeter membrane. The outer membrane can be internal to the body membrane.

typically penetrating injuries caused by projectiles made mobile by the dynamic pressure phase and blast wind. These can include missiles from the exploding device or other debris in the immediate surrounds such as glass and fragments of destroyed buildings or vegetation. Mayo and Kluger, (2006) claimed, secondary injuries were the major cause of death in blasts caused by terrorist bombs. Any type of flying object can penetrate any part of the body, thus a myriad of possible injuries can occur at both close range, and at a distance from the detonation point.

When a victim is flung from one position to another by the blast wind, the incumbent injury is classified as a tertiary injury; blunt speed-driven injuries such as spinal or head injuries and traumatic amputations are common (Lavery & Lowry, 2004; Ripple & Phillips, 1997; Sattin et al. 2008; Wightman & Gladish, 2001). The blast wind typically travels at approximately 4 m/s and it is made turbulent by the development of the negative pressure phase before the forward moving phase is complete.

As previously discussed, this negative pressure phase produces a vacuum effect that sweeps back toward the explosion's point of origin, in direct competition with the forward moving blast wind, creating a turbulent environment. Injuries incurred at this time occur amidst extreme environmental wind turbulence (Stuhmiller, 1997). This is a sub-atmospheric pressure environment as such it may leave a victim vulnerable to the formation of microscopic gas emboli, therefore understanding the injuries originating during the negative pressure phase is crucial to this project.

In the past, quaternary or thermal injuries were included with tertiary injuries or defined simply as additional injuries. These injuries are typically the burns resulting from blast,

which can be extensive. According to Kauver et al. (2006), 52% of the casualties from combat explosives incidents during the United States-led invasion of Iraq in 2003 sustained burns. Kauver et al. (2006) asserted that this rate is typical in combat. The extent of the burn injury in a blast incident depends on the environment in which the explosion is set, the victim's distance from the detonation point, and any active conflagration in the area. The burn may be evident on the skin or internal if inhaled. Inhaled burns are dire, as the respiratory tree is compromised through loss of integrity and tissue oedema, thus affecting ventilatory function (Ikonomidis et al. 2012; Kauver, 2008).

A flash burn from blast is caused by the heat produced by the initial flame and intense flash of light produced at detonation; it is a radiant heat burn (Kauver, 2008). While the initial temperature may reach 3,000 Celsius it is very short-lived, those close to the detonation point are the most vulnerable (Kauver, 2008; Mellor, 1997). Thermal blast injuries are a major problem when the explosion is derived from chemicals or the explosive device is fuel-laden (eg: incendiary, crashed aircraft) or nuclear in origin. Burns may also be caused by the surroundings burning such as a building or from ignited clothing (Video World News, 2009; Video World News, 2013). Fires fuelled by the surrounding infrastructure can produce carbon monoxide poisoning from timber structures in particular, notwithstanding inhalation burns may also ensue from the direct heat (Dunn et al. 2013).

Mayo and Kluger, (2006) proposed a new classification of quinary injuries, consisting essentially of the hyper-inflammatory state caused by toxins released from the explosive device and absorbed through the victim's skin. The hyper-inflammatory response was not related to the severity of injury, but might be related to the non hypovolaemic unresponsive cardiovascular collapse described in 1999 (Irwin et al. 1999). Irwin et al. (1999) described a

similar physiological non hypovolaemic cardiovascular collapse response in a randomised control trial with an animal model. Their results showed conclusively that the changes in haemodynamic status were the result of vagal nerve mediated reflex. However, Kluger et al. (2004) proposed that the inflammatory condition (producing similar haemodynamic compromise) in their patients related to toxic substances embedded in the bomb, justifying their claim because the condition was only identified in patients that had skin injuries. In reality both the physiological changes are credible. Further work is required to test the toxin theory.

The concept of a quinary category is yet to be adopted in the literature, and has not appeared in the published literature since its proposal in 2006. It is surprising that this category has not been given more credibility given that mustard gas and sarin gas (chemical weapons) delivered by bomb are device laden toxins and are well documented in the literature (Okudera, 2002; Wattana & Bey, 2009). Nevertheless, the blast injury categories have changed since their inception so the concept of a new category is not unrealistic in time.

Modern technology has enabled more extensive and refined cellular level examinations of the immuno-chemical changes that occur in blast. Since the late 1990's, work has progressed on changes that occur at the cellular level when the cell is disrupted by the blast shock wave in the case of lung injury (Elsayed & Gorbunov, 2008; Gorbunov et al. 2004; Gorbunov et al. 2005). Elsayad and Gorbunov (2008) presented work indicating that a myriad of bio-active compounds are released as a result of primary blast lung injury. They claimed the production of free radicals in blast injury progresses onward to a cascade of reactions. They believe the process, involving various pathways, ultimately produces events that impair immuno-chemical haemostasis, and this may result in an inflammatory response such as would be

observed by a clinician (Elsayed & Gorbunov, 2008). Surbatovic et al. (2007), had previously generated evidence of immune-cytokine response in blast injured casualties of war. The apparent demise of immunomodulating factors is the principal focus of their blast research (Elsayed & Gorbunov, 2008; Gorbunov et al. 2004)

Despite nuances in authors' categorisation, blast classification has assisted clinical management since its inception in the 1940's. It is widely accepted that blast primary injury carries the greatest mortality because of its effects on vulnerable, essential organs such as the lungs; notwithstanding this, victims of primary injury generally incur multiple other injuries because of their proximity to the detonation point and exposure to all levels of the blast pressure differentials. Any classification injury caused by blast carries greater mortality than the same injury caused by other trauma and the concomitant injuries increase mortality even further in as much classifying blast injury assists clinicians with pertinent medical treatment (Kluger, 2004; Sattin et al. 2008).

3.6 Clinical manifestations of arterial microscopic gas emboli

3.6.1 Blast emboli in clinical practice

Emboli in blast are known to be a significant cause of death through obstruction of blood flow to vital organs (Benzinger, 1950; Clemedson & Hultman, 1954; Cooper et al. 1983; Ho, 2002; Horrocks & Brett, 2000; Tsokos et al. 2003) as such identification of them in blast-exposed patients should be uppermost in researchers' and clinicians' minds. Unfortunately, the lack of research to discover their origins suggests this is not the case. This author's communication

(since 1995) with specialist clinicians in both military and civilian arenas who faced blast injury on a regular basis during war time deployments, revealed a lack of acknowledgement of the microscopic gas emboli phenomenon (author's experience between 1995 and 2013; personal communications G. Lavery, 2000; P. Thomas, 2004 & 2009; D. Read 2006; K. Billett 2007; M. Terry, 2009). This inattentiveness to the emboli phenomenon shows a disconnect between morbidity or mortality potential and clinical practice; a situation that should encourage research if practice is to be genuinely based on evidence.

That emboli are clinically significant is supported by evidence collected over a period of ten years which suggested that patients without primary injury showed ischaemic electrocardiography (ECG) changes that may have resulted directly from embolism in the coronary vessels (Harding, 1996). Harding presented a multiple case report from different blast events, over a six month period, describing ischaemic electrocardiograph (ECG) changes with 12 lead global perspective ECG's in survivors of secondary and tertiary blast injury only, not primary blast injury. Importantly, these ECG's showed ischaemic changes by specific regions of the heart using 12 lead ECG tracings. This is contrary to other reports on ECG changes in blast exposure that only recorded rhythm disturbances (Guy, Watkins & Edmonstone, 2000); rhythm disturbances are not a diagnosis for ischaemic cardiac tissue (Conover, 2002). The ECG tracings by Harding (1996) are an indirect assessment of emboli and this assessment method is discussed further in the following section.

3.6.2 Detecting emboli

Confirmation of centrally located microscopic gas arterial emboli in blast has been achieved directly or indirectly. Searching for evidence of emboli in blast survivors is not routinely

considered in clinical practice according to this author's experience and first hand reports from clinicians working directly with blast injured patients (K. Billett 2007; G. Lavery, 2000; D. Read 2006; M. Terry, 2009; P. Thomas, 2004 & 2009). In the past, direct identification of emboli in blast victims was only possible through swift post mortem examination (Benzinger, 1950; Clemedson & Hultman, 1954; Rossle, 1950; Tsokos et al. 2003).

Emboli have been confirmed by indirect examination using tools such as ECG's to measure effect on the myocardium when an ischaemic event is otherwise unexplained (Harding, 1996; Rossle, 1950). Retinal artery embolisation can also be assessed indirectly, in the living, via retinal examination where acute otherwise unexplained blindness or impaired vision following blast exposure (Horrocks, 2001; Sharpnack, Johnson & Phillips 1991). The most recent comprehensive examination (via post mortem) of blast related emboli evidence was described by Tsokos et al. (2003). However, in recent years diagnostic tools such as angiography, echograms and Doppler assessment have facilitated direct identification of emboli in the living (Wightman & Gladish, 2001).

Increased use of modern emboli detection methods in blast victims is a desired outcome of this thesis, that may be realised once an alternative theory for blast emboli is established. Figure 3.2 shows that emboli formation in a cerebral blood vessel of a human patient is easily detected via an anatomical search with the appropriate tools.
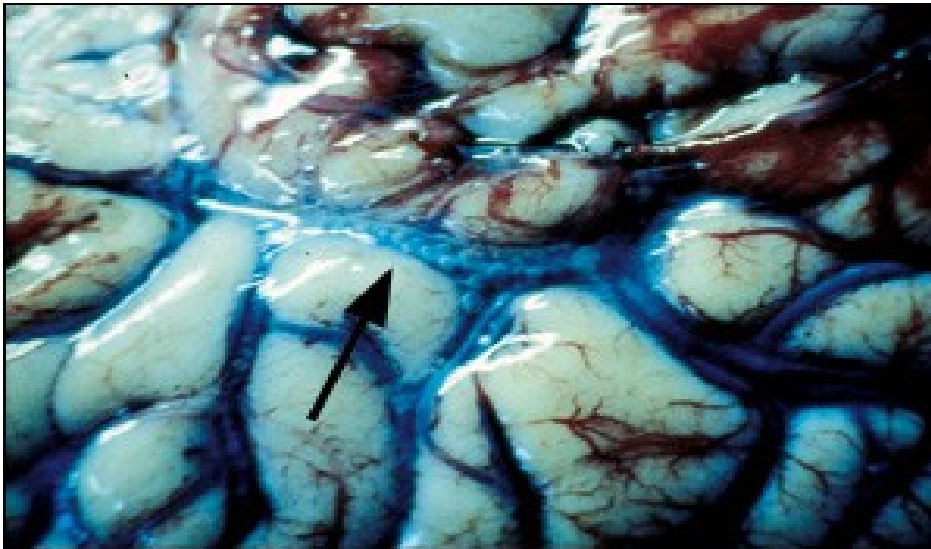
Figure 3.2 The black arrow indicates a row of air emboli found in a cerebral vessel. (http://www.aafp.org/afp/2001/0601/p2211.html Viewed 5 October, 2010).

There is indisputable evidence that emboli resulting from blast exposure occur, and with today's technology are identifiable, however, any search for them in blast survivors rests with clinicians considering that they may occur in survivors as well as the deceased. As the vast majority of clinicians believe microscopic gas emboli originate from lung trauma they are not likely to search for microscopic gas emboli victims suffering a non-primary blast injuries.

Despite an extensive literature search, there appears to be no current literature assessing emboli exposure specifically in live blast victims. It is logical that clinicians will not test for a phenomenon unless there is research evidence to suggest a link between it and a meaningful clinical outcome. Since there is no solid research evidence about blast emboli, beyond the fact that they exist, and that victims can die, this project provides that preliminary information. Likewise, while blast emboli stem from the effects of an explosives event they are none the less emboli, and may behave similarly to emboli originating from other pathophysiology.

3.6.3 Non-blast emboli: general effects and inflammatory response

Exploring what is already known about the effects of embolism created in non-blast events, is a first step in understanding emboli development and their consequences as a phenomenon. There is substantial evidence that any embolus will produce a mitochondrial inflammatory response, that is not related to infection and that emboli are traumatic to endothelium (Kapoor & Gutierrez, 2003; Mitchell & Gorman, 2005; Zhang et al. 2010).

Iatrogenic emboli, a form of non blast emboli, can result from clinical procedures such as cardiac bypass surgery or the removal of central venous catheters (Kapoor & Gutierrez, 2003). Physical signs observed in patients suffering iatrogenic arterial embolism include sudden onset of signs related to the organ(s) impaired by circulatory obstruction, as well as generalised symptoms such as mild headaches, dizziness, disorientation or minor motor weakness and even severe illness requiring anti-seizure medication and/or intensive respiratory support (Kapoor & Gutierrez, 2003). According to Muth & Shank, (2000) the more severe the sign on presentation the more gas is assumed to be embolised. ECG changes may reflect coronary embolisation resulting in myocardial ischaemia or infarction, as they do in blast if embolised in the coronary artery (Benzinger, 1950; Guy et al. 1998; Muth & Shank, 2000; Rossle, 1950).

Arterial emboli can cause pathologic changes by impeding perfusion to forward micro-circulation and organs (Benzinger, 1950: Mitchell & Gorman, 2005). They are also problematic because of the direct inflammatory effect the emboli makes on vessel endothelium, which may result in a systemic inflammatory response (SIRS) as discussed earlier (Kapoor & Gutierrez, 2003). An arterial embolus produces an inflammatory response

by way of the embolus' assault on the endothelial vessel wall, disrupting the local intracellular and extracellular environment.

Research suggests iatrogenic emboli may cause a 'non-severe trauma' or 'non-septic' induced SIRS (Kapoor & Gutierrez, 2003; Muth & Shank, 2000). Immuno-chemical alterations found in the presence of non blast emboli also produce a systemic inflammatory response syndrome commonly produce a SIRS (Kapoor & Gutierrez, 2003; Muth & Shank, 2000). These two points may mean that a SIRS and the immuno-chemical responses to emboli may be linked, which logically may lead to the theory that blast emboli may have a similar if not the same effect as non blast emboli on endothelium and the immuno-chemical response process.

SIRS can present as a mild inflammatory illness or it may become an overwhelming pathological process requiring intensive care support. Emboli effects on the endothelium are well recognised in the literature; in 2004, Suzuki and his team showed that surfactant can reduce arterial gas embolus adhesion to the endothelial wall and this may in some way protect the endothelium (Suzuki, Armstead & Eckmann, 2004). According to Branger & Eckmann, (1999), microscopic arterial emboli cause local arteriolar constriction. These authors found that emboli distort in shape, from round to sausage shape, increasing the surface area in contact with the endothelium which purportedly inhibits re-absorption rate.

More recent work attributes this to the changes occurring specifically in the glycocalyx[4] located at the endothelial wall of blood vessels (Eckmann & Armstead, 2006). It is unknown if these changes at the glycocalyx itself bring about specific local and/or systemic response.

---

[4] Glycocalyx is a glycoprotein-polysaccharide that is found on the surface of vascular endothelial cells in a blood vessel, it has many roles including inflammation regulation (McKinley & O'Loughlin, 2012).

Nevertheless it is known that the increase in local fluid causes the cells to become oedematous and the surrounding affected neurons cause a vasogenic oedema presumably producing a cytokine storm initiated by the bubble irritating the endothelial lining (Cavanagh & Eckmann, 1999; Muth & Shank, 2000).

It would seem that both local and systemic effects are at play in embolic events, and because neurons are affected the emboli effect might extend to neurological dysfunction. According to Drew et al. (1995), arterial gas embolism and fat embolism affect the brain (in rabbits) differently; essentially the gas embolus has a shorter time frame of effect than the fat embolus, but the brain is affected presumably not only directly as Drew, Helps and Smith, (1995) describe but indirectly through cytokine effect at the local neuron level, as described by Muth and Shank, (2000). From this it would seem that even a short-lived embolus that absorbs before identification would still leave behind the pathological repercussions of its presence at the cellular and tissue level and potentially initiate the inflammatory response as discussed.

3.6.4 Inflammatory response and blast research

The inflammatory response in blast victims has been at the forefront of blast research in recent years (Elsayad & Gorbunov, 2008; Gorbunov et al. 2004; Gorbunov et al. 2005; Gorbunov et al. 2008; Kirkman & Watts, 2010; Zunic et al. 2000). There is a developing understanding of the inflammatory pathway in primary blast injury, with a trail of pro-inflammatory mediators (cytokines) having been identified along the way (Elsayad & Gorbunov, 2008). Overproduction of nitric oxide, also a feature of blast injury, is a developing area of research pertaining to the inflammatory response to blast (Elsayad & Gorbunov, 2008; Kirkman & Watts, 2010; Zunic et al. 2000).

Nitric oxide, has long been realised to be a participant in cell function, immune response, vasodilation and apoptosis in mammals including humans (Bogdan, 2001; Brune, Van Knethen & Sandau, 1999; Gorbunov et al. 2008). The overproduction of nitric oxide and the consequential reduction of oxygen delivery to tissues has been shown to worsen outcomes of blast treatment regimes in animal models, as such, it indicates that links between nitric oxide and the inflammatory process in blast are significant (Garner et al. 2009; Zunic et al. 2000). Further prospective case report evidence describes immune-cytokine response in blast injured casualties of war, where survivors presented with an overall inflammatory response effect (Surbatovic et al. 2007).

3.6.5 Blast emboli, blast inflammatory responses, mild traumatic blast brain injury: is there a link?

Understanding emboli pathology in general is pivotal to this research as it was designed to generate exploratory evidence about whether emboli in blast evolve from a source other than translocation from primary blast lung injury. If another source is found to be possible it means microscopic gas emboli are more common in survivors of blast, and with that would be identified in the greater blast injury classifications, not just primary injury. This factor is crucial for the clinical management of blast injury.

Clinical case reports and research detailing the consequences of arterial air emboli in iatrogenic/non blast incidents may assist in defining previously unknown emboli effects in blast because the effect at the cellular/endothelial level may be similar (Branger & Eckmann, 1999; Cavanagh & Eckmann, 1999; Kapoor & Gutierrez, 2003; Muth & Shank, 2000).

The work in blast inflammatory response and the work in emboli inflammatory response is crucial to developing not only that itself, but also a possible link with blast emboli pathology, because the two may be linked if the origins of microscopic gas emboli are discovered. In addition, the inflammatory process may have a role in unlocking the mystery of mild traumatic blast brain injury, which immuno-chemical research is striving to answer (Gorbunov et al. 2008; Ling et al. 2008; McDonald et al. 2011). Woodcock & Morganti-Kossmann, (2013) detailed the various bio-markers identified in typical brain injury; determining any specific markers for blast brain injury would enable demarcation between blast and non blast brain injury. This in turn would allow research to focus on a potential link between blast brain injury and blast emboli. At present a link between the two is not perceivable because it is believed that microscopic gas emboli only form by way of translocation; if it is learned that emboli develop by other means it would be reasonable to consider emboli pathology in non primary blast injury as well.

The major impetus of this thesis was to determine whether microscopic gas emboli can only form by way of translocation, secondary to primary blast lung injury. Using this premise, emboli may occur in non-primary blast injury, and it follows that a blast inflammatory response might be discovered in non-primary blast injury, and that this may be emboli pathology? The question that must be answered is whether the chemical pathways for the inflammatory process in blast are similar to, the same as, or completely different from those of emboli induced inflammatory pathways?

Since blast and iatrogenic emboli are linked by location (in arteries), future research into blast emboli will benefit from work on generic emboli. Such research could identify similar pathways in the inflammatory process if they exist and identify a possible specific cytokine

for blast embolus which sets off the inflammatory response process, then prevention or treatment may be a reality.

3.6.6 Treating air embolism

Emboli can be treated with modern clinical practice and technology. Supportive care such as the provision of oxygen, maintaining the body's fluid balance and actively minimising the inflammatory response, is the central focus in emboli management and any treatment depends on the severity of the physical response. The priority for response to arterial air emboli is resuscitation for the protection of major systems (cardiovascular, respiratory, nervous and renal) (Muth & Shank, 2000). The following treatment options are recommended in the literature:

1. Hyperbaric therapy - to lessen the size of the emboli by raising ambient pressure and building a hyper-oxygenic environment and facilitating a large diffusion gradient to assist with emboli absorption. Treatment of blast emboli using hyperbaric therapy has been advocated in military health care guidelines for some time, but is often impractical in the field and high flow oxygen therapy is a common substitute (Muth & Shank, 2000; United States Department of Defense, 2005)

2. Maintaining normovolaemic status – to optimise micro-circulation and flow because of the likelihood of the emboli causing haemoconcentration thus compromising the micro-circulation (United States Department of Defense, 2005).

3. Corticosteroid therapy and anticoagulant therapy remain controversial but instances of successful use are documented (Muth & Shank, 2000).

3.7 Summary

This chapter outlined the scientific foundations of blast injury, explaining each injury classification and its relationship to the blast pressure wave. Emboli and inflammatory responses to blast exposure were also explored leading to the premise from which blast emboli research could develop once an alternative theory to translocation is proposed.

Chapter 4

Three theories of microscopic

gas emboli development

4.1 Outline

That microscopic gas emboli occur in the bloodstream following blast exposure is beyond doubt. Emboli are responsible for deaths occurring at the blast scene (sometimes due to fatal cardiac arrhythmia) as well as the later consequence of end organ failure resulting from cardiac, retinal, renal or cerebral arterial embolisation (Benzinger & Rossle, in Rossle, 1950; Clemedson & Hultman, 1954; Guy, Glover & Cripps, 1998; Guy, Watkins & Edmonstone, 2000).

Since World War II, researchers have sought to answer fundamental questions about the health outcomes of blast exposure, including its effect on the passage of emboli in the bloodstream and how blast produces cardiac rhythm changes, examining chest wall pressure wave velocity, and post blast respiratory function, all of which has helped refine our knowledge of biophysical injury potential, however the actual origins of blast emboli were not explored specifically (Axelsson et al. 2000; Clemedson, Hultman & Gronberg, 1953; Clemedson & Hultman, 1954; Guy et al. 1998; Guy, Watkins & Edmonstone, 2000; Irwin et al. 1999; Weiss et al. 1999).

Recent research has identified additional pathological consequences of microscopic gas emboli in iatrogenic scenarios, including direct endothelial injury and SIRS (Branger & Eckmann, 1999; Cavanagh & Eckmann, 1999; Kapoor & Gutierrez, 2003; Muth & Shank, 2000). In 2006, it was found that air emboli contribute to the degradation of the glycocalyx (at the endothelium), which in turn may produce an inflammatory response (Eckmann & Armstead, 2006). Despite these developments in the understanding of emboli arising from the non-blast literature evidence into the source of microscopic gas emboli in blast remains scant.

Three existing theories purport to explain the development of emboli in a blast scenario but as the forthcoming, comprehensive examination of the literature reveals none is based on convincing evidence and adequately explains the mechanism by which microscopic gas emboli form during blast exposure. As a result practice guidelines for the treatment of blast victims are based on dated assumptions that have not been proven within a satisfactory margin of error by today's standards (Tsokos et al. 2003). The aim of this project was to dispel some of the mystery surrounding microscopic gas emboli and make a preliminary but important advance in scientifically-based evidence about the formation of microscopic gas emboli in blast exposure.

4.2 The translocation theory

4.2.1 Overview

As previously stated, the prevailing belief in the medical community is that gas emboli form by 'translocation', whereby gas from alveoli moves to pulmonary capillaries when the high over-pressures from a blast wave impose significant loading that disrupts the pulmonary capillary interface in the lung, thus enabling the gas to 'leak' or 'be sucked' through microscopic alveolar-capillary fistulae (Benzinger, 1950; Clemedson & Hultman, 1954; DePalma et al., 2005; Horrocks & Brett, 2000; Mayo & Kluger, 2006; Rossle, 1950; Weiler-Ravel, Adatto & Borman, 1975; Wightman & Gladish, 2001). While the theory as it stands is physiologically plausible, the mechanism by which the gas moves has not been identified. As

such the postulated mode of gas transfer is an inductive theory[5], so it is scientifically inconclusive (Popper, 1934).

The pressures imposed in blast lung injury and damage typically incurred are substantial (Benzinger, 1950; Clemedson & Hultman, 1954). Despite this, Clemedson and Hultman, (1954) were unable to correlate blast air emboli and blast lung injury, and this situation remains unchanged today (Ho & Ling, 1999; Tsokos et al. 2003). The translocation theory requires that a primary blast lung injury is a necessary precursor to emboli formation, leaving the two events inextricably linked, a relationship the literature cannot substantiate (Tsokos et al. 2003). The translocation theory precludes any possibility that emboli formation outside a primary blast lung injury scenario occurs, and its general acceptance has held back research into the true origins of microscopic gas emboli.

The 'translocation theory' was first suggested following work by Benzinger's group including Rossle and Desaga between 1940 and 1950 when they identified gas emboli in central arterial circulation by post mortem examination (Benzinger, 1950; Rossle, 1950). Their discovery of gas emboli in pulmonary circulation on post mortem examination of dogs is not in doubt, however, their supposition as to how they came to be there was not; Rossle (1950) proposed, under guidance from allied scientist colleagues, that emboli 'penetrate the membrane of the capillary wall within a very short time if the static pressure is sufficiently high.' (Rossle, 1950, p. 1267). Unfortunately, Rossle did not qualify his comment beyond this subtle link to a Valsalva manoeuvre.

During the literature debate, in the early 1950's, Clemedson and Hultman could not find any

---

[5] Inductive theory is based on constructing a hypothesis from conclusions reached from current knowledge and predictions (Popper, 1934).

clear correlation between blast lung injury and air emboli; they did not consistently find air emboli in severely injured animals and not exclusively in severely injured animals. They did not account for any differentiation in the type of lung injury incurred, for example any relationship between broncho-pleural fistulae and emboli volume. However, Clemedson and Hultman were precise and detailed in their experiences, showing the extent to which emboli can occur describing many bubbles with '…the appearance of a rope of pearls…' (Clemedson & Hultman, 1954, p. 430). They further described air emboli as being found in central arterial circulation only as far as the brain, in basal arteries and choroid plexus, as well the retinal vessels and coronary vessels.

While fundamental work on blast effects was conducted in field experiments in the fifties and sixties (Chiffelle, 1966; Clemedson, Hultman & Gronberg 1953; Clemedson & Hultman, 1954), more recent work focused on the pursuit of the clinical consequences of blast exposure, such as prolonged hypotension and latent physiological dilemmas, by exploring cardiovascular-reflex activation (Guy et al. 1998; Irwin et al. 1999) and the immune-histochemical or toxicological responses (Gorbunov et al. 2005; Surbatovic et al. 2007; Tsokos et al. 2003). This was useful, but left the origins of blast emboli unexplored.

4.2.2 Proposed reasons for translocation theory's popularity

The three main reasons why the translocation theory's popularity continued in the literature are listed below, accompanied by discussion that offers an alternative stance using evidence from physiological and allied disciplines for similar phenomena not usually considered in the blast research literature.

1.   Blast emboli have only ever been found in pulmonary circulation and central arterial circulation, including vessels supplying the heart, lungs, head and brain.   Why this occurs was discussed in the literature following Benzinger's discovery of emboli in 1950 (Benzinger, 1950; Rossle, 1950).   The proposed mechanism was the translocation effect.   Over the years the speculative nature of the discussion dissipated and the theory became accepted (Alfici, Ashkenazi & Kessel, 2006; Argyos, 2007; Ciraulo & Frykberg, 2006; De Palma et al. 2005; Riley, Clark & Wong, 2002; Rosenfeld & Ford, 2010; United States Department of Defense, 2005; Wightman & Gladish, 2001; Wolf et al. 2009).

The historical journey of the translocation theory began when Clemedson and Hultman (1954) supported Benzinger's (1950) as to why emboli were only found in lung circulation that had passed the oxygenation process; they added credence to the translocation theory by noting that they too had only found emboli in central arterial circulation, with the exception of one observation they dismissed as experimental error.   Clemedson and Hultman (1954) stated that past theorists (Gaudin, 1887; Scher, 1941) were absolutely wrong in their support for autologous emboli formation:

'Since the hypothesis of the dissolution of excess amount of gas in the blood during the high pressure phase of blast (Gaudin, 1887; Scher, 1941) has been refuted it is now clear that the blast damaged lungs must be the sole entrance of the air and that air embolism in blast injury is of the arterial or systemic type.' (Clemedson & Hultman, 1954, p. 433).

This exert implies Clemedson and Hultman had proved their predecessors wrong, presumably by their declaration earlier in the paper that the blast wave is too fast for any dissolved gas to be released.   No experiments were undertaken to test this, but argued strongly later in the paper that the combination of the pressure differentials inflicted by the blast and the lower

76

pressures within the pulmonary vessels allowed air through the damaged micro architecture of the lung parenchyma (Clemedson & Hultman, 1954).

Mason and his team (1971), subjected a dog to a near-lethal blast and observed emboli movement using Doppler examination; the dog survived to the 5th day when he was euthanased for post mortem examination purposes. The experiment monitored the effects of spontaneous breathing on the blast victim and emboli movement, and a logical assumption was made that the air was being tracked in as the dog breathed, a common consequence of broncho-pleural fistulae, (which this may have been). Unfortunately, post mortem examination of the lungs was limited to gross examination only 'The lungs showed bilateral residual haemorrhage typical of early resolution after severe blast injury', (Mason et al. 1971, p. 1253). The post mortem examination took place five days after the initial injury, meaning the original injury may have partially resolved, particularly as the animal progressed reasonably well; the authors claimed the lung showed signs of early resolution of injury.

Injuries such as a broncho-pleural fistula or pneumothorax are consequences of non-specific lung trauma and each is known at times to entrain air into the circulation through torn interfaces, but neither falls into the same category as air emboli evolving from proposed alveolo-venous fistulae. (These discrete physiological differences are discussed in detail later in this chapter). As Mason et al. (1971) undertook only gross lung examination they made no progress on discovering the origins of blast emboli. The authors confirmed that the blast emboli were arterial and short lived but wrote that their experiment provided sufficient evidence to accept the translocation theory. Their conclusion that the animal tolerated the emboli well is dubious, as they justified this with the animal's survival to five days with no real impact on other systems, but work undertaken in 1947 shows dogs are more tolerant of

air emboli than humans therefore translating emboli tolerance from dog to human may be invalid (Durant, Long & Oppenheimer, 1947).

2. Translocation is perceived to be physiologically reasonable. Emboli have only been found in pulmonary and arterial circulation, and are considered to be as one and the same as broncho-pleural fistulae; it does not consider effects that may occur from trans-pulmonic pressure changes, such as one finds in the discipline of diving medicine. Experimental work on diving injuries has shown that the lung damage that occurs might be considered similar to that typically sustained in blast injuries. Lung injuries sustained during diving are caused by a dramatic change in trans-pulmonic pressures of 95 – 110 cm of water, which disrupts pulmonary parenchyma (Malhotra & Wright, 1960; Neuman, 2004; Schaeffer, Nulty & Carey, 1958). In the diving literature the prospect of emboli escaping into circulation from alveoli is considered speculative, because the phenomenon of air transference relies on a normal breath being taken and air emboli have only been identified before ascent to the surface where breathing has only taken place via diving apparatus (Neuman, 2004).

Ho, (2002) suggested a simplified conceptual approach to translocation. His explanation revolves around the fact that whilst we know air and blood mix in the alveoli (because patients may present with blood stained and frothy sputum), he hypothesised that the reverse is inevitable when positive pressure ventilation is instituted. Ho's reverse pressure gradient theory was overturned the following year by the identification of emboli found in cases that were not resuscitated (Tsokos et al. 2003), and this overturning was supported by similar findings in cases observed in Israel by Mayo and Kluger, (2006). Although the description appeared simple, Ho's (2002) theory is not as simple as it may appear.

According to Wightman and Gladish, (2001), once an alveolus is ruptured it has exceeded its tensile strength and as such is irreversibly damaged making it incompetent. It may nonetheless be possible that a ruptured alveolus behaves like a starling resistor[6] and should an instantaneous peak pressure in the airway exceed interstitial pressure the alveolus may allow air to escape. However, given the known rapid increase in interstitial pulmonary pressure in blast this idea is improbable (West, 2005). West's (2005) work used the same principles (pressure changes) as the diving literature does in refuting the feasibility of the translocation effect.

Whether disrupted alveoli, upon their collapse, and before interstitial pressures rise, permit gas into the circulation at the time of injury is pivotal to the translocation debate. However, other than Ho's (2002) attempt, the medical literature is devoid of any discussion of pulmonary pressure changes and the consequent validity of the translocation theory.

3. A third reason the translocation theory is so entrenched lies in its careless citation in the literature since its arrival in the 1950's. In addition, numerous authors have linked translocation in blast to broncho-pleural fistulae injury with few or no citations to support their argument. As noted earlier, Clemedson and Hultman referred to the theory in published work of 1954, citing Benzinger's (1950) argument which was entirely appropriate conduct, and since that time other authors have followed appropriately (Guy, Glover & Cripps, 1998; Stuhmiller, 1991). However, over subsequent years, other authors referred to it as sound theory but gave no citation, leading the reader to regard it as accepted fact (Argyros, 1997;

---

[6]A starling resistor occurs when the alveolar pressure is greater than venous pressure in the lungs (but still less than arterial pressure); the capillary at the downstream end would then collapse. The same mechanism occurs in forced expiration, where the intra pleural pressure collapses the downstream airways, limiting airflow. (West, 2005).

Horrocks & Brett, 2000; Lavonas & Penndardt, 2006; Mason et al. 1971; Mayo & Kluger, 2006; Wolf et al. 2009).

While these authors may have passively promoted the acceptance of the theory, others implied that translocation is a fact by citing a past author(s) who, on investigation, had only suggested it as a possibility (Irwin et al. 1999; Rosenfeld & Ford, 2010; Wightman & Gladish, 2001). Admittedly it is difficult at times to determine whether the authors were referring to broncho-pleural fistulae (in which the great vessels and the larger airway tree are involved as found in many varieties of lung injury), or translocation at the microscopic level (alveolus-capillary). Nevertheless, by not being clear they imply the injuries are one and the same yet they cite references that specifically address the microscopic form of emboli, confusing the whole situation (Irwin et al. 1999; Wightman & Gladish, 2001).

Riley, Clark and Wong, (2002), added to this confusion in several ways. They used two authors as evidence for their support of translocation. Wightman and Gladish, (2001), cited earlier, whose position was that translocation is a fact, even though they cited a colleague who regarded it as a theory. Riley, Clark and Wong's, (2002) other citation is Ho and Ling, (1999), who in the introduction of their review paper clearly state that 'The incidence of SAE (systemic arterial emboli) in primary pulmonary blast injury is unknown.' (Ho & Ling, 1999, p. 4)

Riley, Clark & Wong, (2002) used Ho & Ling's, and Wightman and Gladish's (2001) research to support the following statement: 'Systemic air embolism occurs after lung trauma. The primary blast of an explosion can lacerate the air passages, lung alveoli and blood vessels resulting in direct communication between the structures (Ho & Ling, 1999, Wightman &

Gladish, 2001).′ (Riley, Clark & Wong, 2002, p. 4)

The Riley team's, (2002) discourse is confusing. Firstly, they do not directly reference their specific injury comment, they fail to define their use of the statement by applying it as a premise for a referenced opinion, in doing so they implied a connection between blast lung injury and air emboli, a concept not supported by research subsequently cited further in their text (Clemedson & Hultman, 1954; Tsokos et al. 2003). Secondly, their paper implies that the air emboli known to occur in broncho-pleural fistulae in non specific lung trauma are one and the same to those they assume to occur through translocation in blast at the microscopic level (alveolar-capillary gas translocation), and that this concept is supported by the cited earlier work. Examining the cited works shows this is not the case.

Failing to define the difference between broncho-pleural fistulae emboli and microscopic emboli within the context of a blunt trauma injury is commonplace across a wide range of literature (Alfici, Ashkenazi & Kessel, 2006; Ciraulo & Frykberg, 2006; DePalma et al. 2005; Horrocks & Brett, 2000; Mayo & Kluger, 2006; Weiller-Ravell, Adatto & Borman, 1975). Two of these examples, Alfici, Ashkenazi and Kessel, (2006) also Mayo and Kluger, (2006), make an additional claim that a link exists between blast lung injury and emboli, whereas the actual link for emboli is in blast injury; although emboli can certainly be a consequence of blast injury, it is not yet established that they occur in blast lung injury (Benzinger 1950; Ho & Ling, 1999; Tsokos et al. 2003).

Broncho-pleural fistulae in blast injury are well documented, and are the result of the blunt trauma (resulting from the overpressure). The blast overpressure loading causes a shear injury where larger airways and great vessels can be lacerated by the force of the blast wave

and/or produce pneumothoraces as identified by Ho and Ling, (1999). As Ho and Ling (1999) explain, these injuries and the related fistulae do not necessarily relate to the microscopic emboli question (Ho & Ling, 1999).

Ho and Ling, (1999) made a clear differentiation between the two pathophysiological processes, declaring that firm evidence about how or if gas emboli form at the microscopic level in blast was scant, but they proposed translocation as a logical explanation. They stated that broncho-pleural fistulae are well recognised in other types of lung trauma and occasionally in blast, but stressed that they occur predominately as a consequence of penetrating trauma, not blunt trauma (as primary blast injury is categorised). Riley, Clark and Wong, (2002) failed to acknowledge this explanation by Ho and Ling, (1999), and in doing so erroneously represented the theory of translocation as factual.

Poor use of evidence, implied proof, tautological arguments and confusing paragraph structuring are academic issues, nonetheless, they have an important result that they allowed translocation to be accepted as fact, supporting a relationship between emboli and blast lung injury despite no empirical evidence.

4.2.3 The effect of the theory's acceptance on research and clinical practice

Since a theoretical relationship between blast lung injury and emboli has been accepted as fact in the literature, it logically follows that not only research is based on unproven assumptions but so too are clinical practice guidelines for care. Patient management strategies are best developed using evidence to support the practice with the principle of helping clinicians treating blast casualties.

Advice on clinical practice for blast victims has been provided in a plethora of review papers on blast over many years (Alfici Ashkenazi & Kessel, 2006; Argyros, 1997; Ciraulo & Frykberg, 2006; DePalma et al. 2005; Riley, Clark & Wong, 2002; United States Department of Defense, 2004). Some of these authors offer warnings to colleagues on the pitfalls of early artificial positive pressure ventilation (PPV) in blast lung injury for fear of causing emboli, or at least aggravating the situation by sending more of them into the circulation through the use of PPV; thus leading readers to believe translocation of emboli in blast is primarily either due to artificial ventilation or is made worse by artificial ventilation. In fact the combination of PPV and any lung injury can cause air embolism (Ho & Ling, 1999).

In some of the cases the authors represent translocation as fact, often without citation. In the following examples Argyros (1997, p. 110), writing specifically about blast lung injury suggested that: 'Only casualties with clinical evidence of pulmonary contusion will be at risk for air embolism.' This was followed by a warning linking air embolism and primary lung injury, again without citation: 'It is important to reiterate that air emboli will only be seen in casualties with clinical evidence of pulmonary contusion.' (Argyros, 1997, p. 111).

Because air embolism can occur as a complication of PPV in general blunt trauma (although according to Ho and Ling, (1999), mostly in penetrating trauma), the authors' intention was ostensibly good in that he was forewarning colleagues to problems they may incur in their practice. However, the way this relationship is portrayed, particularly given Argyros, (1997) and Riley, Clark and Wong's, (2002) focus is specifically on blast injuries, conveys the assumption that PPV following blast injury causes or worsens emboli (Argyros, 1997; Riley, Clark & Wong, 2002). It is true that emboli can be an iatrogenic complication of PPV in a number of clinical scenarios but Argyros' (1997) depiction of it causing emboli in blast, has

been refuted by strong evidence in recent years (Tsokos et al. 2003).

There is evidence that the clinician-authors seek to offer a genuine attempt to minimise the adverse effects of positive pressure ventilation in lung injury and applying the same strategies used for general lung injury to blast lung injury is a reasonable strategy. However, in doing so in this way authors misrepresent the basis by which PPV may be a problem in the case of blast trauma, and as such implies translocation as fact.

Clinicians treating all lung injuries, including blast lung injuries, apply protective ventilation strategies that minimise the adverse effects of artificial ventilation on the injured lung and maximise lung function (Lavery & Lowry, 2004; Marini & Truwitt, 1998; Putensen et al. 2009; Stocker, Lenzlinger & Stover, 2014). They focus on minimising airway pressures, promoting oxygen uptake by improving pulmonary circulation, and optimising alveolar function. In practice this is achieved by clinical staff tolerating higher than normal arterial blood carbon dioxide tensions, using specialist nursing strategies to minimise oxygen demand, promote oxygen uptake and maintain general homoeostasis by patient positioning, rest, passive feeding for optimal nutrition and other ordered treatments such as positive pressure ventilation or extracorporeal lung support (Stacy, 2009).

Protective ventilation strategies are standard practice in the treatment of all lung injuries, and evidence supporting the practice is deeply embedded in the critical care discipline (Lavery & Lowry, 2004; Marini & Truwitt, 1998; Putensen et al. 2009; Stacy, 2009; Stocker, Lenzlinger & Stover, 2014). Any advice, such as that advising against PPV because of perceived emboli formation in blast lung injuries is not supported by evidence.

To date it is clear that the public domain literature is a mainstay in facilitating the direction of a theory. Misrepresentation, albeit unintentional, of a theory's credibility, at any stage will distort the knowledge trail and potentially result in a misdirected research focus and potentially misdirected education.

4.2.4 Evidence opposing translocation to date

Although no research opposing translocation directly exists there is little literature evidencing opposition.

Ho and Ling (1999), dismissed the idea of a correlation between blast lung injury and blast gas emboli (Ho & Ling, 1999). In 2003, Tsokos and his team published their post mortem examination of blast victims, within this paper they cited three pivotal papers describing animal studies of emboli and calculated their results inconclusive that lung injury and emboli may be linked (Tsokos et al. 2003). No subsequent data in the public domain challenges this assessment of the relationship between blast emboli and lung injury.

Harding, (1996) reported ischaemic electrophysiology (ECG) changes in 12 lead ECG's for five landmine victims. The ischaemic ECG changes were typical of those changes reported in the literature on blast emboli in coronary vessels (Benzinger, 1950; Clemedson & Hultman, 1954). Cardiac arrhythmiae following blast exposure exist, and are evident in the literature but these are not evidence for emboli and subsequent ischaemic changes because only one

dimensional rhythm tracings were used in these experiments (Guy, Watkins & Edmonstone, 2000; Irwin et al. 1999).[7]

The cases in the Harding (1996) paper showed that the 12 lead ECG changes were directly linked to specific regions of the heart, a typical presentation of ischaemia, indicating coronary emboli were obstructing particular coronary vessels feeding a particular region of the heart. This differentiation between rhythm assessment and 12 lead global ECG readings was not made in previous literature. Harding's (1996), report also defined that all the cases of ischaemic myocardium occurred in the absence of primary lung injury; all five patients suffered traumatic amputations and shrapnel injuries (secondary and tertiary injuries). One of the five patients progressed to cardiac failure on day two, requiring pharmacological treatment. It is possible the ECG changes in Harding's (1996) report, are the result of coronary artery embolisation. The changes were unlikely to be due to a pulmonary reflexive triad response to the blast because this is considered to be an immediate and short-lived event, not a latent consequence of blast and, not an end organ consequence of possible mobile emboli (Benzinger, 1950; Irwin et al. 1999). Although only case study level of evidence, the Harding (1996) paper provided an insight previously unrecorded in the public domain and adds to the suspicion that the formation of microscopic gas emboli may not stem from translocation following primary blast lung injury, and as such requires further exploration.

The translocation theory is the accepted explanation of how microscopic gas emboli form in blast-exposed humans. The theory has progressed from a suggestion to explain a phenomenon, to being regarded as fact, despite minimal or weak evidence.

---

[7] A cardiac rhythm uses a one lead view and cannot be used for assessing ischaemic myocardium, a twelve lead ECG tracing is required, showing the heart's global profile (Conover, 2002).

4.3 The spalling theory

Spalling is a physical process that describes a surface failure because an energy source forced upon it overpowers it. The results of spalling depend on the scenario and the space into which it occurs, material or liquid vapour by way of bubbles are common formations. Spalling can result from cavitation, where vapour bubbles form in a flowing liquid or a liquid disturbed by mechanical means such as a turbine or a ship's propeller or another type of solid fragment. Once the bubbles collapse, a higher localised pressure can cause spalling to neighbouring surfaces (Moholkar & Pandit, 1997).

Cavitation can be driven by a shock wave and as such should not be ignored in the blast research arena. A precise measured shock wave is commonly inflicted on human tissue for medical treatment purposes (Auge & Preminger, 2002). The medical application of lithotripsy treatment for renal calculi uses a deliberately focused, measured shock wave (using a laser) to crush a calculus (stone) into tiny fragments, and this procedure may result in spalling (Lindqvist et al. 2006). The laser shock wave's shearing force produces cavitation bubbles surrounding the stone, whereupon these forces crumble the stone into smaller pieces so as they are more easily passed by the patient via the urethra. The treatment can cause endothelial cell damage within the ureter, and cavitation bubbles, but laser lithotripsy involves a carefully focused and measured dose to minimise such side effects (Lindqvist et al. 2006).

Blast injury researchers believe that spalling is a biophysical response to coupling and that microscopic gas emboli form from spalling (Mellor et al. 1997; Phillips & Richmond, 1991; Stuhmiller, 2008). As previously explained, coupling refers to the process of energy dispersion between a high-density medium and a low-density medium, as occurs when a blast

wave impacts with the chest wall, and moves from this higher-density tissue to the lower-density lung parenchyma, causing trauma to the latter (Iremonger, 1997; Mellor et al. 1997).

Spalling is thought to occur as the blast wave is tensioned at the chest-lung interface resulting in (coupling), it is the tensioning of the surface resulting in material being thrown off (Schardin, 1950; Phillips & Richmond, 1991). Little evidence exists to show that spalling actually occurs in internal anatomical architecture, such as the alveoli and blood vessels due to blast exposure. Nevertheless, it seems to be widely accepted that coupling and spalling are likely to occur in blast exposure, and that spalling is part of the mechanism of primary injury in blast because of its relationship to the coupling effect on organs such as the lungs (Mellor et al. 1997; Phillips & Richmond, 1991; Stuhmiller, 2008).

The researcher's conversations with a blast expert in bio-mechanics and engineering disciplines revealed that disciplines belief that positive pressure shock loading to air-filled organs generates a tensile stress wave that, when reflected off internal air spaces, produces vapour bubbles that they say may be emboli (D. Ritzel, personal communication, 2004). No published evidence supports this idea but we know that water vapour is produced at 6kPa at a temperature of 36.6^0C, normal human body temperature (Brady & Holum, 1993). It is therefore possible that water vapour bubbles form in the blood due to the rapid or explosive decompression experienced in the under pressure phase of the blast wave, which exceeds 6kPa. Accordingly, it may also be true that water vapour bubbles are produced by both a positive pressure load and/or a rapid or explosive decompression?

Both spalling theories imply a relationship between lung injury and ensuing emboli formation, either by positive pressure loading and resultant tensile stress (Mellor et al. 1997; Phillips &

Richmond, 1991; Stuhmiller, 2008), or by explosive decompression (Axelsson et al. 2000; Federal Aviation Administration, 2005) however, a relationship between primary lung injury and emboli in blast exposure is not yet established (Benzinger 1950; Ho & Ling, 1999; Tsokos et al. 2003).

4.4 Autologous emboli development

4.4.1 Overview

Autologous formation of emboli during the sub-atmospheric phase of the blast pressure wave - the autologous theory - is a third mechanism by which microscopic gas emboli might form in blast victim's bodies. The premise of this theory relies on Henry's Law, which is described as the solubility of a gas in a liquid is proportional to the pressure of the gas over the solution (or partial pressure). When the partial pressure is increased, more molecules dissolve into the solution and the opposite occurs when the partial pressure is lowered, the solubility of a gas is lowered when the ambient pressure is lowered (Brady & Holum, 1993; Chang, 1998). Henry's Law is derived from Henry's Constant and is true for a consistent temperature, alterations in solute, solvent and temperature alter the rate by which solubility occurs at particular ambient pressures; the warmer the ambient temperature the less soluble the gas and the cooler ambient temperature the more soluble the gas (Brady & Holum, 1993; Chang, 1998) as shown:

$$p = k_H c$$

Henry's Constant equation, where p is pressure, c is concentration, kH is a constant with the dimensions of pressure, divided by the concentration (Chang, 1998).

In the late 19th century, researchers were starting to take note of the physical alterations, including emboli development, that occurred in deep sea divers (Taylor, 2004). Gaudin (1887) proposed that the gas emboli occurring in decompression sickness were due to physical means and simultaneously that gas produce emboli in the blood during blast exposure but chiefly by chemical, not physical changes as they were proposed in diving (Gaudin, 1887). Gaudin based his ideas on similarities and differences in exposed ambient pressures between the victims of blast and diving. He proposed that chemical reactions may evoke release of carbon dioxide from blood to gas form at lowered pressures. Gaudin's theory was that because diving emboli might occur because of exposure to profound pressure changes, so it may also occur in blast.

While Gaudin proposed chemical reactions as a possible mechanism for emboli development as a result of blast exposure in his thesis of 1887, emboli had yet to be identified in blast victims. Gaudin posed that because emboli develop in diving victims because of changes in pressure he suggested that this may also be the case for blast. That gas can emerge from solution in the blood as a result of a chemical reaction when the body is exposed to a rapid decompression effect in blast is plausible, but untested. In 1941, almost a decade before Benzinger's discovery of blast gas emboli, Scher (1941, p. 797) proposed that autologous gas emboli formation in blast was possible:

'The immediate effect of the explosion is to create a very strong positive pressure in its vicinity, and this would increase the air dissolved in the blood considerably. Subsequently there is a rarefaction of the atmosphere. This would lower the pressure in the lungs below normal and lead to a rapid withdrawal of the previously dissolved air in the blood. Hence there would be a strong tendency for air bubbles to form in the blood and possibly also in some of the tissues,...'

Scher's letter was a comment on a previously published paper so may not have attracted much

attention as if it had been part of a full length report. Nevertheless, it represents a theory for microscopic gas emboli based on the effects of pressure differentials.

The sub atmospheric phase of a blast wave as it relates to injury or emboli has received little research attention (Latner, 1942; Scher, 1941; Zhang et al. 1996) relative to over-pressure as previously outlined in this thesis, as such pertinent literature is scant. In light of this deficiency, the following three sections explore literature and scientific principles that support autologous emboli formation in blast exposure.

4.4.2 Injury observed in the sub-atmospheric environment

As outlined in Chapter 2, the sub-atmospheric phase is the phase of an explosion in which pressure is below one atmosphere, and occurs immediately after the over-pressure phase. The length of time below atmospheric pressure and the depth of pressure reached are proportional to the positive values in the over-pressure phase. The injuries it causes, particularly in the lungs, are similar to those found in the victims of decompression sickness, which demonstrates that the injuries are due to pressure alterations (Latner, 1942; Zhang et al. 1996).

The under-pressure phase of a blast involves two important injurious factors. Firstly, the under-pressure phase is powerful enough to overcome gravity by disrupting concrete slabs and uprooting surrounding vegetation, thereby creating mobile debris (Krauthammer & Altenberg, 2000; Latner, 1942; Zhang et al. 1996). Secondly, the blast generates a latent blast wind effect, causing turbulence as the blast wind moves forward. Concurrently the under-pressure phase sets in as the pressure effect reverses with the net movement of gas back to the detonation point producing the overall vacuum (Mellor et al. 1997). This environment is

where secondary and tertiary injuries occur; some authors claim such injuries cause much more mortality and morbidity in blast trauma than previously reported (Mayo & Kluger, 2006).

Latner (1942) specifically examined the negative phase of blast, finding subjecting rats to deep rapid under pressure resulted in similar lung parenchyma injury to that of the over-pressure phase of blast; he likened these effects to the physiological effects from the Davis underwater apparatus (diving gear of the time). Latner did not mention emboli, his work revolved around injury potential, and took place prior to emboli discovery in 1950. Nonetheless, Latner was the first published researcher to allude to the injury potential of the negative pressure phase of blast (Latner, 1942). Latner was also first to advance an hypothesis that blast researcher's continue to use (Cooper et al. 1991; Guy, Glover & Cripps, 1998; Kirkman, Watts & Cooper, 2011; Latner, 1942; Mellor et al. 1997; Schardin, 1950), that the pressure differential between the peak overpressure point, and the deepest under-pressure point reached during the blast event is the most powerful determinant of the severity of the resultant injury.

The lung injury Latner (1942) identified as resulting from rapid decompression was observed again many years later by Zhang et al (1996) who found similar injury results in their experiments directed at lung injury form explosive decompression exposure. Zhang and his colleagues used an animal model and found emboli in both pulmonary arteries and veins. Their application of modern technology enabled more precise depth and time measurements of the exposures than was possible in Latner's research in 1942. The Zhang team's (1996) work provides evidence that emboli are related to the negative phase of blast. Their timings to peak under-pressure ranged between 0.0035 seconds and 0.0074 seconds, meeting the

criteria of explosive decompression according to the nomination used by the United States Federal Aviation Administration (2005).

Kemph and Hitchcock, (1952) similarly identified gas bubbling in their experiments aimed at assessing changes in blood during measured rapid decompression in 1952. Kemph and Hitchcock (1952), found a loss of carbon dioxide alongside emboli in their experiment with an animal model. This evidence strengthens the relationship between a rapid decompression exposure and emboli formation. Their work further supports the prospect that a negative under-pressure may not be required to liberate the gas from solution if the environment is well saturated, a situation Divinis et al. (2004) observed in their research addressing bubble formation in supersaturated liquids at reduced gravity.

4.4.3 Decompression, dissolved gas and gas tension in solution

As a prelude to understanding the blast autologous emboli development theory, an understanding of the pathophysiology of decompression sickness in divers along with any commonality to it in blast emboli development by autologous means is pivotal.

Early in the twentieth century JS Haldane used the basis of Paul Bert's 1878 observations on decompression sickness to refine the theory of gas bubble formation in divers (Kindwall, 2004). He proposed a new diving regime that avoided the development of a nitrogen tension exceeding absolute pressure, thereby preventing the supersaturation ratio exceeding 2:1 and hence the formation of bubbles. Haldane's work was based on the theory that nitrogen is normally circulating in dissolved form from lungs to tissues, so a diver would absorb nitrogen at depth, at first rapidly, then at a slower rate as the tissue nitrogen tension reached alveolar

partial pressure (Kindwall, 2004). He designed a staged decompression formula based around the concept of the rate of nitrogen exchange in the tissues, according to the arterial and tissue (or venous side) perfusion times.

Haldane further claimed that the difference between arterial tension and venous tension of nitrogen reduced by half with each half of the time passed (Kindwall, 2004). He concluded that a staged decompression, not a long lineal ascent, would minimise the evolution of bubbles because the process of staged decompression provides time at decreasing pressure levels for the nitrogen to dissolve back into the blood (Kindwall, 2004).

Haldane's work revolutionised diving practice and remains the basis for treating decompression sickness today.

Importantly for this thesis Haldane postulated that emboli are unlikely to form in arteries because the human lung equilibrates alveolar and arterial gas tensions in one passing (Kindwall, 2004). The current theory of emboli formation in decompression sickness holds that they are formed in venous sinusoids and venous end capillary beds where there is lower hydrostatic pressure and higher gas tension as nitrogen diffuses out of tissue into blood (Francis & Mitchell, 2004). Interestingly, despite the larger volume of blood in the pulmonary system, where blast emboli have been identified, the hydrostatic pressure of this system is even lower (mean pressure 10-15mmHg) than in the venous capillaries (mean pressure of 15 - 30mmHg) (Lough, 2009). The greatest pressure moment, which is at cardiac systole[8], reaches a mean of 20-30 mm Hg, this is within the same low pressure region as the venous capillary pressure described by Francis and Mitchell (2004), therefore providing an environment ready for dissolved gas release (Divinis et al. 2004). While Divinis' team observed bubble production at low gravity, not rapid decompression, the liberation of carbon

---

[8]   Cardiac systole is when the heart reaches its peak pressure (Lough, 2009).

dioxide specifically in an altered atmospheric condition is interesting and encourages focus on bubble formation in altered atmospheric pressures according to the partial pressure of the dissolved gas, not necessarily the time it may take to liberate it.

Lower pulmonary pressures were a key factor of Clemedson and Hultman's (1954) support for the translocation theory. They claimed the lower pressures enabled blood to be 'sucked' into the pulmonary vessels from torn alveoli (Clemedson & Hultman, 1954). The method by which translocation is meant to occur is yet to be expressed rigorously in contrast, it is physiologically reasonable to suggest the pulmonary vessels, with their high capacity and low pressure unlike all other circulating systems in the body, might be vulnerable to de novo emboli formation from solution (blood) given the right physical conditions. So, taking together the low pressure conditions of the pulmonary system, the higher gas tensions of a dissolved gas (supersaturation) resulting from the over-pressure phase, it is possible that the pulmonary system is a birth place for emboli in blast. This may also explain why smaller pulmonary vessels are found to be dilated after blast exposure (Horrocks & Brett, 2000; Tsokos et al. 2003).

At this point the physiological argument suggests that blast emboli could develop in pulmonary vessels during a rapid or explosive decompression event. This then means, the embolus must contain a gas normally dissolved in blood. The most soluble gas dissolved in blood is carbon dioxide (West, 2005).

4.4.4 Considering the gas emboli content

The review of the literature in the preceding passage surrounding the autologous theory of

emboli development in blast exposure has lead to the consideration that the gas within the emboli might be carbon dioxide. This deliberation is based on the premise that a gas embolus formed in the bloodstream through explosive or rapid decompression would form from a dissolved gas already in the bloodstream and that carbon dioxide is the most soluble of all dissolved compounds in human blood (Brady & Holum, 1993; Chang, 1998; West 2005).

Linking concomitant issues revolving around emboli formation and decompression within the context of the blast related autologous theory may produce a more coherent and structured theory from where research may proceed. This section describes gas emboli formation by way of examining the response of dissolved gases to environmental pressure alterations, blast emboli longevity, and finally emboli mobility along with observed locations emboli have been found in.

1.   Scher (1941) proposed that gas could saturate in blood during the over-pressure phase and be liberated at the sub-atmospheric or under-pressure phase, in contrast, in supporting Benzinger's (1950) emboli findings Clemedson and Hultman (1954, p. 426) advocated translocation as the most realistic avenue for emboli formation stating that given 'the very short duration of both the positive and negative phase of the shock wave it is highly improbable that any gas can be solved and set free as gas bubbles in the blood during the passage of the shock front.'

The argument opposing autologous emboli formation was based on the speed of the decompression event. Even though only two years lapsed between their publications, there was no acknowledgement of Kemph and Hitchcock's (1952) research where they found that subjecting blood to a rapid decompression of (0.02 seconds) produced a foamed sample that

was also lower in carbon dioxide content when compared to the pre test measurement. It appears, by using time as an issue, Benzinger's argument was based on the fact that emboli in diving sickness are generally a slow process in which a slowly dissoluble gas, such as nitrogen, causes emboli.

Nitrogen is a highly insoluble gas, in diving sickness the time submerged prior to a rapid ascent is the key factor for development of circulatory and tissue emboli (Francis & Mitchell, 2004). However, 'Henry's Law is concerned with the amount of gas in solution when equilibrium is reached. It specifically does not address how rapidly that state is reached.' (Taylor, 2004, p. 31). Henry's Law is defined as 'The concentration of gas dissolved in a liquid is proportional to its partial pressure.' (West, 2005, p. 76). When gas tension, or the concentration of dissolved gas, is high it is more readily released from the solution as gas if conditions suit such as, for example, when the surrounding pressure is lowered (Taylor, 2004; West, 2005).

In stating that the timing of the blast wave was the important factor rather then the change in exposed pressure Clemedson and Hultman, (1954), redirected focus away from a possible association between diving sickness and blast emboli. Even so, the authors failed to consider the differences in the solubility of gases normally dissolved in blood. By considering a gas, more soluble than nitrogen, that is dissoluble within the time frame of a blast under-pressure wave may have refocused emboli in blast research in 1954. Although it has been known since 1954 that venous emboli are normally found in people experiencing diving sickness, arterial emboli have been identified at very, rapid ascents (Francis & Mitchell, 2004). Thus, the emboli in blast and the emboli found in divers following very rapid ascents may both be the result of rapid decompression. It would follow that because different gases dissolve at very

different rates, emboli that develop due to blast exposure may not be nitrogen; similarly the emboli identified in divers at very rapid ascents might not contain nitrogen either.

Several sets of researchers, have articulated the possibility that gas arises from blood solution during a rapid or explosive type decompression event (Francis & Mitchell, 2004; Kemph & Hitchcock, 1952; Scher, 1941; Zhang et al. 1996). The possibility that emboli form as a direct result of pressure alterations has not been explored in the blast specific literature. The theory, of autologous emboli formation during the blast sub-atmospheric phase, suggests that gas tension in the blood is liberated if a decompression occurs rapidly, such as it occurs in a blast decompression environment during the under-pressure phase, this idea needs to be tested.

If supersaturation of carbon dioxide occurs in the positive pressure phase of blast exposure, then the theory of autologous emboli formation is plausible. Scientific principles would support this as part of Henry's Law, but the differences between blast emboli and diving emboli, also known as dysbaric emboli, must be explored to test the theory.

Henry's Law states that the concentration of gas dissolved in a liquid is proportional to its partial pressure - 'Dissolved carbon dioxide, like oxygen obeys Henry's Law…' (West, 2005, p. 80.) As carbon dioxide's solubility is twenty times that of oxygen in blood, it easily changes from solution to gas, just as bubbles occur when opening a soft drink (West, 2005).

The concept that this is how emboli form during the under-pressure phase of blast is physiologically plausible, and accords with literature suggesting carbon dioxide is removed from blood during rapid decompression (Kemph & Hitchcock, 1952). Carbon dioxide is the most soluble of all blood gases which means rapid changes of state can occur. If blast emboli

contain carbon dioxide this would mean the emboli are short-lived as they would dissolve rapidly once the environmental pressure returned to normal. Table 4.1, shows that carbon dioxide converts from solution to gas almost 10 times faster than other gases found in blood thereby making it the most logical gas to arise from solution during the short decompression episode.

Table 4.1 Solubility coefficient table for common gases carried in human blood (Brady & Holum, 1993; Chang, 1988).

Carbon dioxide	0.570
Nitrogen	0.012
Oxygen	0.024
Helium	0.008

Carbon dioxide can be found in all three physical states depending on differing specific environments as explained by the following Figure 4.2. Carbon dioxide cannot maintain a liquid state below its critical point of 7,380 kPa and 304 K (Brady & Holum, 1993; Chang, 1988) which is well above atmospheric pressure. While carbon dioxide can theoretically supersaturate under extreme over-pressure, it follows that it would be impossible during the under-pressure phase for it to exist as anything other than a gas even with a short time frame of 0.02 seconds.
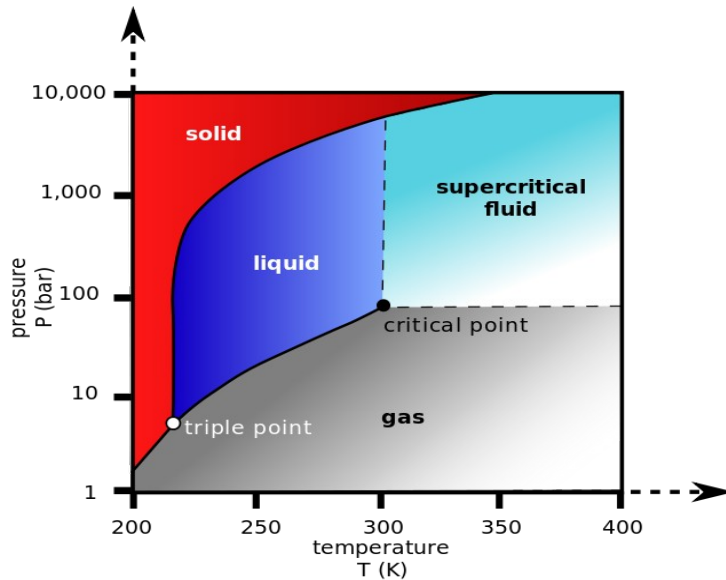
Figure 4.2 Diagram illustrating the conditions favouring a change of state for carbon dioxide. (Chang 1998, p. 440).

2.   Defining the lifespan of the embolus remove the need to investigate some gases any further. Clemedson and Hultman, (1954) described the lifespan of blast emboli as 'short lived' in their 1954 paper, this was further supported by Mason et al. (1971), using Doppler ultrasound to track emboli in a dog model. Neither Clemedson and Hultman (1954), nor Mason's team (1971), quantified the term short lived but it does not logically equate to well tolerated given any emboli has the potential to induce a major embolic event (Benzinger, 1950; Clemedson & Hultman, 1954; Cooper et al. 1983), or systemic inflammatory response (Branger & Eckmann, 1999; Elsayad & Gorbunov, 2008; Gorbunov et al. 2008; Kirkman & Watts, 2010).

Decompression diving sickness emboli survive longer than the short lived emboli produced in blast, and affect the peripheral circulation and tissues for weeks at times (Francis & Mitchell, 2004). These emboli also tend to become trapped in the venous circulation and do not readily move into arterial circulation due to the lungs' filtering capability, unless in rare cases the

lungs are overwhelmed (Neuman, 2004). Why might decompression sickness emboli lifespan differ from that of blast emboli? If that reasoning above about their gaseous composition is correct, blast emboli lifespan is shorter because they contain carbon dioxide rather than nitrogen of the decompression sickness emboli, and thus dissolve back into blood more readily as their environment changes.

In Benzinger's original paper of 1950, he claimed the blast wave time frame was insufficiently long enough to solve the gas and then liberate it, and Clemedson and Hultman supported this contention (Benzinger, 1950; Clemedson & Hultman, 1954). Blast emboli are not likely to contain nitrogen because nitrogen emboli (in decompression sickness) require time to reach the appropriate gas tension that is simply not available in the blast environment. The short life span of the blast emboli, supports carbon dioxide, if emboli occur in blast autologously.

3. This final issue for discussion is emboli mobility along with the locations in which they have been found. Understanding their mobility may also assist in determining any potential for local or systemic pathological consequence of blast emboli, such as injury to the vessel endothelium, or the release of biochemical agents that influence inflammatory pathways (Branger & Eckmann, 1999).

Blast emboli have been found in central arterial circulation and pulmonary circulation: both venous and arterial vessels (Benzinger, 1950; Clemedson & Hultman, 1954; Phillips & Richmond, 1991; and Tsokos et al. 2003). Emboli by definition are mobile and move with the circulation flow where they may occlude vital organs such as the heart, so the theory as to whether they all form in the pulmonary vessels from translocation is currently unsubstantiated

by their mobility factor. There is no literature or clinical evidence to say that victims of blast suffer similar symptoms to the decompression sickness patient. Thereby supporting the concept that emboli in the blast victim are not peripheral or move to the peripheral circulation.

To sum up, there is some evidence that blast emboli have a shorter lifespan than decompression sickness emboli; it is also known that they have only been located in pulmonary and central arterial circulation, whereas decompression sickness emboli are generally found in venous circulation and tissue spaces such as joints. The theory of autologous emboli development due to blast exposure has some strength, particularly in its physiological logic, however carefully planned research is needed to verify or refute it.

4.5 Summary

Each of the three theories alluding to the origin of microscopic gas emboli is physiologically plausible, but none is backed by sound research. Such a situation leaves the search for a solution realistic where a solid conclusion will provide a platform for future work in one of three directions.

Chapter 5

Methodology

5.1 Outline

The previous chapters described the science behind blast injury and the three existing theories of microscopic gas emboli in blast injury. As was shown, none of the three theories explaining how emboli form in the blast scenario are sufficiently explained or researched. The working hypothesis of this project is that the theory pertaining to autologous emboli formation resulting from rapid or explosive decompression exposure is correct. The methodology underpinning this project is explored, then the research question is determined, and a scientific hypothesis is proposed, where key elements driving the methods are conferred.

The process of selecting a research question and hypothesis is based on an assessment of the existing evidence, and designing the experimental program using methods that can answer the question and test the hypothesis. A major focus of the project was the mechanism by which microscopic gas emboli develop in the course of a blast wave exposure. To date, the discussions and evidence for our current understanding of the way blast microscopic gas emboli form is wanting. In particular, the generally accepted theory of translocation is not well supported by the extant research, therefore, it is important to evaluate alternative solutions.

5.2 Informing the research question and hypothesis

Evidence from science, research and the literature has informed the research question and hypothesis, the following points of reference were keystone to that process. The literature

review showed that microscopic gas emboli in blast trauma are under-researched. The translocation theory came to be accepted, but has not been tested. Leading researchers and clinicians alike believe they only stem from translocation associated with lung injury produced by the positive pressure impact from the blast wave (Argyros, 1997; DePalma et al. 2005; Horrocks & Brett, 2000; Mayo & Kluger, 2006; Riley, Clark & Wong, 2002; Rosenfeld & Ford, 2010; Weiller-Ravel, Adatto & Borman, 1975; Wightman & Gladish, 2001; Wolf et al. 2009).

The possibility that emboli are caused by cavitation and spalling action from the shock wave was also explored through the work on surgical laser shock wave treatment (Lindqvist et al. 2006; Sonden et al. 2002). Whether this information can be translated to blast emboli and possibly endothelial injury, leading to biochemical and inflammatory responses, is yet to be determined, because to date the research on blast wave endothelial injury is based on primary lung injury (Elsayad & Gorbunov, 2008; Gorbunov et al. 2005; Surbatovic et al. 2007).

Exploring emboli pathology began with iatrogenic emboli which cause an inflammatory response (Kapoor & Gutierrez, 2003; Muth & Shank, 2000), as can a blast wave injury also (Elsayad & Gorbunov, 2008; Gorbunov et al. 2005; Surbatovic et al. 2007), however there is no research studying blast emboli and any link that may exist between them and the inflammatory responses (known to occur at the vessel endothelium) identified in blast. Identifying the origins of blast emboli may better the current research on endothelial injury in blast to another level.

The autologous theory represents a scientifically plausible explanation of emboli development in blast exposure. The only time during a blast event where autologous emboli development

might occur is during the under-pressure (or sub-atmospheric phase). The under-pressure phase, as is understood in a blast event, is an explosive or rapid decompression event, exposing a victim to a negative ambient pressure within the time frame of between < 0.1 to 0.5 seconds respectively (Federal Aviation Administration, 2005). While little is known of the effects of the blast negative pressure wave in isolation, we know that emboli can form in some animal species, including mammals, from exposure to rapid decompression (Francis & Mitchell, 2004; Hill, Miller & Tucker, 1994; Kemph & Hitchcock, 1952).

Overall, the literature review showed that the extant literature focus in blast injury and blast emboli development was on blast over-pressure effects. The overarching aim of this project was to discover whether any autologous emboli development originating in the under-pressure phase could be identified after a blast event, using a deductive experimental approach.

5.3 Inductive versus deductive theory

The literature review showed that the logic applied to the theory of translocation to date is inductive, because it relies on suppositions, not evidence. The existing arguments are formulated by experience, something Karl Popper, described as reasonable, but merely a step towards a finite answer to a question; ultimately all theories require deductive testing (Popper, 1934). The translocation theory has never been tested, empirical evidence linking emboli to blast lung injury is lacking, nor has it been applied formally to any scientific principle to aid its credibility.

The inductive argument is that because emboli are found in arterial circulation following blast

exposure, air (theorists assumed the emboli was air) must have translocated across damaged lung infrastructure because this is known to happen in other lung injuries, even though those are commonly penetrating rather than blunt injuries (Ho & Ling, 1999). Theorists have failed to consider that the lungs' capacity to filter gas emboli from the venous system may explain why the emboli have only been found in arteries of the central circulation (Francis & Mitchell, 2004). This is further supported by past observations that the blast emboli were short lived (Clemedson & Hultman, 1954; Mason et al. 1971). This characteristic itself raises the prospect that blast induced emboli might not be air as commonly assumed, but be a gas type not considered by Benzinger (1950) and may be faster solving.

Ho (2002), continued this inductive argument for translocation by proposing a conceptual model of how translocation takes place. He did not follow up with deductive research; as such it remains theory, supported only by his inductive process. Following Popper's (1934) deductive philosophical approach, researchers must first acknowledge the theory of translocation has deficiencies, not accept it simply because as Ho (2002) stated, alternatives are too difficult to understand (Ho, 2002).

Importantly, though, Popper's (1934) approach to testing a theory is dependent on the strength of the scientific argument being used to test it. The lack of a solid evidence linking blast lung injury and blast emboli weakens the translocation theorists' argument. Other aforementioned issues such as embolus lifespan and the lungs filtering capability suggest an alternative explanation exists, but they are not sufficient scientific evidence to conclude that microscopic gas emboli do not form by translocation; only experimental evidence can resolve the controversy.

The test used for this project applied a law of physics to the problem: Henry's Law (or Henry's Constant) (Brady & Holum, 1993; West, 2005). Thus the test to uncover microscopic gas emboli origins was based on scientific evidence not conjecture or experience. Considerations such as current evidence, literature examination and exploring aligned areas of research such as blast physics and the blast wave, together with clinical phenomena that affect both blast victims and victims of general emboli, such as systemic inflammatory response, makes the process of this experimental program rooted in the scientific method.

5.4 Scientific method

5.4.1 The deductive research approach

The autologous theory does not require primary lung injury as a precursor, and because there is no empirical evidence supporting a link between primary blast lung injury and blast gas emboli, the translocation theory is a justifiable focus of examination. In addition, the autologous theory is based on the application of known laws of physics within the blast and blast simulated environment.

Deductive reasoning begins with an hypothesis. An hypothesis can be tested when the deductive argument follows from the premise of the research question. Only a certain level of knowledge, that is limited to evaluating a proposition of abstract observation, can be acquired from inductive reasoning alone (Alexander, 1995; Popper 1934; Thompson, 2001). The post-positivist philosophy is the pursuit of objectivity by acknowledging the possible effects of biases (Alexander, 1995), from where theories can be tested using an experimental process

and disproved using deduction creating room for an ever expanding knowledge base for the future (Horgan, 1992; Popper, 1934).

Because the theory of microscopic gas emboli origins in blast is under-explored, as is the under-pressure phase of blast in general, this project employed an exploratory, staged approach using two hypotheses tested in two separate experiments to test the autologous emboli development theory.

5.4.2 Research question

Is the decompression effect that occurs during the negative pressure phase of blast sufficiently powerful to liberate dissolved gas from blood to bubble?

The paradigm for this project is quantitative because the question demands an answer that can only be determined by experimentation. The research question can therefore be expressed as a testable scientific hypothesis.

5.4.3 Hypothesis

The rapid decompression, such as that in blast, liberates a dissolved gas from blood to a gas bubble.

The hypothesis proposes that bubbles form in blood when subjected to an explosive or rapid decompression event. It implies that there will be a measurable difference between the

content of the most easily soluble gas - carbon dioxide - in experimentally exposed active samples with the control samples. The testing of a hypothesis should proceed by a falsification of a null hypothesis. In this case the null hypothesis proposes that:

Bubbles of carbon dioxide do not form autologously in an isolated sample when exposed to a rapid decompression.

5.5 Research design

Previous studies assessing the injurious effects of the negative phase of a blast event have concentrated on assessing lung injury, using live animal models, not assessing emboli development specifically (Latner, 1942; Zhang et al. 1996). Other non-blast research, also using a live animal model, has shown blood will foam and carbon dioxide can be lost when exposed to a sudden decompression event (Kemph & Hitchcock, 1952). Whether the bubbling formed by autologous means is inconclusive because a live animal was used and any gas inspired by the animal was not identifiable in any way for example through 'radiological tagging'. Radiological tagging of inspired gas is a research method for future consideration because it would reveal gas moving through injured pulmonary interface, however at this point it is premature without preliminary laboratory research to justify the use of animal models. While lung injury was unlikely with the depths and proposed speeds generated in their experiment, it cannot be excluded from being the cause of the emboli through any possible lung injury (Kemph & Hitchcock, 1952).

Despite the differences in research design and objectives, the past under-pressure studies as described earlier have some benefits for this project by assisting with generator design. Testing for autologous bubbling in blood can be performed using the premise that a blast-induced over-pressure phase generates a super saturated environment of dissolved compounds in the blood and the subsequent under-pressure phase liberates some compounds as free gases (Brady & Holum, 1993). In this project the gas in question is carbon dioxide because it is naturally occurring in blood, and is the most soluble of all dissolved gases found in blood (West, 2005).

This research employed a two staged approach, using laboratory simulation followed by a live blast event. The rapid decompression phase of blast was examined in isolation in the first instance, and because the over-pressure phase precedes the under-pressure phase this could only be done using simulation. The second stage, using a field experiment, was scheduled to proceed if the simulation data supported further investigation.

A rapid decompression event, in which pressures and duration would simulate a rapid decompression event, identified in the negative pressure phase of blast, was made possible by a custom designed apparatus. The exposed blood was assessed for bubbling, and tested for loss of carbon dioxide immediately after exposure. Results obtained from the rapid decompression simulation were compared against those from blood samples exposed to live blast, in which over-pressure was also a factor.

Ultimately, the objective of these experiments was to generate new evidence about the role of the under-pressure phase of blast in the development of microscopic gas emboli in blast and therefore assess the validity of the autologous theory of microscopic gas emboli development.

5.6 Experimental program

Answering the research question required that the experiments should test blood, that had not been exposed to possible translocation or spalling at the pulmonary interface, for loss of carbon dioxide and visible bubbling following exposure to a rapid decompression effect. The two experimental stages (simulation and explosives experiment) involved the following seven key components that explain the importance of each to the overall experimental program. Each key is again highlighted as it pertained to each type of experiment, for example laboratory simulation or live blast event.

5.6.1 The blood samples

Collecting blood from volunteers was an essential starting point for a number of reasons. Firstly, the sample should be fresh and testing undertaken immediately so as blood gas analysis could be taken to determine any changes in the dissolved carbon dioxide. Secondly, as a fundamental experimental program the simulation event precluded the use of a live animal model. Finally, isolating the blood sample from a living model was additionally advantageous in excluding any possibility that translocation effect from damaged lungs was in part an influence on the results because lungs were not present in the isolated experiment.

A human volunteer sample group supplied venous and arterial blood. Venous sampling was used in the first instance because it is safer to sample and more practical.

5.6.2 Exposing the blood sample to an effective decompression event

In this project isolating the negative pressure wave by producing a rapid decompression event in a controlled environment was designed to test Henry's Law with blood and dissolved carbon dioxide, as such relates directly to the research question and scientific hypothesis. Exposing a blood sample to a rapid decompression event in less than 0.5 seconds was essential to simulate what occurs with a live blast event.

A custom-made rapid decompression generator was required for the laboratory phase of the program where the timing and depth of the decompression of each sample was recorded. The decompression generator used for the experiments is described in greater detail in the following chapter which describes the laboratory experiment specifically.

Experimental tools to test the physiological effects of rapid decompression have been used in research to date, but work related to blast specifically is rare (Kemph & Hitchcock, 1952; Latner, 1942; Zhang et al. 1996). Simulation of blast over-pressure waves is more common, in both blast and high impact blunt trauma experimentation. It has been possible to isolate the over-pressure wave since the development of the 'air driven shock tube' (Duff & Blackwell, 1966). Because the tube itself is a simple cylinder it cannot produce a negative pressure wave that can be modelled to a pure form of a blast wave, but it has been used successfully for high impact trauma and blast over-pressure examination for many years (Duff & Blackwell, 1966; Jaffin et al. 1987; Januszkiewicz et al. 1997; Long et al. 2009; Reneer et al. 2011). Figure 5.1 shows the shock tube's basic design.
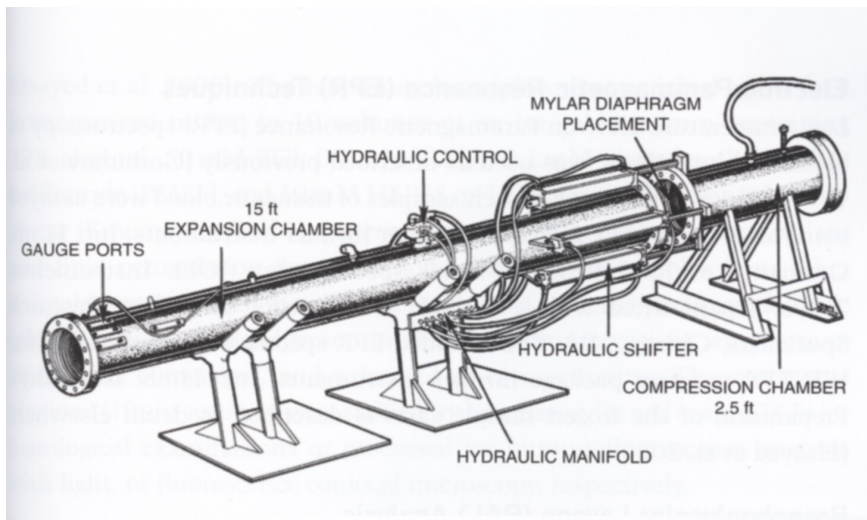
Figure 5.1 A typical gas-driven shock tube, used to produce a positive pressure load and to represent a positive blast wave in laboratory experimentation (Elsayad & Gorbunov, 2008, p. 269).

The 'gas driven shock tube' is a popular experimental tool because it can achieve similar results to a blast over-pressure waveform without the encumbrances of using an explosive event. A negative pressure waveform has not yet been registered with the gas driven shock tube, even though there is some evidence this perspective is developing (Reneer et al, 2011). A rapid decompression event has been known to cause emboli in a variety of live animals (Hill, Miller & Tucker 1994; Kemph & Hitchcock, 1952; Zhang et al. 1996), the laboratory experiment in this project was an opportunity to observe the consequences of a rapid decompression event on dissolved gas independent of over-pressure influence.

A simulation model to reproduce a pressure-time line that best represents the phase of blast under-pressure would ultimately be fixed to the timings and depth parameters expected from a typical blast wave under-pressure pressure time line. Few models for this work exist in the literature. In 1996, Zhang and his colleagues subjected live anaesthetised rats to rapid

decompression using a purpose-built under-pressure generator; they recorded peak levels of under-pressure between -45.5kPa and -86.5kPa with duration times between 0.0213 seconds and 0.0019 seconds. The time to peak under-pressure ranged between 0.0035 seconds and 0.0074 seconds, no mean value was provided. Their under-pressure generator was the only one identified in the literature that is specifically designed to simulate blast under-pressure and recorded depth and duration times. Importantly, their work sought information on gross organ damage, hence the design, structure and materials are somewhat different from those of the current study.

5.6.3 Evidence of bubbling

Macroscopic bubbling is evidence of the blood sample responding to the physical effects of rapid decompression; macroscopic bubbles logically imply the existence of microscopic bubbles. Evidence of bubbling was sought visually in each active sample, and the control sample for comparison, following the active samples exposure to the rapid decompression event and the explosives experiment.

5.6.4 Testing for carbon dioxide

Each exposed sample was tested for carbon dioxide loss, during rapid decompression. There are limited ways in which to analyse the partial pressure of gas in blood. Mass spectrometry was considered briefly because it would detect the presence of a compound in the blood sample. However, it was discounted because the technology cannot precisely distinguish the amount of compound in different physical states. Bristol University chemistry department

provides a simple illustration of the working mechanism of a mass spectrometer, this is shown

as Figure 5.2.
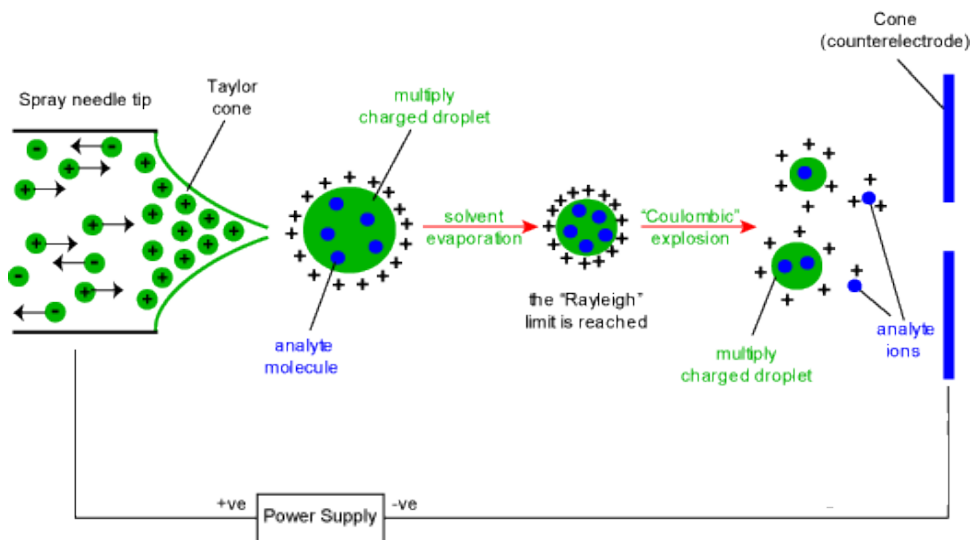
Figure 5.2 Diagram illustrating the ionisation process undertaken within a mass spectrometer,

http://www.bris.ac.uk/nerclsmsf/techniques/hplcms.html: viewed June 2014.

No matter what process is used, whether chemical ionisation, or laser desorption ionisation, or

electron ionisation, mass spectrometry as a process measures the ions of the whole sample.

The process cannot define a molecule or compound in different states, for example a fluid or

gas state adequately, thus the discreet delineation required for this test is lost (Herbert &

Johnstone, 2002; Kebarle & Tang, 1993).

Equally problematic was the time required to apply the test to a spectrometer as the sample

would metabolise over a short time frame. The time limits available for this experiment were

strictly limited because of the metabolising factor of blood.

Blood gas analysis, commonly used in clinical practice, involves the Stowe-Severinghaus

electrode gas analyser technique[9] (Severinghaus & Astrup, 1986; Severinghaus & Bradley, 1958). The blood gas analyser measures dissolved gas tension in samples of blood, commonly human blood, for clinical analysis and treatment. Unlike the mass spectrometer, the blood gas analyser only measures the gas dissolved in the blood, thus providing a difference between experimental active and control samples.

In this project, a clinical blood gas analyser was used to test the blood samples for changes in carbon dioxide levels between the active samples and the control samples, in each of the laboratory and field (blast) experiments. This project was the first known use of this technique to test specifically for carbon dioxide loss in blast based research.

5.6.5 Preserving the blood sample: applying a time frame

Sample preservation was imperative for data integrity. Preservation of each blood sample was achieved in two ways: by cooling it, and imposing a time frame on the experiment time. Relatively excessive metabolism of any sample might have obscured any changes the rapid decompression or blast produced between the samples groups over time.

Once extracted from the volunteers, the blood samples were either used immediately (in the arterial blood laboratory experiment and the field experiment) or preserved in ice slush (in the venous sampling laboratory experiment), to prevent excessive metabolism within the sample. Further details on the preservation methods are given in the following chapters pertaining directly to each experiment's experience.

---

[9] In 1954, Stowe described the CO_2 electrode and a rubber membrane permeable to CO_2 for measuring dissolved CO_2 in the blood. Severinghaus developed this device further to its current model in 1958. (Severinghaus & Astrup, 1986)

5.6.6 Exposing blood samples to a blast

Blood samples were also exposed to a live blast to assess whether the laboratory results from the simulated decompression event were transferable to a real blast event. As noted earlier, live blast generates an over-pressure phase prior to the under-pressure phase, so what effect that would have on the blood samples was a burning question.

The premise of the research question was that emboli might occur by autologous means in blast injured victims; it was therefore essential to test Henry's Law which suggests, subjecting blood to a rapid over-pressure should induce a supersaturation of carbon dioxide that would be available for release as gas during the ensuing under-pressure phase. In theory, dissolved carbon dioxide would resolve to gas from blood during the ensuing rapid decompression or sub-atmospheric phase of the blast wave. Using the blood samples for the blast rather than live animals minimised the margin of error that would exist if translocation was possible during the event.

5.6.7 Ethics considerations

Radiological tagging of an animal's inspired gas would be an ideal experimental design to test this research hypothesis, but without preliminary research such as this supporting the argument ethics approval for a study involving exposing live animals to explosions was deemed impossible to obtain. This program of experiments, involving human blood samples was acceptable to the relevant research ethics bodies. Recently an animal model has been used to study resuscitation following blast exposure (Garner et al. 2009); testing the results presented in this thesis in a similar animal model is an obvious future research opportunity.

5.7 Summary

This chapter has summarised what is currently known about emboli development in blast trauma and presented an outline of an experimental process designed to test one theory of their development using the scientific method.

Ultimately in answering the research question and testing the hypothesis this research program improves the body of knowledge for microscopic gas emboli in a blast scenario and as such offers opportunities for future work in the field.

Chapter 6

Rapid decompression events

Experimental design

and materials

The null hypothesis

Bubbles of carbon dioxide do not form autologously in an isolated sample when exposed to a rapid decompression.

6.1 Outline

The essential goal of this research is to outline and test an alternative theory of microscopic gas emboli development in blast (alternative meaning one that does not involve translocation as described by the translocation theory). The experimental program described herein was designed to determine if microscopic gas emboli form as a direct result of exposure to a rapid decompression event, such as occurs during a blast wave's sub-atmospheric phase. This chapter describes the first of three experiments focused on whether dissolved gas is liberated from solution during decompression as Henry's Law would suggest (West, 2005).

6.2 Objectives and research questions

The overarching objective of this experiment was to determine if carbon dioxide is liberated from a sample of blood when exposed to a rapid decompression event. To pursue this objective the following specific research questions were addressed:

- Is development of emboli evident by bubbling?

- Can the content of emboli be discovered using a blood gas analyser?

- Do changes in carbon dioxide occur due to exposure to a rapid decompression event?

6.3 Under-pressure generator experiments design

To test the hypothesis blood samples, collected from human volunteers, were subjected to a rapid decompression to simulate the sub-atmospheric phase of a blast event. This was achieved using a purpose designed pressure generator and exposing blood samples from human volunteers to a rapid decompression event.

Clinical blood gas analysis was used to test the dissolved gas content of the blood before and after decompression. Changes in carbon dioxide content in active samples when compared to control samples meant that dissolved carbon dioxide in the blood had been liberated from the solution (blood) during the decompression event. The rapid decompression exposure not only assisted in identifying the compound, but employed a unique and modern approach to the subject matter.

Using the sub-atmospheric generator isolated the blood sample from experimental variables such as over-pressure and translocation effects that would be unavoidable if using a blast event or an animal model. A blood gas analyser was used to measure the change in carbon dioxide in the blood samples. The venous tests were a 3 - stage program, including 2 pilot experiments that demonstrated how minor protocol refinements for them informed the final Venous Test group. A final experiment, using arterial blood (Arterial Test group) with the under-pressure generator was also undertaken, to determine any difference between the two blood types when exposed to a rapid decompression.

6.4 Materials : Equipment

The materials used in these experiments consisted of a clinical blood gas analyser, venepuncture and arterial blood sampling equipment and a custom made under-pressure generator. The custom made under-pressure generator design, function and validation with other research tools is described in detail. The remaining materials used are described in the context of their use for blood sampling and testing of the blood samples where they were pertinent to each of the validation tests and experiments.

6.4.1 Under-pressure generator - development and design

The under-pressure generator for this experiment was inspired by a commercial device used in gastric surgery, as shown in Figure 6.1. When the device was filled with water and suction was applied, bubbles appeared, suggesting the negative pressure liberated gas from the water. Although the device could be locked to prevent air entry once suction was applied it could not be used for the simulation experiment because the water could not be removed and neither the depth nor the duration time was recordable, however it did provide the baseline design concept.
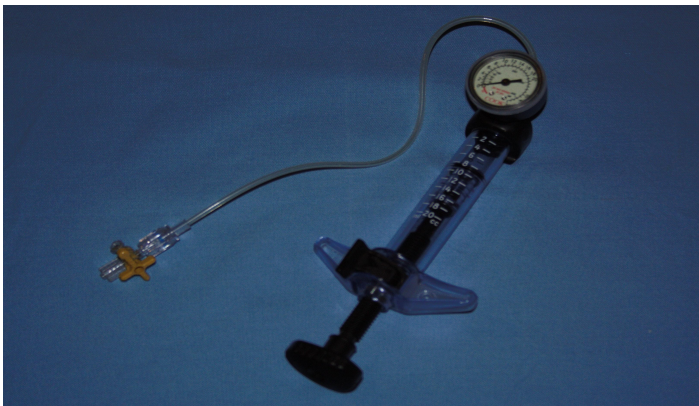
Figure 6.1 Prototype surgical equipment - The gastric balloon dilation syringe.

The generator built for this experiment was designed for testing a number of different types of effects relating to blast impact on components of blood. These experiments used only one of the three stations available because it alone produced the rapid decompression effect. Figure 6.2 shows the original schematic layout. The purple shaded blood sample chamber is seen in lower left position of the apparatus.

PROVISIONAL COPY
20JUL05
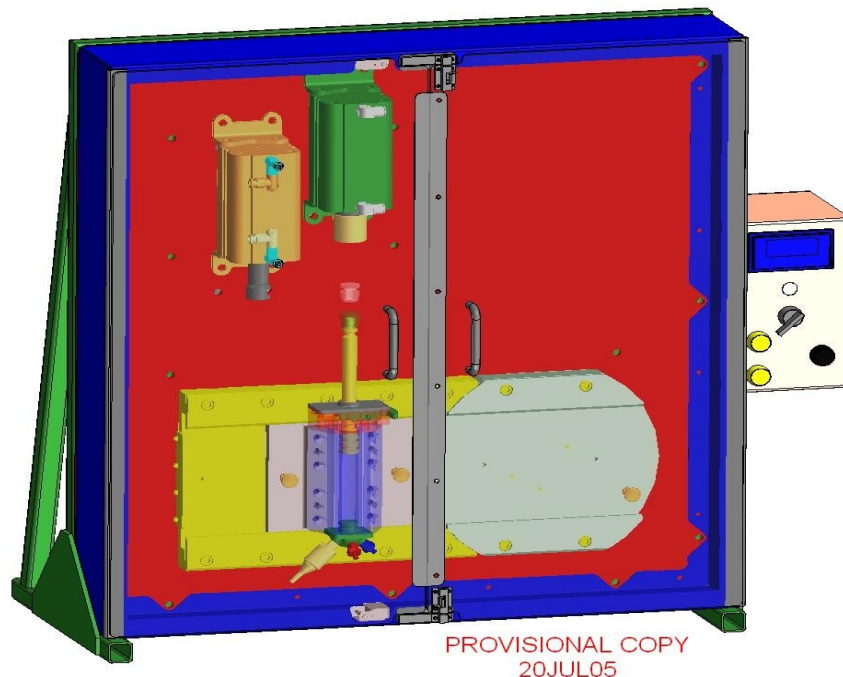
Figure 6.2 Schematic diagram of the under-pressure generator front view.

The generator was modified for these experiments to increase its under-pressure capability, including increasing the depth of each decompression, shortening the duration time and applying computerised capability for data acquisition. The parameters were adjusted to meet the parameters fitting a -100kPa depth and a rapid decompression event, where the duration time to peak depth for a rapid decompression event is a maximum of 0.5 seconds (Australian Transport Safety Bureau, 2009; Federal Aviation Administration, 2005). The transducer was also upgraded in line with the new capability, for measuring the new rapid negative pressure.

124

The transducer was a four-wire piezo-resistive 100psia Endevco 8530C 100psia, (where a = absolute, meaning the pressure is relative to a vacuum rather than ambient atmospheric pressure). This transducer used 10Vdc excitation and was specifically chosen for blast work because it measures static pressure or vacuum with a frequency response to hundreds of kHz. This differentiates research assessing blast dynamic pressure.

Two photographs of the apparatus are available as Figures 6.3 (a) and (b). The sample holding chamber could be locked or unlocked from the apparatus via a rod attached to a ring pull pin as observed in Figure 6.3 (a). This dual action enabled the blood sample to be inserted and removed without impacting on the sample. In the photograph, (Figure 6.3 (a)), the ring pull pin at the top of the photograph shows the chamber is secured to the apparatus, ready for decompression. In this way, the sample holding chamber could be locked to the apparatus and thus pull the sample chamber upward, generating decompression.

The transducer line is shown emerging from the apparatus at the lower left corner of the photograph in Figure 6.3 (a), and the sample chamber is attached to the leur lock port at the right of the transducer line. The decompression was produced by the upward action on the sample chamber, which was generated by compressed air, the facilitation of which is shown by the high pressure tubing in the following photography, Figure 6.3 (b).

125

Figure 6.3 (a) The sample holding chamber in locked action position.

Figure 6.3 (b) Front view showing the extensive high pressure lines (blue), the primary functional mechanism of the apparatus.

The blood sample chamber, shown in the form of the draft layout in Figure 6.4 , while fixed to the apparatus, it was unlocked for insertion and removal of the blood sample via a pin-lock attached at the top of the plunger. Blood was inserted through a leur lock port at the base, so as not to exert undue pressure or a vacuum effect to a test sample.
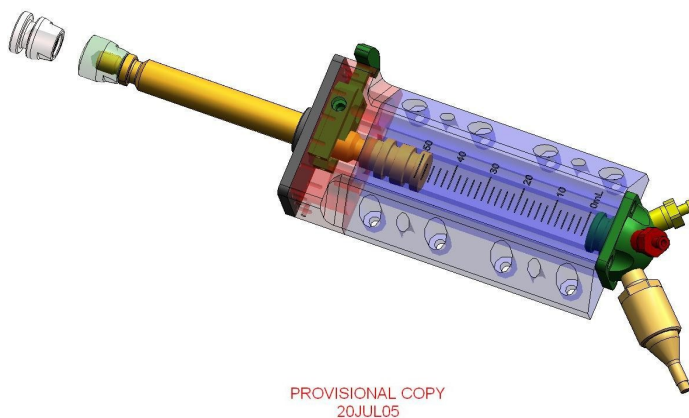
Figure 6.4 Schematic drawing of the blood sampling chamber prior its fix to the apparatus, showing the transducer connection at lower left at the base of the chamber, and syringe ports in red in the middle, and another in yellow above.

Blood samples were inserted gently via a blood gas syringe while supporting the chamber plunger and removed by pressing gently on the plunger so as a vacuum was not exerted on removal. With the blood sample in the blood sample chamber (shaded purple in figure 6.4) the locking cover was then brought down to meet the plunger to lock it in with the pin: this prevented any movement of the chamber once the blood sample was inside the blood sample chamber. The neutrality of this system during sample application and removal is proved through statistical validation for the tool and technique later in this chapter.

The blood sample chamber component was made of heavy duty Perspex rounded and smoothed on the inside, preventing spalling and cavitation to arise within during the experimental process. All tap connections were fixed with leur locking and further sealing was assured with water-proof silicone seal around all leur-locked connections on the outside. The seals were assessed for leaks regularly throughout the experimental process.

The key elements of the apparatus's mechanics include the syringe attachment via a leur lock system, the pressure driven mechanics and tubing enabling the rapid decompression event and the lock and unlock capability in the blood holding chamber system to prevent any undue load on the blood samples being applied.

The data acquisition system used alongside the generator included a personal computer with SCSI hard disk controller with analogue to digital converter card, 16 channels, 16-byte 100kHz sample rate, and a clock card with a Lynx OS Real Time Unix with an Xwindows operating system. The data record and analysis programs were written especially for the experiment by the technical team of the Defence Science Technology Organisation (DSTO), Edinburgh, South Australia. Data obtained from this technology included nominal readings for peak under-pressure, time to peak under-pressure and total under-pressure time. Through analysis, a pressure time line graph was produced for each event, an example is shown as Figure 6.5, illustrating the generators capability.
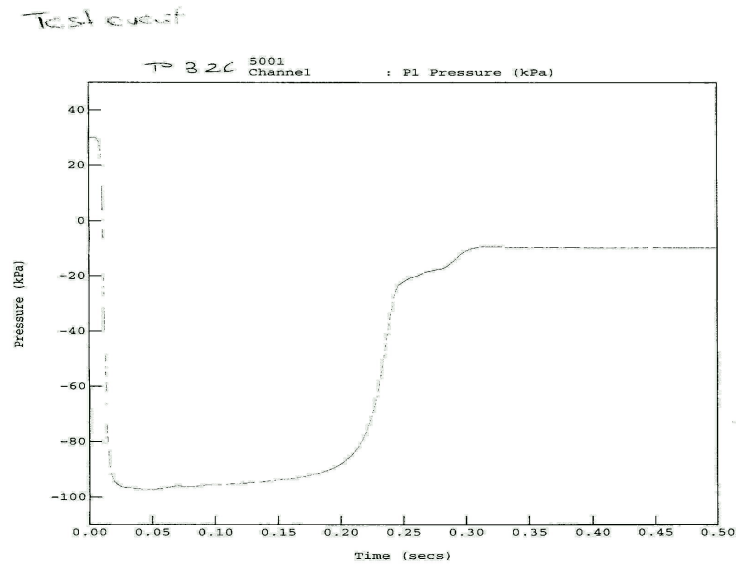
Figure 6.5 Example of a pressure time line produced by the under-pressure generator

6.5 Validating the under-pressure generator

6.5.1 Comparing the under-pressure generator with similar tools and live blast events identified in other research

As explained, the parameters for the under-pressure generator were matched to a rapid decompression event in both timing to peak depth and depth to a sub-atmospheric level. To compare the tool's capability further other researchers experiences were also referenced. Past

129

experiments where rapid decompression effects were key were examined and while these were described in the literature review data relating to their experimental tools are revisited.

In 1942, Latner identified lung injury in rats when subjected to a rapid onset of lowered ambient pressure, he expressed his events as likened to the under-pressure phase of blast. Latner's (1942), research did not include measurements of depth and timings of the exposure.

In 1996, Zhang and his colleagues, used a small animal model to demonstrate a link between under-pressure and lung blast injury by subjecting mammals to rapid decompression using a purpose built under-pressure generator.   The group recorded a peak level of under-pressure between -45.5kPa and -86.5kPa with an overall duration time between 0.0213 seconds and 0.0019 seconds. The time to peak under-pressure ranged between 0.0035 seconds and 0.0074 seconds, no mean was provided. Their under-pressure generator designed specifically for blast simulation, recorded both depth and duration time, and is the only one identified in the literature at this time.

The focus of effort for Zhang et al. (1996) was on assessing gross physical injury to the lung, where speed of decompression is pivotal (Iremonger, 1997; Stuhmiller et al. 1991).   In contrast to Zhang et al. (1996), the experiment for this project is designed to assess possible gaseous alterations in blood that may occur without lung injury, and if an explosive decompression event can occur at particular distance from detonation point so then can a rapid decompression event if the appropriate distance is laid between the victim and detonation point. Because both duration times are a realistic occurrence in blast exposure and the objective was to determine the risk of emboli development in the absence of lung injury, then a rapid decompression event was deemed most appropriate for this experiment.

Using a rapid decompression time frame was judicious also because blood integrity for analysis after exposure was more predictable.

A further decompression study, although not designed for assessing blast specific under-pressure, Kemph and Hitchcock's (1952) apparatus was designed to assess the effects of a rapid decompression on a small animal (mammal) model. The depth they produced extended from 99.9kPa to 3.999kPa from atmospheric pressure of 101 kPa, a total time of under-pressure was not recorded but a time to peak decompression was 0.02 seconds. Kemph and Hitchcock's (1952) work was of interest for this project in that they successfully identified significant alterations in blood including foamed samples and a decline in blood carbon dioxide levels through rapid decompression, thus indicating carbon dioxide loss in blood may take place well before the blast wave falls below the zero atmospheric point.

Further complementing this information from the literature, is the database of blast waveform measurements, taken from a series of blasts at DSTO, Adelaide, 2005. Introduced in Chapter Two, and shown as Appendix I, the information provided additional under-pressure measurements to guide the development of the under-pressure generator for the laboratory experimental phase.

As explored previously, blast wave signatures vary considerably from the unencumbered free field wave. Measuring peak under-pressure that is meaningful is not a standard process. The key element for over-pressure is the peak or static pressure and to some degree duration time, because it relates to injury, but the implications of under-pressure focuses on the speed that the waveform drops below atmosphere to reach a point where it then recovers, not necessarily how deep it falls.

An example of an individual blast wave signature from this Database is shown (compressed in time) as Figure 6.6, and is an example of a classic free field wave form, however not all the waveforms produced a significant under-pressure wave such as this one.
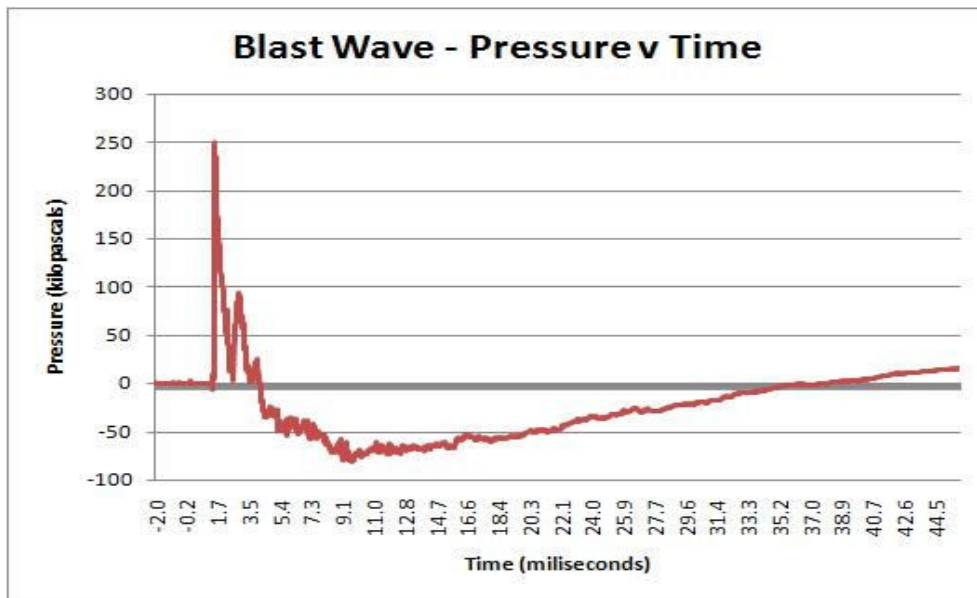
Figure 6.6 An example of a blast wave signature taken from the blast signatures database, raw data supplied by DSTO, Adelaide, 2005.

As specified in chapter 2, alongside (Figure 2.4, p. 31) the under-pressure wave may fluctuate, these fluctuations are significant for determining the under-pressure measurements.

No guideline was available in the literature for measuring key elements of the under-pressure wave. In formulating this database the under-pressure measurements were made from the time the signature dropped below the zero point (or *x* axis) to the depth of its relative peak under-pressure, this provided time to peak under-pressure, and then the total under-pressure time was measured. The difference in time between the time to peak under-pressure, and the total under-pressure time can be substantial for any one wave signature. It is defining two

separate areas over the curve, just as the over-pressure defines area(s) under the curve (Burton, 2005).

There are two points along the waveform signature which may be relevant to human injury, the first is the segment of the wave where there is the greatest pressure differential, *(*Krauthammer & Altenberg, 2000, this is between the peak over-pressure and the peak under-pressure of the wave on its first descent below the *x* axis (or atmospheric pressure). The second point is the fastest decompression descent along the waveform that may, in some cases, fluctuate between positive and negative pressure over time. Where multiple fluctuations occurred during the under-pressure phase, the most common and fastest descent was that which travelled from the peak over-pressure to the first descent below the *x* axis. However, on occasion, the faster descent below the *x* axis followed a brief return to ambient pressure after this first descent. To assist in illustrating the presence of wave fluctuations, the amount of fluctuations are provided alongside other relevant data in the database included as Appendix I. The shortest time-frame to that signature's peak under-pressure was selected as data for this database, because once the ambient pressure has dropped below atmospheric pressure it is that time frame which defines the point as to whether it is a rapid or explosives decompression event, or not. Total decompression time may not always meet the time-frame criteria for an explosives or a rapid decompression.

The duration times of the Blast Signatures database fell inside the parameters defining either explosive decompression or rapid decompression (Australian Transport Safety Bureau, 2009; Federal Aviation Administration, 2005). The time to the peak under-pressure varied from

between 0.0001 seconds to 0.2191 seconds. Those waves that did not meet the criteria for explosives decompression met the criteria for rapid decompression (< 0.5 sec).

As a tool, the simulation apparatus (under-pressure generator) delivered a time to peak decompression level (M 0.02 - 0.04 sec), consistent with a rapid decompression (Australian Transport Safety Bureau, 2009; Federal Aviation Administration, 2005). The total time of under-pressure exposure was within 0.5 seconds, a borderline duration time for explosives decompression, but well within the rapid decompression time frame. The rapid decompression generator was considerably more consistent than a live blast explosion subject to extreme measurements. This design optimised the experimental process and volunteers blood samples by avoiding sample destruction during the experimental process.

The parameters from the literature and those from the DSTO database are presented in Table 6.1, alongside the measurements obtained from the apparatus constructed for this project's laboratory experiments (cited as the 'DSTO under-pressure generator'). No mean values were provided by Zhang et al. (1996).

Table 6.1 Key under-pressure measurements from historical literature, DSTO blast signatures database and the experimental apparatus used for these experiments (DSTO under-pressure generator)

	Time to peak under-pressure (seconds) M = mean	Total under-pressure time (seconds) M= mean	Peak under-pressure value (kPa)
Kemph & Hitchcock, 1952	M = 0.02	Not recorded	3.999
Zhang et al. 1996	0.0035 - 0.0074	0.0213 - 0.0019	-45.5 to -86.5
DSTO (Adel.) Blast Signatures Database (2005)	0.0001– 0.2191 (M = 0.01)	0.0011 - 0.4929 (M = 0.15)	M = -163.23
DSTO- Adel. Under-pressure generator (venous blood sample groups)	M = 0.04	M = 0.26	M = -95.43
DSTO- Adel. Under-pressure generator (arterial blood sample group)	M = 0.02	M = 0.14	M= -94.24

While the parameters from the under-pressure generator were procured from the experiments yet to be described in this thesis, displaying them here show that the under-pressure generator

used in this research was a fitting tool when compared with other researchers data and a rapid decompression event.

6.5.2 Validating the experimental tool with the blood sample application technique –

venous samples

NOTE
Ethics approval for this experimental program was obtained prior to these validation testings because blood sampling from volunteers was required to validate the apparatus as a tool. The ethics approval process is detailed accordingly in the following chapter (Chapter 7, 'Methods').

This assessment experiment was designed to validate the apparatus and the technique applied to insert and remove the blood sample from the blood sample chamber.   Vacutainers, lined with lithium-heparin were used to extract 10mls of blood from participants to test the technique for inserting and removing the blood sample from the under-pressure generator.

The generator was designed so as a 3ml blood sample could be inserted via a syringe through a lockable access port on the side of the blood sample holding chamber, and then removed by pressing the top of the plunger downward in one gentle action so as not to impact negatively on the final result. Testing of this process was required before the experiment was attempted.

Fifteen samples of venous blood from human volunteers were used. Each sample was divided into an active and a control volume. Using two new sterile lithium-heparin lined syringes the

samples were drawn from deep within the vacutainer tube to avoid bubbling. Each sample was then labelled active or control. Randomisation using the website "randomizer.org" was applied to each control and active sample for their order of entry into the blood gas analyser.

To counteract the possibility of air reaching the sample holding chamber, and generating cavitation, a protocol outlining sample management was designed for all samples. All samples were air sealed and inspected for bubbling at the following points prior to insertion into the generator: upon division of the sample into the two (active and control) and immediately prior to the active sample's insertion. Each active and control sample was held with the syringe port uppermost and a small amount of blood was ejected to ensure no dead-space was in the syringe port before the active sample was inserted into the generator.

The generator plunger was supported firmly while the active sample was injected into the sample holding chamber slowly and with constant gentle pressure to mitigate against bubble formation. Once in the chamber, the insertion port was locked and a small amount of blood was released from the generator using the additional port at the base of the chamber with a separate empty syringe attached, the port is shown coloured red, in Figure 6.4, that port was then locked to ensure there was no dead-space in the communicating access line between the sample's syringe and the sample holding chamber. This process is referred to as the 'dead-space check'.

The control sample was set aside, and the active sample was carefully inserted into the under-pressure generator's blood holding chamber through the access port. No sample was subjected to rapid under-pressure at this time. Once the active sample was in the chamber, a time lapse of 30 seconds was made to account for the decompression action. The top of the

plunger was left unlocked from the apparatus itself, disabling the upward movement of the apparatus that initiated the decompression effect, while simultaneously enabling the user to gently push down so a vacuum effect was not applied to the contents during removal and the blood simply flowed from chamber to syringe. Once the active sample was removed from the chamber, both samples were taken to the blood gas analyser.

There was no visual evidence of bubbling in any sample retrieved from the blood chamber and no changes in the carbon dioxide content, or related chemistry between the active and control samples. The conclusion drawn from this was that the technique for the experiment and the under-pressure generator design would not adversely affect the results. The data for this direct comparative validation test is shown as Table 6.2.

Table 6.2 Venous blood samples - technique and generator validation test.

	CO_2 (mm Hg)		HCO_3 (mmol/l)		pH	
	Active	*Control*	*Active*	*Control*	*Active*	*Control*
MEAN	53.33	53.51	28.40	28.13	7.35	7.34
STDEV	4.56	4.58	1.69	1.75	0.02	0.02
SEM	1.44	1.45	0.53	0.55	0.01	0.01
95% CI	50.5-56.2	50.7-56.4	27.4-29.4	27.0-29.2	7.33-7.36	7.33-7.35
p value	0.93		0.73		0.60	

6.5.3 Validating the experimental tool with the blood sample application technique – arterial samples

Fifteen arterial samples were used in this validation test. Arterial blood was taken from a subset of the original volunteer group. The arterial blood sampling was achieved using standard clinical arterial line configurations and equipment including arterial catheters, 0.09% sterile sodium chloride flush bags and arterial line sets with pressure bag applied producing a 3ml/hour drip rate in accordance with Australian clinical standards. Samples were removed from the in-line tap at 15cm from the artery insertion site. Samples were removed using a lithium-heparin lined vacutainer, as it was in the venous sampling tests, following a 5ml blood discard from each aspiration to avoid saline contamination of the sample.

Each large sample of 10ml was divided into two small samples of 3ml each using new sterile syringes lined with lithium heparin, and drawn from deep within the vacutainer blood. Samples were labelled, and the randomisation schedule was applied, as it was for all venous experiments. The active sample was inserted into the blood sample chamber of the under-pressure generator using the same insertion technique as for the venous validation tests, the control sample was set aside. Thirty seconds was taken to replicate the decompression action as it was in the venous validation test timing. The active sample was removed, according to the protocol. No rapid decompression was activated.

Table 6.3 shows no significant differences between the control and active samples when not subjected to rapid decompression. There was no bubbling or change in carbon dioxide content or related chemistry between the active and control samples. Again, the conclusion drawn was that the technique would not adversely affect results.

Table 6.3 Arterial blood samples - technique and generator validation test.

	CO_2 (mm Hg)		HCO_3 (mmol/l)		pH	
	Active	*Control*	*Active*	*Control*	*Active*	*Control*
MEAN	33.01	33.34	21.33	21.37	7.42	7.41
STDEV	0.80	1.03	0.72	0.86	0.01	0.01
SEM	0.21	0.26	0.19	0.22	0.003	0.003
95% CI	32.6-33.4	32.8-33.9	21.0-21.7	20.9-21.8	7.41-7.42	7.41-7.42
p value	0.34		0.89		0.63	

6.6 Summary

This chapter has described and rationalised the experimental design and equipment required for the upcoming experiments. The tests in this chapter have provided quantitative validation for the custom made equipment and the technique required to use it for the laboratory experiments undertaken in the next stage of the experimental process.

Chapter 7

Rapid decompression events

Methods

7.1 Chapter outline

This chapter describes how the experimental process took place. From ethics approval, to the management and application of blood samples to the experimental apparatus, the purpose of the blood gas analyser and the development and refinement and customisation of the experimental protocols.

7.2 Ethics

Ethics approval is a necessary process in any research study particularly when using human or animal tissue. The requirement for ethics approval encourages an ethical approach to research because the applicant must demonstrate justification for the research. Overall, this prevents research that is either unethical, unjustified or premature.

Because research assessing the possible development of emboli resulting from decompression effect in blast is not common, a simulation program of research was an appropriate start Using simulation with blood samples provided the human tissue required without harm to volunteers.

Ethics approval was obligatory because experiments required live human tissue (blood) provided by volunteers. The original application as described below was made in 2005. Minor changes were made as the program progressed and experiments were conducted according to each of the experiment's specific requirements. The volunteer requirements for

each experiment were similar, it was the type of blood (venous or arterial), that varied after the first experiment phase.

Ethics approval was sought with The University of Adelaide Human Research Ethics Committee, Research Ethics and Compliance Unit with approval number: H-116-2005. Blood was collected from volunteers between February 2006 and August 2006 (venous sampling) and further in February 2010 (arterial sampling). State occupational health and safety guidelines were applied for all blood sampling, disposal of used samples and equipment (Work, Health and Safety Act, SA, 1986, reviewed 2001 & 2009) with the following 2 points of exclusion criteria:

1.   If the volunteer felt unfit to provide blood samples on the day requested, he/she could decline to participate that day but re enter when recovered.

2.   If the volunteer was recently prescribed drugs that impact on safe and easy blood sampling, such as anticoagulants or drugs affecting blood coagulation such as Heparin, Warfarin, Aspirin or any non steroidal anti-inflammatory agents, or natural agents affecting blood clotting such as Ginkgo Biloba, or the prescription of thrombolytic drugs in the past 3 months such as Streptokinase or tissue plasminogen activators.

Volunteers were sought from healthy nursing and medical staff of the Intensive Care Unit (ICU), Retrieval Service of the Royal Adelaide Hospital and The Discipline of Anatomical Sciences of The University of Adelaide. An information poster was used to recruit volunteers in the ICU and Retrieval Service between January and July 2005.

Venous blood samples of 10ml were collected at times and locations (within the hospital and university campus) that suited the participants in their work day. Blood was not collected on consecutive days as a courtesy 'rest' from the venepuncture procedure. Twenty seven (27) people volunteered for the laboratory experiments, including both the principal investigator of this study. Three of the total 27 volunteers were excluded after recruitment as two separate attempts at sampling from them were unsuccessful, leaving a final 24 volunteers. All blood samples showed normal clinically accepted data for all the blood test results. All blood samples were collected using commercially produced vacutainer sampling tubes.

Arterial cannulations were performed by an Australian qualified and registered consultant anaesthetist (a Fellow of the Australian and New Zealand College of Anaesthetists) and care for the arterial line set-ups was attended by an Australian registered critical care nurse (author). All arterial blood results were within the healthy normal range. Commercially produced vacutainer collecting tubes were used for all blood sampling.

7.3 Methods – venous groups

7.3.1 Blood sample management – venous samples

Venous samples were obtained through venepuncture technique from healthy volunteers as described previously, in accordance with the Ethics approval.

Each experimental cycle included one decompression event, and one set of blood gas analysis testing including baseline, active and control. Each experimental cycle required one 10ml

sample of blood, using a lithium-heparin lined vacutainer, from one volunteer. Each experimental cycle was repeated numerous times within three separate venous testing groups (Pilot One - 14 experimental cycles, Pilot Two - 14 experimental cycles, and Venous Test Group - 40 experimental cycles). Each experimental cycle collected three different types of data: the decompression event data, visible bubbling observation and the blood gas analysis data.

Baseline blood gas analysis was taken from the main 10ml sample and was performed to confirm the samples integrity and preservation over the time of the experiments cycle, when compared with the control sample at the end of the experimental cycle. Blood sample preservation was maintained through cooling and imposing a time restriction for each experimental cycle. Cooling was achieved by keeping the samples in an ice/slush within a closed insulated container.

Following the baseline test, the vacutainer blood sample was divided into active and control samples (3ml each). Using two new sterile lithium-heparin lined syringes blood was drawn from deep within the vacutainer with the plunger held firm into the base of the syringe, eliminating the possibility of air inside the syringe. Following this inspection the syringe was sealed and the protocol described to ensure a complete air seal was implemented. Each sample was then labelled active or control. Randomisation using the website "randomizer.org" was applied to each control and active sample for their order of entry into the blood gas analyser, as it was for the validation testing. The samples were then placed in the ice/slush bath container. Cooling the blood samples extended the blood sample's preservation from 10 minutes to 20 minutes.

To counteract possible cavitation from bubbles in the sample during rapid decompression, all samples were rigorously monitored for bubble development prior to rapid decompression, and the 'air seal protocol' was implemented, as described in chapter 6 for the validation tests. Each sample was air sealed and inspected for bubbling at the following intervals: upon sample division, upon entrance into warming bath (if warmed), upon retrieval from the bath (if warmed) and again immediately prior insertion into the generator. Here, the syringe cap was removed and a small volume of blood was ejected from the syringe, with the syringe port uppermost, to ensure the entire syringe and syringe port contained blood, not air. The active sample was then injected into the sample holding chamber steadily and slowly, using the dead-space check.

Pilot Two and Venous Test group samples were rewarmed prior to decompression. All samples were at room temperature during the decompression time. Both active and control samples were then taken immediately for blood gas analysis and applied according to the randomisation schedule.

A time log was maintained for each experimental cycle. The average times taken for each experimental cycle are shown in their respective testing groups: Pilot One, Pilot Two and Venous Test Group. Timing began at the collection of the 10ml blood sample from the volunteer, and timing ended at the end of the final blood gas analysis following the decompression event.

Active samples were assessed visually for bubbling and foaming; the latter was defined as continuous bubbling in one third or more of the sample volume. All active samples were compared to its partnered control sample when assessing bubbling and foaming.

The temperatures of samples and the timings of each experimental cycle were integral to the protocol refinements as such also the methods used in these experiments and thereby the results of those are described in the following sections as they relate to each experiment.

7.3.2 Pilot One : (14 experimental cycles)

As this experiment was the first examination and the initial pilot experiment, Pilot One involved 14 samples of venous blood to determine whether a trend would occur. Sixteen samples were collected from the volunteers, 2 were lost to error and spillage, leaving 14 remaining for testing. The samples in this group were all cooled and were kept cool in the ice/slush within a closed insulated container, no rewarming of the samples occurred in this group.

The temperature of each active and control sample was assessed immediately prior to the active sample's application to rapid decompression. The control sample was left aside at room temperature: making the cooling time the same for both. The cooled temperature range for the active samples was $13.5^0\,C - 17.9^0\,C$ (M $16.1^0\,C$) and $13.7^0\,C - 17.8^0\,C$ (M $16.0^0\,C$) for the control samples.

The total time for each experimental cycle in this group was between 14 and 22 minutes (M 17 min).

The active samples were applied for rapid decompression, according to the protocol. Active samples were then assessed visually and a direct comparison was made with the paired

control sample for evidence of bubbling.

The low range temperatures of this group were not comparable to normal body temperature, this cannot stand as a definitive experiment, however, the bubbling and loss of carbon dioxide in the active samples encouraged a refinement of the protocol for the next experiment. Results from 'Pilot One' experiment are detailed in the following chapter (Results and Discussion).

7.3.3 Pilot Two : (14 experimental cycles)

Fourteen samples were used for Pilot two. A power analysis was performed using Pilot one data, indicating that N = 12 samples were required for the study to be adequately powered to 80%. Seventeen samples were collected, 3 were lost to temperature error or spillage leaving 14 samples for testing and analysis.

The same protocol as Pilot one was used, except that both the active and control samples were warmed using a temperature-controlled water bath. For each experimental cycle the sample was initially cooled in the ice-slush container upon extraction from the participant as it was in Pilot one. The cooled temperatures for the active samples were $14.8^0\,C - 18.0^0\,C$ (M $16.5^0\,C$), and $12.2^0\,C - 17.1^0\,C$ (M $15.2^0\,C$) for the control samples.

The water bath was warmed to a constant 37.4^0C so as the samples could warm to body temperature to replicate core body temperature. The active and control sample syringes, secured with a locking cap were immersed into the water bath for one minute. At 60 seconds

each sample was checked for temperature and returned to the water bath again if the temperature had not reached the required temperature range 36.5^0 C – 37.2^0 C (core body temperature), it was returned to the bath for further warming and checked again at intervals depending on the individual sample's temperature advancement.   The post-warming temperature range of the active samples was 36.5^0 C – 38.0^0 C (M 37^0 C), and 36.4^0 C – 38.6^0 C (M 37.4^0 C) for the control samples.

When samples had not reached the targeted temperature range and were returned to the water bath for further warming, this meant the temperatures of some samples were checked three or four times, when only a pre-warming and post-warming check were planned, increasing the overall test time, risk of spillage and interference with the sample. These factors could have affected the samples and therefore the experiment's results, so the protocol was refined for the next final venous experiment.

The overall time for each experimental cycle was 17 – 21 minutes (M 19 minutes).

All active samples were applied for rapid decompression according to the protocols, followed by visual observation of any bubbling, a direct comparison was made with the paired control sample for evidence of bubbling.

This Pilot two protocol was useful in providing an optimum time for the warming process. The desired sample temperature range (36.5^0 C – 37.2^0 C) was reached within an average time of 1.26 minutes. As such 1.26 minutes was used for the next and final 'Venous Test group' rewarming time.   After the decompression event, bubbling and foaming were visually assessed according to the protocol outline, and carbon dioxide was once again given off on

blood gas analysis. The results from Pilot 'Two' are detailed in the following chapter (Results and Discussion).

7.3.4 Venous Test group : (40 experimental cycles)

A power analysis was undertaken using the changes in each of the pilot studies as a guide. This analysis determined that a sample size of N = 10-12 would provide adequate power with an alpha value of 80%. Histograms were also constructed for this final group to assist in determining the number of experiments required for a statistical significance. The histograms are shown as Figure 7.1.

Figure 7.1 Histograms Venous Test group.
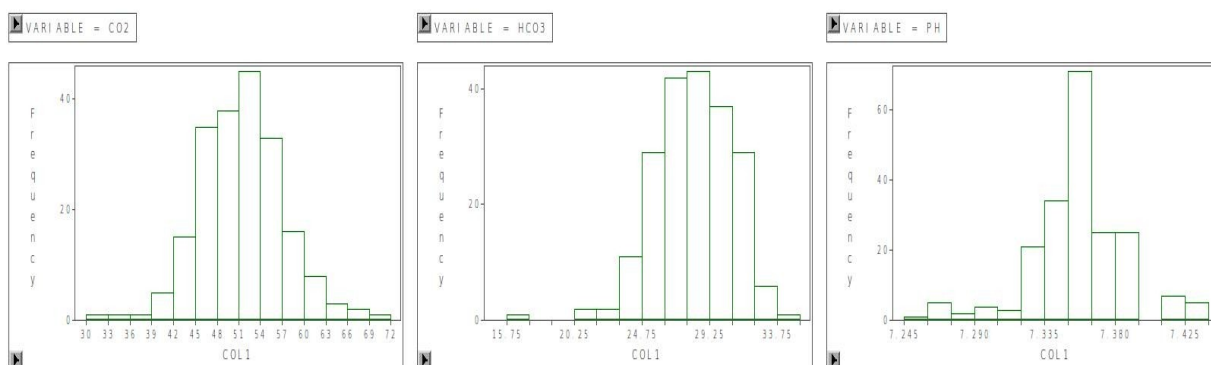
Although 42 samples were logged, only 40 samples were used in the final Venous Test group because 2 samples were lost to protocol error. Sample #56 was rejected from the blood gas analyser for the pH reading (the blood gas analyser instrumentation indicated 'excessive noise' from the foaming sample), average pH values were calculated for 39, all other data was calculated for 40 samples.

The samples were cooled in an ice slush as they were for Pilot One and Two. The warming protocol continued, but the temperatures of the samples were not checked before the warming process began and a standard 1.26 minutes was used as the warming time. The warmed temperatures for the active sample in this Venous Test group were between 35.1^0 C and 38.3^0C (M 36.7^0 C), and 35^0 C – 37.9^0 C (M 36.5^0 C) for the control sample. Samples between 35^0 C and 38.5^0C post-warming temperature were accepted, because this range reflected the range of survivable human temperature. Both samples were removed from the water bath whereupon the samples were subjected to the air seal protocol and the active sample the dead-space protocol before application to the decompression test, while the control sample was set aside at room temperature.

The total time taken for each experimental cycle was between 17 and 21 minutes (M 18 minutes).

A visual inspection of the active samples for bubbling was made following the rapid decompression exposure, a direct comparison of bubbling was simultaneously made with the paired control sample.

Chemical changes naturally occur in blood when one component of the acid-base maintenance system is altered. The significant differences in carbon dioxide losses in active samples in this Venous Test group, described in the following chapter, called for an examination of the aligned chemistry related to acid-base balance. Serum bicarbonate and pH were assessed, as well as potassium.

In human physiology serum potassium plays a minor role in acid base balance as it exchanges

for hydrogen ions at the cellular level (Mayne, 1994), but it also provided evidence against a haemolysed blood sample (Shara & Ayling, 2009). Potassium is the major intracellular cation, it is in greater concentration inside the cell, and low in concentration in the bloodstream (Mayne, 1994; Shara & Ayling, 2009). Serum potassium rises in blood samples that have undergone haemolysis; a haemolysed sample would mean the intracellular contents of the erythrocyte, including potassium and carbon dioxide, had leaked into the extracellular blood sample.

In these experiments this would render the gas tension results questionable because other compounds, such as carbon dioxide, also leak from damaged cell walls when blood is haemolysed resulting in an increase in carbon dioxide tension in the blood (Shara & Ayling, 2009). Knowing the serum potassium levels provided additional information on acid base balance and offered evidence as to whether the cellular membrane remained intact, known as a non-haemolysed sample.

The results and discussion from the Venous Test group are explored in depth in the following chapter describing the laboratory experiments.

7.3.5 Venous sample summary

Data obtained from the venous sampling groups were assessed as three individual experimental groups - two pilot groups, and one main experimental group. Sixty-eight complete venous experimental cycles were attended following the generator validation testing (Chapter 6), seven in total were discarded due to protocol error or accidental spillage, leaving 68 samples tested for blood gas analysis following the rapid decompression test, over the

three venous groups. Although the basic experimental protocol remained the same across the three groups, minor refinements in both Pilot groups informed the final Venous Test group protocol.

Data included visualised bubbling and foaming, blood gas tension measurements, and acid-base chemistry data. Data for each of these three experiments stand alone because their collection processes varied, however, the trend in carbon dioxide loss and visible bubbling is consistent with the overall research hypothesis. Technical data describing the parameters for the under-pressure generator were grouped together as venous blood sample groups because changes made to each experimental groups protocol would not have made any difference to the under-pressure generator data output. These data are listed in Table 6.1, in chapter 6.

7.4 Methods - arterial group

7.4.1 Blood sample management – arterial samples

Blood was retrieved from participants via an arterial line set up as described for the validation tests (chapter 6). As it was in the venous experiments, each experimental cycle included one decompression event, and each decompression event required one 10ml sample of blood from the volunteer, which was repeated 25 times. Each experimental cycle collected three different types of data: the decompression event data, evidence of visible bubbling and the blood gas analysis data. Each experimental cycle used 10ml of volunteer blood collected in 10ml laboratory vacutainer tubes via arterial line access. Each tube contained lithium-heparin to prevent clotting.

A shorter duration time for each experimental cycle was predicted with this experiment because the gas analyser and the under-pressure generator were located in the same room as the cannulated participants, the samples were tested for both blood gas analysis and rapid decompression within minutes of collection so chilling for preservation and subsequent rewarming was not necessary.

Following the baseline test, the vacutainer blood sample was divided into active and control samples (3ml each). Using two new sterile lithium-heparin lined syringes blood was drawn from deep within the vacutainer with the plunger held firm into the base of the syringe, eliminating the possibility of air inside the syringe. The exact same protocols for mitigating possible bubble formation in the samples syringes prior to testing and upon insertion into the generator were applied to the arterial test samples. Each sample was then labelled active or control. Randomisation using the website "randomizer.org" was applied to each control and active sample for their order of entry into the blood gas analyser, as it was for the validation testing and venous blood experiments.

7.4.2 Arterial Test group : (25 experimental cycles)

A power analysis was undertaken using the changes in the Venous Test group as a guide. This analysis determined that again a sample size of N = 10-12 would provide adequate power with an alpha value of 80%. Arterial blood was collected in 10ml commercially available laboratory vacutainer sample tubes. Each tube contained lithium-heparin to prevent clotting. Once the preliminary sample protocol was attended as outlined, the active sample was applied to the rapid decompression event via the under-pressure generator while the control sample was set aside. All samples were kept at room temperature.

As in the venous tests, bubbling or foaming of the active sample following decompression were assessed through visual inspection. A direct comparison was made at this time with the paired control sample.

Each sample experiment cycle was timed. Timing began at the collection of the 10ml blood sample, and timing ended at the end of the final blood gas analysis following the decompression event. A time log was maintained for each experimental cycle and was between 4.5 minutes and 6.2 minutes (M 5.4 minutes).

7.4.3 Arterial sampling summary

Data obtained from the arterial experimental cycles included 25 rapid decompression events, bubbling and foaming observations, blood gas tension measurements, and acid-base chemistry. Data for these experiments stand alone because their collection processes differed from the venous collection data, however, the trend in carbon dioxide loss, acid base alterations and visible bubbling remained consistent with the Venous Test group.

The arterial samples were tested with minimum interference and there was no change made to their temperature, this factor made each experimental cycle more time efficient than the venous experimental cycles.

7.5 Statistical analysis – Rapid decompression experiments

Statistical   analysis was carried out using Microsoft Excel Extended Statistical analysis Library Statistics (Microsoft Corporation, Redmond, Washington, USA).   The statistical testing for these experiments was a comparative base. All p-values were derived from the two sample t-test, assuming unequal variances.

Chapter 8

Rapid decompression events

Results and discussion

8.1 Outline

This chapter presents the results of the experiments described in Chapter 7 and discussion of them. Two pilot experiments were attended (Pilot One and Pilot Two) to determine if exposing a small blood volume to a rapid decompression event would liberate carbon dioxide from the blood. Then, the main venous blood experiment was conducted using the refinements made to the protocols such as replication of body temperature in the blood samples and optimal timings for a blood gas analysis. The final experiment involved arterial blood, using the same testing process as for venous blood, by way of rapid decompression generator and blood gas analysis. The results for all experiments are provided, then the Venous Test group and the Arterial Test group are compared and discussed.

8.2 Venous groups

Changes were observed in each of the three venous sample, groups after exposure to a rapid decompression: the samples bubbled, and their carbon dioxide levels and aligned acid-base chemistry were altered. The loss of carbon dioxide was a consistent trend in the two pilot groups and the loss was statistically significant in the final Venous Test group and the Arterial Test group.

8.2.1 Pilot One

The Pilot One group contained 14 samples, all cooled and tested cold. The tests involving this group were designed to assess whether it was reasonable test the hypothesis with the

experimental tools selected and produce information that could be used to adjust the protocol if required. The average experimental cycle duration time for this group was 17.1 minutes. The average temperature for the active samples was 16^0C and 16^0C for the control sample, a temperature log for each active and control sample for Pilot one is shown as Appendix II.

The decompression level for this group was between -97.13 kPa and -99.10 kPa (M -98.11 kPa), the time to peak under pressure was between 0.031 seconds and 0.041 seconds (M 0.038 seconds), the total decompression time was between 0.312 seconds and 0.378 (M 0.332 seconds). Graphs of the signatures produced for Pilot one are shown as Appendix III.

Visible bubbling was observed in 56% of the active samples in this group (9/14). Bubbling was not observed in any control samples.

There was no difference in carbon dioxide, bicarbonate or pH between baseline and control samples indicating a preserved sample. This is shown as Table 8.1, with t-test p values provided.

Table 8.1 Preserved samples, Pilot One.

	CO_2 (mm Hg)		HCO_3^- (mmol/l)		pH	
	Baseline	*Control*	*Baseline*	*Control*	*Baseline*	*Control*
MEAN	54.45	53.99	30.36	29.76	7.35	7.35
STDEV	4.22	4.32	1.83	1.88	0.04	0.04
SEM	1.13	1.15	0.49	0.50	0.01	0.01
95% CI	53.2 - 57.7	51.7 - 56.2	29.0 - 31.3	28.8 - 30.8	7.33 - 7.37	7.34 - 7.37
p value	0.37		0.40		0.92	

In this Pilot One group there was a loss of carbon dioxide content in the active samples when compared with the controls. Thirteen of the 14 active samples contained less carbon dioxide than their corresponding control sample. The baseline samples had a mean carbon dioxide content of 54.45 mmHg (95% CI 53.2 - 57.7), control samples had a mean carbon dioxide tension of 53.99 mmHg (95% CI 51.7 - 56.2) and the active samples had a mean carbon dioxide tension of 52.06 mmHg (95% CI 49.7 - 54.5). Figure 8.1 shows the carbon dioxide loss in active samples when compared with baseline and control samples groups.

The t-test comparing the carbon dioxide in the active with the control samples showed this was not a statistically significant difference between the two samples (p = 0.262).
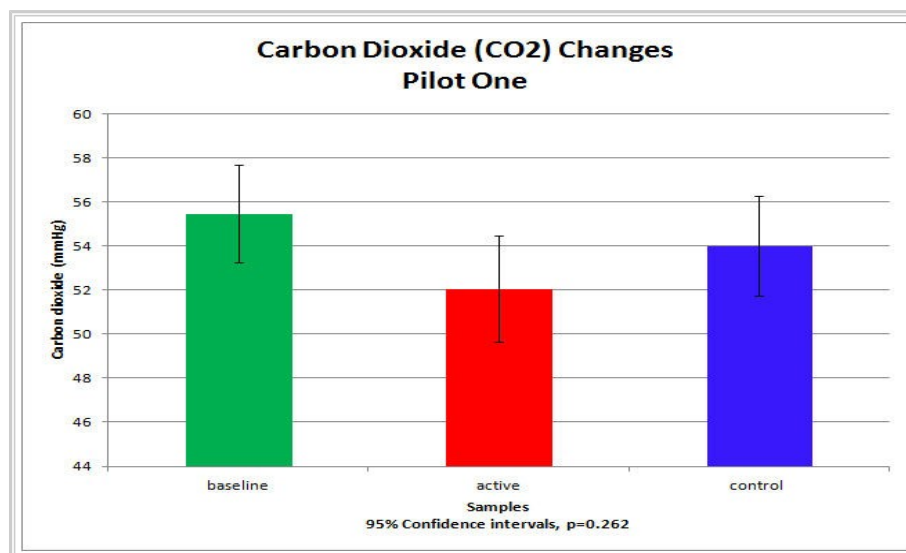

Figure 8.1 Carbon dioxide content in baseline, active and control samples, Pilot One group. The p value relates to the active versus the control samples.

Discussion – Bubbling occurred in the majority of active samples (56%), no bubbling was identified in control samples. Upon direct active sample comparison it was observed that

carbon dioxide loss occurred without evidence of bubbling, but bubbling correlated to carbon dioxide loss. The carbon dioxide losses in 13 of the 14 active samples when compared with the control sample, suggest the rapid decompression event caused dissolved gas in the blood to leave solution.

The constant in Henry's Law equation is temperature, when the temperature of the solution is increased, dissolved substances are liberated from the solution, the reverse happens when the solution is cool. Cooling these samples for the experiment may have contributed to the lack of statistical significance.

Because of the low temperatures of the samples this group has limitations in applying any outcome to humans as a stand alone experiment. Notwithstanding, there was evidence of a trend showing a loss of carbon dioxide in active samples, this trend was not observed in the preserved samples. The consistent levels of carbon dioxide in the baseline and preserved control samples showed the preservation protocol was viable. The results overall justified further examination using larger sample sizes and a protocol more representative of real-world situations.

8.2.2 Pilot Two

This group of 14 samples was re-warmed using a water bath with the temperature controlled at 37.4^0C. Active and control blood samples were in sealed syringes and placed into the water bath immediately following the baseline blood gas analysis and division of the sample into two as it was in Pilot One. The average experimental cycle duration time for this group was 19.6 minutes.

Each sample was also checked for a pre-warming temperature prior to immersion. The pre-warming temperatures of each active and control sample are presented alongside the post-warming temperatures in Appendix IV. Average temperatures following the re-warming process for this group was 37.0^0C for the active samples and 37.4^0C for the control samples. The aim was to re-warm the cooled samples to body core temperature within the 20 minutes time frame limitation for the entire experimental cycle.

The re-warming process posed a problem in that some samples had not reached a human body temperature after time in the water bath. This protocol (described in chapter 7) led to added time to the overall test, problems with spillage, as well it subjected the samples to constant interference.

Three samples were discarded from Pilot Two group for overheating or excessive exposure to interference. Excessive interference was pre-determined where more than three temperature checking attempts were made as these factors may have affected the integrity of the samples and ultimately the results. From this experience, a refined protocol was devised for the next and final venous blood experiment (Venous Test group).

Despite the problems, the re-warming protocol enabled a target sample temperature alongside a time frame to reach it. Timing the re-warming process was essential because it identified an ideal warming time. The ideal temperature (37.2^0C) was reached within an average time of 1.26 minutes. The warming times are available as Appendix V. This warming process added an average of 2.5 minutes to each experimental cycle when compared with Pilot one.

The decompression levels reached for this group were between -96.04 kPa and -98.57 kPa (M -97.64 kPa), the time to peak under-pressure was between 0.032 seconds and 0.040 seconds (M 0.035 seconds), the total decompression time was between 0.309 seconds and 0.34 seconds (M 0.32 seconds). Graphs of the signatures produced are shown as Appendix VI.

Visible bubbling was observed in 57% of the active samples in this group (8/14). Bubbling was not observed in any control samples.

Sample preservation was assessed in the same manner as in Pilot One. The Pilot Two group showed no changes between the baseline and control samples over the time of the experiment. There was no difference in carbon dioxide, bicarbonate or pH between baseline and control samples, indicating the samples were well preserved. This is shown as Table 8.2, with t-test p values provided.

Table 8.2 Preserved samples, Pilot Two.

	CO_2 (mm Hg)		HCO_3 (mmol/l)		pH	
	Baseline	*Control*	*Baseline*	*Control*	*Baseline*	*Control*
MEAN	54.41	52.26	29.74	28.96	7.35	7.36
STDEV	4.99	5.64	2.36	2.68	0.03	0.03
SEM	1.33	1.51	0.63	0.72	0.01	0.01
95% CI	51.8-57.0	49.3 - 55.2	28.5-31.0	27.6 -30.4	7.33- 7.36	7.35-7.37
p value	0.29		0.42		0.42	

In this Pilot Two group, carbon dioxide was lost in 14/14 samples (100%) when compared with both baseline and control groups. This consistent loss is noteworthy but the amount lost was not statistically significant.

Mean carbon dioxide tension in the baseline samples was 54.41 mmHg (95% CI 51.8 - 57.0), in the control samples 52.26 mmHg (95% CI 49.3 - 55.2), and in the active samples 49.96 mmHg (95% CI 47.3 - 52.6). The t-test comparing the active with the control samples showed a lack of significance p = 0.88. A graph demonstrating these findings is shown as Figure 8.2
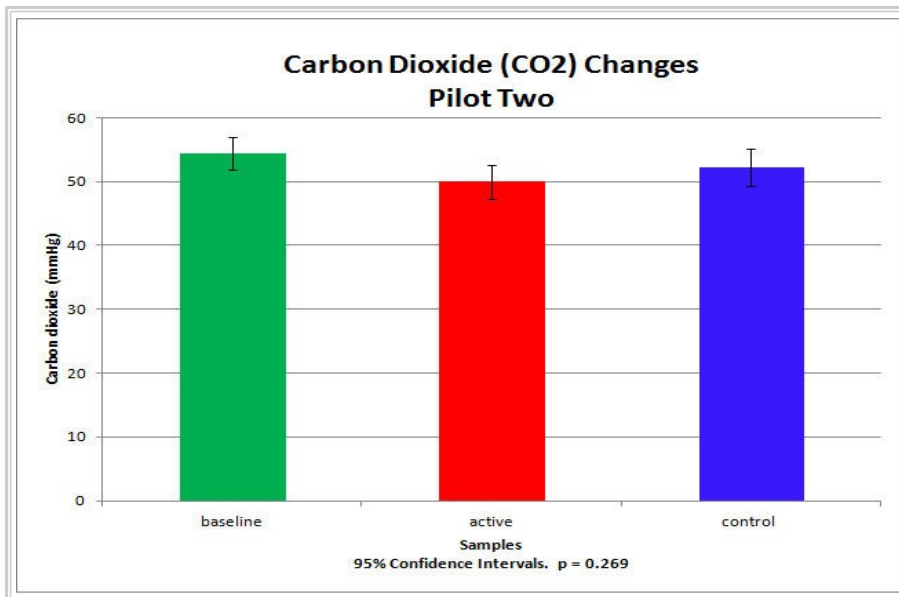

Figure 8.2 Carbon dioxide content in baseline, active and control samples, Pilot Two group. The p value relates to the active versus the control samples.

Discussion - The high proportion of active group samples that lost carbon dioxide suggests that the decompression event liberated dissolved gas from the blood. The mean difference in

carbon dioxide loss between active and control samples in this group is greater than was observed in Pilot One. This could be due to the warmer blood temperatures, consistent with Henry's Law. As it was in Pilot One where direct active sample comparison was made carbon dioxide loss correlated to bubbling.

The Pilot Two group is a stand alone experiment, however, the trend in loss of carbon dioxide remains consistent with Pilot One. Although plagued by sample interference and longer time intervals between the baseline blood gas analysis testing and the final blood gas analysis tests, Pilot Two was crucial in determining whether warmed samples would behave similarly or differently from cooled samples, thereby relating the test conditions to those of human physiology. Equally important was the establishment of the optimum blood sample warming time (described in Chapter 7), which enabled refinements of the protocol for the main experiments. This time of 1.26 minutes was used in the definitive sample group the Venous Test group.

8.2.3 Venous Test group

The Venous Test group included 40 samples. All were re-warmed, with one post-warming temperature assessment per sample; no sample temperatures were taken prior to the warm bath immersion. Forty samples were used in the blood gas analysis testing, one pH value from sample #56 could not be tested due to excessive foaming following rapid decompression exposure. The average experimental cycle duration time for this group was 18.82 minutes.

All samples were cooled following extraction; the baseline measure was obtained, samples were divided into control and active parts, returned to the cooler, randomised for blood gas

analysis, and monitored for bubbling using the same protocols as the pilot experiments. All samples were immersed in the bath to warm for 1.26 minutes. The average testing temperature for the active samples was 36.7^0C and 36.4^0C for the control sample. A temperature log for each active and control sample's temperature following warming is shown as Appendix VII.

The decompression levels reached for this group ranged between -86.24 kPa and -96.84 kPa (M -95.43 kPa), the time to peak under-pressure was between 0.033 seconds and 0.050 seconds (M 0.04 seconds), the total time taken for the decompression ranged between 0.28 seconds and 0.41 seconds (M 0.35 seconds). Graphs of the signatures produced are shown as Appendix VIII.

Bubbling was observed in 29 of 40 samples (72.5%). Bubbling was not observed in any control samples. Table 8.3 shows the bubbling and foaming data for the Venous Test group.

Table 8.3 Observed bubbling sample data for Venous Test group.

Bubbling	Foaming
72.5% (29/40)	20.6% (6/29)

Sample preservation was confirmed by comparing the baseline and control samples. Table 8.4 shows there was no difference between baseline and control samples, with t-test p values provided.

Table 8.4 Preserved samples, Venous Test group.

	CO_2 (mmHg)		HCO_3 (mmol/l)		pH	
	Baseline	*Control*	*Baseline*	*Control*	*Baseline*	*Control*
MEAN	52.66	50.95	28.62	28.00	7.35	7.36
STDEV	5.63	5.43	2.03	2.31	0.03	0.03
SEM	0.89	0.86	0.32	0.36	0.00	0.00
95% CI	50.9-54.4	49.3-52.6	28.0-29.3	27.3-28.7	7.34-7.36	7.35-7.37
p value	0.17		0.21		0.19	

The Venous Test group showed a loss of carbon dioxide in 36/40 samples (90%). The baseline samples had a mean carbon dioxide tension of 52.66 mmHg (95% CI 50.9 - 54.4), control samples showed a mean carbon dioxide tension of 50.95 mmHg (95% CI 49.3 - 52.6), and the active samples showed a mean carbon dioxide tension of 46.86 mmHg (95% CI 45.0 - 48.7). Mean carbon dioxide tension in active samples was significantly lower than in control samples, strongly suggesting the active decompression event released dissolved gas from the blood.

The t-test comparing the active with the control samples showed a significant difference between the active and control samples (p < 0.001). Tabled data showing the difference in carbon dioxide between active and control samples is shown in Table 8.5. Changes in all 3 sample groups are shown by graph in Figure 8.3.

Table 8.5 Carbon dioxide loss in active samples compared with the control samples, Venous Test group.

	CO_2 (mm Hg)	
	Control	*Active*
MEAN	50.95	46.86
STDEV	5.63	5.43
SEM	0.89	0.86
95% CI	49.3-52.6	(45.0 - 48.7)

Figure 8.3 Carbon dioxide content in baseline, active and control samples, Venous Test group. The p value relates to the active versus the control samples.

Serum bicarbonate levels were analysed in addition to carbon dioxide tension because they are closely related to each other as part of the acid-base balance system. Bicarbonate levels were low in active samples when compared with control and baseline samples. Mean bicarbonate levels for the baseline samples were 28.6 mmol/l (95% CI 28.0 - 29.2), the control mean was 20.0 mmol/l (95% CI 27.3 - 28.7) and the active mean was 25.0 mmol/l (95% CI 23.6 – 26.6). The t-test showed a significant difference between active and control bicarbonate samples (p <0.001). A graph demonstrating these findings is shown as Figure 8.4.

Figure 8.4 Bicarbonate levels in baseline, active and control samples, Venous Test group. The p value relates to the active versus the control samples.

The pH levels in the Venous Test group were aligned with the changes in carbon dioxide and bicarbonate content. Mean baseline pH was 7.35 (95% CI 7.34 - 7.36), the mean control pH was 7.36 (95% CI 7.35 - 7.37), and the mean active pH was 7.35 (95% CI 7.34 - 7.36). The t-test between the active and control samples showed no difference between the two samples (p = 0.189). A graph demonstrating these findings is shown as Figure 8.5.
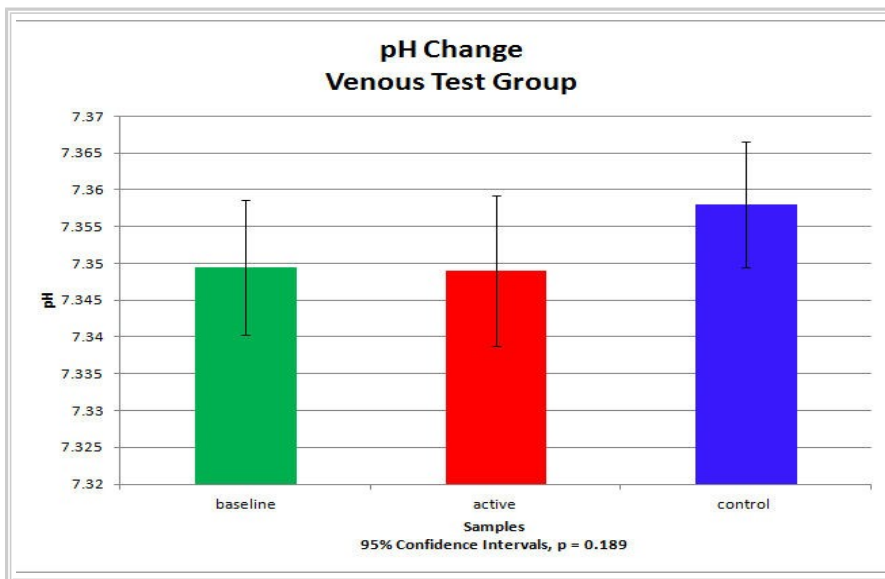
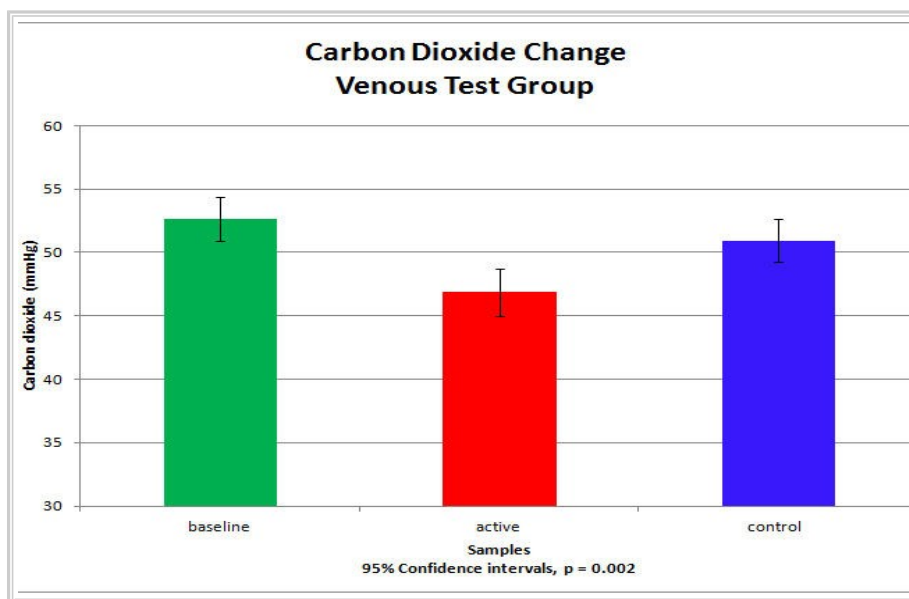**Figure 8.5** The pH in baseline, active and control samples, Venous Test group. The p value relates to the active versus the control samples.

Finally, serum potassium levels were assessed for both cellular wall integrity and the minor role it plays in acid base balance. The baseline samples showed a mean potassium level of 3.91 mmol/l (95% CI 3.87 - 3.95), the mean control 3.92 mmol/l (95% CI 3.87 - 3.96), and mean active 3.78 mmol/l (95% CI 3.72 - 3.83). The t-test showed there was a loss of serum potassium in the active sample when compared with the control sample ($p < 0.001$) but no difference was found between the baseline and control samples. A graph demonstrating these findings are shown as Figure 8.6.
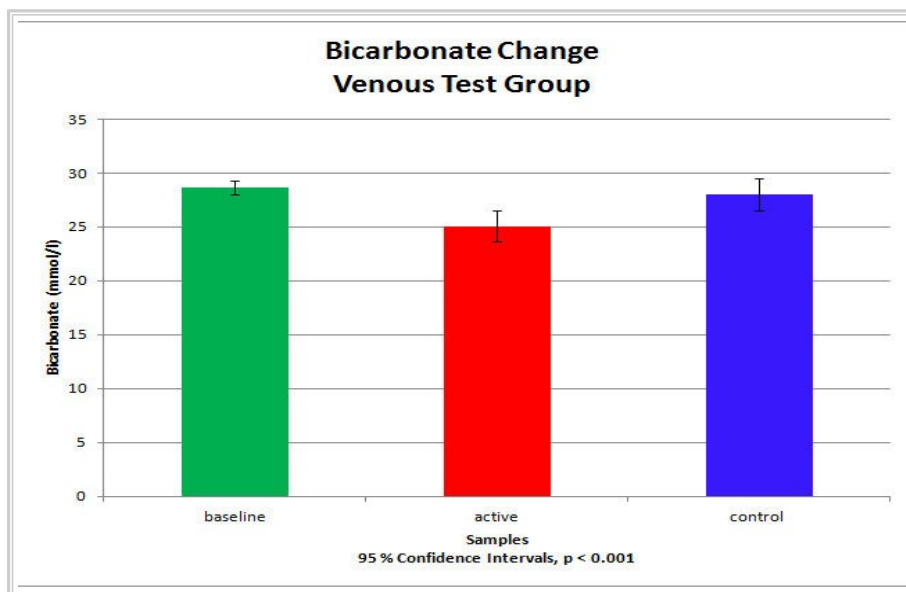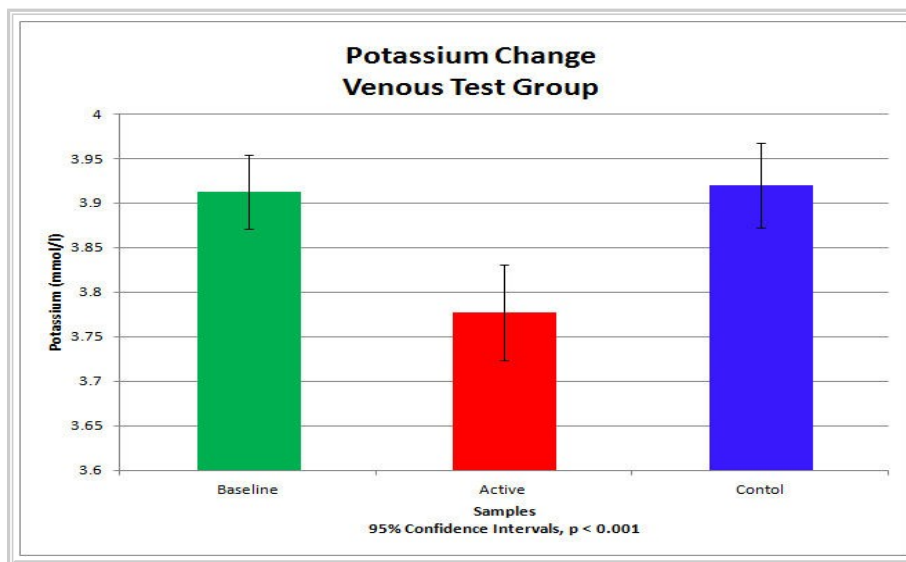

Figure 8.6 Potassium levels in baseline, active and control samples, Venous Test group. The p value relates to the active versus the control samples.

Discussion - This experiment showed that the bubbling formed in the active samples was a direct result of the rapid decompression event, as a demonstration of Henry's Law. The significant mean decline in carbon dioxide tension in the active samples suggests that the observed bubbles are carbon dioxide released from the blood (solution).

171

In this test the relationship between carbon dioxide and bubbling samples correlating was consistent with the pilot groups in all but one sample. Direct active sample comparison showed carbon dioxide loss occurred in a sample without evidence of bubbling, but bubbling always correlated to carbon dioxide loss, except in one sample: Active sample #40 bubbled and the carbon dioxide was 51.3 mmol/l, the corresponding control sample was 50.5 mmol/l. This anomaly cannot be explained.

The trend in the loss of carbon dioxide in this Venous Test group is significant. The contributing factor to this greater trend is most likely due to the streamlined re-warming process which impacted preferably on each experimental cycle's timing.

The chemical response to the decline in carbon dioxide tension is further evidence that the bubbles are carbon dioxide because the acid-base chemistry of the active group was adjusted accordingly, with significantly lowered bicarbonate levels in the active samples when compared to the controls.

The chemical response to the loss in carbon dioxide tension prevented the sample becoming alkaline, and in doing so it drove bicarbonate and hydrogen ions back to weak carbonic acid, thus maintaining a normal pH level. The chemical mechanism for this is explained in the Henderson Hasselbach equation as shown below (Brady & Holum, 1993, p. 711).

$$\mathbf{HCO_3^- + H^+ \leftrightarrow H_2CO_3 \leftrightarrow CO_2 + H_2O}$$

There was no statistical difference in the pH level of the three samples in the Venous Test group. In human physiology the pH is balanced by the concentration of hydrogen ions, the

difference between these groups is not statistically significant therefore the pH has balanced the loss of carbon dioxide by buffering the bicarbonate.

The loss of potassium ions cannot be explained beyond the potassium playing a small role in the acid-base balance exchanging with hydrogen ions to stabilise the pH, the result of which is evident (Lee Hamm, Hering-Smith & Nakhoul, 2013). On the contrary, a rise in potassium would have signalled a haemolysed sample, and as potassium moves out from a damaged cell into the bloodstream so too does carbon dioxide and in this experiment the carbon dioxide was not raised, but lowered (Mayne, 1994). The biological significance lies in the fact that the potassium was not raised, because that confirms the erythrocyte wall was intact, and as such further substantiates the other data reported.

8.3 Arterial group

In human physiology, the carbon dioxide concentration is higher (increased partial pressure) in venous blood than in arterial blood. As a demonstration of Henry's Law the liberation of dissolved carbon dioxide from arterial blood may differ when exposed to a rapid decompression event because of this difference. The Arterial Test group experiment was designed as a comparison to the Venous Test group.

The protocol varied from the venous groups because there was no cooling or rewarming of the blood samples. The samples in this group were subjected to testing immediately following

extraction from the participant because the volunteer and the apparatus were co-located in the laboratory.

8.3.1 Arterial Test group

Twenty five samples were analysed for this experiment. No sample was cooled, because all samples were applied for testing and analysis within a tighter time frame than the Venous Test group. Baseline, active and control samples were collected for this experiment using the same protocol measures as in the Venous Test group.

Each cycle was achieved rapidly, largely due (as described in Chapter 7) to the proximity of the participant to the testing apparatus. The average experimental cycle duration time for this group was 5.4 minutes. No erroneous results occurred in this group and all samples were accepted by the blood gas analyser.

The decompression levels attained for this group were between -93.40 kPa and -95.48 kPa (M -94.24 kPa). The time to peak under-pressure was between 0.020 and 0.023 seconds (M 0.020 seconds). The total time of the decompression was between 0.135 and 0.141 seconds (M 0.138 seconds). Graph signatures of arterial decompression events are shown as Appendix IX.

Arterial samples bubbled, 20/25 (80%) samples bubbled and 7 of those 20 foamed. No bubbling was identified in the control samples. Although the bubbling was fine foaming at times, as defined by the protocol in Chapter 6, no sample was rejected from gas analysis.

All foaming samples were allocated to visible bubbling data and allocated further as a sub-set to that group as it was in the Venous Test group, provided as Table 8.6.

Table 8.6 Observed bubbling sample data for the arterial group.

Bubbling	Foaming
80% (20/25)	35% (7/20)

Even though samples were not artificially preserved (by cooling) a comparison was made between the baseline and control data to attest for sample conservation for the duration of each experimental cycle. Sample preservation was confirmed by comparing the baseline and control samples. Table 8.7 shows there was no difference between baseline and control samples, with t-test p values provided.

Table 8.7 Sample preservation comparing baseline with control, Arterial Test group.

	CO_2 (mm Hg)		HCO_3^- (mmol/l)		pH	
	Baseline	Control	Baseline	Control	Baseline	Control
MEAN	32.82	32.91	21.07	21.12	7.42	7.42
STDEV	1.04	0.90	0.65	0.65	0.01	0.01
SEM	0.21	0.18	0.13	0.13	0.00	0.00
95% CI	32.4 - 33.2	32.6 - 33.3	20.8 -21.3	20.9-21.4	7.41-7.42	7.41-7.42
	0.76		0.78		0.98	

The Arterial Test group showed a loss of carbon dioxide in all 25 samples. Mean carbon dioxide tension changed significantly between the active and control groups. The mean

carbon dioxide tension of the baseline samples was 32.82mmHg (95% CI 32.4 - 33.2), control samples 32.91 mmHg (95% CI 32.6 - 33.3), and the active samples 31.0mmHg (95% CI 30.3 - 31.6). As with most of the venous experiments direct active sample comparison showed carbon dioxide loss occurred without evidence of bubbling, but bubbling correlated to carbon dioxide loss.

The t-test comparing the active with the control samples confirmed the significance of the difference between the active and control samples (p < 0.001). A data table showing the difference in carbon dioxide between active and control samples is shown in Table 8.8. Changes in carbon dioxide all 3 sample groups are shown by graph in Figure 8.7.

Table 8.8 Carbon dioxide loss in active samples compared the control sample, Arterial Test group.

	CO_2 (mm Hg)	
	Control	*Active*
MEAN	32.9	31.0
STDEV	0.9	1.6
SEM	0.2	0.3
95% CI	32.6 - 33.3	30.3 – 31.6
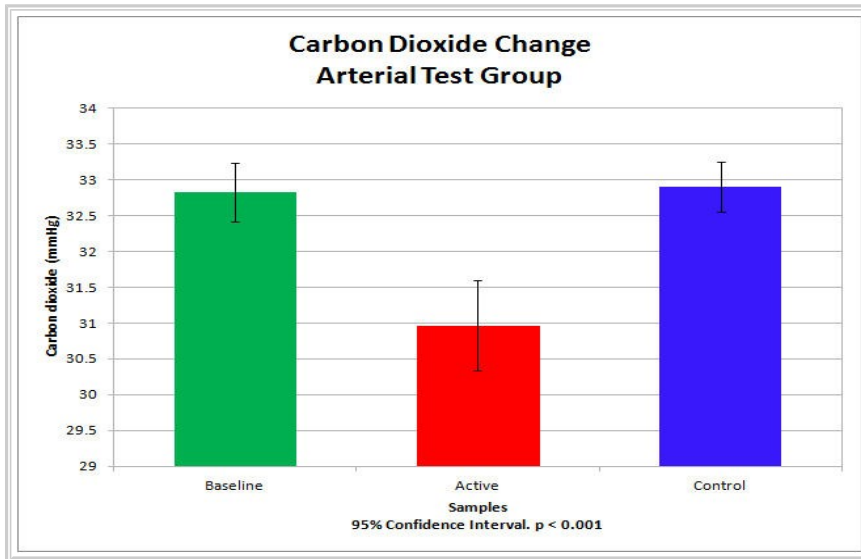
Figure 8.7 Carbon dioxide content in active, baseline and control samples, Arterial Test group. The p value relates to active versus control samples.

The bicarbonate baseline samples showed a mean bicarbonate level of 21.1 mmol/l (95% CI 20.8 - 21.3), control samples 21.1 mmol/l (95% CI 20.9 - 21.4), and active samples 20.6 mmol/l (95% CI 20.2 – 21.0).

The t-test confirmed a significant difference between the active and control bicarbonate levels (p = 0.035). This is shown as Figure 8.8.

Figure 8.8 Bicarbonate levels in active, baseline and control samples, Arterial Test group. The p value relates to active versus control samples.

The baseline samples mean pH level was 7.41 (95% CI 7.41 - 7.42), the control 7.41 (95% CI 7.41 - 7.42), and active 7.43 (95% CI 7.42 - 7.44). The mean baseline and control pH's were unchanged, but the confidence intervals show a significant difference existed in the mean pH of the active versus the control samples (p < 0.001). This is shown as Figure 8.9.
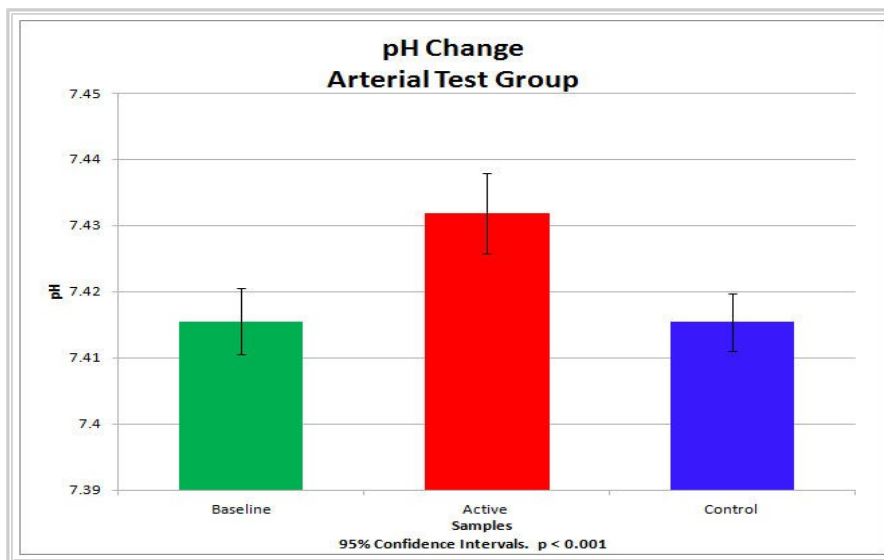
Figure 8.9 pH in baseline, active and control samples, Arterial Test group. The p value relates to the active versus the control samples.

The Arterial Test group's potassium levels were not raised as would be expected if the sample haemolysed. The active sample group's mean potassium levels were lower than the control groups. The baseline sample mean potassium was 3.91mmol/l (95% CI 3.86 - 3.96), the control was 3.91mmol/l (95% CI 3.85 - 3.97), and active sample was 3.79mmol/l (95% CI 3.72 - 3.86). The t-test showed a significant decrease between the active and control samples ($p < = 0.015$). The potassium levels are presented by graph in Figure 8.10.

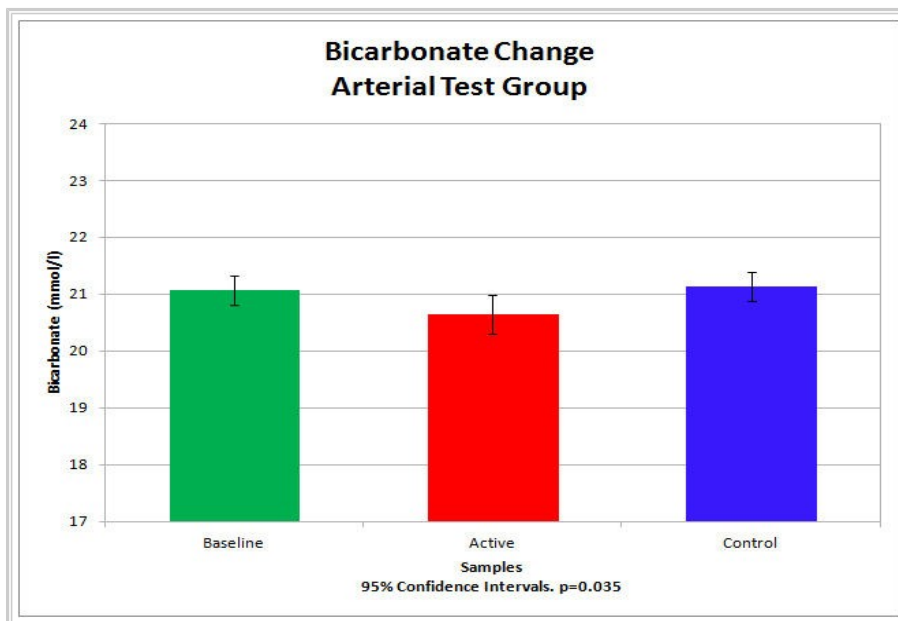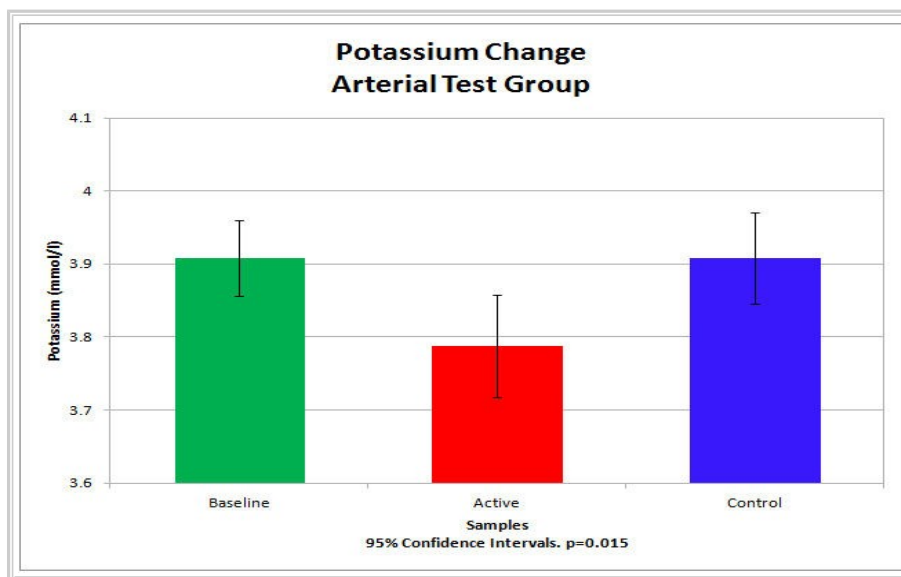**Figure 8.10** Potassium levels in baseline, active and control samples, Arterial Test group. The p value relates to active versus control samples.

Discussion - The experimental results from the Arterial Test group reinforced the information learned from the Venous Test group. The significant changes in the carbon dioxide of the active samples relative to the control samples supports the hypothesis that rapid decompression causes bubbling because it releases carbon dioxide from the blood.

The loss of carbon dioxide precipitated an immediate chemical response to the loss of

hydrogen ions to maintain the blood pH. The significantly lowered bicarbonate levels attest an acid-base balance has been achieved to counter the loss of carbon dioxide ensuring a normal pH is maintained. The pH in the active samples for this group is higher than the baseline. This anomaly cannot be explained, but it is notable that the pH in the active Arterial Test group samples remained within the clinically acceptable range of 7.35 – 7.45, which means the acid base balance was maintained (countering the loss of carbon dioxide), through a combined bicarbonate loss and potassium exchange with hydrogen ions as the significantly lowered potassium levels ($p \le 0.015$) evidence shows. This may explain the borderline confidence intervals, despite significance ($p = 0.035$), in the active and control bicarbonate measurements.

The lowered potassium in active samples resulted from a shift of potassium into cells as hydrogen ions were released to lower the blood pH, a further compensation for the loss of carbon dioxide (Lee Hamm, Hering-Smith & Nakhoul, 2013). While potassium played a small role in adjusting the pH balance, the potassium levels demonstrated that the erythrocytes were intact.

8.4 Discussion - the under-pressure generator experiments – Venous and Arterial groups

The samples from both major test groups exposed to the rapid decompression, bubbled and lost carbon dioxide. This finding was supported by the aligned acid-base adjustment made to compensate for the lowered carbon dioxide. Further measurement of serum potassium indicated the erythrocyte cell wall was intact.

Both pilot experiments were important steps because they allowed progressive refinement of the protocol for the Venous Test group. Using cooled blood (Pilot One) enabled a preliminary assessment of the response of the blood gas composition to rapid decompression without the complicating factors of the samples metabolising, as is the natural course of events. Pilot Two involved warmed blood samples and required frequent temperature checking, thus was relatively time consuming; it produced the same pattern of carbon dioxide loss as in Pilot one, but the extra handling made the protocol too prone to error. A refined, streamlined cool and rewarm protocol was used for the Venous Test group. Finally, the Arterial Test group showed further consistency. The Venous Test group (N=40) samples and the Arterial Test group (N=25) provided data that allowed the study's hypothesis to be tested.

Four key observations were made from these two final experimental groups: high proportions of visible bubbling/foaming, carbon dioxide loss, acid base adjustment and red blood cell wall integrity. These points are discussed in turn below followed by how the data overall relates to Henry's Law.

8.4.1 Visible bubbling

Visible bubbling was observed in active samples from each of the groups tested. Over 50% of each sample group bubbled, and some samples foamed. There was no bubbling identified in control or baseline samples. All but one of the bubbling samples, in the Venous Test group, showed a loss in carbon dioxide, not all samples with a lowered carbon dioxide showed evidence of bubbling.

The Arterial Test group exhibited the highest proportion of foaming of all the laboratory test groups, including the pilot groups. The decompression timings to peak under-pressure and overall duration times were also faster than the venous times. The shorter decompression times may have caused the higher proportion of bubbling and foaming in each decompression, despite historical opinion opposing the concept as too fast. Alternatively, the bubbling might be due to the more stable blood temperature in the arterial experiments, without further research this is an interesting observation but a conclusion cannot be drawn at this time.

The protocols mitigating bubbling during the laboratory experiments were rigorously adhered to prior to rapid decompression, despite this, cavitation from the apparatus cannot be ruled out. Nonetheless, the significant results of bubbling correlating with carbon dioxide loss and aligned acid-base balance would not be expected in a cavitation scenario, so are sufficient evidence to encourage further research.

8.4.2 Carbon dioxide loss

While more samples returned a loss in carbon dioxide in the Arterial Test group than the Venous Test group, the Venous Test group returned a greater loss in carbon dioxide overall. The difference in carbon dioxide loss between the two groups supports Henry's Law because more carbon dioxide was liberated from the venous group that has a greater partial pressure of carbon dioxide at the onset of the experiment than the arterial group. The difference is statistically significant but the different sizes in the sample groups (40 venous samples versus 25 arterial samples) should be a consideration when qualifying the significance. The t-test showed a significant difference in the loss of carbon dioxide in the active samples between the Venous Test and the Arterial Test group ($p = 0.002$). This data is shown in Table 8.9.

Table 8.9 Differences in carbon dioxide between the Venous Test group and Arterial Test group. P value relates to arterial versus venous groups.

	CO_2	
	Arterial	*Venous*
MEAN	-1.9	-4.1
SD	1.0	4.0
SEM	0.2	0.6
95% CI	-2.3 -1.5	-5.3 - -2.3
p value		0.002

8.4.3 Lowered bicarbonate, potassium-hydrogen ion exchange and pH maintenance

Acid-base maintenance is a naturally occurring chemical activity, any change in one parameter will result in compensation by a buffer element or compound. In response to lowered carbon dioxide concentration bicarbonate concentration fell in the active samples in both Test groups. In both groups this reduction in bicarbonate level was statistically significant and supported by the pH levels. The existence of acid-base buffering was further evidenced by a significantly lower potassium level than the control samples alongside the normal pH. Even though both test groups produced a similar trend of data, each group with significant results, the amount to which these differences were was statistically significant. These changes are shown in Table 8.10.

Table 8.10 Differences in the changes effecting acid-base balance between the Venous Test group and the Arterial Test group. P value relates to arterial versus venous groups.

	pH		HCO3$^-$ mmol/l		K$^+$ mmol/l	
	Arterial	*Venous*	*Arterial*	*Venous*	*Arterial*	*Venous*
MEAN	0.02	-0.01	-0.5	-2.93	-0.12	-0.14
SD	0.01	0.02	0.82	4.33	0.18	0.16
SEM	0.002	0.002	0.16	0.68	0.04	0.03
95% CI	0.01-0.02	-0.01-.00	-0.08 - -0.16	-4.27- -1.58	-0.18- -0.05	-0.19- -0.09
p value	<0.001		0.001		0.61	

The chemical changes described above attest to the action of the Henderson-Hasselbach equation as it works to equilibrate acid-base alterations in the body once dissolved carbon dioxide was lost to gas (Mayne, 1994).

8.4.4 Henry's Law or cavitation?

The experimental process and the results from these experiments provide considerable evidence to support an alternative theory to the translocation theory. Spalling and cavitation development are alternative explanations for the bubble production observed in these experiments however the compliant internal surface within the sample chamber to produce cavitation means this is unlikely. The possibility of bubbles being in the sample to cause cavitation prior to the rapid decompression is minimised by the strict adherence to the protocol laid out in Chapter 7 (Methods). No bubbling was detected in the active samples and none was in the blood holding chamber at any point prior to the active sample's administration into the generator. Because of the smooth holding chamber lining, cavitation bubbling, if it occurred, would have evolved from widespread cell rupture, resulting in fragments from which spalling may occur (because the internal lining of the syringe was smooth) but the

potassium levels indicated that all cell membrane walls were intact. According to P. Winter (written communication, 2010) cavitation bubbling from surface tension would contain water vapour, which means carbon dioxide tension within the blood sample would be unchanged. As outlined earlier, the volume of carbon dioxide loss correlating to the samples with the higher partial pressure at the experiment outset is also interesting, and supports Henry's Law, this is unlikely to be evident if cavitation was the cause of the bubbling.

8.5 Synopsis: laboratory experiments

These experiments have shown that a rapid or explosive decompression event, such as one might experience through blast wave exposure, causes bubbling, and significantly reduces carbon dioxide tension within blood samples. The visible bubbling and the significant loss of carbon dioxide with corresponding acid-base adjustments in the active samples support the hypothesis that microscopic gas emboli form in blood when exposed to a blast event. Further support is seen in the corresponding lowered potassium levels indicating an intact erythrocyte. Additionally, the samples temperatures were within normal physiological range at the time of testing, which is consistent with the constant required for Henry's Law. These experimental samples were decompressed below 101.3 kPa within a time frame accepted as a rapid decompression by extant literature and government policy.

The experiment is an expression of Henry's Law (Brady & Holum, 1993). These experiments inform and justify progressing to a live explosives experimental program.

Chapter 9

Explosives events

Experimental design

and materials

The null hypothesis

Bubbles of gas do not form autologously in an isolated blood sample when exposed to an explosion.

9.1 Outline

Both historical and contemporary blast researchers have studied live blasts to generate understanding of the biophysical impact of a blast wave on humans using animal models, (Bowen, Fletcher & Richmond, 1968). Some researchers contend that such methods are now outdated (Bass, Rafaels & Salzar, 2008). The development of computer modelling techniques means live blast experimentation is less common today (Przekwas, 2008). Despite its current relevance in blast research, computer modelling is limited to researching biomechanical injury which uses mathematical-engineering tools, blast injury causing physiological disturbance such as biochemical alterations cannot be determined using biomechanical engineering tools. Live blast experimentation is still a useful research technique, particularly for determining the effects on aspects of biochemical changes that may be altered by blast such as chemical changes, cytokine responses, inflammatory responses and alterations in blood gases (Garner et al. 2009).

For practical reasons live blast experimentation typically follows simulation experiments, much like drug trials in humans. In this project simulation was used to test the hypothesis that emboli form in response to the rapid decompression effect of the sub-atmospheric phase of blast. As described in chapter 8, the results were encouraging, providing sufficient evidence to justify taking the research into the field using live blast experiments.

Live blast experimentation requires considerable preliminary work to minimise the inherent risks. These risks include risk of physical harm to the researchers and risk of damage to the environment surrounding a designated explosives range that may include sensitive native bushland, particularly in Australia (Metherill, 2013).

This chapter describes the objectives, planning, design, methods and labour of 3 explosives events: one pre-experiment validation test and two live blast experimental tests. The experiment was intended to replicate the changes in blast exposed blood identified in the simulation experiments, and hence test the hypothesis.

9.2 Objectives

As noted above, the primary objective of the live blast experiment was to determine whether exposing an isolated blood sample to a live blast event would produce the same changes as observed in the under-pressure simulations.

9.3 Experiment design

This experiment was designed to determine if the data captured in the simulation experiments could be captured in a live blast where the samples were exposed to both the positive pressure load and the rapid decompression effect of an explosion. This explosives experiment meant replacing the rapid decompression exposure in the laboratory with a live explosive exposure

but the same blood sampling and blood gas analyses were required before and after the exposure event.

Laboratory and field experimentation differ in logistics and time availability therefore considerations for these different circumstances were made. The experiment design and protocols used in the simulation laboratory experiments were reviewed, revealing three main issues for consideration: time frame limitations, identifying a valid sample container and mitigating excessive radiant heat and energy transfer of the blast wave to the blood samples.

9.3.1 Time frame considerations

In the previous simulation experiments each of the three samples: baseline, active and control were tested within each experimental cycle, (as described in chapters 6 and 7), conversely, this experiment required these same samples to be tested within the one experimental cycle time frame of 15 minutes because explosives events were limited, where ten samples were exposed in each event. This increased volume of blood gas analyses within the same duration time (an expected 30 blood tests overall for each experimental cycle or explosion) required a revision of the protocol used for the laboratory experiments. These revisions included additional assisting personnel, additional blood gas analysers, minimising the baseline sample testing protocol, and finding a blood holding container for efficient extraction of the blood for testing.

9.3.2 Selecting a sample container: an examination of three models

Blood samples had to be collected, transported, and stored safely within the Defence explosives range site. The experiment itself required a sample storage container that could remain sealed throughout the live blast exposure, while still permitting a blast wave to affect the blood sample. It was also important that the sample container was transparent (for visual assessment) and provide easy access for extracting the blood from it for blood gas testing, thus minimising any consequent metabolism or contamination of the sample.

Three potential sample containers were tested for preliminary suitability. The first, a ten centimetre piece of silicone tubing with a diameter of 5 mm, sealed at both ends. Second, a standard intravenous extension line used in clinical practice with locking clips at each end and, finally a standard blood gas syringe also used in clinical practice sealed with a rubber stopper.

The latter two devices, commonly used in clinical practice, were made from polyethylene; the extension line was a pliable form, the syringe was not. None of the three containers was hydrophobic or hydrophilic, and each could hold the small volume of blood (3ml) required without an air gap.

Trials were conducted with each container, using water, to measure the time required to conduct the experiment. Ten samples were used. Both tubing containers proved to be too slow to load with blood, create an air lock seal, and then remove the blood sample after the blast event for analysis. Retrieving the sample from tubing without subjecting the sample to a

moderate vacuum via a syringe proved impossible in 60% of attempts. Piercing the tubing with a needled syringe rather than opening the cap quickened the process slightly, but this increased the vacuum in the syringe as the blood was withdrawn and the samples bubbled. Using the tubing models, the time taken to process a group of ten samples (from mock initial testing through to final post blast testing) ranged between 30 and 32 minutes. Only the clinical blood gas syringe met the sampling time criteria (15 min). Hence, the same type of syringe used for the laboratory experiments was selected for preliminary testing to determine its endurance to withstand a blast exposure. Only one explosives event was available for a syringe validation test.

9.3.3 Syringe validation test

Six syringes, each containing 4ml of water, were set at designated distances from an open air blast. Each syringe with secured stoppers, was strapped to a pole using heavy duty duct tape, positioned 1.0 metres from ground level, and at 2 metre interval distances from the detonation point, starting with 4m and up to fourteen metres, from the epicentre of the blast. The blast was conducted in the open air at an Australian military testing range. A 20kg TNT equivalent explosion was used. This validation test was obtained by using space within the field of another experiment undertaken by Defence Science Technology Organisation (Melbourne), no choice of explosive charge size was available and ethics approval was not required for this test because no blood sampling was undertaken.

The results showed bubbling occurring within intact syringes at 6, 10, 12 and 14m. The syringe held at 4m was destroyed and the stopper was disrupted. The syringe at 8m was damaged, the stopper was torn and water leaked out.

The syringe model held up to the explosives event and showed signs that the blast wave had passed through the container, demonstrated by the bubbling. Protecting the water inside the syringe from radiant heat was not necessary in this validation test, the integrity of the syringe was under examination; if the syringe stood up to exposure without protection from the radiant heat, then it would be suitable for the experiment when protection would be employed for the blood sample.

Because the syringe was made from polyethylene, it was resilient enough to withstand the blast at 4m but also allowed for passage of the blast wave through it. The tensile strength of polyethylene is greater than human blood vessels so does not represent them perfectly but this is unavoidable; blood vessels can tear during blast exposure causing spillage and air contamination of the blood, rendering them useless for this experimental purpose. Polyethylene syringe has a low friction co-efficient (Ogle, 1951), which both aids in maintaining the blood sample's integrity and minimises the risk of the cavitation phenomenon occurring.

9.3.4 Mitigating the blast effects on the blood samples removed from their natural environment

Because the blood samples were removed from their normal environment, this experiment included several strategies to mitigate the radiant heat while still appropriating normal blast energy transfer effect on them. Firstly, a relatively small material weight of 5 kg was selected. Secondly, based on the results of the syringe validation tests the samples were positioned 12m from the explosive device. Thirdly, a tissue simulant block (detailed in the following section) reduced the direct sample exposure to the heat. Finally, the experiment was conducted in

open air, avoiding the increased local temperature that occurs in a blast event in a confined space.

Radiant heat produced by an explosion can cause flash burns to a body surface facing the blast wave but the flash burn rate inflicted by an explosion varies considerably (Kauver et al. 2008). This variation reflects both the momentary flame produced at the detonation point, and the rapid deterioration of heat as it moves away from the detonation point. Even though the heat generated by a large conventional blast can be up to $3,000^0$ C at detonation point, the blast wave produced by an explosion is typically a rapid transient heat effect that dissipates rapidly as it moves away from the blast epicentre (Cooper et al. 1983; Kauvar et al. 2008). The effects of blast induced heat on blood are not known. Previous authors, including Gorbunov at al. (2008) and Tsokos et al. (2003), who studied how human blood cells respond to blast did not report blood temperature or boiling.

9.4 Materials

The materials used in these experiments varied slightly from the laboratory simulation experiments because of the time restraints of the field experiment. These amendments included: 3 clinical blood gas analysers to expedite gas analysis duration time for the 23 samples tested (3 baseline, 10 control and 10 active) per experimental cycle, sterile clinical venous access equipment including winged infusion sets for multiple sample extractions from an individual, vacutainers and lithium-heparin lined syringes. Materials specific to the explosion event included 2 tissue simulant blocks and 10kg cyclotrimethylene-trinitramine explosives material (commonly known as C-4) in 2 separate experiments (5 kg each). In view

of the logistics and the timing limitations due to the nature of the experiment, 2 research assistants were recruited to assist with blood sampling and blood gas analyser testing.

Five kilograms of C-4 were used for each blast event. The custom made tissue simulant block is described in detail, the remaining clinical blood sampling materials used are described in the context of their usage in the following Chapter 10, 'Methods' .

9.4.1 Tissue simulant block

Tissue simulant mimics human internal tissue, acting as a close material barrier between the blast wave front and the syringes, thereby allowing the blood samples to be exposed to an energy transfer of magnitude and nature similar to a real world explosion. It acts in a similar manner to ballistic gelatine but without the latter's climate control requirements, a consideration made for the experiment location. The tissue simulant blocks were manufactured by AT&E Systems, Adelaide, an approved Defence Department supplier. Each block weighed 27kgs and was 600mm long, 300mm wide and 150mm deep. Access slots were made within the block to hold the syringes inside the simulated human internal environment.

9.5 Summary

This chapter has outlined the preparations made in design and materials required for the explosives experiments to follow.

Chapter 10

Explosives events

Methods

10.1 Outline

This chapter describes the process by which the explosives experiment was undertaken. The experiment included two separate explosives events using the exact protocol for each. Ethics approval for an explosives experiment was complex, a number of organisations interests and compliances were required.

10.2 Ethics

An extension to the original ethics proposal was granted by the University of Adelaide permitting the progression of blood sampling from participants using venous blood for explosives testing on an Australian Defence demolition range through the Human Research Ethics Committee, Research Ethics and Compliance Unit with approval number: H-116-2005. Additional ethics approval was sought and received from the Australian Defence Human Research Ethics Committee (ADHREC), approval number: 648-11. A senior member of Defence was required to act as 'Defence Sponsor' for the activity: Colonel John Shanahan (Royal Australian Engineers) accepted the request, and supported the experiment.

Two blast events were scheduled at an Australian Defence Force demolition range located in north Queensland, with the approval of both the University of Adelaide Ethics Committee and ADHREC.

ADHREC's ethics approval included the requirement that blood splattering from the test samples should be kept to a minimum to limit human exposure to blood contamination. Moreover, defence members not listed as investigators or participants were not permitted to handle blood samples or contaminated material.

Range safety was managed by the Defence range safety officer, the responsibilities included all personnel present, the range proper and the surrounding environment.

10.3 Methods – Explosives group

10.3.1 Blast zone layout

The blast zone layout had to take account of the expected transit of the blast wave front, followed by a negative pressure wave, the blood sample had to be exposed to the blast wave without being compromised. The goals of designing the field layout were to minimise reflection interference, and to position the tissue simulant block at a distance from the epicentre of the blast that would ensure exposure of the block to an unimpeded free field waveform.

The tissue simulant block, loaded with blood samples as shown in Figure 10.1 (a and b) was secured with duct tape, and placed 1.2m above the ground in a tripod stand, the slots and syringe face facing away from the detonation point to eliminate unwanted heat from the blast.

Figure 10.1 (a) Laying the sample syringe inside the tissue simulant block.
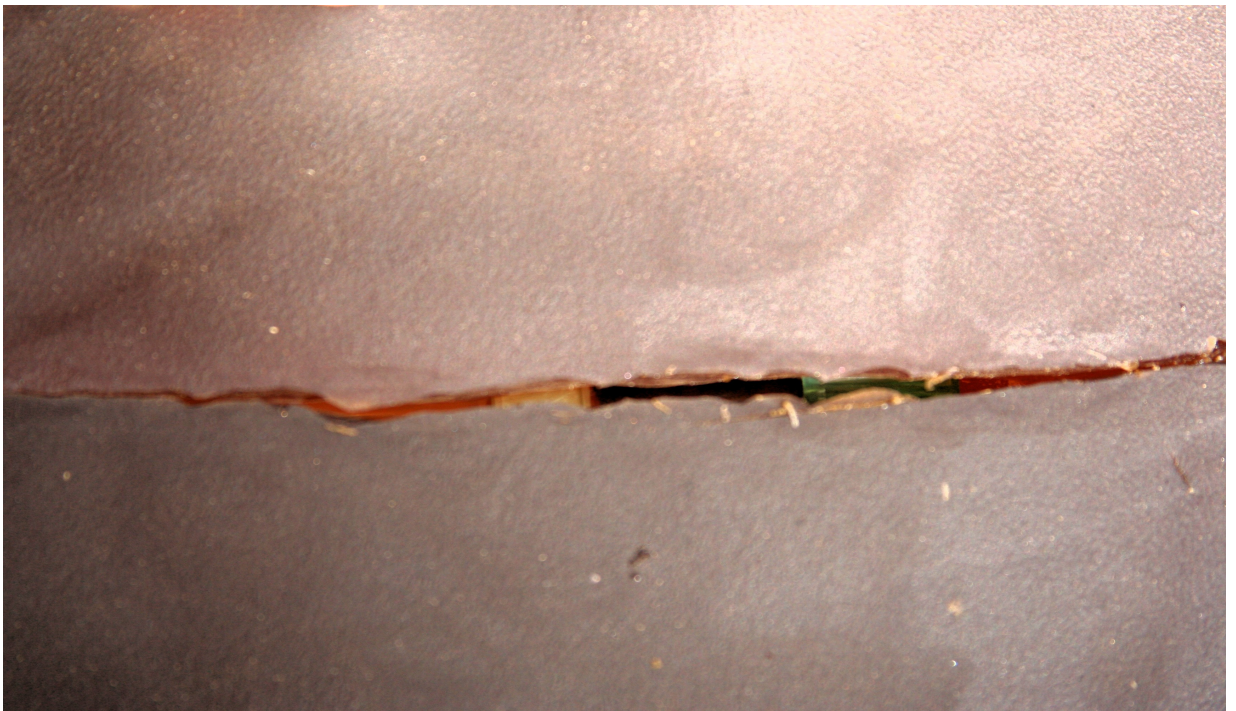
Figure 10.1 (b) The syringe lays snug and enclosed within the tissue simulant block.

The explosive source (5kg C-4) was suspended at the same height above ground, 1.2m, with a distance of 12m between each tripod, providing for an open air, free field, unimpeded exposure within the injury zone as described in Chapter 2 (Bowen, Fletcher & Richmond, 1968; Iremonger, 1997; Stuhmiller, 2008; Yelverton, 1997). Figure 10.2 illustrates the blast zone layout including key distances.
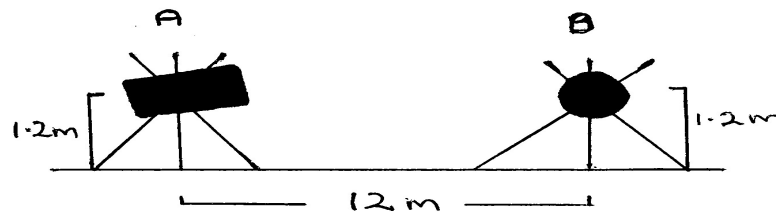
Figure 10.2 Blast zone layout diagram. Where point A- is the block containing 10 active samples, and point B- is the explosives (detonation point).

10.3.2 Explosives events (2 experimental cycles)

The power analysis using the changes in the Venous and Arterial Test groups as a guide was used to determine this experiment's overall sample size. This analysis determined that a sample size of N = 10-12 and would provide adequate power with an alpha value of 80%

Two explosives events were performed, 10 samples were exposed in each event. Each experimental cycle included one explosives event, bubbling observation and blood gas analysis testing. Each syringe was tucked within the tissue simulant block through the slots where upon the material closed around it, keeping the syringe encased inside. Duct tape was strapped around the block to further ensure syringes remained in place during the blast event.

As the active samples were laid in the simulant block, the control samples were set aside in the shade 500m away from the blast zone. Once the explosive was detonated and the 'all clear' safety requirement of 3 minutes was established, the block was retrieved.

10.3.3 Blood sample management - Explosives Test group (2 experimental cycles, 44 blood samples)

Twenty venous blood samples of 8ml each were collected from 3 human volunteers using winged infusion sets and vacutainer collecting tubes. Samples were not chilled in an effort to maintain a natural environment consistent with a field experiment and because, like the arterial tests the blood gas analysers were co-located at the explosives range.

As previously outlined in Chapter 9, the logistic and time constraints meant an adjustment to baseline measurement was necessary. Multiple active and control samples were derived from 3 baseline samples for each of the 2 experimental cycles, taken from a sample from each of the 3 participant's multiple donations: 6 baseline samples overall). This change in protocol was validated by the findings in the four laboratory experiments where sample preservation was consistent, so the same was expected with this experiment within a similar time frame.

Once a baseline sample was obtained, the samples were drawn from the vacutainers into two new sterile syringes, lined with lithium-heparin, from deep within the sample ensuring the syringe plunger was firm, preventing air inside the syringe.

The samples were labelled active or control, randomised as they were in the laboratory experiments, and tagged further to identify each with their respective pair (active and control

from the same sample division). Active samples were secured within the simulant block for explosion testing, the controls were set aside 500m away outside the designated blast zone.

Following the explosion, visual observation was used to detect bubbling. Then both active and control samples were entered into the blood gas analyser, in their respective pairs, and in accordance with their randomisation tag as it was with the laboratory sample groups. Both active and control samples were kept in the same temperature conditions at all time. While queued for the gas analyser, the blood samples were kept inside air-conditioned vehicles alongside the blood gas analysers because of the tropical conditions outside.

Each active and control sample was tested for changes in carbon dioxide content, acid-base adjustment and potassium. One control samples was spilled, that sample and its paired active sample was removed from the data count prior to gas analysis. Forty-four samples overall were tested for blood gas analysis using 3 blood gas analysers with 3 operators.

10.4 Statistical analysis

Statistical analysis was carried out using Microsoft Excel Extended Statistical analysis Library Statistics (Microsoft Corporation, Redmond, Washington, USA). Statistical testing was a comparative base as it was for the previous experiments. The data from the 2 experimental cycles were analysed together as one group. All p-values were derived from the two sample t-test, assuming unequal variances. Average values for the baseline data were calculated from 6 samples, averages for all other values were calculated from 19 remaining samples.

Chapter 11

Explosives events

Results and discussion

11.1 Outline

This chapter outlines the results obtained from the explosives experiments, undertaken at the Defence explosives range. A discussion of the findings is provided with an analysis of the results in the context of clinical blood chemistry changes brought about by exposure to an explosion.

11.2 Explosives events

Two explosives events were performed on the same day, at the same location, within two hours of each other, using the same volume and type of explosive material (5kg C-4 equivalent explosive material). One blast zone site was used for both events. No sample was cooled.

11.2.1 Explosives Test Group

Data from the two explosions, involving 10 active and 10 control samples for each explosion, were analysed simultaneously in two experimental cycles. One control sample was spoiled, it and its paired active sample was removed from the data collective, leaving 19 active and 19 control samples over all. Data collected in the two explosives events collectively included 6 baseline blood gas analyses, the experiment time, 19 active and 19 control post blood gas analyses. Average values were calculated accordingly from 6 baseline, and 19 active and 19 control samples.

Due to logistic and time constraints during the performance of the live blast experiments multiple active and control samples were derived from 6 baseline samples.

The duration time for each of the two experimental cycles was 14.40 minutes and 15.52 minutes respectively. The breakdown of one blood gas analyser in the second cycle accounts for the longer duration time in the latter.

The experimental data are described with respect to their visual appearance and biochemical characteristic as it was in the under-pressure simulation experiments. Visual data included observation of the active samples post-exposure and the results of comparison with the corresponding control samples.

Dissolved carbon dioxide tension was measured in each sample, tested via blood gas analysis, and tested for aligned bicarbonate concentration, pH and serum potassium levels (to assess red cell wall integrity), and these data were compared across active and control samples.

No clotting was evident in the active samples suggesting they were not affected by radiant heat, (as one would expect in a high protein solution if boiling had occurred). From this the standard visual observation was made.

Sixteen (84%), of the active samples contained some bubbling following exposure to the explosion. No bubbling occurred in the control group. Of the 13 bubbled samples, 4 (25%) had foamed according to the assessment protocol laid out in Chapter 6. This was macroscopic evidence of gas bubble formation.

Table 11.1 outlines the bubbling type described.

Table 11. 1 Observed bubbling and foaming phenomenon, Explosive Test group.

Bubbling	*Foaming*
73% (14/19)	28% (4/14)

11.2.2 Sample preservation

As previously described, in Chapter 9, one baseline test was made for each of the three participants blood sample. Sample preservation was confirmed by comparing the baseline and control samples. Table 11.2 shows there was no difference between baseline and control samples, with t-test p values provided.

Table 11.2 Chemical composition of baseline and control samples, depicting preserved samples. Explosives Test group.

	CO_2 (mm Hg)		HCO_3 (mmol/l)		pH	
	Baseline	*Control*	*Baseline*	*Control*	*Baseline*	*Control*
MEAN	44.18	42.71	26.00	24.5	7.37	7.37
STDEV	6.93	7.89	3.78	3.59	0.02	0.05
SEM	1.59	1.90	0.87	0.79	0.00	0.01
95%CI	41.1-47.3	39.2-46.3	24.3-27.7	22.9-26.2	7.36-7.38	7.35-7.39
p value	0.55		0.23		0.98	

11.2.3 Effects of exposure

There was a statistically insignificant (p = 0.809) change in carbon dioxide tension between the active and control groups. The mean carbon dioxide content of the baseline was 44.18mmHg (95% CI 41.06 - 47.30), control samples 42.71mmHg (95% CI 39.16 - 46.26), and the active samples 43.34mmHg (95% CI 39.74 – 46.93). A data table showing the difference between active and control samples is shown as Table 11.3. All 3 sample groups are shown by graph in Figure 11.1, on the following page.

Table 11.3 Data demonstrating the difference in carbon dioxide between active and control samples, Explosives Test group.

	CO_2 (mm Hg)	
	Control	*Active*
MEAN	42.71	43.34
STDEV	7.9	8.0
SEM	1.9	2.1
95% CI	39.2- 46.3	39.7 - 46.9

Figure 11.1 Mean carbon dioxide content in baseline, active and control samples, Explosives

Test group. The p value relates to the active versus the control samples.

Bicarbonate levels were measured to determine whether any acid-base adjustment had occurred. The baseline samples had a mean bicarbonate level of 26.00mmol/l (95% CI 24.30 - 27.70), the control samples 24.55mmol/l (95% CI 22.93 - 26.16), and the active samples 25.02 mmol/l (95% CI 23.22 - 26.81). There was a small loss of bicarbonate in both the active and control samples when compared with the baseline, but no significant change was evident. The t-test comparing the active and control samples returned p = 0.705. These results are shown in Figure 11.2.
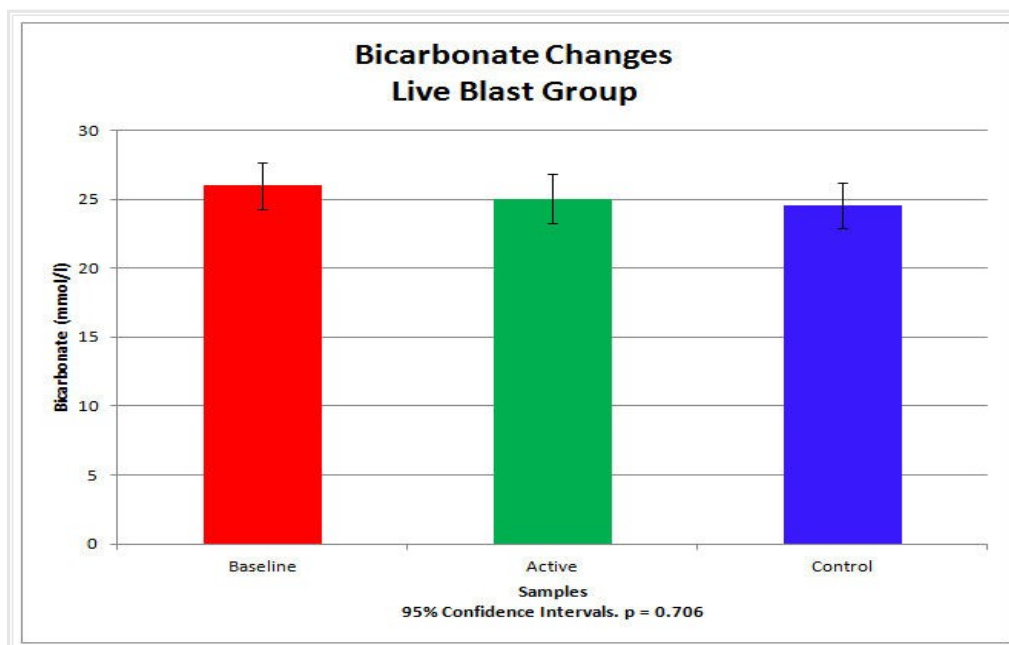
Figure 11.2 Mean bicarbonate levels in baseline, active and control samples, Explosives Test group. The p value relates to the active versus the control samples.

The mean pH values of the active and control samples were not significantly different (p=0.827). The baseline samples' mean was 7.37 (95% CI 7.36 – 7.39), control samples 7.37 (95% CI 7.35 – 7.39), the active samples 7.37 (95% CI 7.35 – 7.39). These data are shown as Figure 11.4.

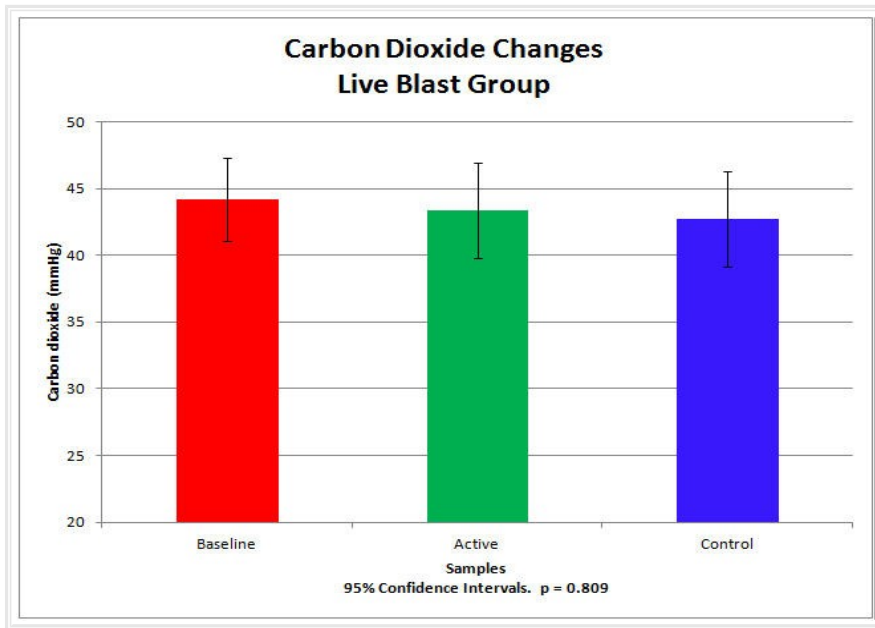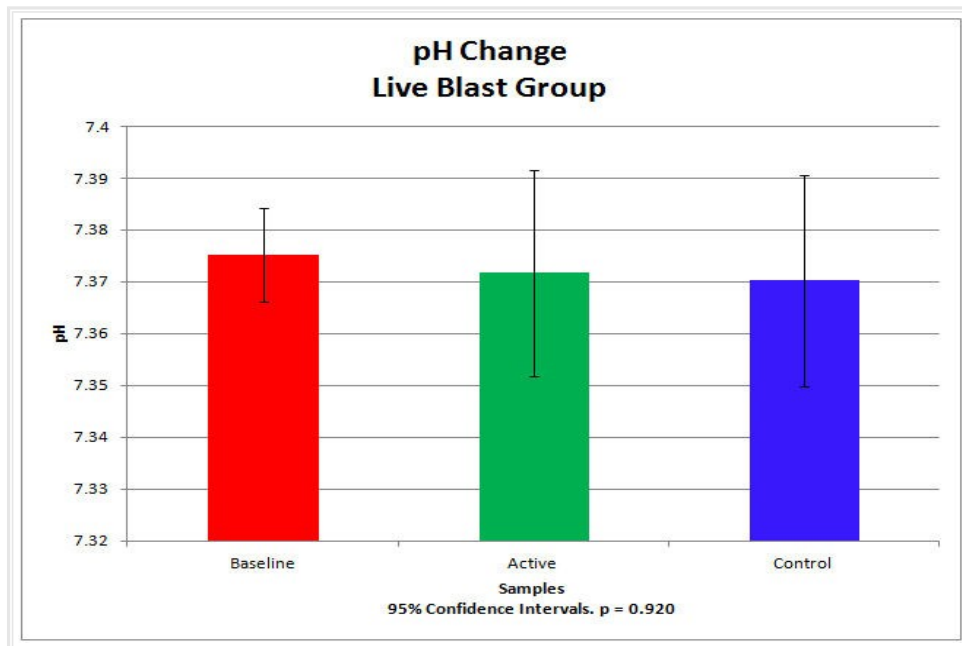**Figure 11.4** Mean pH values in baseline, active and control samples, Explosives Test group. The p value relates to the active versus the control samples.

Serum potassium content increased significantly in the active samples when compared with the control samples. The mean baseline potassium level was 3.93mmol/l (95% CI 3.84 – 4.02), control 3.93mmol/l (95% CI 3.86 – 3.99), and active 5.92mmol/l (95% CI 5.56 – 6.29). A t-test showed there was a significant difference between the active and control potassium levels (p <0.001). This is depicted in Figure 11.5.
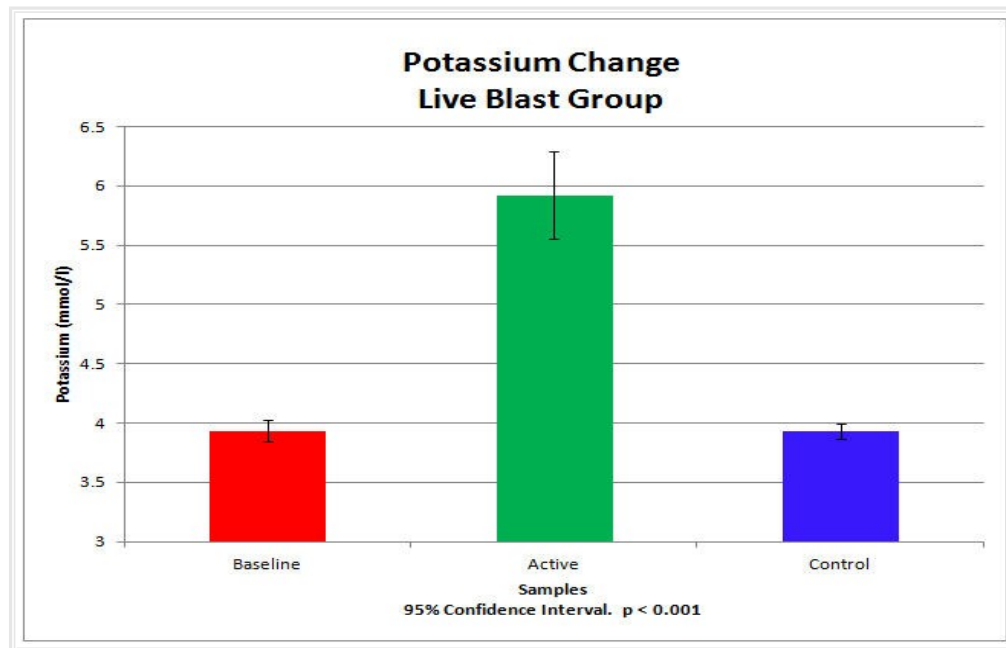
Figure 11.5 Mean potassium levels in baseline, active and control samples, Explosives Test group. The p value relates to the active versus the control samples.

11.3 Discussion – explosives experiment

The blood samples exposed to both the overpressure and under-pressure phases of a blast showed few significant changes when compared with the control samples. The only possible explanation for the differences between the results of this live blast experiment and the

laboratory simulation experiments is the effects of the over-pressure phase. The laboratory simulation experiments supported the hypothesis that carbon dioxide is liberated from blood when exposed to a rapid decompression event demonstrating Henry's Law in action. However, the subtle signal from the under-pressure phase, discernible in the laboratory experiments, was overwhelmed by the positive pressure load during the live blast wave. This is demonstrated by the fact that the erythrocyte walls were damaged in the live blast experiments (proved by the significant change in active sample potassium content) but not in the simulations. Specific results of the live blast experiments and their implications are discussed below with reference to previous research.

11.3.1 Observation of the bubbling

Visual observation was a vital component of the experiment because it provided immediate evidence that blood consistency changed following exposure to the blast wave (bubbling and foaming). However, this does not constitute proof that emboli are formed as a consequence of the under-pressure in the live blast experiment because the blood samples were exposed to both over-pressure and under-pressure in this experiment. Alternative explanations for the bubbling are posited below.

The first possibility is that the over-pressure wave produced in the explosives event was able to force gas past the plunger of the syringe and cause direct mechanical foaming. However if this had occurred, it would inevitably cause a large pressure increase inside the barrel of the syringe dislodging the stopper from the other end. It seems unlikely that the pressure wave acting on the syringe stopper from the outside could resist the hydraulic effect of the fluid column inside the syringe whereas all syringe caps were in place following the experiment.

In effect the column of blood inside the syringe would have become a hydraulic ram and overcome any opposing forces acting on the other end. The syringe validation test exercise, where bubbles were found in the water filled syringe following the explosion exposure also argues against this explanation.

A second possible explanation arises from the idea that bubbling is thought to result from spalling and cavitation during an explosive event, as described in Chapter 4 (Schardin, 1950; Phillips & Richmond, 1991). Cavitation is a precursor to spalling, in which a solid object or debris within a liquid generates turbulence. The inside lining of the syringe barrel used in this experiment was a flat, smooth surface; this product was used in a deliberate effort to counteract cavitation. Despite efforts to counteract the problem, spalling and cavitation might have resulted from cell fragments after erythrocyte rupture during the over-pressure phase, leading to foaming. Visual inspection alone cannot determine if erythrocytes were ruptured.

This visual examination of blood exposed to a blast wave gives rise to two reasonable explanations: either the bubbling occurred by autologous means, caused by the under-pressure wave, or was caused by cavitation and spalling originating from cellular debris resulting from the over-pressure wave. While the latter position is theoretically possible, it does not explain how the bubbling occurred in the laboratory experiments when no over-pressure was applied and the red blood cells remained intact. Either of these explanations offer an alternative theory to the translocation theory.

11.3.2 Carbon dioxide content

The carbon dioxide levels measured in the explosives experiments were not significantly different in the active samples when compared with the control samples. The carbon dioxide measured was that dissolved within the blood; carbon dioxide contained within the erythrocyte was not included. The positive pressure load of the blast damaged the erythrocyte walls enabling cell contents, including carbon dioxide, to leak into the extracellular blood sample, muddying the comparison of active and control samples.

As noted in chapter 2, Tsokos et al. (2003) studied the type and degree of damage to human red blood cells in blast providing the most detailed scrutiny of structural change to date. However, the level of damage in volume and consequence to that damage was not explored. Determining the precise degree of damage to red blood cells and the consequence of that requires research using an animal model or a simulated blood vessel and would be of benefit in the future. Observations made of the potassium level, further onward in this chapter, assist in explaining red blood cell integrity.

11.3.3 Raised carbon dioxide, raised bicarbonate and stable pH.

Lack of significant change in dissolved carbon dioxide concentration as a result of the blasts was matched by the lack of significant change to bicarbonate levels and a stable pH, however the subtle direction of the shift (slight rise when compared with the control) in the bicarbonate was consistent with its natural tendency to buffer as a consequence of the rise in carbon dioxide, and the pH was maintained. As was argued following the simulation experiments

214

described in chapter 8, these outcomes suggest the carbon dioxide change, although noet significant, in the active samples was true.

11.3.4 Serum potassium rise

The rise in serum potassium content in the active samples showed haemolysis of the sample occurred. Thanks to Tsokos et al. (2003), we know blood cells can rupture during blast exposure and thereby increase a potassium level (Shara & Ayling, 2009). As previously described in the simulation experiments where there was no rise, this rise in potassium is the marker that explains why carbon dioxide is raised and not lowered in the blast events.

11.4 Synopsis – explosives experiment

The results presented in this chapter do not preclude the possibility that a portion of carbon dioxide dissolved in the blood has been liberated as during the under-pressure phase.

While some evidence for emboli development due to blast was observed in the foaming samples, the live explosive experiment did not provide statistical evidence as to the gas the bubbles (or emboli in pathological terms) contained. As noted above, the dissolved carbon dioxide measurements in this experiment cannot confirm that the gas was released from the blood because the carbon dioxide content in the active samples may have increased by other means. Equally, the experiment cannot confirm that cavitation and spalling did not play a role in the bubbling phenomenon because the erythrocyte walls were disrupted leaving the environment ripe for spalling and cavitation from the cell fragments.

The foaming in the active samples, suggests that emboli are not a consequence of pulmonary infrastructure injury as most of the blast injury literature assumes, and the alternative autologous theory and the effects of the under-pressure wave in blast are reasonable targets for future research.

While this one experiment fell short to prove the gas liberated from the blood sample was carbon dioxide, the experiment has offered evidence that translocation via damaged lungs may not be the only source of gas emboli evolution in blast. This area of blast injury demands further research using a deductive processes such as this research work has. Determining changes in blood using a gas analyser in explosives research is not previously documented in the literature, thereby this new information, using this technique provides a valuable stepping stone for future blast research.

Chapter 12

Summary

12.1 Outline

This research produced a new fundamental understanding of blast and blast injury as it relates to microscopic gas emboli. It began with a comprehensive review of the literature, focusing on three theories of the development of microscopic gas emboli, and their veracity. The review demonstrated deficiencies in the evidence and our knowledge of how microscopic gas emboli form in humans due to blast exposure, revealing the weakness of the currently accepted theory.

The focus of the thesis then turned to the most biologically plausible of the three remaining theories of microscopic gas emboli formation, the autologous theory, and how that might be tested in an experimental program. The hypothesis formed on the basis of the literature review was that bubbling forms in blood as a direct result of its exposure to a rapid decompression such as is experienced during the under-pressure phase of a blast wave.

The experimental program involved three information components: a database, laboratory experiments and live explosives experiments. Data from existing experimental work were combined into a database of blast wave parameters. In conjunction with the parameters for a rapid decompression event (a rapid drop in surrounding pressure within 0.05 seconds) (Australian Transport Safety Board, 2009; Federal Aviation Administration, 2005), these parameters were used to support the design of the simulation apparatus for the laboratory experiments.

To examine the effect of a rapid decompression on human blood, samples of blood were exposed to a rapid decompression using an under-pressure generator in laboratory conditions.

Finally, field-work using live explosions was conducted, also using human blood samples. The simulation phase showed that a sub-atmospheric exposure could liberate carbon dioxide from its dissolved state in blood. The live blast phase produced less controlled but more natural observation, and enabled evaluation of the influence of over-pressure, on the results.

This research advanced our understanding of how microscopic gas emboli form in human blood as a result of blast exposure, in particular by showing that the rapid decompression effect liberates a dissolved gas from blood to gas bubble, hence supporting the autologous theory and providing evidence against the translocation theory. The details of the findings and their specific contributions to knowledge are described in the following sections.

12.2 Blast parameters – the database

The primary step was the collation of baseline data for the experimental phase from blast researchers at DSTO, Adelaide. Analysis of the database confirmed that the under-pressure phase of a blast wave lasts considerably longer than its over-pressure counterpart; the database parameters were consistent with previous researchers' findings. It also confirmed that the under-pressure phase wave approaches a vacuum state, -100 kPa or -1 atmosphere. In addition, analysis of the DSTO blast database enabled the determination of parameters of a variety of points of interest along the blast wave timeline, revealing complexities of the sub-atmospheric wave that have been the subject of little or no investigation, and are hence poorly understood despite their potential importance to clinical medicine.

12.3 Under-pressure simulations - the laboratory experiments

Samples of human blood visibly bubbled when subjected to the rapid sub-atmospheric pressure in a custom made rapid decompression generator. Comparison of blood gas analyser measurements of the control and active samples showed a significant loss of dissolved carbon dioxide and corresponding acid-base adjustment.

The results of the simulated blast exposure, in the absence of lung injury (because the blood samples were isolated from a live/active lung), underscore the weakness of the translocation theory because bubbling was observed and a dissolved gas was liberated from the blood.

12.4 The explosives experiments

Samples of human blood exposed to live explosions bubbled visibly inside their sealed containers; however, the mean carbon dioxide content of the active samples post exposure was not significantly different from that of their partnered controls. The dissolved carbon dioxide measurement was obscured by the effects of the positive pressure phase, which caused the erythrocytes to rupture and release intracellular ions and compounds into the extracellular compartment. Although this finding did not provide clear evidence in support of the autologous theory for microscopic gas emboli formation (due to the effects of the positive pressure phase, as just mentioned) it justifies continuation of the search for mechanisms of emboli development other than translocation because the bubbling in blood could not possibly have resulted from translocation via damaged pulmonary architecture.

12.5 The project's accomplishments

Previous research on microscopic gas emboli in blast has been retrospective, consisting mostly of post-mortem examination within the context of primary lung injury. The research described in this thesis is the first to test both the effects of rapid decompression on human blood, and directly assess the resulting carbon dioxide changes in exposed human blood using blood gas analysis.

The use of blood gas analysis in this project revealed substantial physical and chemical alterations in blood resulting from blast pressure differentials strongly suggesting that translocation is not the likely mechanism of microscopic gas emboli development in blast, and that instead the autologous theory is a scientifically plausible alternative. This translates to the real possibility that emboli are more common in blast victims than previously thought, because it shows primary lung injury is not a necessary pre-cursor for microscopic gas emboli. In this way the evidence arising from this project implies that a greater proportion of blast victims will develop microscopic gas emboli than previously thought, because emboli are likely to arise in those suffering secondary and tertiary blast injuries.

Finally, this research's confirmation that the emboli generated in blood through rapid decompression consist of carbon dioxide is important because this information enables prediction of emboli behaviour. Emboli cause immediate damage to the endothelial environment: knowing that they are composed of carbon dioxide means their presence is most likely to be noted only retrospectively, leaving a search for them through the pursuit for a specific biomarker, this may forge new research pathways and assist clinicians in their management of victims.

12.6 Prospects for future work

By showing that the accepted translocation theory is unlikely for all emboli development in blast, and simultaneously providing some evidence for the plausibility of the autologous theory, and doing so using a novel but simple and reproducible method, this research provides a sound platform for further work on blast emboli development in human survivors. Two future research opportunities are outlined in the following section.

12.6.1 The translocation theory and the cavitation theory

The logical extension of the research described in this thesis would be to provide conclusive evidence about the veracity of the translocation theory of microscopic gas emboli development using a live animal model. Blood gas analysis of carbon dioxide loss in the blood of a live animal given a blunt (not penetrating) non primary blast injury would augment the evidence that this thesis has produced. A further refinement would be to tag the animal's inspired gas with a radiological marker to highlight any gas transfer across alveoli and pulmonary capillaries, thereby providing a definitive test of the translocation theory.

Future blast research addressing cavitation, in the absence of lung injury, would also be advantageous given that this thesis has shown the evidence for the translocation theory is weak, and the bubbling in the explosives tests was inconclusive. The long held belief is that cavitation occurs in primary lung injury, if the bubbling in the explosives experiments was caused by cavitation from cell fragments, then this is a new research opportunity.

12.6.2 Mild traumatic blast brain injury and biomarkers

The number of military personnel who survive blasts continues to grow, thanks to improved protective technologies and sophisticated treated regimes; somewhat paradoxically, this means minor blast injury or exposure to blast is a growing clinical problem. The mechanisms of mild traumatic blast brain injury resulting from blast exposure are the subject of continuing research.

Recent work has uncovered a link between mild traumatic blast brain injury and immuno-chemical responses in the circulatory system, (Gorbunov et al. 2008; Ling et al. 2008; McDonald et al. 2011). As mentioned previously, it has been known for some time that emboli cause endothelial damage alongside a systemic inflammatory response (Kapoor & Gutierrez, 2003). Research continues to discover new information about the response of the glycocalyx and its related blood vessel endothelium to inflammatory responses, and most recently fluid movement (Johansson et al. 2011; Van den Berg et al. 2006; Woodcock & Woodcock, 2012).

Microscopic carbon dioxide emboli, if forming in capillaries or possibly within the glycocalyx itself, would cause transient disruption to the endothelium whilst simultaneously setting up a cascade of inflammatory response for a time after emboli resolve as the gas returns to solution. Multidisciplinary research into blast induced emboli and the associated inflammatory responses, building on recent findings about glycocalyx function, may prove beneficial to uncovering the origins of mild traumatic blast brain injury.

12.7 Conclusion

This research tested the validity of the translocation theory, long held to be the only explanation for the development of microscopic gas emboli in blast exposure. Applying a basic scientific principle to the problem, by way of Henry's Law, provides credibility to the autologous theory when no absolute explanation was available supporting the translocation theory. These results were obtained through simulation and live blast experimentation, including the use of modern blood analysing techniques not previously used in blast or blast related research, and produced physical changes in exposed human blood and evidence that emboli consisted of carbon dioxide. These findings, show that the translocation theory is unlikely to be correct, simultaneously suggesting the autologous theory is plausible. Incidental observations have found that the spalling and cavitation theory in blast may benefit from further research as well.

The research involved the first known live blast experimentation using human blood in Australia; it offers a pathway for further exploration of live blast experimentation. It is hoped future research will lead to even greater improvements in our knowledge of microscopic gas emboli formed during blast exposure and methods of improving the health outcomes of blast victims.

Reference List

References

Alexander, J 1995, *Fin De Siecle,* Social Theory: Relativism, Reductionism and The Problem of Reason, Verso, London.

Alfici, R Ashkenazi, I Kessel, B 2006, 'Management of victims in a mass casualty incident caused by a terrorist bombing: treatment algorithms for stable, unstable and in extremis victims', *Military Medicine,* vol. 171, no. 12, pp. 1155-1162.

American College of Surgeons, 2008, 'Chapter I: Initial assessment and management', in American College of Surgeons (eds), *Advanced trauma life support for doctors; student course manual*, 8th Edition, Chicago, Il. USA.

Argyros, G 1997, 'Management of primary blast injury, *Toxicology,* 121, pp. 105-115.Arnold, J Halpern, P Tsai, M 2004, 'Mass casualty terrorist bombing: A comparison of outcomes by bombing type', *Annals of Emergency Medicine,* 43, pp. 263-271.

Auge, B Preminger, G 2002, 'Update on shock wave lithotripsy technology', *Current Opinion in Urology,* vol. 12, no.4, pp. 287-90.

Australian Government, Defence, Bushmaster Australia, Australian Government, viewed 20 November 2011,

http://www.army.gov.au/Our-work/Equipment-and-clothing/Vehicles/Bushmaster

Australian Transport Safety Bureau, Aviation Research and Analysis Report, 2009, Australian Government, viewed 22 October 2013.

http://www.atsb.gov.au/media/27376/ar2008075_2.pdf

Axelsson, H Hjelmqvist, H Medin, A Persson, J Suneson, A 2000, 'Physiological changes in pigs exposed to a blast wave from a detonating high explosive charge', *Military Medicine,* February, 165, pp. 119-126.

Axelsson, H Yelverton, JT 1996, 'Chest wall velocity as a predictor of non auditory blast injury in a complex wave environment', *Journal of Trauma*, 40, S31-37.

Bass, CR Rafaels, KA Salzar, RS 2008, 'Pulmonary injury risk assessment for short-duration blasts', *Journal of Trauma*, vol. 65, no. 3, pp. 604-615.

Benzinger, T 1950, 'Chapter XIV-B, Physiological effects of blast in air and water', *German aviation medicine, World War II,* vol. 2, Department of Air Force, Washington DC, USA.

Bellani, G Guerra, L Musch, G Zanella, A Patroniti, N Mauri, T Messa, C Pesenti, A 2011, 'Lung Regional Metabolic Activity and Gas Volume Changes Induced by Tidal Ventilation in Patients with Acute Lung Injury', *American Journal of Respiratory and Critical Care Medicine*, vol. 183, no.9, pp. 1193-1199.

Bochicchio, G Lumpkins, K O'Connor, J Simard, M Schaub, S Conway, A Bochicchio, K Scalea, T 2008, 'Blast Injury in a civilian setting is associated with a delay in diagnosis of traumatic brain injury', *The American Surgeon,* vol 74, no.3: pp. 267-270.

Bogdan, C 2001, 'Nitric oxide and the immune response', *Nature Immunology,* 2, pp. 907-916.

Bowen, I Fletcher, E Richmond, D 1968, *Estimate of man's tolerance to the direct effects of air blast,* Headquarters defense atomic support agency, Washington DC, 20305, Lovelace Foundation for Medical Education and Research, Albuquerque, New Mexico, USA, 87108, DA-49-146-XZ-372

Brady, J Holum, J 1993, *Chemistry the study of matter and its changes,* Wiley & Sons, New York.

Branger, A Eckmann, D 1999, 'Theoretical and experimental intra-vascular gas embolism absorption dynamics', *Journal of Applied Physiology,* 87, pp. 1287-1295.

Brune, B Van Knethen, A Sandau, K 1999, 'Nitric oxide: an effector of apoptosis', *Cell Death and Differentiation,* vol. 6, issue 10, pp. 969-975.

Burton D, 2005, *The history of mathematics: an introduction,* 6th Edition, McGraw-Hill, New York.

Cannon, L 2001, 'Behind armour blunt trauma - an emerging problem', *Journal of the Royal Medical Corps,* vol. 147, pp 87-96.

Cavanagh, D Eckmann, D 1999, 'Interfacial mechanics of stationary bubbles in flows in inclined tubes', *Journal of Fluid Mechanics,* vol. 398, pp. 225-244.

Chaloner, E 2005, 'Blast injury in enclosed spaces', *British Medical Journal,* vol. 331, p. 119.

Chang R, 1988, *Chemistry,* Random House, New York, 3rd Edition.

Chapman, G 2010, Viewed September 15, 2010.

http://news.theage.com.au/breaking-news-world/california-gas-explosion-kills-one-destroys-homes-20100910-154i1.html

Chen, H Wang, Z Ye, Z 2000, 'Rabbit lung injury induced by explosive decompression', *Chinese Journal of Traumatology,* vol. 3, no. 2, pp. 81-84.

Chiffelle, TL 1966, *Pathology of direct air blast injury,* Technical Progress Report, Lovelace Foundation for Medical Education and Research, Department of Defense, Albuquerque, New Mexico, United States of America.

Ciraulo, D Frykberg, E 2006, 'The surgeon and acts of civilian terrorism: blast injuries', *Journal of the American College of Surgeons,* vol. 203, no. 6, pp. 942-950.

Clemedson, CJ Hultman, H Gronberg, B 1953, 'Respiration and pulmonary gas exchange in blast injury', *Journal of Applied Physiology,* vol. 6, pp. 213-220.

Clemedson, CJ Hultman, H 1954, 'Air embolism and the cause of death in blast injury', *Military Surgeon,* vol. 114, pp. 424-437.

Conover, M 2002, *Understanding Electrocardiography*, Eighth Edition, Mosby Inc. Harcourt Health Sciences, St Louis.

Cooper, G Maynard, R Cross, N Hill, J 1983, 'Casualties from terrorist bombings', *Journal of Trauma*, vol. 23, no. 11, pp. 955 – 967.

Cooper, G Townsend, D Cater, S Pearce, B 1991, 'The role of stress waves in thoracic visceral injury from blast loading: modification of stress transmission by foams and high density materials', *Journal of Biomechanics*, vol. 24, no. 5, pp. 273-285.

Department of Human Services -Victoria, 2007, Terrorist Medical Training Database.

Desaga, H 1950, 'Chapter IV-D, Blast injuries', *German aviation medicine, world war II*, vol. 2, Department of Air Force, Washington DC, USA.

DePalma, R Burris, D Champion, H Hodgson, M 2005, 'Blast injuries', *The New England Journal of Medicine*, vol. 352, pp. 1335-1342.

Divinis, N Karapantsios, T Kostoglou, M Panoutsos, C Bontozglou, V Michels, A Sneep, M De Bruijn, R Lotz, H 2004, 'Bubbles growing in supersaturated solutions at reduced gravity', *American Journal of Chemical Engineers*, vol. 50, no. 10, pp. 2369-2382.

Dixon, C Millar, B 2014, viewed January 7, 2014,

http://www.theage.com.au/victoria/gas-explosion-a-devastating-end-to-a-routine- police-callout-20140106-30dm6.html

Dobnik, V 2014, viewed March 14, 2014,

http://www.theage.com.au/world/new-york-buildings-collapse-searchers-scour-rubble-for-survivors-after-gas-explosion-kills-7-20140314-hviht.html

Drew, P Helps, S Smith, E 1995, 'Cerebral arterial fat embolism in the rabbit', *Journal of the Neurological Sciences,* vol. 134, pp. 15-20.

Duff, R Blackwell, A 1966, 'Explosive driven shock tubes', *Review of Scientific Instruments,* vol. 37, no. 5, pp. 579-586.

Dunn, K Shulman, S Stock, A Naeher, L 2013, 'Personal carbon monoxide exposures among firefighters at prescribed forest burns in southeastern United States', *Archives of Environmental and Occupational Health,* vol. 68, no. 1, pp. 55-59.

Durant, T Long, J Oppenheimer, M 1947, 'Pulmonary venous air embolism', *American Heart Journal,* vol. 33, no. 3, pp. 269-281.

Eckmann, D Armstead, S 2006, 'Influence of Endothelial Glycocalyx Degradation and Surfactants on Air Embolism Adhesion', *Anaesthesiology,* vol. 105, no. 6, pp. 1220-1227.

Elsayad, N Gorbunov, N 2008, 'Chapter 8, Biochemical mechanism(s) of primary blast injury: the role of free radicals and oxidative stress', in Elsayad, N Atkins, J (eds), *Explosion and Blast Related Injuries,* Elsevier Academic Press, London.

Encyclopaedia Britanica, 2013, viewed 11 December 2014,

http://www.britannica.com/EBchecked/topic/416842/Alfred-Bernhard-Nobel

Federal Aviation Administration, 2005, *Operations of aircraft at altitudes above 25,000 feet MSL and/or mach numbers (MMO) greater than 7.5,* Department of Transportation, United States of America.

Francis, T Mitchell, S 2004, Chapter 8, 'Pathophysiology of decompression sickness', in Bove, AA (ed), *Davis & Bove's Diving Medicine,* Elseiver Inc., Philadelphia, PA.

Frykberg, E 2002, 'Medical Management of Disasters and Mass Casualties from Terrorist Bombings: how can we Cope?', *The Journal of Trauma, Injury, Infection and Critical Care,* vol. 53, no. 2, Aug, pp. 201-212.

Garner, J Watts, S Parry, C Bird, J Kirkman, E 2009, 'Development of a large animal model for investigating resuscitation after blast and haemorrhage', *World Journal of Surgery,* vol. 10, no. 33, pp. 2194-2202.

Gaudin P, 1887, 'On the pathogenesis of accidents in consecutive explosions in the firedamp', (Sur la Pathogenie des Accidents Nerveux Consecutifs aux Explosions du Grisou), Le Doctorat en Medecine, Paris.

Gorbunov, N McFaul, S Van Albert, S Morrissette, C Zaucha, G Nath, J 2004, 'Assessment of inflammatory response and sequestration of blood iron transferrin complexes in a rat model of

lung injury resulting from exposure to low-frequency shock waves', *Critical Care Medicine,* vol. 32, no. 4, pp. 1028-1034.

Gorbunov, N McFaul, S Januszkiewicz, A Atkins, J 2005, 'Pro-inflammatory alterations and status of blood plasma iron in a model of blast induced lung trauma', *International Journal of Immunopathology and Pharmacology,* vol. 18, no. 3, pp. 547-556.

Gorbunov, N Asher, L Elsayad, N Atkins, J 2008, 'Chapter 9, Inflammatory response in primary blast injury', in Elsayad, N Atkins, J (Eds), *Explosion and Blast Related Injuries,* Elsevier Academic Press, London.

Grimshaw, J Eccles, M Lavis, J Hill, S Squires, J 2012, 'Knowledge translation of research findings,' *Implementation Science,* 7 : 50, pp. 1-17, viewed 20 January 2014,

http://www.implementationscience.com/content/pdf/1748-5908-7-50.pdf

Guy, R Glover, M Cripps, N 1998, 'The pathophysiology of primary blast injury and its implications for treatment. Part I: The thorax', *Journal of the Royal Naval Medical Service,* vol. 84, no. 2, pp. 79-86.

Guy, R Kirkman, E Watkins, P Cooper, G 1998, 'Physiologic responses to primary blast', *The Journal of Trauma, Injury Infection and Critical Care,* vol. 45, no. 6, pp. 983-987.

Guy, R Watkins, P Edmonstone, W 2000, 'Electrocardiographic changes following primary blast injury to the thorax', *Journal of the Royal Naval Medical Service,* 86.3, pp. 125-133.

Harding, JE 1996, 'ECG changes in victims of anti personnel land-mines', Proceedings of Australia and New Zealand Intensive Care Scientific Meeting, October, Melbourne, Australia.

Harding, JE 2000, Personal diary, East Timor, Officer in Charge – Intensive Care Unit.

Herbert, C Johnstone, R 2002, Chapter 1, The ionisation process, *'Mass spectrometry basics'*, CRC Press, USA.

Hill, R Miller, C Tucker, A 1994, 'Influence of carbon dioxide on venous gas emboli production during altitude decompression in goats'. *Aviation, Space and Environmental Medicine*, vol. 65, no. 2, pp. 139-143.

Hirshberg, B Oppenheim-Eden, A Pizov, R Sklair-Levi, M Rivkin, A Bardach, E Bublil, M Srung, C Kramer, M 1999, 'Recovery from blast lung injury, one year follow up', *Chest,* vol. 116, no. 6, pp. 1683-1688.

Ho, A Ling, E 1999, 'Systemic air embolism after lung trauma', *Anaesthesiology,* vol. 90, no. 2, pp. 564-575.

Ho, A 2002, 'A simple conceptual model of primary pulmonary blast injury', *Medical Hypothesis,* vol. 59, no. 5, pp. 611-613.

Hoge, C McGurk, D Thomas, J Cox, A Engel, C Castro, C 2008, 'Mild traumatic brain injury in U.S. soldiers returning from Iraq', *The New England Journal of Medicine,* vol. 358, no. 5, pp. 453-463.

Horgan, J 1992, 'The intellectual warrior', *Scientific American,* November, pp. 20-21.

Horrocks, C Brett, S 2000, 'Blast injury', *Current Anaesthesia and Critical Care,* vol. 11, pp. 113-119.

Horrocks, C 2001, 'Blast injuries: biophysics, pathophysiology and management principles', *Journal of the Royal Army Medical Corps,* vol. 147, pp. 28-40.

Ikonomidis, C Lang, F Radu, A Berger, M 2012, 'Standardizing the diagnosis of inhalation injury using a descriptive score based on mucosal injury criteria', *Burns,* vol. 38, no. 4, pp. 513-519.

Iremonger, M 1997, Chapter 15, 'Physics of detonations and blast waves', in (Eds), Cooper, G Dudley, H Gann, D Little, R Maynard, R *Scientific Foundations of Trauma,* Butterworth & Heinmann, Oxford, United Kingdom.

Irwin, R Lerner, M Bealer, J Mantor, P Brackett, D Tuggle, D 1999, 'Shock after blast wave injury is caused by a vagally mediated reflex', *Journal of Trauma-Injury, Infection and Critical Care,* vol. 47, no. 1, pp. 105-110.

Jaffin, J McKinney, L Kinney, K Cunningham, J Moritz, D Kraimer, J Graeber, G Moe, J James, S Harmon, J 1987, 'A laboratory model for studying blast overpressure injury', *Journal of Trauma-Injury, Infection and Critical Care,* vol. 27, no. 4, pp. 343-458.

Januszkiewicz, A Mundie, T Dodd, K 1997, 'Maximal exercise performance-impairing effects of simulated blast overpressure in sheep', *Toxicology,* vol. 121, no.1, pp. 51-63.

Johansson, P Stenseballe, J Rasmussen, L Ostrowski, S 2011, 'A high admission syndecan-1 level, a marker of endothelial glycocalyx degradation, is associated with inflammation, protein C depletion, fibrinolysis, and increased mortality in trauma patients,' *Annals of Surgery,* vol. 254, issue 2, pp. 194-200.

Kapoor, T Gutierrez, G 2003, 'Air embolism as a cause of systemic inflammatory response syndrome: A case report', *Critical Care,* vol. 7, pp. r98-r100.

Katz, E Ofek, B Adler, J Abramawitz, H Krausz, M 1989, 'Primary blast injury after explosion in a civilian bus', *Annals of Surgery,* vol. 209, no. 4, pp. 484-488.

Kauver, D Wolff, S Wade, C Cancio, L Renz, E Holcomb, J 2006, 'Burns sustained in combat explosions in operations Iraqi and enduring freedom (OIF/OEF) explosion burns', *Burns,* November, vol. 32, no. 7, pp. 853-7. E-publication, viewed 8 August 2008.

Kauver, D 2008, 'Chapter 6, Quaternary blast injury: burns', in Elsayad, N Atkins, J (eds), *Explosion and blast related injuries,* Elsevier Academic Press, London.

Kebarie, P Tang, L 1993, 'From ions in solution to ions in the gas phase: the mechanism of electro spray mass spectrometry', *Analytical Chemistry,* vol. 65, no. 22, pp. 972A-986A.

Kemph, J Hitchcock, F 1952, 'Changes in blood and circulation of dogs following explosive decompression to low barometric pressures', *American Journal of Physiology*, vol. 168, no. 3, pp. 592- 600.

Kilner, T 2000, 'Chapter 1, Pre Hospital Care', in Dolan, B Holt, L (eds), *Accident and emergency: theory into practice*, Bailliere Tindall, Edinburgh.

Kindwall, E 2004, 'Chapter 1, A Short History of Diving Medicine', in Bove, AA (ed), *Davis & Bove's Diving Medicine*, Elseiver Inc., Philadelphia, PA.

Kirkman, E Watts, S Sapsford, W Sawdon, M 2008, 'Chapter 5, Effects of blast injury on the autonomic nervous system and the response to resuscitation', in Elsayad, N Atkins, J (eds), *Explosion and blast related injuries*, Elsevier Academic Press, London.

Kirkman, E Watts, S 2010, 'Characterization of the response to primary blast injury', *Philosophical Transactions of the Royal Society – Biological Sciences*, vol. 366, no. 1562, pp. 286-290.

Kirkman, E Watts, S Cooper, G 2011, 'Blast injury research models', *Philosophical Transactions of the Royal Society – Biological Sciences*, vol. 366, no. 1562, pp.144-159.

Kluger, Y Peleg, K Daniel-Aharonson, L Mayo, L 2004, 'The special injury pattern in terrorist bombings', *Journal of American College of Surgeons*, December, vol.199, no.6, pp. 875-879.

Krauthammer, T Altenberg, A 2000, 'Negative phase of blast effects on glass panels', *International Journal of Impact Engineering*, 24, pp. 1-17.

Latner, AL 1942, 'The low pressure phase of blast', *The Lancet*, vol.11, pp. 303-304.

Lavonas, E Pennardt, A 2006, 'Blast injuries', *eMedicine,* pp. 1-11, viewed 11 January, 2006, http://www.emedicine.com/emerg/topic63.html

Lavery, G Lowry, K 2004, 'Management of blast injuries and shock lung', *Current Opinion in Anaesthesiology,* Apr., vol. 17, no. 2, pp. 151-157.

Lee Hamm, I Hering-Smith, K Nakhoul, N 'Acid-base and potassium homeostasis'. *Seminars in Nephrology,* 2013, 33, pp. 257-64, viewed 5 November, 2014. http://acutecaretesting.org/en/journal_scanner/journals/on-the-relationship-between-potassium-and-acid-base-balance

Leung, L VandeVord, P Dal Cengio, A Bir, C Yang, A King, A 2008, 'Blast related neurotrauma: a review of cellular injury', *Molecular and Cellular Biomechanics*, vol. 5, no. 3, pp. 155-168.

Lindqvist, K Holmberg, G Peeker, R Grenabo, L 2006, 'Extra corporeal shock-wave lithotripsy or ureteroscopy as primary treatment for ureteric stones: A retrospective study comparing two different treatment strategies', *Scandanavian Journal of Urology and Nephrology,* vol. 40, no. 2, pp. 113-8.

Ling, G Bandak, F Grant, G Rocco, A Ecklund, J 2008, 'Chapter 4, Neurotrauma from explosive blast', in Elsayad N, Atkins J (eds), *Explosion and blast related injuries*, Elsevier Academic Press, London.

Long, J Bentley, T Wessner, K Cerone, C Sweeney, S Bauman, R 2009, 'Blast overpressure in rats: recreating battlefield injury in the laboratory', *Journal of Neurotrauma*, vol. 26, no. 6, pp. 827-840.

Lough, M 2009, 'Unit III: Chapters 16, 17, 18', in Urden, L Stacy, K Lough, M (eds), *Thelma's Critical Care Nursing*, 6th Edition, Mosby Inc. USA.

Malhotra, M Wright, C 1960, 'Arterial embolism during decompression and its prevention', *The Royal Society*, B154, pp. 418-427.

Marini, J Truwitt, J 1998, 'Chapter 13, Monitoring the respiratory system', in Hall, J Schmidt, G Wood, L *(eds), Principals of Critical Care*, 2nd Edition, McGraw-Hill, USA.

Mason, W Van, H Damon, E Dickinson, A Nevison, T 1971, 'Arterial gas emboli after blast injury', *Experimental Biology and Medicine,* vol. 136, pp. 1253-1255.

Maynard, R Coppel, D Lowry, K 1997, 'Chapter 17, Blast injury of the lung', in Cooper, G Dudley, H Gann, D Little, R Maynard, R (eds), *Scientific Foundations of Trauma,* Butterworth & Heinmann, Oxford, United Kingdom.

Mayne, P 1994, 'Chapter 4', in *Clinical chemistry: diagnosis and treatment,* 6th Edition, Arnold Publishers, London.

Mayo, A Kluger, Y 2006, 'Blast induced injury of air containing organs', *Australian Defence Health,* vol. 7, pp. 40-44.

McDonald, C Johnson, A Cooper, D Nelson, E Werner, N Shimony, J Snyder, A Raichle, M Witherow, J Fang, R Flaherty, S Brody, D 'Detection of blast related traumatic brain injury in US military personnel', 2011, *New England Journal of Medicine,* vol. 364, June, 2, pp. 2091-2100.

McKinley, M O'Loughlin, V 2012, *Human Anatomy,* McGraw-Hill, NYC, 3rd Edition. p. 30-31.

Mellor, S 1997, 'Chapter 19, Terrorist bombings: pattern of injury' in Cooper, G Dudley, H Gann, D Little, R Maynard, R (eds), *Scientific foundations of trauma,* Butterworth & Heinmann, Oxford, United Kingdom.

Mellor, S Dodd, K Harmon, J Cooper, G 1997, 'Chapter 4, Ballistic and other implications of blast', in Ryan, J Rich, N Dale, R Morgans, B Cooper, G (eds), *Ballistic trauma: clinical relevance in peace and war,* Arnold Publishing, London, United Kingdom.

Metherill, L 2003, ABC News, viewed 25 Oct. 2013, http://www.abc.net.au/news/2013-10-23/rfs-finds-defence-caused-lithgow-blaze/5041650.

Mitchell, S Gorman, D 2002, 'The pathophysiology of cerebral gas embolism', *Extra Corporeal Technology,* March, vol. 34, no. 1, pp. 18-23.

Moholkar, Vijayanand, S Pandit, Aniruddha, B 1997, 'Bubble behaviour in hydrodynamic cavitation: effect of turbulence', *American Institute of Chemical Engineers Journal,* vol. 43, no. 6, pp. 1641–1648.

Moran, J Bersten, A Solomon, P 2005, 'Meta-analysis of controlled trials of ventilator therapy in acute lung injury and acute respiratory distress syndrome: an alternative perspective,' *Intensive Care Medicine,* vol. 31, pp. 227-235.

Muth, C Shank, E 2000, 'Gas embolism', *The New England Journal of Medicine,* vol. 342, no. 7, pp. 476-482.

Neuman, T 2004, 'Chapter 9, Pulmonary barotrauma', in Bove, AA (ed), *Davis & Bove's Diving Medicine,* 4th Edition, Elseiver Inc., Philadelphia, PA.

Ngo, T Mendis, P Gupta, A Ramsay, J 2007, 'Blast loading and blast effects on structures: an overview' *Electronic Journal of Structural Engineering,* pp. 76-91, viewed 14 July 2013. http:://www.ejse.org

Ogle, RW 1951, Polyethylene disposable syringe, US patent 2693803.

Okudera, H 2002, 'Clinical features on nerve gas terrorism in Matsumoto', *Journal of Neuroscience,* vol. 9, Issue 1, pp. 17-21.

Palmer ED 1954, Investigation of the gastric mucosa spirochetes of the human, Gastroenterology, vol. 27, no. 2, pp. 218-220.

Peleg, K Aharonson-Daniel, L Michael, M Shapira, S and Israel Trauma Groups, 2003, 'Patterns of injury in hospitalised terrorist victims', *American Journal of Emergency Medicine,* vol. 21, no. 4, July, pp. 258-262.

Petri, N Dujella, J Definis-Gojanovic, M Vranjkovic-Petri, L Cuculic, D 2001, 'Diving fatalities caused by underwater explosions: a report of two cases', *The American Journal of Forensic Medicine and Pathology,* vol. 22, no. 4, pp. 383-386.

Phillips, Y Richmond, D 1991, 'Chapter 6, Primary blast injury and basic research: a brief history', in *Textbook of military medicine,* P*art I: warfare weaponry and the casualty, vol. 5: conventional warfare: ballistic, blast and burn injuries,* The Office of Surgeon General, Dept. of Army, United States of America.

Pizov, R Oppenheim-Eden, A Matot, I Weiss, Y Eidelman, L Rivkind, A Sprung, C 1999, 'Blast lung injury from an explosion on a civilian bus', *Chest,* vol. 115, no. 1, pp. 165-172.

Pomara, C D'Errico, S Riezzo, I Perilli, G Volpe, U Fineschi, V 2013, 'Blast overpressure after tire explosion: a fatal case', *American Journal of Forensic Medicine & Pathology*, vol 34, no. 4, pp. 306-310.

Popper, K 1934, *The logic of scientific discovery,* Routledge Classic: Kindle Edition, May 20 2007, (First printed edition 1934, first translated to English 1959).

Przekwas, A 2008, 'Chapter 7, Multiscale computational modelling of lung blast injuries', in

Elsayad, N Atkins, J (eds), *Explosion and blast related injuries*, Elsevier Academic Press, London.

Putensen, C Theuerkauf, N Zinserling, J Wrigge, H Pelosi, P 2009, 'Meta-analysis: ventilation strategies and outcomes of the acute respiratory distress syndrome and acute lung injury', *Annals of Internal Medicine, v*ol. 151, no. 8, pp. 566-576.

National Health and Medical Research Council, n.d.

http://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/ea16.pdf 2012.

Ramasamy, A Hill, A Hepper, A Bull, A Clasper, J 2009, 'Blast mines: physics, injury mechanisms and vehicle protection', *Journal of the Royal Army Medical Corps,* vol. 155, no. 4, pp. 258-264.

Reneer ,D Hisel, R Hoffman, J Kryscio, R Lusk, B Geddes, J 2011, 'A Multi-Mode shock tube for investigation of blast-induced traumatic brain injury,' *Journal of Neurotrauma*, vol. 28, no. 1 , pp 95-104.

Riley, D Clark, M Wong, T 2002, 'World Trade Centre terror: explosion trauma-blast, burns and crush injury,' *Topics in Emergency Medicine,* June, vol. 24, no. 2, pp. 47-59.

Ripple, G Phillips, Y 1997, 'Chapter 20, Military explosions', in Cooper, G Dudley, H Gann, D Little, R Maynard, R (eds), *Scientific foundations of trauma,* Butterworth & Heinmann, Oxford, United Kingdom.

Rosenfeld, J Fitzgerald, M Kossmann, T Pearce, A Joseph, A Tan, G Gardner M Shapira, S 2005, 'Is the Australian hospital system adequately prepared for terrorism?' *The Medical Journal of Australia,* vol. 183 (11/12), pp. 567-570.

Rosenfeld, J Ford, N 2010, 'Bomb blast, mild traumatic brain injury and psychiatric morbidity: A review' n.d. http://www.noninvasiveicp.com/node/73

Rossle, R 1950, 'Chapter XIV-C, Pathology of blast effects', in *German aviation medicine, World War II, Vol 2,* Department of Air Force, Washington DC, USA.

Rudin, W 1976, Principles of mathematical analysis, 3rd Edition, McGraw-Hill, New York.

Sattin, R Sasser, S Sullivent, E Coronado, V 2008, 'Chapter 1, The epidemiology and triage of blast injuries', in Elsayad, N Atkins, J (eds), *Explosion and blast related injuries*, Elsevier Academic Press, London.

Schaeffer, K Nulty, W Carey, C 1958, 'Mechanisms in development of interstitial emphysema and air embolism on decompression from depth', *Journal of Applied Physiology,* vol. 13, pp. 15-29.

Schardin, H 1950, 'Chapter XIV-A, The physical principles of the effects of a detonation', in *German aviation medicine, World War II, vol 2,* Department of Air Force, Washington DC, USA.

Scher, S 1941, 'Air embolism and blast', *The British Medical Journal,* Correspondence: letter, May 24, p. 797.

Scherer, M Burrows, H Pinto, R Somrack, E 2007, 'Characterising self reported dizziness and ostovestibular impairment among blast injured traumatic amputees: a pilot study', *Military Medicine,* vol. 172, July, pp. 731-737.

Severinghaus, J Astrup, P 1986, 'History of blood gas analysis. III. Carbon dioxide tension', *Journal of Clinical Monitoring,* vol 2, Issue 1, pp. 60-73.

Severinghaus, J Bradley, A 1958, 'Electrodes for blood PO_2 and PCO_2 determination', *Journal of Applied Physiology,* 13, pp. 515-520, n.d.
 http://jap.physiology.org/cgi/reprint/13/3/515

Shamir, M Rivkind, A Weismann, C Sprung, C Weiss, Y 2006, 'Conventional terrorist bomb incidents and the intensive care unit', *Current Opinion in Critical Care,* vol. 11, pp. 580-584.

Shara, J Ayling, R 2009, 'Correcting potassium concentration of haemolysed samples', *Annuals of Clinical Biochemistry*, published online 4th March 2009, viewed 20 July, 2013.
http://acutecaretesting.org/en/journal_scanner/journals/correcting_potassium_concentration_of_hemolyzed_samples

Sharpnack, D Johnson, A Phillips, Y 1991, 'Chapter 8, The pathology of primary blast injury', in *Textbook of military medicine, Part I: Warfare weaponry and the casualty, Vol 5: Conventional warfare: ballistic, blast and burn injuries,* The Office of Surgeon General, Department of Army, United States of America.

Sonden, A Svensson, B Roamn, N Brismar, B Palmblad, J Kjellstrom, B 2002, 'Mechanisms of Shock Wave Induced Endothelial Cell Injury', *Lasers in Surgery and Medicine,* vol. 31, pp. 233-241.

Stacy, K 2009, 'Unit IV: Chapters 24, 25, in Urden, L Stacy, K Lough, M (eds), *Thelan's Critical Care Nursing,* 6th Edition, Mosby Inc. USA.

Stocker, R Lenzlinger, P Stover, J 2014, 'Contemporary intensive care treatment for patients with severe multiple trauma', *European Manual of Medicine,* vol. 7, pp. 55-109. n.d. http://link.springer.com/chapter/10.1007/978-3-540-88124-7_7.

Stuhmiller, J Phillips, Y Richmond, D 1991, 'Chapter 7, The Physics and Mechanisms of Primary Blast Injury', in *Textbook of Military Medicine, Part I: Warfare Weaponry and the Casualty, vol 5: Conventional Warfare: Ballistic, Blast and Burn Injuries,* The Office of Surgeon General, Department of Army, USA.

Stuhmiller, J 2008, 'Chapter 10, Blast injury: translating research into operational medicine', in *Quantitative Physiology: Problems and Concepts in Military Operational Medicine,* in Sante, W Friedl, K (eds), Text in production: United States Army Medical Research and

Materiel Command, Maryland, Preview viewed April 3, 2011. http://www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA506843.

Surbatovic, M Filipovic, N Radokovic, S Stankovic, N Slavkovic, Z 2007, 'Immune cytokine response in combat casualties: blast or explosive trauma with or without secondary sepsis', *Military Medicine,* vol. 172, February, pp. 190-195.

Suzuki, A Armstead, S Eckmann, D 2004, 'Surfactant reduction in embolism bubble adhesion and endothelial damage', *Anaesthesiology,* vol. 101, no. 1, July, pp. 97-103.

Taber, K Warden, D Hurley, R 2006, 'Blast related traumatic brain injury: What is known?' *Journal of neuropsychiatry and clinical neurosciences*, vol 18, no. 2, pp. 141-145.

Taylor, L 2004, 'Chapter 2, Diving physics', in Bove, AA (ed) *Davis & Bove's Diving Medicine*, Elseiver Inc., Philadelphia, PA.

Teich, M Gebbeken, N 2010, "The influence of the underpressure phase on the dynamic response of structures subjected to blast loads", *International journal of protective structures*, vol. 1, no.2.

Teland, J 2012, *Review of blast injury prediction models*, Norwegian Defence Research Establishment, viewed 6 June, 2013.

http://rapporter.ffi.no/rapporter/2012/00539.pdf a.

Thompson, M 2001, *Philosophy of Science,* Hodder & Stoughton, United Kingdom.

Tsokos, M Paulsen, F Petri, S Madea, B Puschel, K Turk, E 2003, 'Histologic, immunohistochemical and ultrastructural findings in human blast lung injury', *American Journal of Respiratory and Critical Care Medicine,* vol. 168, pp. 549-555.

Tsokos, M 2008, 'Chapter 3, Pathology of human blast lung injury', in Elsayad, N Atkins, J (eds), *Explosion and blast related injuries,* Elsevier Academic Press, London.

United States Department of Defense, 2005, *Emergency War Surgery, NATO Handbook I: Types of wounds and injuries,* Virtual Naval Hospital, n.d. http://www.vnh.org/EWSurg/ch05/05TreatmentofBI.html

University of Bristol, (The), Life Sciences Mass Spectrometry Facility, Natural Environment Research Council, n.d.
http://www.bris.ac.uk/nerclsmsf/techniques/hplcms.html

Van den Berg, B Nieuwdorp, M Stroes, E Vink, H 2006, 'Glycocalyx and endothelial (dys) function: from mice to men', *Pharmacological Reports,* 58, suppl., pp. 75-80.

Video World News, 2009, video, viewed 15 November, 2013.
http://media.theage.com.au/news/world-news/poland-gas-explosion-starts-massive-blaze-4925327.html

Video World News, 2010, viewed 26 January, 2014,

http://media.theage.com.au/california-gas-explosion-latest-1923551.html

Video World News, 2013, video, viewed 30 July, 2013.

http://media.theage.com.au/news/world-news/propane-gas-explosion-in-florida-4614768.html

Wade, C Ritenour, A Eastridge, B Young, L Blackbourne, L Holcomb, J 2008, 'Chapter 2, Explosion injuries treated at combat support hospitals in the global war on terror', in Elsayad, N Atkins, J (eds), *Explosion and blast related injuries*, Elsevier Academic Press, London.

Warren, J Marshall, B 1984, 'Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration', *The Lancet*, vol. 1, issue 8390, pp. 1311-1315.

Wattana, M Bey, T 2009, 'Mustard gas or sulfur mustard: an old chemical agent as a new terrorist threat', *Pre Hospital and Disaster Medicine*, vol. 24, issue 1, pp. 19-29.

Weiler-Ravell, D Adatto, R Borman, J 1975, 'Blast injury of the chest: a review of the problem and its treatment', *Israel Journal of Medical Science*, vol. 11, pp. 268-274.

Weiss, Y Oppenheim-Eden, A Gilon, D Sprung, C Muggia-Sullam, M Pizov, R 1999, 'Systolic pressure variation in haemodynamic monitoring after severe blast injury', *Journal of Clinical Anaesthesia*, vol. 11, pp. 132-135.

West, JB 2005, *Respiratory physiology: the essentials*, 7th Edition, Lippincott, Williams & Wilkins, Philadelphia, PA, United States of America.

Wightman, J Gladish, S 2001, 'Explosions and blast injuries', *Annals of Emergency Medicine,* vol. 37, no. 6, pp. 664-678.

Wolf, S Bebarta, V Bonnett, C Pons, P Cantrill, S 2009, 'Blast injuries', *The Lancet*, vol. 374, issue 9687, pp. 405-415.

Woodcock, T Morganti-Kossmann, M 2013, 'The role of markers of inflammation in traumatic brain injury', *Frontiers in Neurology,* vol. 4, no. 18, viewed 28 February 2014. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3586682/

Woodcock, T Woodcock, T 2012, 'Revised Starling equation and the glycocalyx model of transvascular fluid exchange: an improved paradigm for prescribing intravenous fluid therapy', *British Journal of Anaesthesia,* viewed 23 June, 2014, http://bja.oxfordjournals.org/content/early/2012/01/29/bja.aer515.full.pdf+html.

Work, Health and Safety Act, Government of South Australia, South Australia, 1986, reviewed/updated 2001 & 2009. n.d.

http://www.legislation.sa.gov.au/LZ/C/A/OCCUPATIONAL%20HEALTH%20SAFETY %20AND%20WELFARE%20ACT%201986.aspx

Yelverton, J 1997, 'Chapter 16, Blast biology', in Cooper, G Dudley, H Gann, D Little, R Maynard, R (eds), *Scientific Foundations of Trauma,* Butterworth & Heinmann, Oxford, United Kingdom.

Zhang, J Wang, Z Leng, H Yang, Z 1996, 'Studies on lung injuries caused by blast under-pressure', *The Journal of Trauma, Injury, Infection and Critical Care,* vol. 40, (3S), March, pp. 77S-80 S.

Zhang, Q Raoof, M Chen, Y Sumi, Y Sursal, T Yunger, W Brohi, K Itagaki, K Hauser, C 2010, 'Circulating mitochondrial DAMPs cause inflammatory responses to injury', *Nature,* March 4, vol. 464, no. 7285, pp. 104-107.

Zhang,T Wang, M Xie, M Jia, M 2011, 'Analysis of traumatic occupational fataliities in China', *Amercian Journal of Industrial Medicine, v*ol 54, no. 7, pp. 560-564.

Zuckerman, S 1940, 'Experimental study of blast injuries to the lungs', *The Lancet,* pp. 219-224.

Zunic, G Pavlovic, R Malicevic, Z Savic, V Cernak, I 2000, 'Pulmonary blast injury increases nitric oxide production, disturbs arginine metabolism, and alters the plasma free amino acid pool in rabbits during the early post traumatic period', *Nitric Oxide,* vol. 4. pp. 123-128.

Personal communications

All personal and email communications are listed by permission.

Billett K, 2007, *personal communication,* RN BN Grad Dip Critical Care, Squadron Leader, Royal Australian Air Force. Deployed East Timor 2000, and as intensive care specialist nurse to Iraq 2004.

Jolly W, 2007, *personal communication*, LTCOL, Royal Australian Corps of Engineers, Australian Army. Deployed Iraq 2004.

Lavery G, 2000, *personal communication,* FRCA, Intensivist, Royal Victoria Hospital Belfast, N. Ireland.

Read D, 2006, *personal communication*, MBBS FRACS CSC, Director of Surgery, Royal Darwin Hospital, Major, Royal Australian Army Medical Corps. Deployed General Surgeon: East Timor 2000, Iraq 2005).

Ritzel D, 2004, *E-mail communications,* Engineer, Explosive Specialist, Dyn-FX Consulting Ltd, Canada.

Ritzel D, 2011, *personal communication in Melbourne, Australia,* Engineer, Explosive Specialist, Dyn-FX Consulting Ltd, Canada.

Terry M, 2009, *personal communication,* MBBS FRACS, FRCS, Royal Navy, Surgeon Commander, General Surgeon. Deployed Iraq 2003, Afghanistan 2007-8.

Thomas PD, 2004 & 2009, *personal communications*, MBBS FICCM FANZCA FRACP OAM, Medical Director Intensive Care Unit, The Royal Adelaide Hospital 1991 – 2008. Colonel, Royal Australian Army Medical Corps. Deployed Intensivist: Rwanda 1994, Bougainville, East Timor 2001, Iraq 2004, Afghanistan 2009.

Winter P, PhD, BSc. Lead Scientist, Weapons Systems Division, Defence Science Technology Organisation, Edinburgh, South Australia. (Ret 2008)

Appendices

Appendix I

Blast signatures database

Blast Signature Database (DSTO, Adelaide, 2005)

	POP (kPa)	total t OP (sec)	PUP (kPa)	t to PUP (sec)	total t UP (sec)	number of positive reflections after first UP
1	113.40	0.0022	-49.35	0.0009	0.0030	4
2	1133.51	0.0024	-107.12	0.0006	0.0029	4
3	266.95	0.0030	-52.85	0.0058	0.0138	7
4	290.47	0.0028	-34.96	0.0055	0.0651	4
5	262.94	0.0042	-82.06	0.0332	0.0426	1
6	246.18	0.0025	-43.00	0.0031	0.0716	2
7	257.16	0.0028	-40.15	0.0032	0.0140	10
8	3750.26	0.0016	-153.21	0.0016	0.4080	3
9	528.79	0.0024	-135.39	0.0040	0.1283	1
10	444.40	0.0043	-64.98	0.0240	0.0549	2
11	520.56	0.0017	-205.35	0.0020	0.1343	1
12	482.58	0.0016	-161.68	0.0017	0.0120	5
13	3938.72	0.0016	-38.96	0.0001	0.0016	0
14	4604.15	0.0015	-872.82	0.0003	0.0016	3
15	577.60	0.0020	-110.15	0.0037	0.0926	2
16	657.17	0.0031	-136.69	0.0200	0.3881	2
17	534.80	0.0018	-141.20	0.0021	0.1058	4
18	540.36	0.0017	-101.73	0.0039	0.0195	4
19	7044.59	0.0036	-71.40	0.0109	0.3060	3
20	1117.41	0.0059	-499.26	0.2191	0.3278	1
21	2221.95	0.0001	-272.35	0.0010	0.0011	2
22	1471.96	0.0013	-927.17	0.0050	0.1225	1
23	2562.91	0.0122	-19.75	0.0126	0.3972	0
24	1251.83	0.0018	-225.63	0.0002	0.0018	0
25	783.19	0.0029	-390.35	0.0033	0.0476	3
26	704.65	0.0029	-386.83	0.0323	0.3544	1
27	459.53	0.0014	-760.09	0.0016	0.1439	5
28	919.32	0.0019	-107.15	0.0003	0.0021	2
29	2878.95	0.0021	-165.36	0.0366	0.1958	0
30	6029.75	0.0083	-33.42	0.0102	0.4013	0
31	4362.72	0.0019	-88.58	0.0027	0.0927	2
32	4196.07	0.0026	-69.88	0.0029	0.4069	3
33	692.47	0.0027	-73.80	0.0082	0.0349	4
34	715.76	0.0031	-219.51	0.0155	0.0411	1
35	2492.03	0.0032	-121.54	0.0157	0.0490	4
36	713.43	0.0015	-171.44	0.0019	0.0520	4
37	663.25	0.0019	-19.80	0.0010	0.0025	4
38	4319.90	0.0015	-125.49	0.0031	0.0984	6
39	4507.51	0.0027	-45.58	0.0030	0.4069	3
40	694.13	0.0030	-84.34	0.0074	0.1757	1
41	666.61	0.0031	-204.36	0.0137	0.4065	4
42	785.79	0.0033	-134.67	0.0136	0.3790	3
43	585.39	0.0015	-218.91	0.0020	0.1616	3
44	671.39	0.0015	-84.36	0.0018	0.0400	1
45	4837.18	0.0060	-59.25	0.0066	0.2315	2
46	744.35	0.0029	-72.96	0.0036	0.0473	2
47	691.86	0.0032	-203.01	0.0158	0.0448	2
48	802.62	0.0034	-144.77	0.0148	0.0465	4
49	4116.16	0.0016	-143.21	0.0031	0.1224	5
50	4457.37	0.0027	-74.44	0.0029	0.0997	2
51	752.67	0.0031	-101.26	0.0037	0.0993	1
52	738.65	0.0029	-225.23	0.0154	0.0479	1
53	802.28	0.0032	-148.47	0.0154	0.3864	2
54	590.01	0.0016	-164.39	0.0020	0.1009	2
55	644.80	0.0018	-63.48	0.0020	0.3564	0
56	4135.36	0.0015	-112.21	0.0030	0.0050	5
57	4619.93	0.0027	-66.85	0.0028	0.0959	2
58	671.38	0.0032	-77.68	0.0034	0.4066	2
59	727.21	0.0030	-203.69	0.0158	0.1813	2
60	820.13	0.0034	-129.28	0.0147	0.0669	3
61	564.78	0.0015	-132.92	0.0019	0.0145	7
62	637.75	0.0100	-116.10	0.0020	0.1009	1
63	4556.13	0.0015	-122.54	0.0020	0.0999	4

64	5098.48	0.0025	-65.33	0.0028	0.0959	2
65	731.03	0.0031	-65.47	0.0058	0.0442	2
66	720.47	0.0032	-214.46	0.0157	0.4062	2
67	811.04	0.0034	-126.92	0.0147	0.0730	1
68	648.06	0.0016	-195.31	0.0020	0.0726	2
69	661.35	0.0017	-89.52	0.0019	0.0084	6
70	4253.47	0.0016	-101.87	0.0020	0.0993	5
71	4296.34	0.0019	-72.92	0.0030	0.1005	6
72	1243.45	0.0035	-50.22	0.0038	0.0961	2
73	721.15	0.0029	-250.48	0.0152	0.4067	1
74	576.71	0.0016	-167.64	0.0020	0.1008	6
75	686.31	0.0096	-18.99	0.0033	0.1007	2
76	4721.48	0.0017	-87.11	0.0029	0.1007	2
77	4858.45	0.0025	-107.86	0.0030	0.0999	8
78	874.46	0.0032	-62.70	0.0083	0.0992	4
79	665.93	0.0031	-210.08	0.0154	0.4929	1
80	865.92	0.0036	-148.14	0.0137	0.3862	1
81	684.41	0.0017	-134.01	0.0020	0.0748	4
82	761.72	0.0018	-24.14	0.0003	0.0018	4
83	3290.86	0.0012	-441.44	0.0013	0.1012	0
84	3779.80	0.0518	-39.94	0.0506	0.1019	0
85	606.34	0.0031	-657.18	0.0151	0.4065	1
86	1698.84	0.0037	-385.49	0.0041	0.3958	1
87	691.46	0.0278	-70.26	0.0215	0.0746	0
MEAN	1753.11	0.0037	-163.24	0.0103	0.1466	0.88

Legend

OP- over-pressure
UP – under-pressure
POP – peak over-pressure
PUP – peak under-pressure
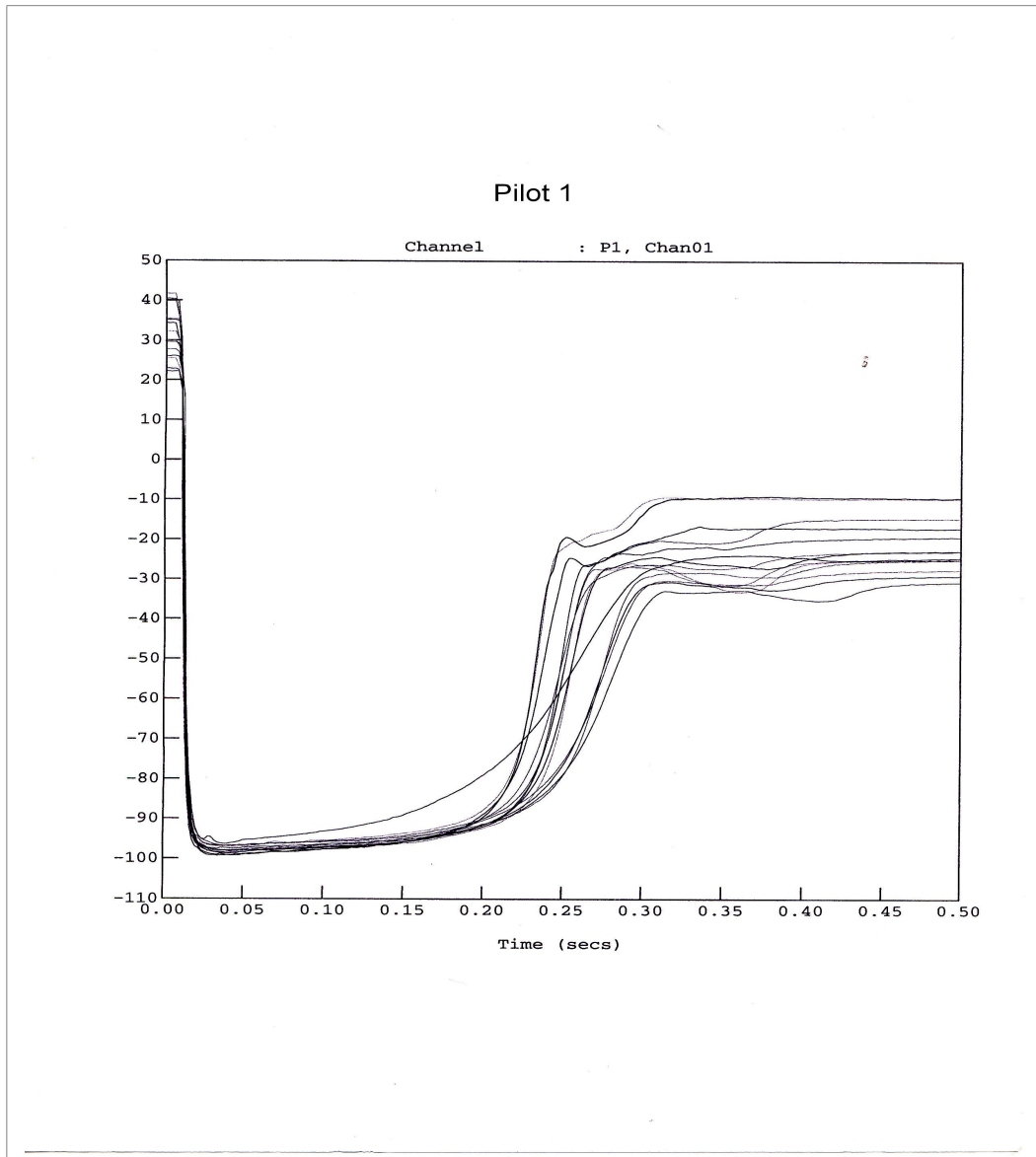sec – seconds
kPa – kilopascals
t - time

Appendix II

Samples temperature log in degree Celsius (c) – Pilot One

Pilot One		
sample	**Active**	**Control**
1	16.2	16.3
2	17.1	16.5
3	18.3	17.8
4	17.4	17.0
5	17.9	16.4
6	15.8	15.3
7	16.3	16.8
9	15.6	16.2
10	16.9	16.6
11	14.7	15.2
13	17.6	17.4
14	13.5	14.0
15	14.9	15.1
16	13.8	13.7
mean	**16.14**	**16.02**

Appendix III

Under-pressure generator signatures – Pilot One

Appendix IV

Samples pre-warming and post-warming temperatures log in degree Celsius (c) - Pilot Two

Pilot Two					
Sample #	**pre Active**	**pre Control**	**post Active**	**post Control**	**temperature checked**
18	16.3	15.1	36.5	37.0	x1
19	16.5	15.0	37.6	37.9	x2
20	15.8	15.5	37.0	38.0	x3
21	16.3	14.2	37.6	37.8	x2
22	17.1	16.5	37.8	38.2	x2
23	17.3	15.9	36.6	37.4	x1
24	18.0	17.1	36.5	37.3	x1
25	15.7	16.5	37.8	38.0	x2
26	15.8	16.0	38.0	38.6	x1
27	14.8	15.1	34.5	36.4	x1
28	16.2	14.2	36.5	37.0	x1
29	18.0	15.6	37.4	36.8	x2
31	16.3	12.2	37.6	37.5	x1
32	17.4	13.6	37.8	36.8	x1
mean	**16.53**	**15.17**	**37.0**	**37.4**	

Appendix V

Samples warming times log (minutes) – Pilot Two

time of warming (min) in warming bath set at 37.4C	
Sample #	**time (mins)**
18	0.6
19	1.3
20	1.6
21	1.5
22	1.6
23	0.6
24	1.1
25	1.3
26	1.4
27	1.1
28	1.4
29	1.8
31	1.5
32	1.0
mean	**1.26 minutes**

Appendix VI

Under-pressure generator signatures – Pilot Two

Pilot 2
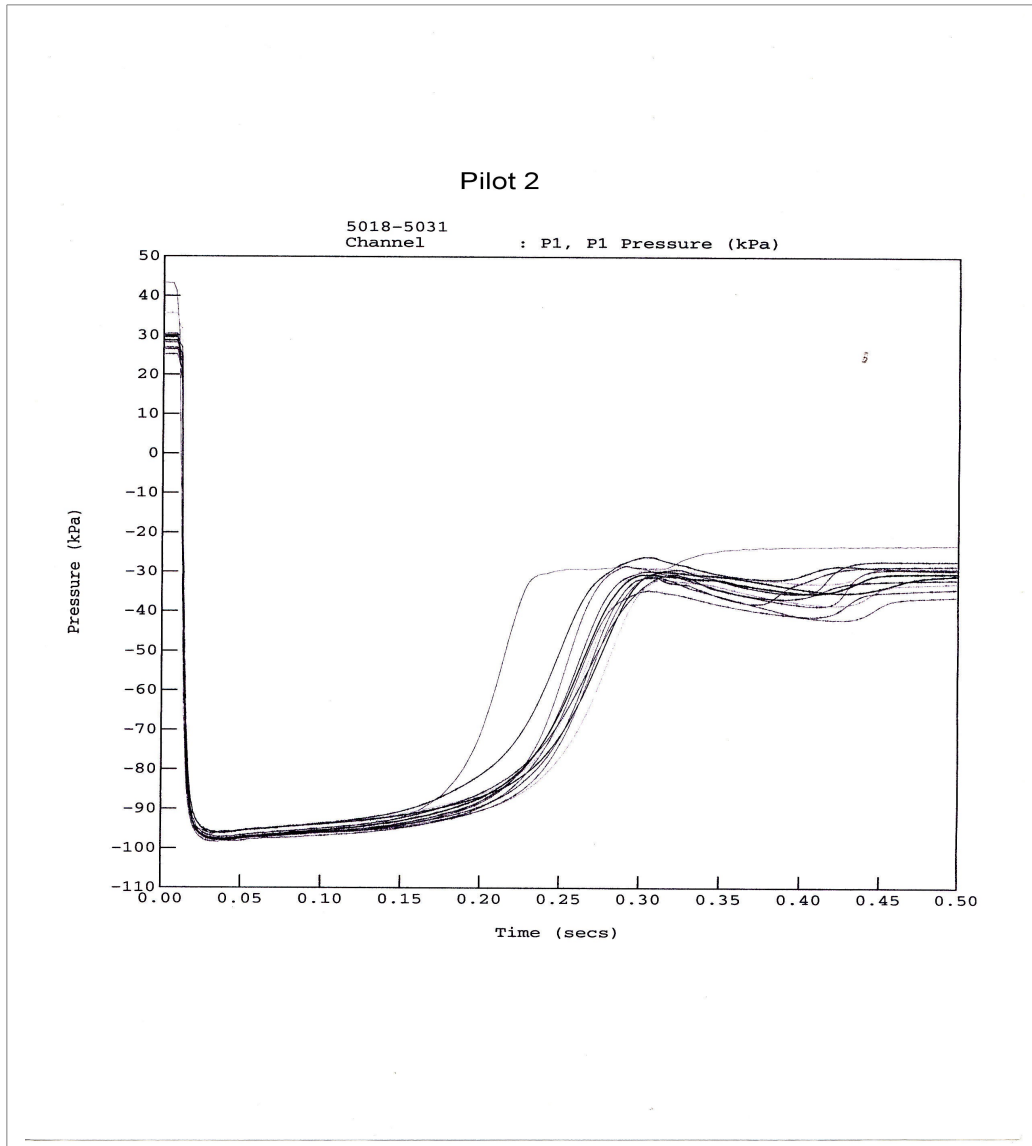
Appendix VII

Samples temperature log in degree Celsius (c) – Venous Test group

Samples temperatures following warming bath immersion using 1.26 minutes		
Sample #	**Active**	**Control**
34	36.9	36.1
35	36.2	36.9
36	35.9	36.2
37	38.2	37.8
38	37.5	37.9
39	38.0	37.8
40	37.5	37.9
41	36.1	36.6
44	36.1	37.0
45	38.3	37.1
46	37.1	36.7
47	35.4	36.2
48	36.1	35.4
49	35.5	35.2
50	35.1	36.3
51	36.2	35.5
52	36.4	36.0
53	35.6	35.7
54	35.1	36.2
55	36.1	36.0
56	36.9	35.8
57	37.8	36.9
58	36.4	36.1
59	36.4	37.2
60	35.9	36.2
61	35.4	36.9
62	37.0	37.2
63	37.3	36.4
64	37.4	36.6
65	37.3	36.2
66	37.9	36.2
67	37.8	36.4
68	36.6	35.9
69	38.2	36.2
70	36.1	35.4
71	37.9	37.0
72	37.2	37.0
73	37.5	36.4
74	36.1	36.0
75	35.8	36.0
mean	**36.7**	**36.4**

Appendix VIII

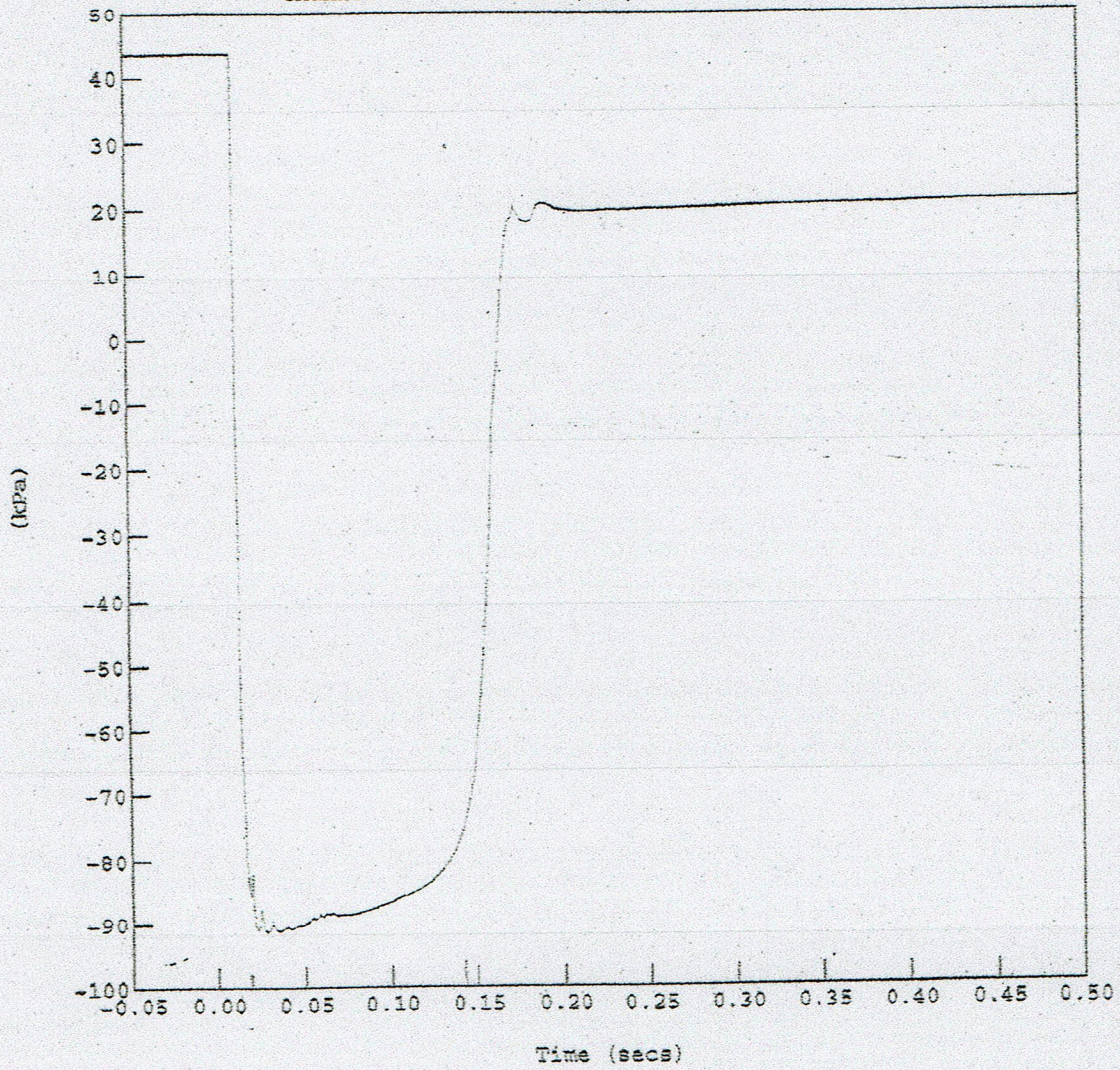## Under-pressure generator signatures – Venous Test group
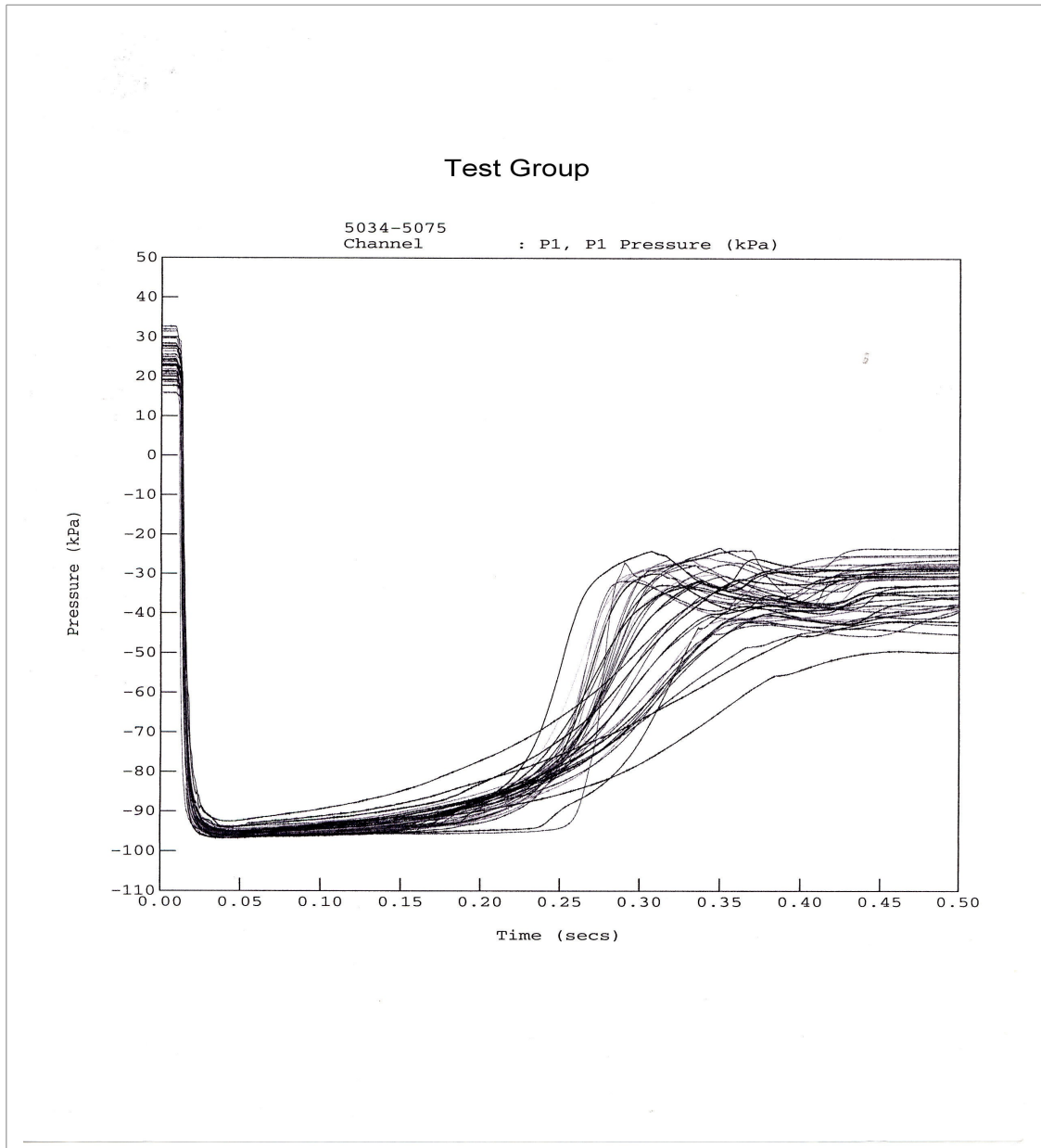
Appendix IX

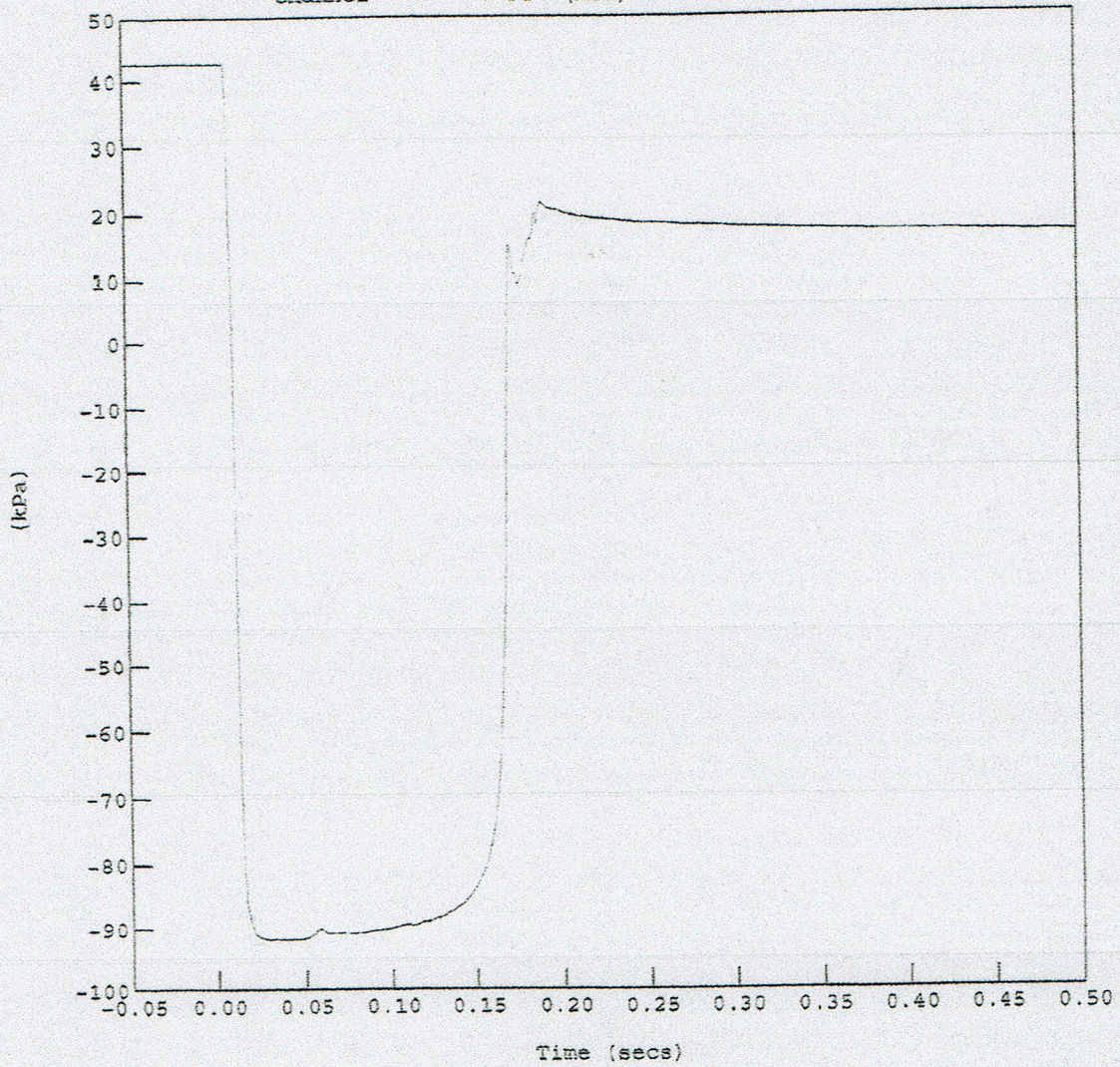**Under-pressure generator signatures – Arterial Test group**

Six tracings demonstrating the apparatus' consistency.

Decompressions: 1, 5, 10, 15, 20 25.
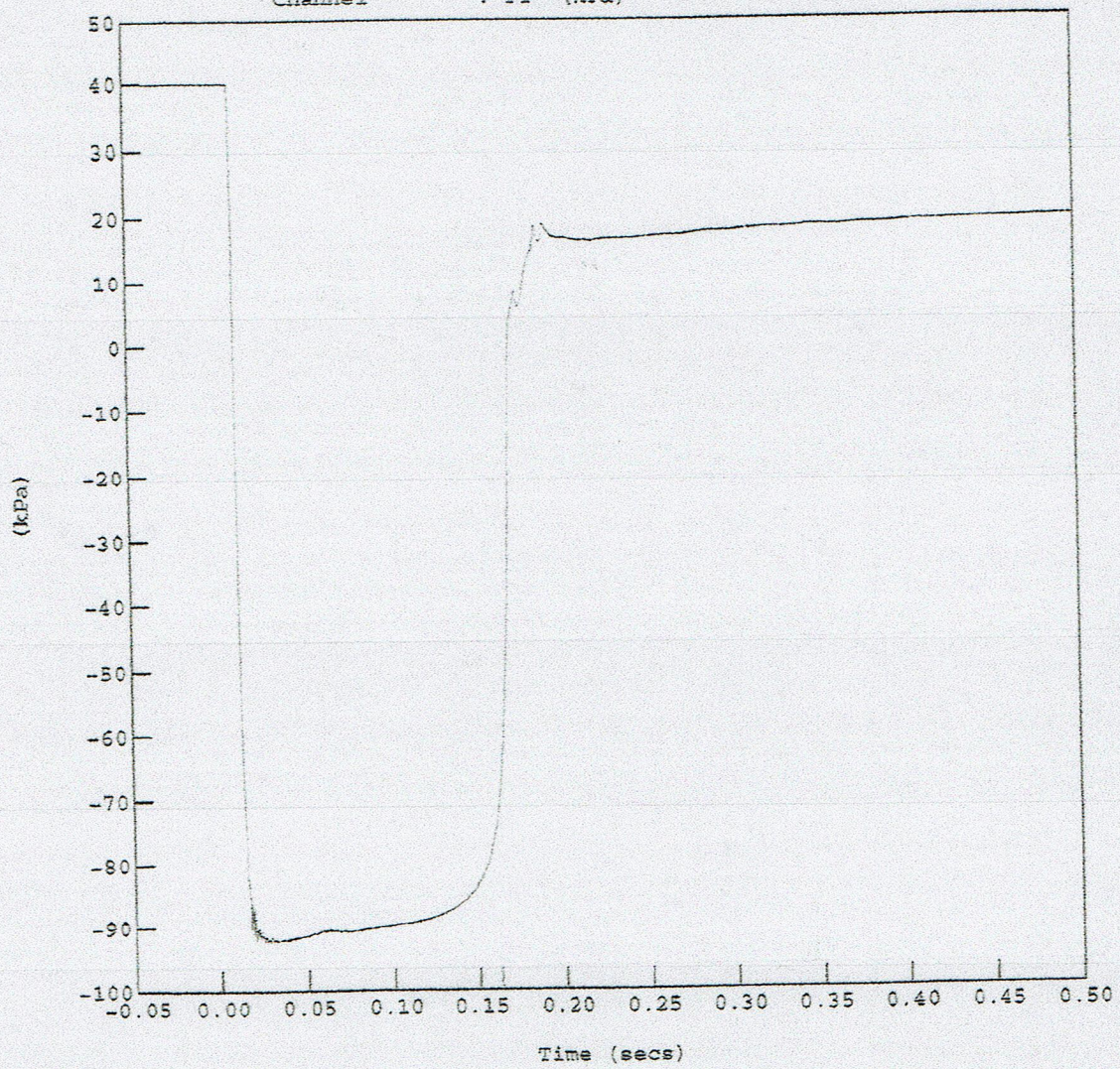
Test Reference : Blood_Rig / 28-1-2011 / 5101
Arterial A1
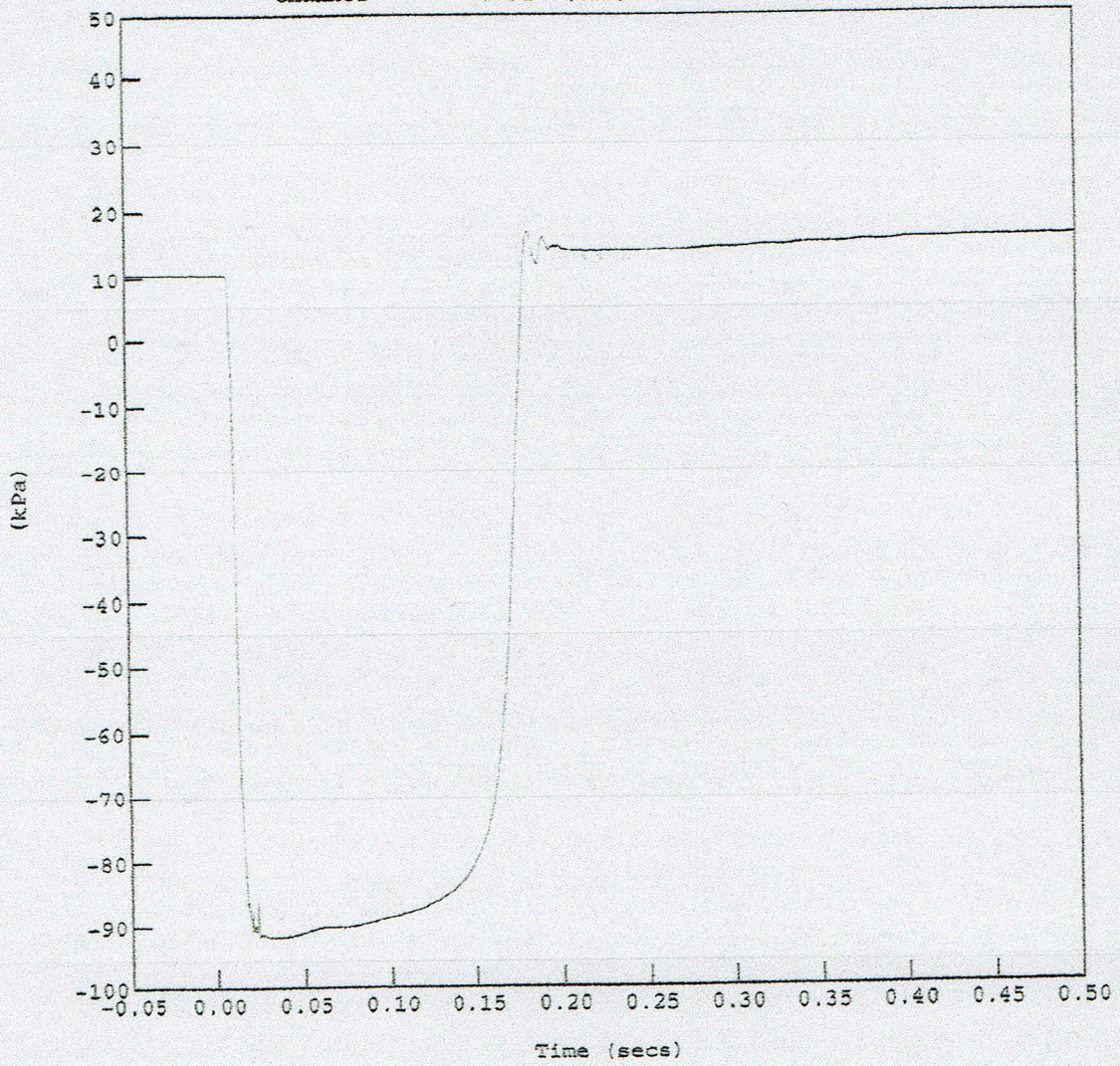Channel   : P1 (kPa)

Test Reference : Blood_Rig / 28-1-2011 / 5105
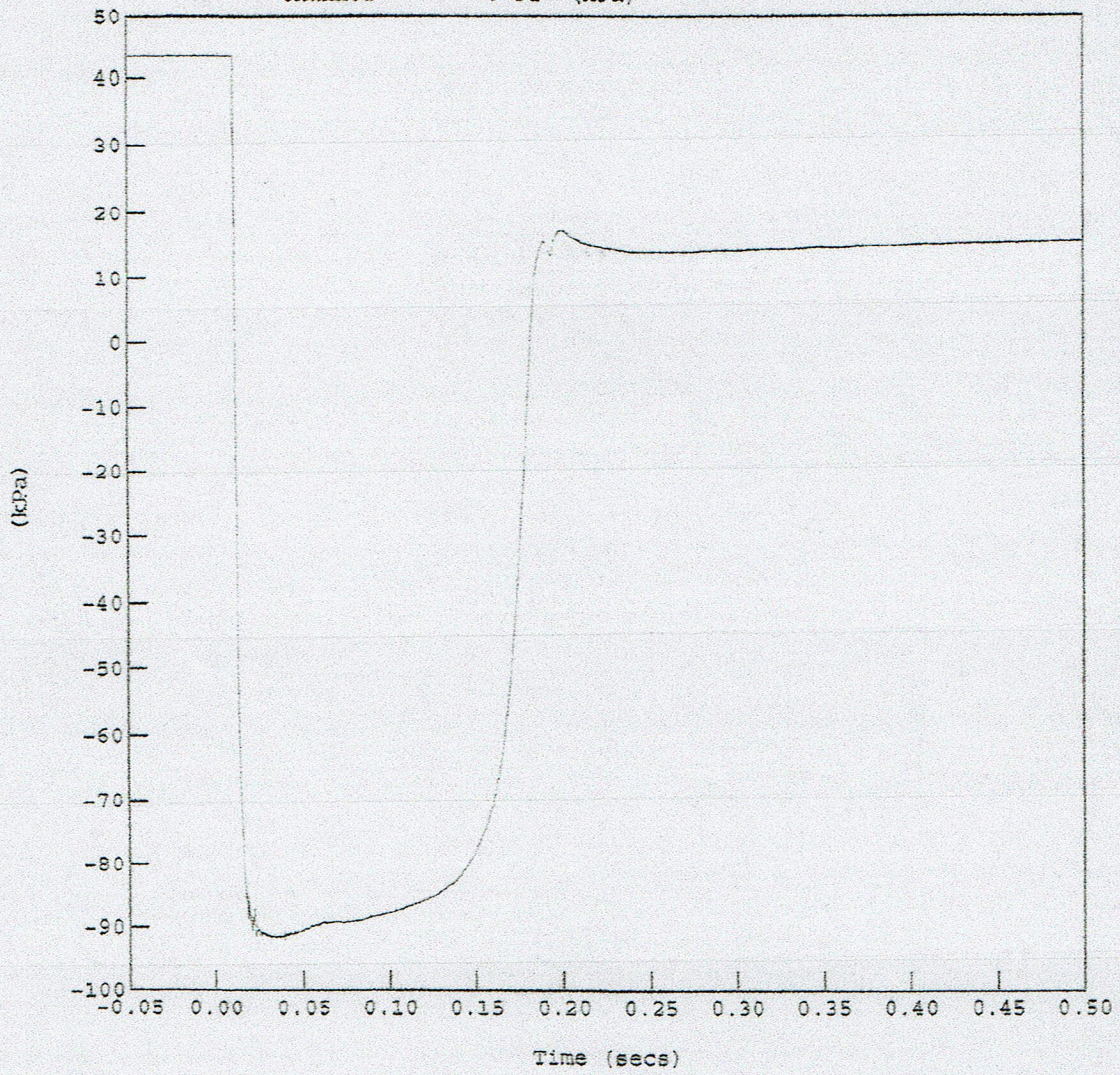Arterial 5
Channel   : P1 (kPa)

267

Test Reference : Blood_Rig / 28-1-2011 / 5110
Arterial 10
Channel   : P1 (kPa)

Test Reference : Blood_Rig / 28-1-2011 / 5115
Arterial 15
Channel   : P1 (kPa)

269

Test Reference : Blood_Rig / 28-1-2011 / 5120
Arterial 20
Channel   : P1 (kPa)

Test Reference : Blood_Rig / 28-1-2011 / 5125
Arterial 25
Channel1   : P1 (kPa)