Vesicant Burns: Is Laser Debridement ('Lasablation') a Viable Method to Accelerate Wound Healing?

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The vesicant agents, Lewisite and sulphur mustard were initially deployed as chemical weapons in the First World War at Ypres in 1917. These agents cause immediate blistering followed by deep dermal to full thickness burns and can be fatal in 1-3% (NATO 1996).

Dermabrasion can accelerate the healing of these types of burn injuries without the need for additional split skin grafting (Rice 1997 and Rice, Brown & Lam et al 1999).

Current laser technology allows accurate laser debridement of burn wounds.

The corollary of this is that it should be feasible to *partially* laser debride a vesicant burn to accelerate healing. This treatment should be more precise, encouraging rapid healing compared to dermabrasion.

This thesis investigated the application of modern CO_2 and Erbium:YAG lasers for debriding Lewisite burns on a representative model. A large white pig model (n=6) was used to investigate the effectiveness of CO_2 and Erbium:YAG lasers in ablation of established Lewisite burns. Burns underwent treatment at four days post-exposure and were histologically assessed at one, two and three weeks thereafter for the rate of epithelial healing.

The re-epithelialisation rates in the laser debrided groups were accelerated by a factor of four compared to untreated, historical controls by the first week. (Anova p = 0.006 for pulsed CO_2 and p = 0.012 for Erbium: YAG).

Ablation of the burn eschar was thought to accelerate the rate of healing by causing partial debridement. This method has been termed *'lasablation'*.

A concurrent study of CO_2 and Erbium: YAG laser debridement of normal skin resulted in a superficial resurfacing burn which healed within a week, regardless of the type of laser. The burn depth was in the order of 150 µm. The superficial nature of these burns allowed for rapid healing, consistent with previous findings.

All work was carried out in accordance with the Animals (Scientific Procedures) Act 1986 with Home Office approval.

Dedication
This is dedicated to my wife Sandra, Daniel my son, and to my parents who have all constantly and selflessly supported me throughout the entire project.

Acknowledgements

Declaration

Presentations from this work
1. Lam DGK , Rice P
'Accelerated wound healing of Lewisite-induced chemical burns with debridement by Ultrapulse & Erbium:YAG lasers'
British Association of Plastic Surgeons 3rd European Appointed Meeting, Birmingham (July 2000)

2. Lam DGK, Rice P
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European Burns Association, 8th Congress, Greece (September 1999)

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1. Lam DGK, Brown RFR, Rice P
The Treatment of Lewisite burns with Laser Debridement - Lasablation. Burns 28(1):19-25, February 2002

2. Rice P, Brown RFR, Lam DGK, Chilcott R, Bennett NJ
Dermabrasion- A Novel Concept in the Surgical Management of Mustard Burns. Burns 26(1):34-40, Feb 2000

Abstracts from this work
1. Lam DGK, RFR Brown, Rice P
Accelerated wound healing of Lewisite induced chemical burns with debridement by Ultapulse and Erbium:YAG lasers. Abstracts from the best regional presentations. Annals RCSE 84(3):214 May 2002

2. Rice P, Bennett NJ, Lam DGK, Brown RFR
The Role of Dermabrasion in Lewisite-induced Skin Injury. In: Proceedings of the 2000 Medical Defense Bioscience Review. Hunt Valley, MD:USAMRICD. p.1179-86

3. Lam DGK, Bennett NJ, Rice P
Accelerated wound healing of Lewisite induced chemical burns with debridement by Ultapulse and Erbium:YAG lasers. European Burns Association 8th Congress Abstract Book p.105, 1999

4. Rice P, Brown RFR, Bennett NJ, Lam DGK
Dermabrasion- A Novel Concept in the Surgical Management of Mustard Burns. European Burns Association 8th Congress Abstract Book p.197, 1999

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The History of Burns Treatment

1.1.1 The Ancient Civilisations

Until two centuries ago it was still believed that fire was one of the essential elements that constituted all matter. The evolution of man has depended greatly on his ability to create and control fire, with *Homo erectus* learning this about a million years ago. *Homo sapiens* and his predecessor *Homo erectus* have developed a healthy respect for fire as a result of being burnt thus probably developing our society's notion of being condemned to the eternal flames of Hell. Certainly heretics and witches were burnt at the stake for this reason.

Neanderthal man treated burns with extracts of plants. The Egyptians used incantations and a mixture of goat's milk, gum, and milk from a lactating woman who had given birth to a son. In the Smith papyrus of around 1500 BC, reports of unusual Egyptian mixtures have been found, most of these consisting of various types of lemon strips soaked in oily preparations. The Chinese were using tinctures and extracts made from tea leaves as early as the 4th Century BC

Hippocrates suggested the following: "Having melted old swine's seam and mixed it with resin and bitumen, and having spread it a piece of cloth and warmed it at the fire, apply a bandage." He also used warm, vinegar-soaked dressings to relieve pain, and later treated burns by tanning with solutions oak bark. The ancient Roman civilisations had a plethora of treatments: Celsus described treatment with a mixture of honey and bran. Pliny the Elder wondered if it would not be better to allow burns to remain exposed to the air rather than to cover them with ointments.

It would appear that in these early civilisations local care was not too far afield from those of this century, such as the use of petroleum jelly gauze dressings, and exposure. An example of the unusual concoctions used by the ancients included:

'The salve of Paracelsus for wounds and burns is even more impossible. It consisted of the fat of very old wild hogs and bears, heated half an hour in red wine, then dropped into cold water which was next skimmed and the fat rubbed up with the roasted angle worms and moss from the skull of a person hung, scraped off during the increase of the moon to which were added blood stone, the dried brain of a wild hog, red sandalwood and a portion of a genuine mummy'!

In the ninth century, the famous Arabian physician, Rhases, recommended the use of cold water for the alleviation of pain. This is now the cornerstone of first aid for burns.

1.1.2 The Middle Ages

Gunpowder burns were a particularly prevalent problem during the Middle Ages. Ambroise Paré, one of the first barber surgeons in the 16th century, wrote at length about 'combustions'. It was described that, "if the burn is superficial, pustules and blisters develop unless proper measures are taken and if the burn is deep, scabs or a crust appear which are burnt flesh. The action of the fire causing combustion thickens the skin, rendering it hard and taut, provoking great sufferings, which draw the humours from the immediate neighbourhood of the burn and distant parts and convert them into serious aquosities, which looking for an outlet....." This was undoubtedly a description of the blister fluid produced.

"A deep burn having caused hard scabs is not so painful as a superficial one, as is proved by daily experience in respect of those who are cauterised. For just after cauterisation, they only feel a little pain. For a great combustion removes the feeling." Paré's observations further led him to cease using boiling oil to cauterise the ends of limb amputation stumps and use salves and clean dressings instead. During the battle of Villaine the supply of oil had been depleted, and Paré was forced to use these conservative measures on amputation stumps. He discovered

that these wounds healed rapidly without the expected complications. Such revolutionary thinking reduced the mortality rates from sepsis, amputation being one of the most common procedures at that time.

Even then he advised what is now known as early excision,"... and to such scabs, so deep a scarification will be made that it will reach the living flesh".

1.1.3 19th Century Onwards

Baron Guillaume Dupuytren, the famous French surgeon of the Hôtel Dieu in Paris, classified burn lesions into six degrees of injury according to depth and probably invented the bath for burns. He also contributed one of the first statistical burn studies in 1828, when he categorised 50 patients who were admitted to the Hôtel Dieu, according to age, sex, surface extent of burns, and subsequent mortality.

Curling of London recognised gastric and duodenal ulceration as a complication of severe burn injury in 1842.

Professor James Syme proposed the use of dry cotton-wool dressings to cover burns in 1827 at the University of Edinburgh. Subsequently, the first burn hospital was operated under his aegis in 1848.

1.1.4 20th Century

It was during the first quarter of this century that the Golden Age of modern surgery began to dawn. The replacement of antisepsis by asepsis and the development of airway control via endotracheal intubation meant that operations could be performed which would previously been impossible. The Second World War

accelerated many of the improvements in burn treatment. A key milestone was in recognising the fluid shift associated with a burn injury and the need for volume replacement, which meant that burn victims could now survive the initial insult.

Gross infection around and within the burn still complicated the overall recovery. With the invention of procedures to reduce cross-infection, wound infection and sepsis, coupled with more powerful antibiotics, it was becoming possible to treat large burns successfully. Initially, Gram-positive and then Gram-negative organisms became controllable. Together with the antibacterial properties of topical silver salts, the rates of infection rapidly reduced.

The next major development was the recognition that it was not appropriate to treat burns in a side room of a General Surgical ward - specialised facilities were required. This lead to the development within the National Health Service of Regional Burns Units housing dedicated Burns Teams comprising of surgeons, anaesthetists, nurses, therapists, nutritionists and other ancillary staff.

a)   Fluid Replacement

Advances in the understanding of fluid losses in burns began with the studies of Frank Underhill of Yale, a professor of pharmacology and toxicology. He studied 20 burn victims in 1921and observed changes in haemoglobin, haematocrit and serum chloride levels. Blister fluid analysis led to the recognition of the importance of protein losses in burns. This research showed that the previously held theory that toxins caused the shock in burns was incorrect: burn shock was primarily due to fluid loss. This was reinforced by the work of Blalock in 1931 on burned dogs, observing the effects of hypovolaemia manifesting as oliguria and hypotension.

Cope and Moore in 1947 demonstrated that some fluid loss occurred within the patient and not exclusively outside, thus providing an explanation for hidden fluid

loss in burns. Evans devised a formula for calculating fluid replacement in burns in 1952. This was based on both the burn surface area and size of the patient that became a widely accepted concept. After utilising the Evans formula for a period of time, surgeons at the Brooke Army Medical Centre modified it and thereafter it was called the Brooke formula. This modified formula was based on the same overall quantity of fluid but changed the proportions of colloid and non-colloidal solutions. Shires and Baxter at the Parkland Hospital devised their Parkland formula utilising only lactated Ringer's solution during the initial 24 hours after the injury.

b) Chemotherapeutic Agents

The search for an effective topical agent for burn wounds continued throughout the early 1900s. Edward Clark Davidson originated the use of tannic acid spray in 1925 by modifying the Chinese concept of using tea leaves (a rich source of tannic acid). This was in order to dry off the eschar thus decreasing fluid losses. Aldridge suggested gentian violet in 1933 as an escharotic because he thought it had a bacteriostatic effect. Later, 5 per cent silver nitrate was added as an additional escharotic.

In 1942, Harvey Allen popularised the additional use of petrolatum gauze dressings combined with strict immobilisation.

c) Exposure

Wallace, of Edinburgh, reintroduced the exposure method in Great Britain in 1949 of allowing the burn wound to slough, awaiting the appearance of granulation tissue. During that period there were a great debates concerning the open and closed methods of management. Many surgeons in warmer climates favoured the exposure method because the development of crust and eschar provided a

physiological covering to the wound. Surgeons in northern climates favoured bulky dressings because they felt that the patients were uncomfortable with exposure. The development of infections hidden beneath dressings converted most surgeons to at least some type of exposure treatment in large burn wounds for hospitalised patients.

d)   Antibiotics

Septicaemia was the primary cause of death in burns and that many of these deaths were due to staphylococci. As improved antibiotics against the gram-positive organisms became available, pseudomonas sepsis became the primary cause of death in most burn patients. The virulence of this gram-negative sepsis placed great emphasis on finding agents that would penetrate the eschar and minimise bacterial growth under the eschar.

Lindsay discovered Sulfamylon™ (mafenide acetate) in the 1950's and established that this antibacterial agent was unique in that it penetrated the dead tissue. At about the same time, Moyer proposed the use of 0.5 per cent silver nitrate wet dressings. These were found to protect against the growth of bacteria in and beneath the burn wound. Thus, silver nitrate soaks became a very popular method of treatment. Charles Fox of New York combined sulphur and silver into a compound known as silver sulphadiazine, which is still used to this day as Flamazine™.

1.1.5 History of Skin Grafting and Closure of the Burn Wound

The Tilemaker caste in India is believed to have recorded the earliest reports of skin grafting. Free grafts of skin were utilised, including the subcutaneous fat taken from the gluteal region which was beaten with wooden slippers until a considerable

amount of swelling had taken place! The earliest work depicted shows grafting to injuries of the nose. Baronio published the first treatise on experimental plastic surgery in 1804. Free full-thickness autografting was demonstrated by experiments performed on sheep.

The first epidermal graft was performed by the Swiss surgeon, Reverdin, in 1869. He reported transferring two small pieces of epidermis in a patient who had lost the skin of his thumb. Thiersch, in 1874, described the use of deeper pieces of skin. He deliberately transferred some dermis along with the epidermis. These split thickness skin grafts are still referred to by his name.

In 1875, Wolfe reported the plastic repair of a lower eyelid defect with a free full-thickness graft from the arm. This is probably the first report of a full thickness graft and justifies the association of his name with this type of graft. In 1914 John Staige Davis, of Baltimore, modified Reverdin's idea to produce what he called the small, deep graft. Instead of being the thinnest bit of superficial skin which could be cut, the full thickness of skin was included at its centre. This graft proved to be great practical value and became known the pinch graft.

Large, split-thickness Thiersch grafts were harvested by the freehand use a long, thin knife. A modified knife with a roller in front became known as the Humby knife in England. The currently popular drum-type dermatome was developed by Padgett and Hood in 1939 at the University of Kansas. Harry M. Brown conceived the electric dermatome while he was a Japanese prisoner during World War II and this became one of the great advances in the treatment of burns. With this instrument, wide sheets of graft could be cut with great ease.
The Brown dermatome was modified by Hargest in 1965, who replaced the electric motor and cable with an air-driven motor which increased the speed of the blade and made for a smoother cut.

1.1.6 The Dawn of the 21st Century (Rose & Herndon 1997)

A review from the Shriner's Burns Institute in Texas, USA, has shown that in the last two decades burn mortality rates have rapidly declined due to advances in treatment as shown in **Table 1.1.1**

In 1952, a teenage burn victim with 50% TBSA burns would have had a 50% chance of surviving the injury. Today, a child would have a 50% survival rate for even 98% TBSA burns. These are due to advances in the understanding of the pathophysiology of burns regarding fluid resuscitation, infection control, nutritional support, early wound closure and rehabilitation.

a) Pathophysiology
The biochemical reactions caused by burn eschar are better understood. Specific roles played by arachadonic acid, oxidants and cytokines contribute to arteriolar and venular dilatation followed by platelet aggregation, resulting in vascular stasis. High levels of thromboxane A$_2$ (TXA2) have been found in burn wounds and specific inhibitors of TXA2 are able to improve perfusion within the zone of ischaemia. Anti-oxidants such as xanthine oxidase inhibitors modulate the degree of vascular permeability, thus controlling the degree of reperfusion injury.

Cytokines are involved in tissue inflammation, haemodynamic changes, immune defences, alterations of metabolic rate and wound healing. TNF (tumour necrosis factor), IL (interleukin)-1, IL-2, IL-4, IL-6, IL-8 and IL-12 along with IFN-γ (gamma interferon) have been identified in burn tissue. Manipulation of the effects of these can lessen the severity of injury and stimulate healing.

b) Fluid resuscitation
Increased fluid requirements are well recognised, although more specific formulae have been developed. With children in particular, weight based formulae tend to fluid overload and hence the Shriners Burns Institute at Galveston, Texas has

developed surface area formulae. The presence of an inhalational injury will usually mean increased requirements by up to a third. Both type and quantity of fluid used differ in each resuscitation formula, with crystalloids such as normal saline and Ringer's lactate recommended. Colloidal solutions including dextrans (Gentran [®]), gelatins (Haemaccel [®] and Gelofusine[®]), fresh frozen plasma and human albumin solution are used in addition to cope with increased needs. Invasive cardiac monitoring should be utilised for severely burnt patients to optimise the level of resuscitation particularly if patients already have some degree of pre-existing disease (Lam et al 1999). Ischaemic lesions in the gastrointestinal tract have been ascribed to decreased splanchnic blood flow with inadequate fluid resuscitation. This is associated with bacterial translocation across the gut into mesenteric lymph nodes with distant spread.

c) Inhalational Injury

This is one of the most serious complications of a burn injury since, untreated, it can progress to ARDS (Acute Respiratory Distress Syndrome) with 50% mortality. Increases in the permeability of lung parenchymal tissue have been recognised as contributing to the pulmonary oedema and atelectasis seen with these injuries. However, anti-inflammatory agents such as ibuprofen can reduce the amount of fluid formed.

d) Infection Control

Topical antimicrobial agents such as mafenide acetate, silver nitrate and silver sulphadiazine are still the mainstay. The development of bacteria such as Enterococcus, Staphylococcus and Pseudomonas with multiple antibiotic resistances has meant that administration of systemic antibiotics has to be judicious. The emphasis is now on augmenting immune function to prevent development of burns sepsis. Experimental administration of IL-12, an immunopotentiator, reverses the immunosuppressant cytokine profile translating into improved survival and increased resistance to bacterial infection.

e) Metabolic response

The mechanisms involved in the metabolic response to injury and stress have been elucidated. These have been termed the ebb and flow phases. The basal metabolic rate (BMR) can be doubled with a burn and a concomitant increase in resting energy expenditure (REE) ensues. The central core temperature is reset and this is associated with increased amino acid and glucose turnover to cope with demands. Growth hormone acts in an anabolic manner preventing loss of vital muscle mass and has been shown to accelerate donor site healing. It induces formation of a complementary hormone, IGF-1 (insulin-like growth factor) which reduces protein catabolism.

f) Nutrition

Early enteral nutrition is beneficial, decreasing the amount of catabolic hormones induced, maintaining gut mucosal integrity and improving nitrogen balance. Total parenteral nutrition has not been shown to be as useful since there is an array of metabolic and immunological complications.

g) Burn Excision and Wound Closure

Surgical debridement to achieve tangential excisions (Janzekovic 1970, Jackson & Stone 1972) of burn eschar was an important landmark. This embraced the concept of early tangential excision (ETE) and grafting of the burn wound, after initial resuscitation and by the fifth post-burn day. This method contrasted with earlier techniques of excising only small deep burns, and delaying the grafting of larger burns until spontaneous separation of slough had occurred and the wound was granulating. Janzekovic's technique and its variants have subsequently become the norm for the surgical management of burn patients, and this shift towards earlier surgery has been widely credited as a major factor in improved outcome with lower blood loss and rates of infection. When applied to full thickness burns, tangential excision preserved any viable subcutaneous tissue thus minimising post-graft deformity.

Novel debridement concepts have included mechanical dermabrasion (Lorthioir 1963, Krant & Arons 1977, Holmes and Muir 1983) and laser debridement (Stellar et al 1971, Levine et al 1974, Fidler 1975) to achieve clearance to a healthy tissue bed.

The evidence base for earlier surgery has been an amalgamation of many centres' experience. In 1974, Burke et al reported a much shorter hospital stay in children treated by early excision compared with those treated conservatively. The same group also demonstrated unprecedented survival in children who had extensive burns when the wounds were excised in one stage and covered with allograft. Similarly, a large retrospective study from Boston (Tompkins et al 1986) showed a significant fall in mortality in paediatric burn patients following the introduction of prompt burn wound excision in stages. A decreased length of stay was also noted. A randomised controlled trial of total burn excision within 24 hours of injury versus exposure treatment showed no difference in mortality in any of the stratified patient groups, but shorter hospitalisation and fewer infective episodes in patients with small burns of less than 15% body surface area (Sorensen 1984).

In contrast, a controlled trial from the Shriner's Burns Institute in Texas comparing conservative exposure treatment with ETE revealed a subgroup of decreased mortality for young adults (17-30 years) with no inhalation injury, undergoing ETE of burns over 30% body surface area. However, these patients *did not differ* significantly on morbidity measures such as length of stay, number of septic episodes and total number of operative procedures. Patients older than 30 or with a concomitant inhalational injury did not fare any differently (Herndon et al 1989).

The evidence thus appears to favour some policy of surgical excision for burn wounds that are unlikely to heal promptly, rather than a conservative approach, with delayed grafting. There is no conclusive evidence to discriminate between early *total* burn excision versus *staged* excision, beginning after resuscitation.

Some authors have reported that areas of deep dermal burn can be stabilised by dressing with a cream containing silver sulphadiazine and cerium nitrate, allowing surgery to be delayed (Boeckx et al 1992).

h) Skin Substitutes and Reconstruction

The use of cadaveric skin and allograft sheets has been shown to be life-saving where there is insufficient autograft in patients with large TBSA burns. New developments have included synthetic sheets such as Biobrane®, potato peels (sic) and Dermagraft-TC® which is populated with neonatal fibroblasts that help incorporate the sheet into the burn wound. Bilaminar skin substitutes such as Integra® and Alloderm® have been developed which provide the essential neodermis whilst autograft can be harvested at leisure. This approach allows the ability to debride patients with massive burns or physiological compromise without the need for immediate skin graft cover. The indications for the use of these materials are ever increasing since they are now used in post-burn reconstruction. Cultured epidermal cell sheets have proven to be disappointing in use, with a predisposition to long-term bulla formation.

The introduction of tissue expansion and advances in microsurgical free tissue transfer has meant that burn reconstruction is possible with either the use of skin substitutes or autologous tissue. Pressure garments and plastic face and neck moulds are used to reduce the degree of hypertrophic scarring and contracture.

1.1.7 Conclusion

Burn injuries of skin can vary from a mild blistering lesion with superficial epidermolysis to a full thickness eschar that has considerable morbidity. The diagnosis and treatment of burns is a constantly evolving area since there is scope for both clinical judgement and investigations to overlap in their usefulness, reproducibility and reliability. Injury to the skin can be graded according to the

depth of burn providing both a prognostic indicator and guide for the surgeon as to how best to treat the lesion.

Burn care has progressed from dressing with salves, through to burn wound exposure, improvements in burn physiology awareness and the refinement of the concepts of both early and tangential burn wound debridement.

The physical sequelae of burn injury survival is manifested most commonly as hypertrophic scarring which can lead to contractures across limbs and digits, with resultant loss of function. Modern management includes revision surgery by scar excisions, the use of local flaps, skin substitutes, tissue expansion and free tissue transfer.

In this last decade burn care has improved such that it is now possible for patients with 80% body surface area burns to frequently survive.

Burns

1.2.1 Anatomy of the Skin

The skin is the largest organ in the body with a surface area of approximately $0.025m^2$ in a neonate, to $1.8m^2$ in an average adult. Principally consisting of two functional layers, epidermis and dermis, the thickness for the former ranges from 0.05mm (eyelid) to 1mm (sole of foot) for epidermis. The underlying dermis is usually ten times thicker. Hence burn depth is very different for areas exposed to the same degree of thermal injury.

The skin is very thin in infants and gradually increases until age 30 to 40 years after which there is a gradual thinning. Males generally possess a thicker skin with the average skin thickness in the order of 1-2mm.

Principle functions of the skin are:

- Protective. Against the outside elements including radiation, external trauma, infectious and noxious agents.

- Homeostatic. For fluid, protein and electrolyte balance. The excretions of these are controlled by skin.

- Thermoregulatory. Control of core temperature is maintained through vasodilatation and sweating during exertion.

- Neurosensory. The dermis contains fibres which detect noxious stimuli, and can differentiate between hot and cold.

- Synthetic. Manufactures Vitamin D (1, 25 dihydrocholecalciferol) in response to sunlight. Melanin production can be increased to cope with exposure to ultraviolet radiation

- Immunological. Assists the presentation of antigens to immune cells and provides antibacterial properties in sebaceous secretions. Desquamation reduces the bacterial load on the skin surface.

- Social. Aids social and interpersonal interactions (e.g. emotions)

The epidermis originates from ectoderm and is made up of keratinocytes (epithelial production), melanocytes (melanin production), Langerhans cells (immune function) and Merkel cells (mechanoreceptors). It is subdivided into five layers **(Fig.1.2.1)**:

1. Stratum germinativum (basal layer). A high mitotic rate in this single cell thickness layer aids in the germination of newly formed cells which migrate outwards.

2. Stratum spinosum. Named due to the fact that the cells have a prickled appearance, keratin synthesis occurs whilst active mitosis has ceased.

3. Stratum granulosum. Cells begin to specialise in keratin production

4. Stratum lucidum. Cells lose their nuclei and flatten to join the next layer, typically seen in thicker skin.

5. Stratum corneum. Flattened cells and cellular debris, plus keratin are constantly shed from this protective layer. It is thinnest around the eye, flexor surfaces of the forearms, scrotum and axillae.

The entire process from germination to desquamation takes an average of 27 days in man. Structures that penetrate through the epidermis include hair follicles, sebaceous glands and eccrine sweat glands. These originate from the dermis and are termed as adnexal structures.

The dermis originates from mesoderm and consists of fibrous connective tissue. The ground substance is made up of glycosaminoglycans and proteoglycans through which cellular nutrients can diffuse. The main cell type is the fibroblast
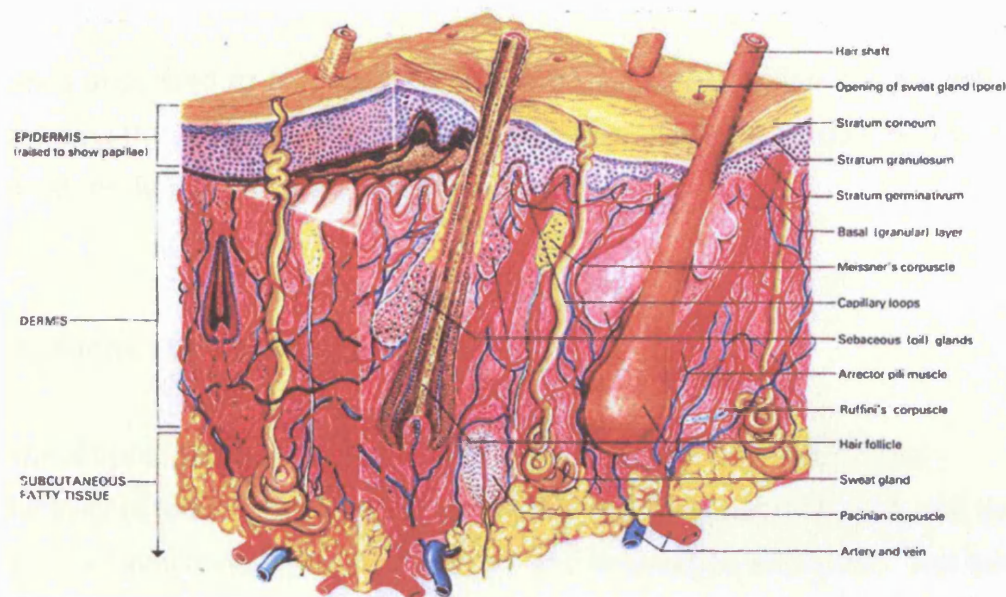
Fig. 1.2.1 The General Structure of the Dermis

for both collagen and elastin synthesis. The former is secreted into an extracellular matrix, undergoing cross-linkage and coiling to form strong fibres that allow stretching coupled with tensile strength. The latter forms elastin fibres that contribute to the resting tension of the skin. A constant process of remodelling occurs which has a basal rate when the skin is resting, increasing with chronic mechanical stress or healing.

The two functional divisions are a thin papillary dermis and a thicker reticular dermis. A subdermal plexus of blood vessels sends branches up to separate these two layers, the so-called dermal plexus. Smaller vessels constitute the papillary plexus.

Other structures within the skin include nerve endings which may be bare or receptor-associated and lymphatic channels.

1.2.2 Definition of a burn

A burn is described as a coagulative lesion on the body surface, the causative factor being transfer of thermal energy to the skin structure. Once the heat is removed, no further necrosis is sustained.

a) Mechanisms of Injury

A thermal transfer of energy to the skin is involved that depends on the conductivity of the skin, the rate of absorption and dissipation of heat, and the presence of insulating layers such as hair and sebaceous secretion. The tissue water content and amount of surface pigmentation are important, as is the presence or absence of clothing.

The duration of contact is important in the history of a burn. The skin is able to tolerate short bursts of high temperatures or modest rises in skin temperature over a longer period. This is due to the ability to dissipate heat. Where absorption exceeds the rate of dissipation, then cellular damage will occur. Temperatures of up to 44°C can be tolerated for up to six hours. Between 44°C and 51°C the rate of cellular damage approximately doubles per degree in temperature rise. Above this, the epidermis will be easily destroyed with total tissue destruction at

temperatures of 70°C or above. The temperature involved alters the immediate response seen. Endothelial cells are damaged at temperatures up to 100 °C causing uncontrolled capillary leakage of exudates; this is a typical picture seen with scalds. Flame burns develop temperatures of between 200 °C and 1000 °C leading to rapid coagulation of tissues and vessels with minimal exudate.

After such overheating, the rate of cooling is dictated by the effects of circulating blood, insulation by the oedema fluid collected around the burn and the evaporation of surface water, which shields the skin from the burning agent. Cooling the affected part is a basic first aid measure. Non-cooled skin rises to a higher temperature post-burn and the temperature returns to normal more slowly. The use of ice immediately lowers the subdermal temperature, reducing the blood flow through vasoconstriction of the deeper, undamaged vessels. This has been found to promote the rate of healing (Moserova et al 1975). The vasoconstrictive effect of cooling does initially limit oedema formation, although post-cooling oedema may be even more extensive as a result of reactive hyperaemia (Jakobsson & Arturson 1985).

Inflammatory cells are stabilised by cooling, with subsequent release of inflammatory mediators being reduced in line with a reduction of immediate oedema formation. This accounts for the soothing effect since noxious stimuli are minimised.

b) Visual Appearance

Three recognised zones can be appreciated following flame or scalding injuries (Jackson 1953) **Fig.1.2.2**:

1. A coagulated central zone of tissue which has undergone instantaneous destruction of tissue structure, cells and cellular content.

2. An intermediate zone with recognisable stasis of blood flow seen within two to three hours post-burn associated with gross tissue swelling.

3. An outer zone of least damage with initial hyperaemia that gradually subsides.

Jackson's Burn Wound Model

Coagulation
Stasis
Hyperaemia
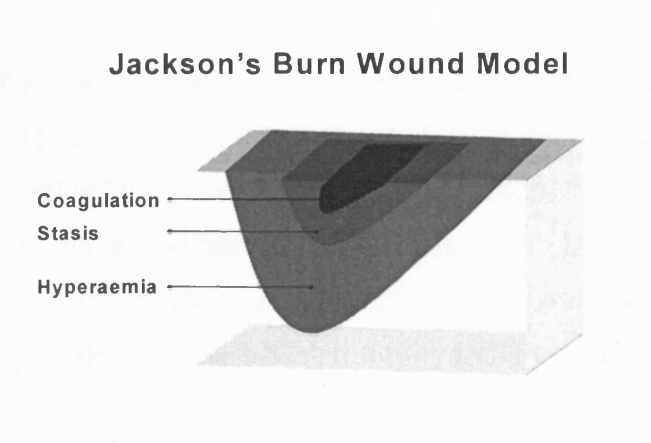
Fig.1.2.2

c)   Depth and Severity of Thermal Burn Injury

This is classed according to the level of anatomical injury **(Fig.1.2.3)**:

- Superficial burns

Only the thin outer epidermis is affected with erythema and discomfort. The pain resolves within 48-72 hours and the damaged epithelium peels off without any residual scarring.

- Partial thickness

The epidermis and variable amounts of dermis are involved, with a further division into *superficial dermal* and *deep dermal* burns. Superficial dermal burns involve the upper third of the dermis with a massive amount of oedema formation. This is due to increased microvascular permeability with formation of a protein-rich

exudate. This lifts off the epidermal layer causing a tense, painful blister since many nerve endings are exposed. With subsequent sloughing of the epidermis, the raw surface will re-epithelialize within two weeks leaving minimal scarring. Deep dermal burns leave only a few viable epidermal cells and healing therefore is only achieved by any remaining adnexal structures. These are not as painful since the majority of nerve endings will have been coagulated. Healing may take many months and results in scarring.

- Full thickness burns

The skin is destroyed at all levels and may involve subcutaneous structures such as muscle, tendon or bone. The residual eschar is avascular and nerve endings have been destroyed with consequent loss of sensation. The colour of the eschar varies from white to black and has a leathery texture. In large quantities, eschar is a potential source of sepsis and must be excised at an early stage. Left untreated, a small burn will heal very slowly, taking many months and leaving a contracted scar.

A combination of the anatomical location, surface area and depth involved, age and pre-existing medical conditions of the patient help to assess the overall severity of a burn injury. Associated inhalational injury further adds to morbidity.

Superficial

Partial –Superficial
Dermal & Deep Dermal

Full thickness

Epidermis

Dermis

Subcutaneous Fat
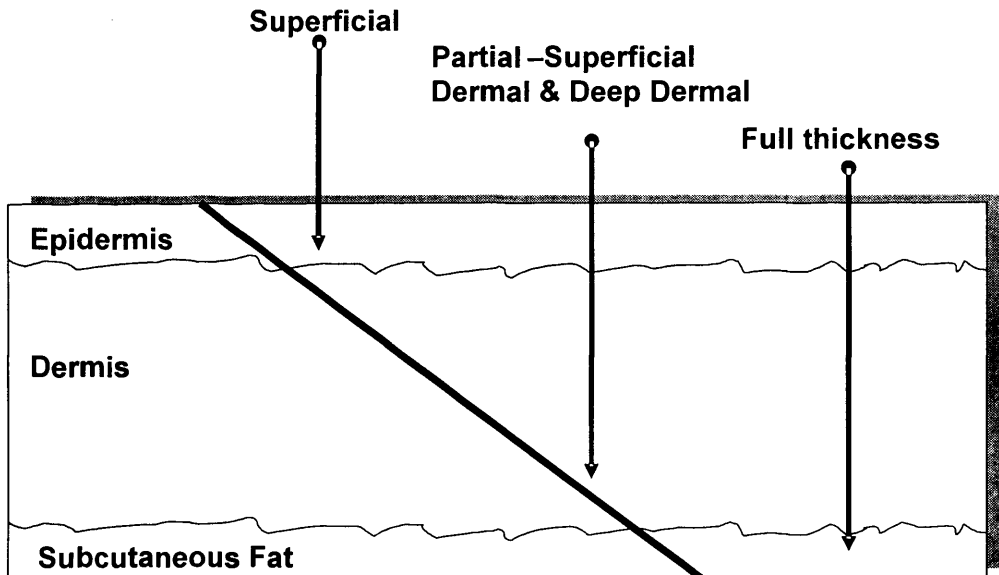
Fig.1.2.3.   Classification of Burn Injury Depth

1.2.3   Other Categories of Burns (Artuson 1996)

a)   Electrical Burns

This accounts for between 3 to 9% of all burns requiring admission to burn centres. High-tension electrical cabling is responsible for the worst of these injuries. Damage arises due to the conversion of heat not only at the point of entry but also during conduction of the current. The type of injury is related to the environment in which the injury occurs, with most domestic accidents involving low voltage. These account for the overall majority of electrical burns. High voltage injuries are seen as a result of industrial accidents where the upper limbs are more commonly affected, often needing amputation.

b)   Radiation

Electromagnetic radiation encompasses a broad spectrum which ranges from radio waves, though to infrared, visible and ultraviolet lights. Even shorter wavelengths are known as ionizing radiation and these comprise of X- and gamma rays. Localised heating of tissues is seen with wavelengths in the middle of the spectrum, for example infra red; however, the shorter wavelengths gamma and X-rays ionize tissue, producing damage via the generation of free radicals. Free radicals are mutagenic to DNA and result in global cellular dysfunction.

Radiation injury is uncommon, although information has been gleaned as a result of nuclear accidents, such as that at Chernobyl in 1986.

c)   Chemical

These comprise 2-4% of burn injuries and often involve less than 5% TBSA. The main categories are acids (40%), alkalis (20%), inorganic agents (30%) and organic agents (10%). This section discusses only those injuries from common toxic chemicals that will be seen in peacetime. Specific chemical weapons will be discussed later in Section 1.6 Vesicant Research.

In contrast to thermal burns where tissue destruction ceases once the heat source is removed, chemical damage persists for as long as the agent is present until it is neutralised by the skin, or washed away. The duration of contact needs to be only for seconds to result in a full thickness burn. The depths of penetration depend on whether the agents are lipophilic in which case they will cross the skin. The various mechanisms involved include dehydration, corrosion, vesication, oxidation and denaturation. The history is vital in ascertaining the type, duration, concentration, and interactions of the exposed chemicals.

Acids tend to undergo an exothermic reaction on contact with moist skin. This generates a thermal burn in addition to a corrosive injury. The resultant dry crust of coagulative necrosis prevents extension of this injury. Of these, hydrofluoric acid

is the most serious since it penetrates deeply, sequestrating intracellular calcium and magnesium leading to the arrest of cellular metabolism. This can result in hypocalcaemia if the doses are large enough. A unique characteristic of these reactions is the intense pain produced on exposure followed by deep, indolent ulcers. Bony decalcification has also been noted from the effects of the fluoride ion.

Alkalis react with proteins and lipids within the skin causing a corrosive, liquefactive necrosis that penetrates deeply. The eschars are soft and soap-like in nature. Due to their prolonged duration of action, the insidious onset of full thickness burns is often missed. Phosphorus spontaneously ignites in air, causing deep thermal burns.

The damage manifests as localised burns with latent, deep extensions. Systemic absorption occurs with the fat-soluble chemicals and may lead to intravascular haemolysis, cardiovascular collapse and multiple organ dysfunction syndrome (MODS). Organic compounds are excreted via the lungs resulting in a chemical pneumonitis.

1.2.4 Burn Pathophysiology

The response to burn injury comprises both local effects at the burnt area and systemic effects. Overall, there is an increased metabolic demand to cope with the initial trauma of the burn, followed by the requirements for wound healing in the aftermath.

1.2.4.1   Local Effects

The coagulative lesion leads to triggering of the inflammatory response with subsequent pain, hyperaemia and oedema. These cause substantial post burn fluid shifts. The release of vasoactive substances such as prostaglandins,

leukotrienes and histamines lead to increased membrane permeability with resultant hypoproteinaemia.

Clinically, the appearance of the burn follows the three zones as described previously: coagulation, stasis and hyperaemia (Jackson 1953). More superficial burns will develop fluid-filled blistering with separation at the dermo-epidermal junction.

A deeper burn lesion will include dermal elements and this accounts for the toughened, leathery appearance of deep burns, termed as eschars.

The zone of stasis will become maximal within two to three days in which the overall eschar size is believed to grow as the burn area demarcates. Eschar will separate off between viable and non-viable tissue. The collection of cellular debris and necrotic tissue provides an effective culture medium with an inherent risk of infection.

1.2.4.2 Systemic Manifestation

a) Cardiovascular

Burn shock plays an important role in the aetiology of cardiac dysfunction after a burn injury. This is due to a combination of the neuroendocrine response to burning and the release of inflammatory mediators from tissue post-injury. Fluid shifts from the intravascular extravascular space persist over the initial 24 hour period as a result of capillary leak over the burn wound. This will manifest as hypotension with decreased cardiac output, tachycardia and oliguria. Ongoing resuscitation should be aggressive based on the patient's response. The role of pressors to artificially maintain a high cardiac index has yet to be fully elucidated; hence early survival from major burns depends strongly on an intact cardiovascular system.

b) Respiratory

Inhalational injuries are sustained when the patient has breathed in fumes directly or been trapped within a confined area where there is a fire. This has a much higher morbidity and the three principal factors are carbon monoxide poisoning, direct injury of the upper aerodigestive tract and inhalation of the products of combustion. Carbon monoxide displaces has a higher affinity for haemoglobin, displacing oxygen, leading to impaired gas transfer. Direct airway burns are less common with steam injury being responsible for the majority of these. Smoke inhalation from burning synthetics includes aldehydes, ketones and other organic agents which are extremely noxious to the respiratory system. Such injury may cause global failure which manifests itself as acute respiratory distress syndrome (ARDS).

c) Metabolic Response

An increased metabolic demand is encountered with stimulation of the sympathetic nervous system inducing a stress response. A cytokine mediated catabolic state ensues, with gluconeogenesis from body muscle and fat, together with rapid glycogenolysis of glucose stores.

An overall negative nitrogen balance will lead to rapid loss of body weight if additional nutrition is not given. Basal metabolic rate (BMR) will alter dramatically with large burns.

In general, the stress response is recognised as comprising two phases. The ebb phase occurs within the first forty-eight hours where there is a decreased metabolic rate, lowered cardiac output with bradycardia and a sugar wasting state with low insulin secretion. Anaerobic metabolism accompanying lipolysis comes into force, and there is an inherent risk of multiple organ failure with a large burn at this stage. This is termed multiple organ dysfunction syndrome (MODS).

The flow phase of the stress response begins after forty eight hours and involves a doubling of the BMR with an increased cardiac output and core body temperature. Increased levels of adrenaline and noradrenaline cause a relative glucose intolerance and insulin resistance, together with high serum cortisol secretion.

An overall catabolic state is produced and the only way to counter this effectively is with supplemental nutrition in addition to normal requirements.

The aims of treatment are then apparent: to restore the integrity of the skin and to reverse the severe metabolic demands placed on the body. These will be discussed later on in Section 1.4 Current Treatment.

Wound Healing

1.3.1 Historical Aspects

A wound is defined as a disruption of the continuity of the integument. Injury to the skin and underlying vasculature triggers a sequence of events that result in inflammation, and the subsequent processes used to restore normality are termed 'wound healing'. The complications can be life threatening, commonly affecting functional ability, and virtually always compromises appearance.

The healing of wounds is through formation of a scar that acts as a patch rather than a replacement for the restoration of structural integrity to the tissue. Problematic scars can be too weak (stretching), too strong (compressing important structures), and too abundant (hypertrophic and keloid scars).

The physiology of wound healing has been a concern of physicians throughout the ages. The earliest medical writings of the Smith Papyrus (1700 BC) dealt extensively with wound care. The ancient physicians of Egypt, India, and Europe practised gentle methods for dealing with wounds, appreciating the importance of foreign body removal, apposition of skin edges with suturing, and protecting injured tissues from the environment with clean materials. With the invention of gunpowder and ensuing gunshot wounds, however, a new approach to wound care emerged that no longer relied on the natural processes of soft-tissue repair. For the next 250 years, surgeons aggressively treated patients who had open wounds with cautery, sealing wounds by boiling oil or scalding water. This still resulted in a great number of septic, and often fatal, wounds. In the 16th century, the great French army surgeon, Ambroise Paré, rediscovered the value of gentle methods of wound care. During the battle of Villaine the supply of oil had been depleted, and Paré was forced to use milder salves and clean dressings on amputation stumps. He discovered that these wounds healed rapidly without the expected complications, and from this modest beginning the modern era of wound care evolved.

Over the next three centuries following Paré's observations, the understanding of the biological processes involved in wound healing was limited to John Hunter's experiments with replantation and his speculations about the difference between wound contraction and contracture. Joseph Lister's writings on wound sepsis, and Alexis Carrel's notes on organ transplantation and tissue preservation furthered this knowledge. The cellular changes in the healing of soft-tissue wounds were not elucidated until this century although the principles of inflammation - tumour (swelling), dolor (pain), rubor (erythema) and calor(heat) had been known since Ancient Greek times.

1.3.2 Cutaneous tissue repair - An Overview (Hunt et al 1997, Cotran et al 1994)

A general schema of the pathways involved is shown (Fig.1.3.1). There are three recognised phases: the initial inflammatory phase, fibroblastic repair and final maturation. A complex mixture of cytokines, growth factors, complement and other inflammatory mediators will be involved in this.
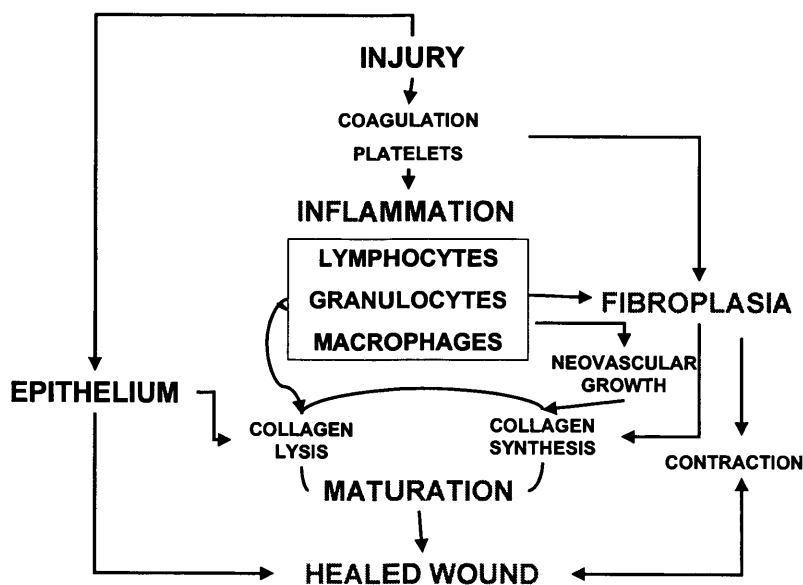
Fig.1.3.1 The Overall Healing Process

The simplest example of wound repair is the healing of a clean, uninfected surgical incision where the edges are approximated by surgical sutures. This is referred to as healing by *primary intention*. The incision leads to localised necrosis of both epithelial and connective tissue cells as well as disruption of epidermal basement membrane continuity. The narrow incisional space immediately is filled with a plug of coagulated blood containing fibrin and blood cells and subsequent dehydration of the surface clot forms the scab that covers and protects the wound. An environment is then created which is both chemo-attractant and growth promoting.

Within 24 hours, neutrophils appear at the margins of the incision, which migrate toward the fibrin clot. The epidermis at its cut edges begins to thicken due to increased mitotic activity of the basement membrane, and within 24 to 48 hours tongues of epithelial cells migrate from the edges and grow along the cut margins of the dermis, depositing basement membrane components as they move. Fusion along the midline beneath the surface scab produces a continuous, thin epithelial layer.

By 72 hours, the neutrophils have mostly been replaced by macrophages and granulation tissue now progressively invades the incision space. Necrotic debris is removed by phagocytosis. Collagen fibres are now present in the incision margins, although initially these are vertically oriented and do not bridge the incision. Epithelial cell proliferation continues, which thickens the epidermal covering layer.

Over the next few days, the incisional space is filled with granulation tissue and the rate of neovascularisation becomes maximal. Collagen fibrils become more abundant and begin to bridge the incision. The epidermis recovers its normal thickness, and progressive differentiation of the surface cells leads to a mature epidermal architecture with surface keratinization.

During the second week, there is continued accumulation of collagen with proliferation of fibroblasts. The inflammatory leukocytic infiltrate and increased vascularity have begun to regress. At this time, the process of blanching begins,

accomplished by the increased accumulation of collagen within the incisional scar , accompanied by the slow regression of vascular channels.

Within a month, the new scar comprises of a cellular connective tissue that is devoid of inflammatory infiltrate and covered intact epidermis. The dermal adnexae that have been destroyed in the line of the incision are permanently lost. The tensile strength of the wound increases gradually thereafter, with about 50% of the final amount reached by six weeks. The provisional matrix is turned over, rearranged and strengthened. It may take months for the wounded area to obtain its maximal strength.

Where there is tissue loss in surface wounds or with complications such as those of infarction, ulceration and abscess formation, the process of repair is more complicated. The common denominator in all these situations is a large tissue defect that must be filled. Regeneration of parenchymal cells cannot completely reconstitute the original architecture. Closure is achieved by granulation tissue that grows in from the margin to complete the repair. This form of healing is referred to as healing by *secondary intention*.

Secondary healing differs from primary healing in several respects:

1. The inflammatory reaction is more intense since large tissue defects initially have   more fibrin, necrotic debris and exudate that must be removed.

2. Larger amounts of granulation tissue are formed.

3. The main difference seen in secondary healing is the phenomenon of wound contraction, which occurs in these large surface wounds. Contraction is attributed to the presence of myofibroblasts - altered fibroblasts that have the ultrastructural characteristics of smooth muscle cells.

4. The aesthetics of these wounds are invariably less desirable than those healed by primary intention.

The proportion of collagen types in healing wounds is altered during remodelling. Initially, Type III collagen is in abundance, but as healing progresses this is converted to Type I with a final ratio of 4:1 of Type I:III. Type V collagen is noted to form in parallel with granulation tissue and tissue vascularity.

Degradation aids in the debridement of injured sites and also in the remodelling of connective tissue necessary to repair any defect.

During this remodelling period, the wound is prone to both contraction and stretching. Wound contraction is attributed to myofibroblasts that are linked together as sheets by a complex of fibrils and filaments known as the fibronexus. This is usually beneficial for open wounds but can become disabling if it occurs across a joint where it is referred to as a contracture. This may need to be overcome with passive stretching and splintage. Stretching of the wound will occur where the tension is great enough overcome contractile forces, leaving a lax scar.

When sutures are removed, usually at the end of the first week, wound strength is approximately 10% of the strength of unwounded skin, but it increases rapidly over the next 4 weeks. This rate of increase then slows down approximately nine weeks after the original incision, plateauing at about 70 to 80% of the tensile strength of unwounded skin. The time-scale is altered by many factors including the length of the initial inflammatory process, site and size of the injury.

Ultimately, an ideal healed wound has a closed surface with an intact epithelium and minimal scar reaction.

1.3.3 Wound Healing in Burn Injuries   (Holmes & Muir 1983)

Although the above applies to all wounds, burns in particular will heal by secondary intention due to their large surface area involvement with epithelial

damage. In superficial burn wounds, the epithelial loss will spontaneously regenerate from the intact epidermal elements. No new connective tissue is formed since the full thickness of the skin has not been transgressed and therefore no resultant scarring is expected.

Partial thickness burns are deeper, involving the papillary and reticular dermis. These will re-epithelialize over a period of time that varies with the depth of the burn. The two main sources for healing are the remaining intact epithelial layers with their dermal appendages (e.g.hair follicles, sweat and sebaceous glands) and new connective tissue from the subcutaneous and dermal-subcutaneous interface. The roots of the dermal appendages are still intact with partial thickness burns hence epithelialization can still be contributed from the remnants **(Fig.1.3.2)**.

Full thickness burn may extend into fat, fascia or even bone and necessitate excision to viable tissue with coverage by split skin grafting. Rpaid healing necessitates removing thin sheets of skin from suitable unburnt areas and placing them over an adequately debrided bed free of any necrotic tissue. Scar severity is dictated by depth of burn and the length of time involved in healing, with untreated full thickness burns inevitably resulting in unacceptable scarring. This is where the wound has healed solely by secondary intention.
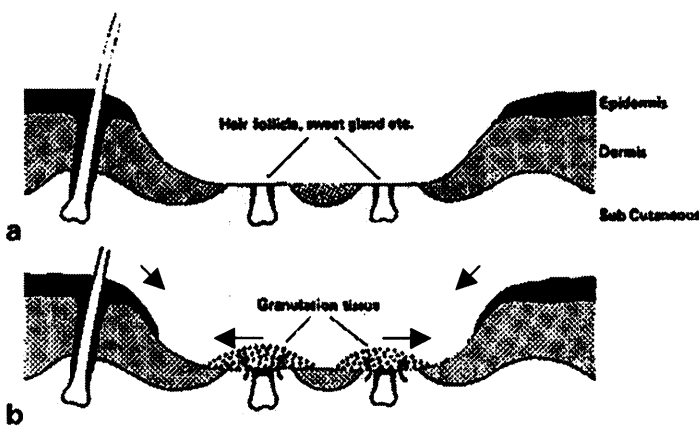
Fig.1.3.2 Sequence a-b represents the spontaneous healing after burn injury. Note epithelialisation from wound edge diminishing wound size. (Reproduced from the British Journal of Surgery 1983;70:611-613. with permission of the publishers, Blackwell Scientific.)

1.3.4 Burn Wound Closure - Uptake of Split Skin Grafts

Split skin grafting **(Fig.1.3.3)** is the most common technique by which the debrided burn wound is closed over. Instead of waiting for secondary intention healing which may take months, effective skin grafting can be successful within days. Containing both epidermal and dermal elements they are able to adhere to the wound surface and provide permanent cover, restoring the protective integrity of the skin.
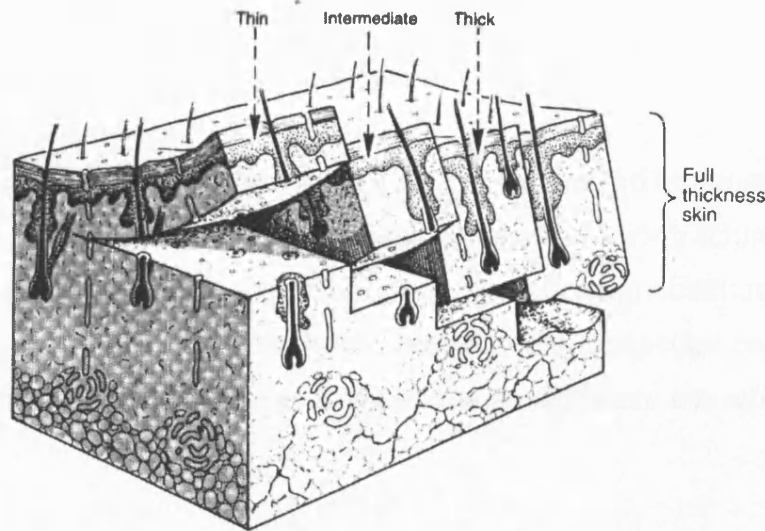
Fig. 1.3.3 Anatomical Levels of split skin graft
(Reproduced from Fundamental Techniques in Plastic Surgery with permission of the publisher, Churchill Livingstone)

Graft uptake is described in three recognised phases: tissue imbibition, inosculation and revascularisation:

- Imbibition of serum prevents the graft from becoming desiccated and enables survival whilst the graft is avascular over the ensuing 48 hour period
- Inosculation involves a fine network of capillary buds growing into the graft
- Revascularisation involves the formation of a functional blood supply to the new graft which is established as early as the sixth day.

The adherence of the graft is initially maintained by fibrin for the first three days

and then subsequently by the fibrovascular ingrowth and neovasculature formation. The graft then undergoes a process of contraction inversely proportional to the thickness of dermis present within the graft.

Contraction is greatest for thin split grafts and least for full thickness skin grafts, the mechanism of which is uncertain. Brown (1990) postulated that this may be due to the inhibitory effect of intact dermal collagen i.e. contact inhibition.

1.3.5 Complications of healing (Su et al 1998)

Scars can become problematic if too loose, too tight or too abundant. Flexion contractures, neuromas, fibromatoses (e.g. Dupuytren's contracture), peri-articular joint stiffness, intra-abdominal adhesions causing obstruction, duodenal ulcer strictures, liver cirrhosis, breast implant capsular contractures, burn scar contractures, hypertrophic scars and keloid scars are all within this group.

1.3.5.1   Hypertrophic scar

This describes raised, erythematous, pruritic lesions within the confines of the original scar. Although all scarring is temporarily hypercellular and contains more extracellular matrix components, this begins to resolve by six months. Persistence of this defines the hypertrophic scar. They are commonly formed in areas of high tension and movement such as anterior chest, neck and shoulder regions along with the flexor aspects of the extremities. Planning the alignment of these scars in the lines of least tension minimises risk of hypertrophy. Trigger factors include infection, dehiscence, minor trauma, haematoma or a foreign body where the wound has to heal by secondary intention. They are highly vascular and contain large amounts of Type V collagen arranged in nodules with myofibroblasts. Chronic inflammation is also a causative factor especially noted with earlobe scars from earrings. Often these are extremely prominent and mistaken for keloid scars, but where normal scarring occurs elsewhere with the patient, then they are best

described as inflammation-induced hypertrophic scars. Regression may be seen as late as two years after the original injury thought to be due to the myofibroblasts present within the scar tissue.

1.3.5.2 Keloid Scar

Where the scar tissue outgrows and exceeds the original wound size, this is then termed keloid scarring. Derived from the Greek *chele*, meaning crab claw, the name aptly describes their invasion into normal tissue. They are similarly erythematous and pruritic, although their growth behaviour is in a manner akin to benign hyperplastic soft tissue tumours. The condition is thought to be a genetic abnormality with the wound healing process and there is usually a family history. Children and adolescents are most affected although this tendency does not always remain in adult life. Le Flore (1980) reports incidences of keloid scars in Negroids between 5 to 15 times that seen in Caucasians. Oriental skin has a lower tendency to keloid formation, with Arnold (1959) reporting an incidence of between 3 to 5 times that of Caucasian skin. Histologically, they consist of thick irregular branches of collagen, which are hypersensitive to growth factors (Cohen 1977) and hormonal influences. Rapid enlargement of keloid scars during pregnancy has been described (Moustafa 1975). They may develop up to a year after the original insult, even with minor trauma such as a scratch or acne furuncle, and rarely undergo spontaneous regression.

1.3.5.3 Contracture

Wound contraction draws normal skin into a site previously occupied by the wound. If this occurs over joints, a functional deformity is incurred, with the limitation of movement due to a taut scar. This is then termed a contracture. The mouth, eyes and neck are less resistant to contractile forces and may develop both a physical and cosmetic deformity.

Current Treatment of Burns

The treatments available for burns can be divided into conservative therapy with dressings alone through to active surgical debridement with the usage of skin or skin substitute to cover the resultant defect.

1.4.1 Assessment & Resuscitation

An accurate assessment of the percentage of the body surface area involved, together with an estimation of burn depth are vital in planning treatment. Standardised charts to evaluate the percentage of total body surface area (TBSA) involved are useful. In 1951, Wallace's described his 'Rule of Nines' dividing major parts of the body into easily calculated segments **(Fig.1.4.1)** which is applicable to patients over the age of fifteen, with subsequent modifications by Lund & Browder for children **(Fig. 1.4.2)**. The latter have larger heads in relation to body size, hence the correction facto

Fig. 1.4.1 Rule of Nines

Fig.1.4.2 Lund & Browder Chart

1.4.1.1 Burn Depth

Rapid changes within the zone of perfusion mean that a mixed depth burn is often difficult to assess for the first 48 hours. Heimbach (1992) reviewed various supplementary aids that have been tried to increase accuracy, including the use of vital dyes, burn biopsy, fluorescein and laser Doppler flowmetry to assess dermal microcirculation. It was concluded that none of these tools were completely successful. Serial clinical assessment still remains as one of the most useful tools in the estimation of burn depth.

1.4.1.2 Burn Resuscitation

Successful management of shock is critical in major burns and has been shown to reduce morbidity (Miller 1994). The aim of fluid resuscitation is to replace that which is sequestered as a result of the injury, best monitored by hourly observations of urinary output. Several formulae exist to calculate the appropriate amount including those by Evans, Muir & Barclay and Parkland (Warden 1992). Formulae based on weight and TBSA, they estimate a quantity of fluid to be infused over the ensuing 36 to 48 hour period to cope with fluid losses. Children will require additional maintenance fluids, again based on weight since they have less reserve.

1.4.1.3 Choice of fluids

Salt replacement is the main factor with crystalloid solutions such as dextrose saline, hypertonic saline and Ringer's lactate. Protein solutions such as albumin and fresh frozen plasma have been used to generate an inward oncotic force to overcome the outward hydrostatic force encountered in the capillary system. Other colloidal solutions such as dextran and hetastarch are more potent in their ability to retain water within the intravascular compartment. There are various regimes used within each Burn Unit depending on local preference.

1.4.1.4 Inhalational injury

Airway injury probably accounts for more mortality than surface burns since it is often latent and overlooked. Two components contribute to this injury: inhaled noxious substances produced by combustion such as aldehydes, ketones, organic acids and carbon monoxide. The lattermost has a much higher affinity for haemoglobin, resulting in lowered oxygen delivery to tissues. Blood levels of carboxyhaemoglobin in excess of 15% are usually indicative.

In addition, injury to the airway mucosa results in oedema that parallels that of the body, resulting in a constricted bronchial tree. The combination of these can be fatal and is responsible for triggering acute respiratory distress syndrome (ARDS) which has a 60% mortality rate. Damage to the bronchial tree with sloughing of dead tissue predisposes the patient to further risk of pneumonia.

Treatment is supportive only with early intubation aimed at maintaining the airway to allow ventilatory support until the injury subsides. Hence a history of smoke inhalation must be treated with extreme suspicion with a low threshold for intubation as necessary.

1.4.1.5 Nutrition

As discussed previously the hypermetabolic state triggered by burn injury necessitates high volume nutritional support for effective repair. Establishment of early enteral feeding, ideally within the first 18 hours post-major burn has been shown to reduce mortality (Raff et al 1997). Feeding is usually enteral until the patient is able to tolerate an oral diet, when the former is gradually tailed off. The patient is monitored with a combination of intake and weight charts along with serum protein levels or urinary nitrogen collection

1.4.1.6 Wound Care

After initial assessment, the burns should be debrided and dressed. Any loose skin and burst blisters should be debrided. These must be performed with

adequate amounts of analgesia. Meticulous preparation of wound surfaces will allow early healing to take place in partial thickness burns, minimise infection and also prepare areas of full thickness burn in readiness for grafting.

1.4.2 Special categories of Burn

1.4.2.1   Electrical

Specific treatment for these types of patient includes protection of the airway, and ventilatory support may be required. A forced diuresis (75-100ml/hr) must be maintained to ensure that the effects of myonecrosis on the kidney are minimised. This is achieved with large volumes of Ringer's lactate, which are often underestimated, since reliance on the total burnt surface area (TBSA) in fluid formulae does not consider the associated, deeper conduction injury. In addition, alkalinization of the urine can be achieved by the addition of 50 ml aliquots of 7.5% sodium bicarbonate (45 mmols, to a maximum of 400 mmols for an adult) to the infusion. This counteracts the acidosis which develops over the next four days. Monitoring of the cardiac rhythm must be observed since cardiac myonecrosis can develop. Elevated cardiac isoenzymes (CK-MB or Troponin-T) aid the diagnosis and should be taken serially to monitor progress.

Demarcation of non-viable tissue may require angiography. Anti-thromboxane agents can limit the extension of the necrosis pending surgical debridement.

Surgical intervention, planned after adequate resuscitation, takes on two forms: immediate injuries requiring escharotomy and fasciotomy to prevent loss of limb, combined with adequate debridement of necrotic tissue and coverage of defects with skin grafts. Associated fractures and trauma must be recognised and treated appropriately. These include intracranial pathology and long bone, spinal and pelvic fractures, since falls are often sustained during these injuries. Secondary, reconstructive surgery aims to maximise function with the remaining healthy tissue. Prostheses may often be required if loss of limb was sustained.

1.4.2.2   Radiation

Radiation sickness begins within 48 hours of exposure with doses in excess of 2 Sieverts. The radiosensitive tissues include the skin, bone marrow, gastrointestinal tract and reproductive organs since these contain cells with a rapid turnover. Initial symptoms include nausea with vomiting, fatigue and disorientation. The skin develops burn lesions, which may be as mild as erythema with vesication but can develop into full thickness burns that lead to ulceration. Although these heal, in later years they may undergo malignant change to become basal and squamous cell carcinomas. Bone marrow suppression occurs, resulting in loss of all germ cell lines leading to immune suppression, anaemia and thrombocytopenia. The sickness is divided into three stages: the initial prodromal phase of 48 hours, the latent period which may last a few weeks, followed by the critical phase (the nadir point of the immune system).

Treatment is supportive with intravenous infusion of fluids and blood products, nutritional support and maintaining a sterile environment. The patient is most vulnerable to sepsis during the first five weeks, after which time the bone marrow will have begun to recover. Total body irradiation is used as a therapeutic modality for haematological malignancy and presents with a similar picture before bone marrow transplant. In the same manner, a patient with a total body irradiation injury can be treated with a bone marrow transplant.

1.4.2.3   Chemical

Treatment involves a careful assessment of the exact nature of the chemical and the conditions under which exposure occurred. Copious irrigation with water followed by application of a specific antidote if available as listed in **Table 1.4.1**

a) Eye Injuries

Ocular exposure requires copious water or saline irrigation, keeping the eyelids forced apart. The intense blepharospasm may need to be overcome with a topical local anaesthetic. Alkalis are particularly dangerous since the cornea is

easily penetrated, leading to saponification of plasma membranes and collagen denaturation. This leads to corneal ulceration with the possibility of blindness. The management of ocular injury takes precedent over skin and warrants transfer to an ophthalmic unit.

b) In General

All contaminated clothing should be removed with irrigation continued for hours if possible, which will minimise any exothermic reaction and dilute any remaining agent. If water insoluble, then soap and water should be used.

Respiratory burns are often sustained after the escape of a cloud of noxious gas and may require oxygen therapy, bronchodilators, steroids and ventilation if extensive. Antibiotic cover is needed if a chemical pneumonitis develops.

Ingested caustics should be treated with milk rather than an emetic since aspiration pneumonitis can further compound morbidity. An ingestion injury also takes precedent over a skin injury since perforation of a viscus is possible mandating laparoscopy or exploratory laparotomy. Phosphorus is unusual since it will continue to burn if exposed to air; wet dressings should be kept on until an antidote is available. Placing the patient in a dark room will enable traces of phosphorescence to be identified. Cyanide poisoning can result in respiratory arrest, requiring oxygen and ventilatory support besides parenteral sodium thiosulphate.

With exposure to high concentrations of hydrofluoric acid, sub-eschar injection of calcium gluconate or even early excision and skin grafting has been proposed. The agent penetrates into the subungual region of the finger with ease. Such is the vasospastic effect of this that digital gangrene is a distinct possibility, requiring removal of the nail and placement of the digit in calcium gluconate gel. Specific antidotes for each chemical are in **Table 1.4.1**

1.4.3 Burns Surgery

1.4.3.1 Escharotomy & Fasciotomy

The obvious immediate priorities are those that are life and limb threatening. Circumferential, full thickness burns of limbs are at high risk of vascular compromise since they can act as a tourniquet. In the initial resuscitation period escharotomy should be performed over such areas. Releasing incisions are designed to avoid major sites of nerve injury.

Chest escharotomy may be required for burns to the torso especially children who rely more on abdominal movement for respiration. The incision separates the abdominal and chest areas, allowing further respiratory excursion.

Early onset compartment syndrome may necessitate formal fasciotomy as well.

1.4.3.2 Excision & Skin Grafting

The burn tissue is in contact with a large surface area of healthy tissue and it is this necrotic load that contributes to the risks of infection. Early tangential excision and burn wound closure controls infection and conserves viable tissue ideally within five days of resuscitation. With deep dermal burns the graft bed may still include dermis and this is less cosmetically disfiguring than with a full thickness excision.

1.4.4 Alternative Debridement Strategies

1.4.4.1 Dermabrasion

Dermabrasion of burn injuries was proposed for deep dermal burns as a method of accelerating healing without the application of skin grafts (Lorthioir 1963). The method involved the use of abrasive discs to remove burn tissue to the point at which the presence of bleeding indicated viability. Removal of all necrotic tissue converted the burn into a shallow ulcer that encouraged

spontaneous epithelialisation via remaining intact dermal appendages. Krant (1977) and Holmes (1984, 1987) described dermabrasion as a method of accelerating the healing of deep dermal burns that had not healed spontaneously by two weeks. Wounds were dermabraded until viable tissue was reached, thus preparing a ready recipient bed for the application of skin grafts. If there was still a degree of intact reticular dermis as evidenced by a honeycomb appearance of the burn surface, then Holmes' practice was not to graft. In some cases, Krant described failure of skin graft take in areas where spontaneous re-epithelialisation had occurred i.e. that grafts were not always necessary. These were most probably the type of deep dermal burns which Holmes' group did not graft anyway.

Holmes believed that the mechanism of accelerated healing lay in the removal of granulation tissue that was competing with the epithelial cells to cover the burn surface. The dermabrasion process would liberate such foci of epithelial cells allowing them to rapidly cover the remaining surface leading to rapid maturation of any granulation tissue present (Fig. 1.4.3). This would lead to a minimising of the risk of scar hypertrophy.

Furthermore, the technique produced similar or superior aesthetic results with less blood loss incurred and excellent graft uptake where needed. Rapid wound healing and more stable dermis resulted since some original elements still remained.

By delaying the first excision in carefully selected burns, this allowed the superficial parts of mixed depth burns to heal spontaneously, thus requiring smaller areas of skin graft if at all required. It combined the benefits of both tangential excision with delayed primary excision.

Although theoretically sound, the method has not gained wider acceptance since it is time-consuming, prolongs hospital stay, and applicable therefore only to smaller surface area burns.
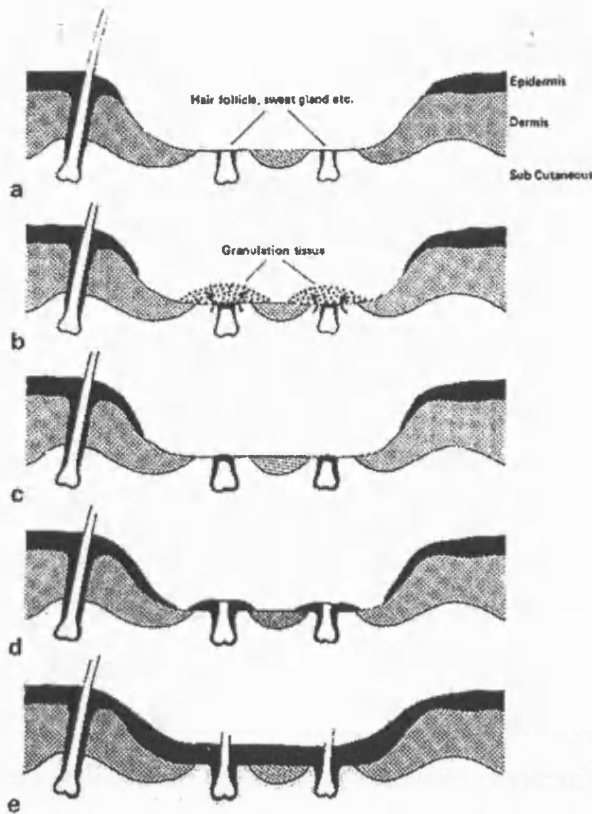
Fig. 1.4.3 Sequence a-b represents the spontaneous healing after burn injury. Note epithelialisation from wound edge diminishing wound size.

c)After dermabrasion, granulation is removed
d,e)Epithelialisation

(Reproduced from the British Journal of Surgery 1983;70:611-613. By permission of the publishers, Blackwell Science.)

1.4.4.2 Laser debridement

Lasers have the effect of being able to coagulate and vaporise tissue in the path of the beam and Hall (1971) first demonstrated the potential for use as a scalpel. However, a residual zone of necrosis was noted at the wound edges and hence the use of the laser for skin incisions was advised against.

The perceived advantages include the prevention of significant haemorrhage with accurate debridement of burn eschar. These will be discussed in detail in Section 1.5.6 Burn Debridement with Lasers.

1.4.5 Closure of Burn Wound (Kelton 1999)

1.4.5.1 Skin Grafts

Skin grafts are portions of skin, which are removed from one anatomical (donor) site and placed in another over a defective (recipient) area. Successful take of the skin graft will ensure rapid healing, minimising any infection and recipient site morbidity.

With a split skin (Thiersch) graft (SSG), a sheet of epidermis and dermis is removed from a cosmetically acceptable site (usually the thigh or buttock) with a thickness of around 300-375µm (Fig.1.4.4). The dermal adnexae, such as the sebaceous glands and hair follicles, remain intact and thus the donor site heals rapidly, normally within two weeks. The SSG can be perforated to aid drainage of any haematoma and if meshed using a dedicated device, can be expanded to cover a surface area of up to six times its original size. With healing, these grafts are likely to undergo excessive contraction and may cause scarring.

Fig. 1.4.4 Split Skin Graft
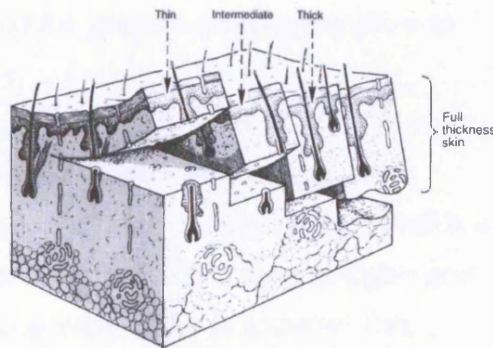
Fig. 1.4.5 Anatomical Levels of skin graft
(Reproduced with permission of the publisher, Churchill Livingstone)

A full thickness (Wolfe) graft (FTSG) includes all the dermis with its associated adnexae (Fig.1.4.5). It is used for the face since it is better matched for cosmetic appearance and can be used to cover exposed neurovascular structures, cartilage and bone (provided the periosteum is intact). The donor

site needs to be closed either directly, which limits the amount of graft which can be harvested or cover with a SSG. Common donor sites include the post- and pre-auricular areas, supraclavicular area, groin and upper arm with obvious limitations as to the size of the grafts that can be taken. Due to the thickness, contraction is less problematic although graft uptake may not be as reliable if the graft is too thick.

Usual clinical practice mandates that these are *autografts*, originating from the patient themselves.

1.4.5.2 Skin Substitutes

Where large amounts of skin are needed for cover, then cadaveric SSG is useful in providing at least temporary cover. There is a minimal risk of viral transmission, namely hepatitis and HIV; hence the use of allografts is limited in clinical practice. Allografts can 'take' on the recipient site, but depending on the immune status of the patient will usually be rejected within a week. Cadaveric allografts preserved with glycerol, amniotic membrane and xenografts such as pigskin have all been tried with variable success and acceptance.

Cultured epidermal autografts have been successful but are expensive and fragile since there is no dermal element, and the grafts are highly sensitive to infection.

Synthetic substitutes have been formulated on a bilaminar structure with a completely synthetic framework or a collagen-synthetic composite. Integra® is a synthetic dermal template consisting of a porous matrix of bovine collagen and shark glycosaminoglycan together with a disposable layer of silicone. This product is applied to full thickness wounds after excision, and becomes vascularised to produce a 'neodermis'. The silicone layer is subsequently removed to allow grafting with very thin split skin grafts according to the availability of donor sites. The principle is to temporise and provide wound cover so that enough autograft can be harvested for full closure. This allows re-cropping of donor sites previously used.

1.4.6 Prevention of Scarring (Su et al 1998)

Superficial burns heal quickly leaving inconspicuous scars, but it is generally agreed that burns which heal in longer than two or three weeks invariably result in some degree of hypertrophic scarring. Careful design of the surgical incision and early closure reduces the inflammatory phase of healing and thus the degree of fibrosis. Early wound closure can be achieved directly or with the aid of a skin graft, flap or biological dressing. The incision should be planned to achieve closure with the minimal of tension from sutures. Careful tissue handling, adequate debridement and meticulous haemostasis also aid in this.

Contractures should be anticipated and treated early with the use of scar massage, compression and static splinting to prevent loss of function. This modulates the reorganisation of newly laid collagen, enforcing an orderly matrix to be formed.

With a high mitotic rate, a healing wound has a likelihood of tumour induction that must be borne in mind. A long-standing ulcer may undergo malignant transformation to become a Marjolin's ulcer.

Wound dehiscence can occur within the first few weeks post-surgery with excessive tension. Lesser degrees will result in stretching of the scar especially in the presence of concurrent disease such as diabetes and malnutrition (hypoproteinaemia and vitamin C or zinc deficiency).

1.4.7 Treatment of Scars

Conservative measures include compression therapy to limit burn scar hypertrophy described by Linares (1993) at the Shriner's Burns Institute, and silicone gel sheeting which probably provides a similar pressure effect (Mercer 1989). Low-dose ionizing radiation as an adjunct to the excision of keloid scars was shown to be useful as early as 1961 by Cosman, although the acceptance

of the procedure has been cautious due to the risk of inducing carcinogenesis and stunting of growth in children.

Intralesional steroid injections have been shown to reduce the amount of scar tissue formed post-excision of keloid (Murray 1963).

Surgical excision to realign the tensile forces with the use of Z-plasties, flap closure or serial excision are successful although in combination with one of the aforementioned conservative therapies has a higher success rate.

Lasers in Surgery and Medicine

1.5.1 Physical principles

A laser is an instrument designed to generate an intense beam of electromagnetic energy of a given wavelength. Medical applications of this high-energy beam rely on the ability of the laser to coagulate, vaporise and cut tissue.

The origins of the laser are in the essential physics that were developed by Albert Einstein in 1917 as the revolutionary concept of quantum theory. Max Planck postulated that all atomic systems had to be restricted in the energies that they could attain - they were quantised. With this, Einstein proposed the idea of stimulated emission, the inverse process of absorption.

Electrons within an atom are generally in a ground state but according to quantum theory electromagnetic energy may be added to the atom in discrete 'packets', quanta, leading to excitation of the atom. If a photon, a quantum of light, has sufficient energy to bridge the gap between two energy levels then it will be extinguished in the process of doing so - known as photon absorption. In the excited state however, the atom will be stimulated to emit a photon in response - photon emission. With a sufficient stimulus a 'population inversion' occurs where the majority of the atoms are in an excited state. Being identical, these released photons will cause a chain reaction with two photons stimulating two new photons and these four photons going on to produce eight and so on. The light generated by these uniform photons has a minimal spread of wavelength and propagates in a parallel and monochromatic bundle. This is termed as coherent light and the process used to generate this is known as 'light amplification by the stimulated emission of radiation', or **laser**. The medium in which this reaction takes place is termed the lasing medium and is commonly gaseous e.g. Argon or Carbon Dioxide, but can be crystalline e.g. Neodymium Yttrium Aluminium Garnet (Nd-YAG). Other rare earth elements

used for lasing media include Erbium and Holmium.

Laser light can be focused by the use of mirrors and deploying a narrow focal region to concentrate the power generated. Such a concentration of energy over a small area of material that can absorb this radiation will generate intense heat with subsequent vaporisation of the material.

1.5.1.1   Types of Laser

Each type of lasing medium has a different emitted wavelength that determines the degree of absorption in tissue **(Fig. 1.5.1)**. (Crumplin 1984) demonstrated below:
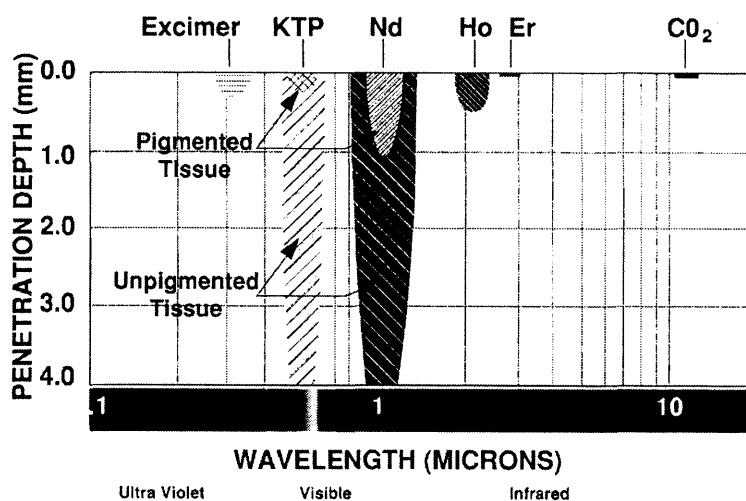
Fig. 1.5.1. The Absorption Lengths for Various Laser Media
This shows the relative depths of absorption for each lasing medium
with only minimal penetration of Erbium(Er) and Carbon Dioxide (CO₂)

- Carbon Dioxide is in the infra red spectrum with a wavelength of 10.6μm (10 600 nm). Since it is rapidly absorbed by water it is ideal for skin surface vaporization and wet drapes may easily be employed for protection. Corneal burns are seen with this type of laser.

- Copper has a wavelength around 510nm and is used in the treatment of vascular lesions since it is able to selectively rupture the feeding vessels to telangectasia and port wine stains.

- Nd-YAG penetrates more deeply with a smaller wavelength of 1.06μm (1060 nm) in the invisible infra red spectrum. Depths of 3-5mm can be achieved leaving a residual eschar of damaged tissue. The above two lasers are invisible to the naked eye and require a guiding laser beam such as Helium-Neon to show the operator the direction of aim.

- Argon generates a wavelength of 490-510nm with blue/green visible light that is absorbed by red pigment. The depth achieved by the last two lasers can cause retinal injury with damage to central vision.

- Erbium-YAG is highly absorbed by water and does not penetrate deeply into tissue with a wavelength of 2.94μm. It is able to vaporise thin sections of skin (~5μm thickness) and mucosa.

1.5.1.2   Laser safety issues

There are specific regulations governing the safe usage of lasers by the British Standards Institute (1977). These relate to setting up and using the equipment together with intra-operative safety. These details are covered in **Appendices 2 and 3**.

1.5.2 Lasers in Surgery & Medicine

The first lasers were used in the 1960's with pioneering ophthalmologists utilising the potential of the laser to treat retinal pathology (Flocks and Zweng 1966). Since then, their usefulness as surgical tools has gained wider acceptance due to the precision, tissue selectivity and haemostatic effect

offered. Hence, modern applications encompass most surgical specialities including neurosurgery, ophthalmology, dermatology, otorhinolaryngology, gynaecology, general surgery, cardiovascular surgery, urology and plastic surgery:

- Gastrointestinal tract. The Nd-YAG laser is used to ablate tumours of the oesophagus and rectum, secure haemostasis in bleeding lesions and in laparoscopic surgery such as cholecystectomy and tumour resection.
- Upper airway. The CO_2 laser is used to ablate tumours of the pharynx, larynx, nasal and sinus cavities.
- Urology. Bladder tumours are amenable to ablation by Nd-YAG laser.
- Ophthalmology. The Nd-YAG laser is used in cataract surgery and in the treatment of glaucoma. Photocoagulation for diabetic retinopathy is also commonplace.
- Gynaecology. The CO_2 laser is used to treat vulval and cervical and uterine lesions.
- Dermatology. Removal of tattoos and vascular lesions such as port wine stains and haemangiomas is possible with copper and argon dye lasers. This is due to the selective ability of the laser beam for certain pigments.
- Plastic Surgery. The CO_2 and Erbium -YAG lasers are used for skin resurfacing techniques such as the ablation of orbital and periorbital rhytids and repair of photodamaged facial skin (Apfelberg 1996, Rhorich 1997, Collawn 1999). Cutaneous lesions such as actinic cheilitis, acneiform scarring, epidermal naevi and carcinomas (both basal cell and squamous) are also treatable.

1.5.3 Laser Terminology (Fitzpatrick & Goldman 1994)

• **Energy**

Energy is the capacity to do work and is expressed as force multiplied by length or mass multiplied by velocity. The unit of measurement is a joule. It is usually calculated in laser surgery as power multiplied by time of application and is a dosage measurement.

• **Power**

Power is the rate of performance, or flow, of energy; it is energy divided by the time of application. The unit of measurement is the watt. One watt equals one joule per second. Power output (the speed of energy emission from the laser tube) is important in limiting the severity of the laser conduction burn, but surgical control is largely a function of the power density and spot size, as well as the repetition rate.

• **Power Density**

Power density, or irradiance, is the rate of energy delivery per unit of target tissue, expressed in watts per square centimetre. It is determined as the power divided by the surface area of the beam or spot size. Since the area of a circle varies with the square of its radius, any reduction in spot size will produce a fourfold increase in energy at the impact site. Increases in power output from the tube, however, result only in a corresponding linear increase in the power density. Power density is the principal determinant of the rate at which tissue is vaporized. High power densities (irradiance) vaporise tissue rapidly. The power density is a static measurement and does not account for time.

• **Fluence**

Fluence (or the energy density) is the total energy divided by the cross-sectional area of the beam and is expressed as joules per square centimetre and is also the product of irradiance and exposure time. When the power density is greater than about 100 W/cm^2, the amount of tissue damage that occurs is proportional to the *time* of application of the beam, not to the irradiance (power density)

.Whereas irradiance determines the rate at which tissue is vaporised, the volume of tissue removed is entirely a function of the amount of energy applied. A given amount of energy to vaporise a given volume of tissue can be obtained by an infinite combination of powers and times. When heat dispersion in tissue is considered, time of application is the most critical factor.

- **Spot Size**

The spot size of the laser is controlled by focusing lenses or by simply moving the hand piece toward or away from the target tissue. It is important to realise that small variations in hand piece-to-target distance may produce dramatic alterations in the diameter of the beam or spot size and consequently in power density. The irradiance across the beam is distributed in a Gaussian fashion, peaking at the centre of the beam and falling off to zero at the edges. A larger spot size allows for smoother, more uniform vaporisation of tissue but requires that a much higher power be used to compensate for the dilution of power density over the increased area of the larger spot size. The smaller the spot size, the greater the tendency to create uneven ridges, furrows, and bleeding.

- **Laser Pulse**

Laser energy from the continuous beam of a laser can be delivered in short pulses of energy by a variety of techniques. The most common is simply mechanically shuttering the beam so that it is physically blocked for short periods, resulting in an on-off repetitive sequence of pulses. Beam chopping permits very short duration mechanically shuttered pulses by employing a fanlike device. Superpulsing and ultrapulsing are processes of delivering very short pulses of very high peak powers by electronically pumping the laser tube. Q-switching employs rotating mirrors and other methods, which result in an accumulation of laser energy generating a giant pulse of very high power and extremely short duration.

- **Duty Cycle**

When a laser is used in a repetitive pulse mode, the duty cycle refers to the time that the laser is actually on. It is the product of the pulse duration and the

repetition rate expressed as a percentage. The average power of the laser can be increased by increasing the repetition rate. When this is done, the duty cycle is increased, but the irradiance (power density) remains the same. The power has not been turned up, but the delivery of that same power per pulse has been accelerated, resulting in a higher average power. The same is true for increasing pulse width. This results in a higher average power but does not alter irradiance.

- **Thermal Relaxation Time, T_r**

The thermal relaxation time of tissue is the time required for the heated tissue to lose 50% of its heat through diffusion. Significant thermal diffusion will not occur if the pulse duration is shorter than the time it takes the heated layer to cool. Minimal thermal damage is therefore expected if there is insignificant diffusion of heat during the laser pulse.

- **Chromophore**

Constituents of tissue have a specific coefficient of absorption which is wavelength dependent **(Fig. 1.5.2)**. Skin is principally 70% water and therefore radiation wavelengths in the 2-12μm range are strongly absorbed by water as shown below. Hence the CO_2 laser operating at 10.6μm targets water as the main chromophore.
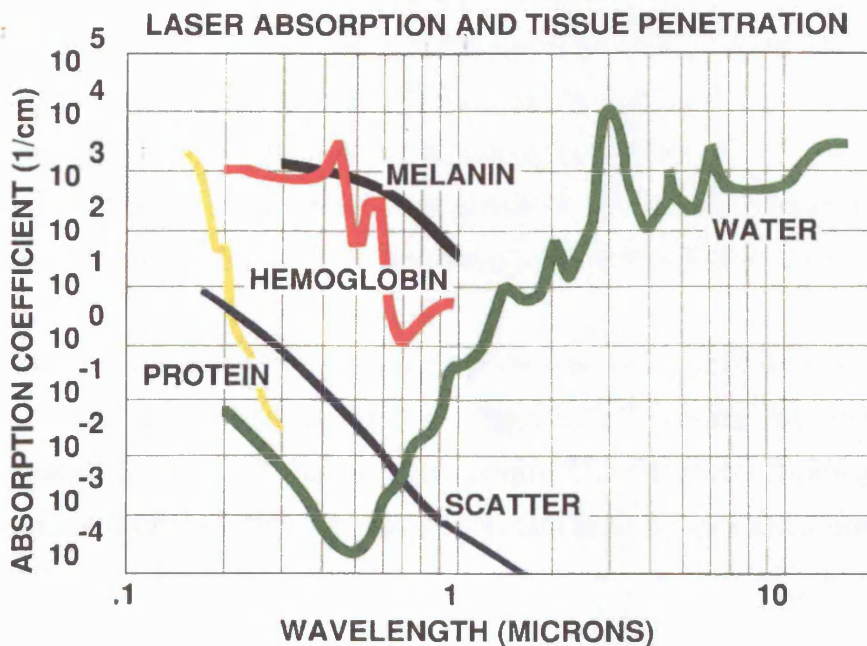
Fig. 1.5.2 Chromophore absorption according to wavelength

1.5.4 Laser-Tissue Interaction (Polanyi 1983, Reid 1991)

When electromagnetic radiation impinges upon a thin layer of matter, part is reflected, part is absorbed and part is transmitted. The essential property of the laser beam is the ability of the absorbed radiation to cause localised heating of tissues via a thermal and kinetic excitation of the skin structures.
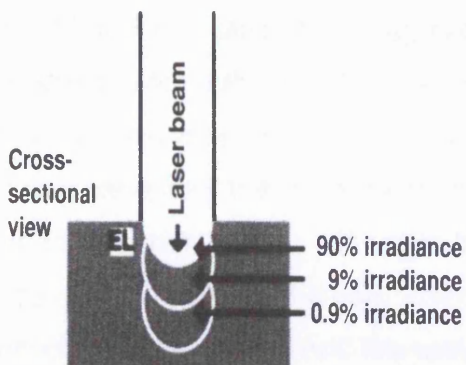
Fig. 1.5.3 Concept of extinction length

The electromagnetic radiation interacts with tissue according to Beer's Law, which states that tissue damage at the site of energy absorption decreases exponentially with increasing distance from the crater edge. Hence epidermal cells at the wound edge remain viable and can therefore contribute to wound healing.

The depth of penetration in skin for CO_2 is around 30µm, the so-called *extinction length* (EL)whereby 90% of the radiant energy is absorbed (Polanyi 1983)**(Fig.1.5.3)**. The remaining 10% will pass through another extinction length leaving 1% of the original to propagate further, and so on. Hence, the majority (99%) of incoming radiation is absorbed within two extinction lengths. EL is a function of both the *chromophore and wavelength* of radiation involved.

Absorption length is another term which describes the depth to which light must penetrate to be absorbed by 63% **(Fig.1.5.4)**. The extinction length is approximately 2.3 times the absorption length. The minimum thickness of tissue damage for a laser lies between the absorption and the extinction lengths (Trost et al 1992).
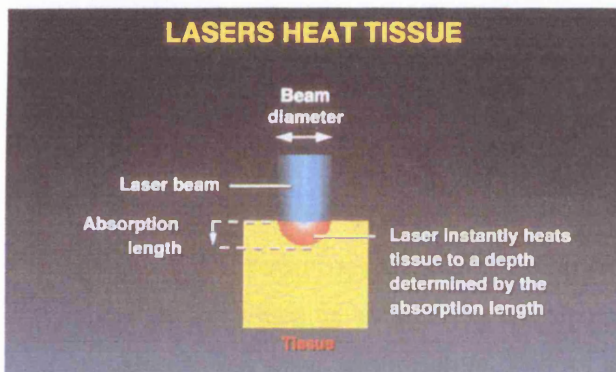
Fig. 1.5.4 Diagrammatic representation of impact zone

The heating of a critical volume of tissue continues until the temperature of the site of impact exceeds the coagulation threshold and then the vaporisation threshold. With sufficient fluence and a pulse duration being *less than the thermal relaxation time of the tissue*, then one critical volume of tissue will be cleanly vaporised *without* thermal damage to the surrounding tissue. However, with low fluence tissues will begin to desiccate and coagulate *before* vaporization is ever achieved. The subsequent charring leaves a residual anhydrous tissue layer and the temperature may exceed 600 °C. Acting as a heat sink this char can cause a large amount of heat to be conducted into deeper tissues causing inadvertent burns.

It follows that two principal mechanisms underlie the manner in which laser tissue destruction occurs: immediate photovaporization and delayed coagulation necrosis (Reid 1991). The thickness of the zone of injury is a crucial balance between adequate tissue removal and an *acceptable* amount of residual tissue damage.

1.5.4.1   Depth of Laser Injury

There are many factors involved in modulating the depth of residual thermal damage. Minimal thermal damage is expected if there is insignificant diffusion of heat *during* the laser pulse.

Pulse width has a linear relationship with depth of thermal damage (Fitzpatrick 1991). Hence, damage can be minimised by shortening the *pulse* duration in which the beam is fired. (McKenzie 1983, Hobbs 1987, Walsh 1988, Domankevitz 1997) even with high fluences. This depends on the thermal relaxation time, T_r of the tissue. If the pulse duration is shorter than the T_r for tissue (~695μs, Walsh 1988), then all heating and vaporisation is achieved *before* sufficient conduction can take place to cause surrounding damage. The challenge, therefore, is to develop a laser capable of producing short burst beams with high fluence and shallow absorption/extinction lengths.

This has implications in the development of laser technology. Thermal damage has been problematic with previous types of laser where the pulse timing was relatively longer than the *Tr* of tissue (Walsh 1988, Schomacker 1990, Fitzpatrick 1991). Mechanisms to shorten the pulse include physical blocking of the beam by a fanlike device, so-called Beam Chopping, and Q-switching with rotating mirrors. Superpulsing and Ultrapulsing are achieved by pumping the laser tube electronically to generate the short pulses.

The depth and nature of injury has been studied by various workers to establish optimal power settings and the effects of the types of laser in human

(Fitzpatrick et al 1991, Stuzin et al 1996) and animal models (Green et al 1990, Zweig et al 1990, Yang et al 1995, Walsh 1988). Continuous wave lasers leave zones of residual thermal damage between 200 and 600µm (Dover 1996) with thinner layers being produced by rapidly scanning the laser over the target (Domankevitz 1997). It then follows that superpulsing causes less necrosis with thermal damage zones measured varying between 25 to 100µm depending on the wavelength and pulse duration selected (Green 1992).

The ablation threshold fluence of skin has been reported as ranging between $2.6Jcm^2$ - 4.75 Jcm^2 (Walsh 1988, Green 1990). The proportion of collagen and water within various tissues dictates the level of damage and in this respect, muscular tissue is more resistant than skin and the cornea (Walsh 1988). Efficient photothermolysis requires a laser capable of generating fluences of at least $5Jcm^2$ which should be able to vaporise skin with no residual thermal damage provided the *Tr* is not exceeded.

1.5.5 Ultrapulse (UPL) & Erbium: YAG (EYL) Lasers - The Latest Generation

These lasers are capable of producing microsecond duration pulses and coupled with a high power density are able to ablate thin sections of skin (5-40µm) with minimal thermal damage to the underlying layers. They have found use in the ablation of benign intraepithelial and dermal tumours, tattoo removal, and in facial skin resurfacing surgery to correct rhytids (wrinkles) around the eyes and mouth, acneiform and hypertrophic scars, and psoriatic plaques. Newer applications may include treatment of diseases limited within the superficial dermis such as lichen sclerosis et atrophicus, xanthelasmata and lichen planus (Fitzpatrick and Goldman 1991). These lasers can ablate squamous and basal cell neoplasias, although the effectiveness of this may be marred by the lack of histological confirmation.

The cutting ability of the CO_2 (10.6µm wavelength) laser is not lost since the

pulse length can be selectively lengthened to utilise this function. In this mode, the CO_2 laser can be used as a simultaneous cutting and coagulation instrument, allowing meticulous haemostasis with a dry surgical field. For aesthetic surgery, the Ultrapulse CO_2 laser (Coherent Medical, Cambridge, UK) can be utilised for skin resurfacing with its ablative ability and then changed to cutting mode to perform a bloodless incision for a blepharoplasty in the same procedure.

Aiming the beam freehand in a raster or 'airbrush' method may cause inadvertent damage if the beam is passed repeatedly over a treated area. With the introduction of a Computerised Pattern Generator (CPG) (**Fig.1.5.5 & 1.5.6**), the operator is able to fire a precise and rapid succession of multiple, patterned pulses providing even coverage and depth (Apfelberg 1998) to lesions, minimising any crossover. The combined effect of multiple beams is such that a larger surface area per shot is attainable.
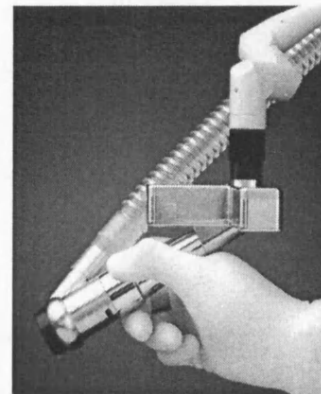
Fig.1.5.5 Hand–held CPG
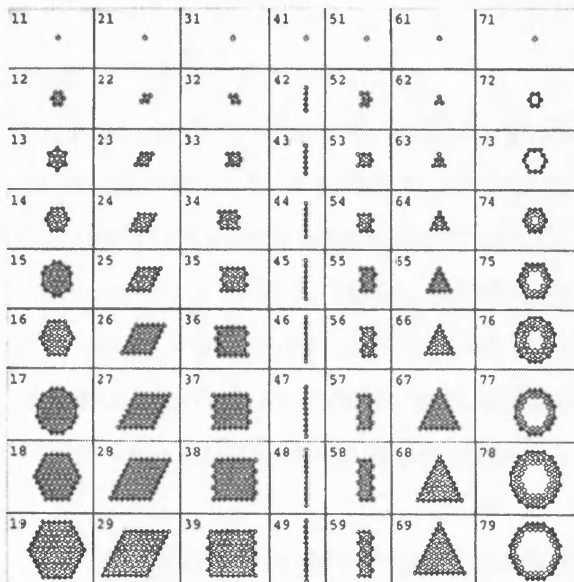
Fig. 1.5.6
Computer Generated
Patterns possible for the
Ultrapulse 5000C CO_2
Laser

The Erbium:YAG laser (Continuum Biomedical, Dublin, California, USA) **(Fig.1.5.7 opposite)** has been developed to maximise on the narrower wavelength of 2.4μm which allows a short extinction length when absorbed by tissue. In conjunction with microsecond duration pulses, it is able to ablate thinner sections of tissue in comparison to the Ultrapulse laser.

The ability of these lasers to serially debride tissue with great accuracy lends themselves to use as a tool in the treatment of vesicant burns which will be discussed in the next section.

1.5.6 Burn Debridement with Lasers

The gold standard of sharp tangential debridement of burn eschar is effective at creating an adequate bed for skin graft uptake at the cost of considerable blood loss. Electrocautery allows for good haemostasis at the cost of a long operative time.

With this background, lasers have been studied for burn wound excision in order to prepare a surface suitable for skin graft uptake. In 1971, Stellar et al reported their preliminary success with a CO_2 laser in debriding full thickness burns on pigs of up to 20% TBSA. Achieving 90 to 100% skin graft take, this was affirmed by the groups' subsequent work in 1973. By using the laser as a scalpel rather than as an ablative tool, decreased blood loss was seen and with similar graft take compared to sharply excised controls.

Levine (1974) at the Brooke Army Medical centre in Texas reported on the use of the CO_2 laser for debridement of a full thickness burn on the neck of a patient with immediate autograft. One half of the burn was sharp excised as a control and there was no difference in graft take between the sides. It was noted that blood loss was dramatically reduced but that operation time was

slower.

The same group performed a comparison of electrocautery, laser and scalpel burn debridements where blood loss with laser was reduced by up to one third with operating time doubled. These results were still superior to that of electrocautery (Levine 1975). Fidler of the Shriner's Institute in Ohio reported a series of fifteen paediatric burn excisions with the CO_2 laser with immediate autografting. Blood loss was only a third of conventional sharp debridement (Fidler 1976).

Jackson (1977) also reported that the technique took longer and was more physically traumatic to the wound with the need for excised burn slough to be pulled off with considerable traction. The graft take in this series was slightly less compared to scalpel tangential excision, attributed to the thin layer of surface necrosis left behind.

Constant refinements have included the argon laser-assisted quartz scalpel which combined the cutting properties of a steel scalpel with the selective haemostatic properties of the laser beam (Heimbach 1980). A pig model showed good graft take of 90%. The Neodymium:YAG laser further reduced blood loss by up to a half, but it was felt that the doubled operating time limited the applicability only to small burns (Hukki 1989). The haemostatic ability of the CO_2 laser was again noted in a series of twelve paediatric burn excisions with blood losses of 40ml in total for excisions of up to 30%TBSA. In this group, it was noted that the application of graft required delay in order for healthy granulation to appear, indicating moderate residual thermal necrosis (Lacheretz & Herbaux 1988).

The main hurdles appeared to be the relatively low power available, the operating characteristics of the laser (i.e. continuous wave), and the physical bulk of the devices. Although a small amount of surface necrosis was beneficial in coagulating blood vessels, allowing an almost bloodless field, excessive residual surface necrosis was also problematic in delaying wound healing and

graft uptake.

With the introduction of pulsed CO_2 lasers the perceived favourable advantages of minimal blood loss and a rapid mode of action were coupled with less thermal damage (Green 1990). Using porcine skin it was established that burn eschar behaved differently compared to skin in that it is already desiccated; therefore slightly higher laser fluences ($3.0 Jcm^{-2}$ versus $2.6\ Jcm^{-2}$) were necessary to effect ablation of tissue already burnt. This was due to the fact that normal skin and burnt tissue contain differing amounts of water, a principal chromophore for $10.6\mu m$ laser radiation. Significantly, Green reported that the zone of residual necrosis was around $85\mu m$ with the pulsed CO_2 laser compared with 500-$800\mu m$ for conventional continuous wave (CW) lasers, thus improving conditions for autograft survival.

Rapid scanning CW-lasers were another modification to overcome thermal damage. Burn debridement with immediate autografting in a porcine model left residual thermal necrosis zones of around $180\mu m$ with this type of laser. Blood loss was virtually nil with graft take comparable between laser debrided and sharp debrided control sites (93% vs. 96%) (Glatter 1998). The subjects were followed up for six months at which time both sites were histologically identical and with no observable difference in scarring.

Following on from this the same group at Harvard Medical School in Boston performed a trial on 21 children with full thickness burns, again comparing the rapid scanning CW-laser with sharp debridement as control, of full thickness burns with immediate skin grafting. Equal graft uptake was noted (94% vs. 94%) with no difference in a scar assessment at up to eight months of follow up (Sheridan et al 1999). Operating times were similar for the small area of wound involved although the authors did admit that the laser would take longer when dealing with circumferential and extremity burns, due to size limitations. The plume generated from the vaporisation was also noted to be extensive, requiring a dedicated evacuation system. It was concluded that the main

advantage of this method would be the virtual absence of significant haemorrhage.

The perceived favourable advantages of these latest lasers include minimal blood loss and a rapid mode of action, coupled with less thermal damage. The ability to deliver large amounts of energy within a short pulse has only been possible recently with the development of the Ultrapulse Laser and similar devices. This ensures efficient photothermolysis with minimal transmitted heat damage to the underlying healing dermis.

Each type of laser has different depths of penetration and operating characteristics that will be discussed in Section 2.

Vesicant Agents

1.6.1 Historical aspects

Poisons have been used as weapons since 600BC when the Assyrians used ergot to poison the drinking water of their enemies. A noxious combination of smoke and flame generated by sulphur and pitch known as Greek Fire incapacitated an Athenian force prior to assault in 500 BC.

In 1346, the Tartar army catapulted the dismembered corpses of bubonic plague victims over the city walls in the siege of Kaffa. This was probably the original route to the spread of Black Death which ravaged 14th Century Europe. Throughout the Middle ages, the use of plague victims to spread disease was commonplace.

The Duke of Wellington vetoed a proposal in 1846 to use self-igniting shells containing cacodyl and cacodyl oxide on the grounds that 'it would not accord with the feelings and principles of civilised warfare'! Indeed, the Brussels Declaration of 1874 prohibited the use of poisons or poisonous weapons, reinforced by the Hague Declaration of 1899.

Chemical warfare agents were first used on a large scale in the First World War. On April 22 1915, the Germans used chlorine gas which lead to a reported 5 000 deaths with 15 000 wounded. Later, sulphur mustard was used on July 12 1917, near Ypres in France with devastating effect. Over 120 000 British casualties were sustained this time, with 2-3% of these dying as a result of exposure to sulphur mustard. The use of these agents was a violation of the Hague Congress agreement originally signed in 1899 by all powers except the United States, where pledges were made to not use poisonous or suffocating gases. Later on, these use of these agents (though not the acquisition or stockpiling), was again banned by the Geneva Protocol of 1925, although Iraq broke this during the Iran-Iraq War of 1980-88 when they were used on troops and Kurdish civilians at the initiation of the Kheibar offensive (Khadivar 1991).

Since 1986 Angola has been using chemical weapons against UNITA soldiers (Heyndrickx 1990). Further use of these weapons was seen during the Gulf War. As a result of this, a United Nations Security Council Resolution was passed in 1991 requiring that Iraq declare its holdings and allow international supervision of the destruction of its weapons of mass destruction. To this date, the Resolution has not been honoured.

With the resolution of the Cold War, the potential threatened usage of chemical and biological weapons is now more likely than a nuclear attack.

The aim of vesicants is not to kill or permanently maim but to incapacitate the enemy with a large quantity of (usually) non-life threatening injuries. This is achieved by blocking lines of communication with casualties or forcing opposing troops to wear cumbersome protective equipment. In this respect, vesicants fulfil these objectives well. However, the lethality rate of 1-3 % must be borne in mind. Epidemiological information about the effects of these agents has been gained from three sources:

- injuries sustained by soldiers exposed to vesicants on the battlefield,
- workers involved in the manufacture of these agents
- unintentional adverse effects to patients exposed to these agents for medical therapy.

Secret testing was conducted between WWI and up to 1975 by the British and U.S. military for which there is scant documentation. Over 60,000 service personnel were involved in these tests. This lack of information prompted an Enquiry Committee from the Institute of Medicine to be set up in 1991. The sole purpose was to try to establish what tests had taken place in the U.S. in order to identify and follow up veterans who had been exposed either during conflict or during secret tests. The lack of records was due to many factors:

- long-term effects which had not been considered since scientific inquiry was directed by the military which were interested only in the acute injuries. This

contributed to the paucity of animal studies after WWII, aimed at elucidation of the long term implications of exposure.

- there was no unified body of information to maintain records due to a lack of appreciation of the seriousness of latent effects.
- with the outbreak of war and change in priorities with expected combat injuries, long term effects were put aside
- continued secrecy was maintained after the war to make it extremely difficult to follow up exposed individuals

The committee came to the conclusion that the lack of information and long term follow up severely diminished the quality and amount of information that could have been utilised in the long term assessment of vesicant injuries. Furthermore, the levels of exposure to Lewisite and sulphur mustard were probably much higher than inferred from the test protocols.

A review of vesicant agent toxicology has summarised the available knowledge from both the open and 'grey' literature for the purposes of knowledge dissemination amongst healthcare workers and emergency planners (Watson & Griffin 1992).

The pathological and toxicological effects have been investigated on in vivo human skin in the early half of last century at the Experimental Station in Porton (Cameron et al 1946). Cutaneous exposures lead to blisters which were noted to be subepidermal with coagulative necrosis of the dermis - full thickness burns. With the subsequent prohibition of human experimentation, ex vivo skin grafts attached to athymic nude mice (Papirmeister 1984), explanted human skin (Lindsay & Rice 1996), and various other animal models have been investigated to find an accurate model for the vesicating action of these agents. The only credible clinical information to date has been from comprehensive reports of the outcome of 65 of the 170 or so casualties evacuated to European hospitals from the Iran-Iraq conflict during 1984-6 (Willems 1989). These were evacuated between 4 to 17 days post-exposure, providing a unique opportunity to treat these casualties with up-to-date therapy. Some of these casualties had

sustained burns of up to 70% TBSA inflicted by a mixture of mycotoxins and conventional chemical agents (sulphur mustard, Lewisite and tabun). United Nations weapons experts visited the war zone to confirm the authenticity of the samples (Kadivar & Adams 1991).

1.6.2 Lewisite

Lewisite is an organic trivalent arsenical compound possessing significant systemic toxicity and marked vesicating properties in man (Goldman & Dacre 1989). It was isolated in pure form by Lee Lewis in 1918 at the Catholic University of America in Washington and has an odour similar to that of geraniums. During the First World War the thrust of chemical weapons research had been towards investigating the potential of substituted arsines, and it was found that Lewisite was highly toxic for this purpose. The first shipment was on its way to Europe when the 1918 armistice was agreed which ended the first World War; this shipment was apparently destroyed at sea (Prentiss 1937). After its use as a chemical weapon was proposed, the former Soviet Union stockpiled significant quantities of Lewisite (particularly as a mixture with sulphur mustard) although it was never actually used in war. Despite this, interest in Lewisite has persisted since it is a lethal compound with the ideal properties for use as a chemical weapon.

It is a cellular poison and is lethal in high doses being a systemic poison absorbed into the bloodstream. Immediate pain is felt on contact and it is readily absorbed through the skin but moist mucosal areas such as the eyes, groins and respiratory tract are particularly vulnerable. It is also thought to act as a carcinogen.

Compared to sulphur mustard, a well-recognised vesicant chemical warfare agent, there has been little research performed to define the natural history of Lewisite-induced skin lesions in either experimental animals or man.

1.6.3 Sulphur mustard

Sulphur mustard was first identified in the late 19th century by various chemists although it was given the name of 'Lost' after Lommel and Steinkopf in 1880 who first suggested its use as a warfare agent. This became the agent that was used to fill the shells that were first fired at Ypres in 1917 by the Germans. Commonly referred to as 'mustard gas', it is, in fact, liquid at room temperature and has a smell similar to mustard or garlic. It is lethal in sufficiently high doses and is a recognised cellular poison and carcinogen. The persistency of droplets that slowly evaporate has been the reason why it is the vesicant of choice in military use. Exposed skin and mucosal surfaces are vulnerable to its effects whether contact is via vapour, liquid droplets or aerosol.

It is a highly efficient alkylating agent, hence the ability to act as a mutagen by damaging DNA. Conversely, in the form of nitrogen mustard it has been used for chemotherapy of carcinomas.

1.6.4 Chemistry of Lewisite and Sulphur Mustard (Perchura 1993)

Lewisite (dichloro(2-chlorovinyl)arsine,$C_2H_2AsCl_3$) is produced by the reaction between acetylene and arsenic trichloride using aluminium chloride as a catalyst. The resulting colourless, oily liquid has a scent likened to that of geraniums and vaporises at low temperatures. The freezing point is between 0^0C to -18^0C, making it more effective over a wider rage of environmental temperatures. It has a low solubility in water, hence there is only intermediate persistency in soil. Heat degradation may be considerable, limiting the usefulness of its deployment via detonating shells. Irritant effects are noted with doses as small as 6-8 mg/m^3.

Sulphur Mustard (bis(2-chloroethyl)sulphide,$C_4H_8Cl_2S$) is a vesicant produced by the interaction between ethylene and sulphur chlorides - the Levinstein process. The odour is similar to garlic. It is liquid at room temperature and has

a freezing point between 13^0C to 15^0C. This ensures that the agent is very persistent since it can remain inactive during cold weather causing contact blistering or slowly vaporising leading to respiratory and skin injuries months after deployment. It also degrades at a slow rate thus preserving longevity of action. On contact, about 80% will evaporate and of the remainder, 10% will become fixed into the skin and 90% is systemically absorbed. Doses in the order of 0.5 mg/m^3 can lead to irritation.

Besides cell membranes, vesicants are able to penetrate through wood, leather and rubber amongst other materials. Fortunately, the action of post-exposure vesicants is brief. No documented reports of injuries to attendants or health care workers have been documented. Similarly, handling of contaminated dressings and skin contact has not been shown to be detrimental (Willems 1989).

1.6.5 Mechanism of Action – Chemical / Molecular Interaction

1.6.5.1 Lewisite

Acting as a cellular poison, Lewisite binds avidly to thiols, which are particularly concentrated in the skin and hair. Subsequently enzymes and other macromolecules with these sulphydryl groups are irreversibly damaged. The formation of acetyl coenzyme A, an intrinsic part of the pyruvate dehydrogenase component of the Krebs cycle is inhibited. This is due to the combination of Lewisite with dihydrolipoic acid which is one of the vital components in the cycle.(**Fig.1.6.1**) The cyclic compound formed interferes with energy production within the cell (Peters 1953). Systemic absorption results in rapid depletion of the glutathione stores in erythrocytes. This results in instability of the cell membrane leading to massive intravascular haemolysis. Little else is known in the literature regarding the chemical target for Lewisite. Although similar in potency to sulphur mustard for inhalational injuries, Lewisite is more rapidly absorbed in the skin and capable of inducing severe toxicity via its metabolic product, Lewisite oxide.

```
C H 2 S H                                           C H 2
  |                                                   |    \  S
C H 2                      H         C I            C H 2    \
  |                        |         |                |      > A s C H = C H C I + 2 H C I
C H 2 S H      +      C I - C  =  C  - A s  - C I  ─→  C H   /
  |                              |                     |    /  S
(C H 2 )4                        H                   (C H 2 )4
  |                                                   |
C O O H                                             C O O H
```
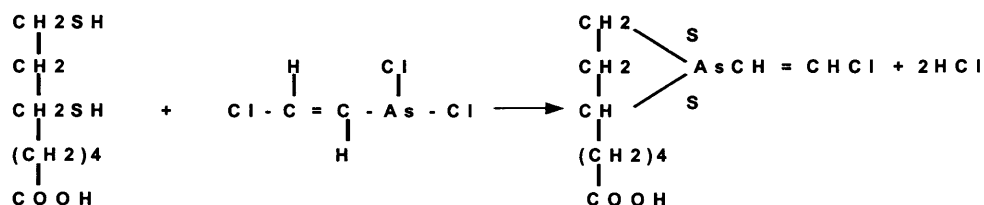
Fig.1.6.1 Lewisite irreversibly binds lipoic acid halting cellular metabolism

'Lewisite shock' has been described following just a 2ml topical dose, with symptoms of fatal cardiovascular collapse. This is similar to the burn shock seen in victims with high TBSA burns (Sollman 1957). For this reason, Lewisite has been coined as the 'Dew of Death'. Excretion of lewisite oxide is via the hepatic and renal systems. Fulminant hepatic and renal failure following ingestion has been induced in animal models with marked necrosis of the liver and biliary tree. These findings are similar to arsenical poisoning although the potency of the arsenic in Lewisite is much greater than with simple inorganic arsenical compounds such as arsenious oxide (Cameron 1946).

Lewisite is intensely hydrophobic, hence its rapid penetration into the skin and mucous membranes. There is a deeper and more rapid penetration with the generation of necrosis at a much earlier stage. In addition, oedema is more extensive.

1.6.5.2   Sulphur Mustard

Sulphur mustard interacts with organic phosphates such as nucleotides and phospholipids and the amino groups in amino acids, peptides, purines and pyrimidines. There is also an affinity for sulphydryl groups, phosphate and pyrophosphate ions. Hence, it is extremely effective in the alkylation of nuclear components including DNA, RNA and other proteins resulting in cell

malfunction. The specificity for binding within the DNA genome will affect structural proteins, cytokines, adhesion molecules and enzymes. The generation of oxygen free radicals has also been implicated as a mechanism of damage (Eldad 1998). It is particularly effective with mitotic cells and may lead to cytostasis, mutation and slow cell death. .

As a bifunctional alkylating agent, sulphur mustard has two carbon chains that are capable of internal cyclization, a process which is necessary for alkylation to occur. Alkylation of complementary DNA bases by a single molecule of sulphur mustard is, therefore, possible and will lead to the formation of interstrand crosslinks. The important chemical steps are summarised in **Fig.1.6.2**

- First cyclization reaction.
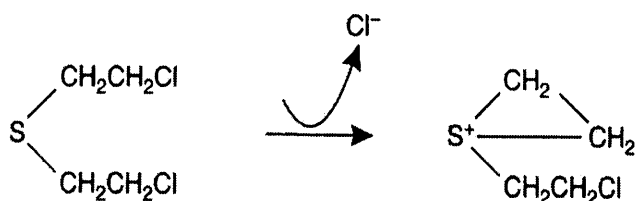
- Alkylation of DNA base:
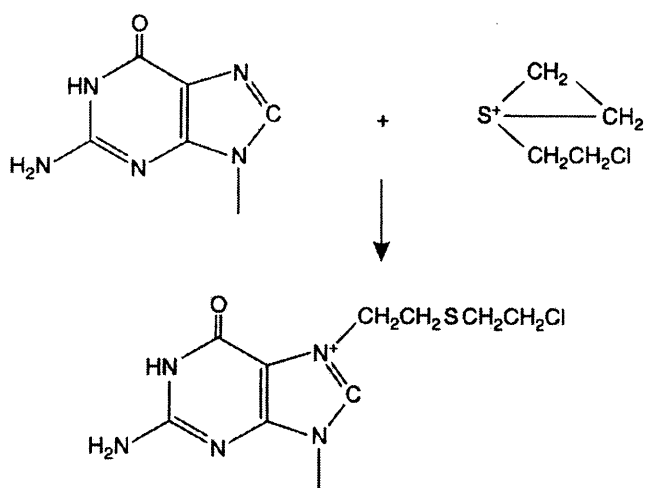
- Second cyclization and alkylation of complementary DNA base resulting in a DNA crosslink:
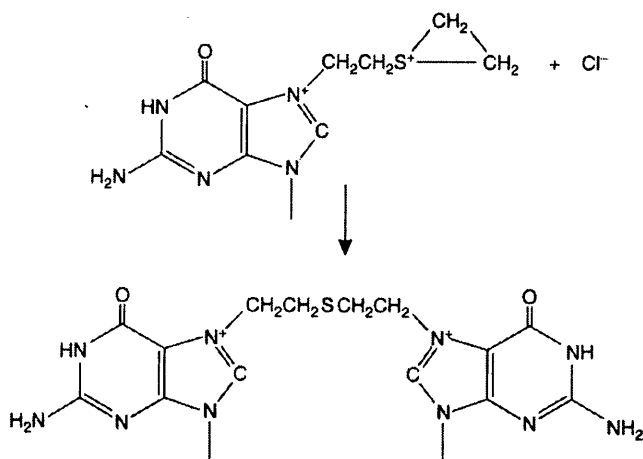
Fig.1.6.2. Formation of Sulphur Mustard (bis(2-chloroethyl)sulphide,$C_4H_8Cl_2S$)

Alkylated guanine residues have a tendency to form base pairs with thymine rather than cytosine (as normal), resulting in coding errors and inaccurate protein synthesis. This may ultimately lead to either non-production or excessive production of key metabolic enzymes and structural macromolecules.

As such, actively proliferating cells of the haemopoietic and gastro-intestinal systems are particularly vulnerable. The effects on these rapidly dividing tissues are particularly severe and have led to profound bone marrow suppression, gastrointestinal damage and spermatogenic arrest in human casualties (Rice and West, 1987; Willems, 1989). In this sense, the sequelae are similar to those of X-rays, hence the term 'radiomimetic' is sometimes applied to mustard compounds. In skin, the sulphur mustard affects the basal cell layer turnover rate. Wound healing is thus severely impeded with delayed extrusion of any eschar.

1.6.6 Post-exposure Physical symptoms

Immediate skin irritation and pain will be produced on contact with Lewisite. In

contrast, sulphur mustard exposure is characterised by a latent period of several hours before chemical burns become manifest. Agent contact does not produce immediate symptoms, with exposed individuals often not promptly decontaminating or requesting medical assistance. At high concentrations, however, there will be cardiovascular collapse with nausea and vomiting before the development of erythema.

1.6.6.1   Ocular Effects of Vesicants

Inflamed and painful eyes occur within 2-3 hours with swollen eyelids, lacrimation, blepharospasm, photophobia and temporary blindness. The eye is exquisitely sensitive, being affected at lower vapour concentrations than any other tissue. By 24 hours, the eyes can become swollen to the extent that they are shut. The conjunctivae become hyperaemic, associated with corneal oedema, iritis and the development of posterior synechiae with severe corneal injury. Heavy neovascularisation can occur and corneal ulceration and erosions are seen.

Secondary infection is common. Recovery is complicated by prolonged photophobia and blepharospasm which can recur over months or years. Mustard induced keratopathy is a delayed sequela occurring between eight to forty years after the original injury.

1.6.6.2   Respiratory Effects of Vesicants

A variety of dose-dependent effects can occur within the respiratory tract, including intense throat discomfort, continuous hoarse coughing, nasal discharges, copious mucus production, and bronchial inflammation. The inflamed mucous membranes become oedematous and then necrotic with desquamation leading to collapse and consolidation. Tracheitis, rhinitis, laryngitis and bronchitis may be seen even with mild inhalational exposures. Pulmonary oedema with pleural effusion is seen with significant injury. Localised atelectasis with compensatory emphysema may be severe enough to lead to pneumothorax. Secondary infection can easily occur in the presence of immune suppression leading to potentially fatal bronchopneumonia. Lung abscesses have also been reported (Newman-Taylor 1991). Respiratory failure will ensue

most probably through the triggering of Acute Respiratory Distress Syndrome (ARDS).

Recovery from a non-fatal injury to allow moderate activity may take up to eight weeks. In the long term, increased rates of COPD (Chronic Obstructive Pulmonary Disease) have been described in those who have survived exposure to vesicants.

1.6.6.3   The Effects of Vesicants on the Skin

Lewisite has an instantaneous action compared to sulphur mustard and both lead to general irritation of the skin, first manifested as an itching rash, can develop (at higher exposures) into large, painful bullae (up to 70cm in diameter) that will spontaneously burst within days with sloughing of the skin. This is due to necrosis of the basal cell layer at the dermo-epidermal junction with a resultant increase in vascular permeability. The fluid contains only trace amounts of vesicant since all chemical activity ceases within a few minutes. This is non-toxic and has no vesicating effect. There is a predilection for moist sites such as the groins and axillae where the full chemical reaction can take place The surface slough provides a rich culture medium and exudating, raw surfaces can become secondarily infected with ease (NATO 1996). The denuded dermis is extremely tender since these are partial thickness burns.

With higher concentrations of exposure, full thickness burns are possible (Cameron 1946, Kadivar & Adams 1991).

The lesions heal by secondary intention with eschar formation, resembling thermal burns, albeit more slowly. Lewisite burns heal more quickly than mustard burns due to the lesser degree of alkylation of epidermal cells in the surrounding region (Rice 1999).

The burn lesions are unlike that seen in thermal, electrical or corrosive (acid/ alkali) burns. There is little or no thrombosis of vessels, with a great degree of moistness of the affected area. The coagulated appearance of thermal injuries is not a feature of vesicant injury. The skin at first is pale but then becomes

erythematous within a few hours of exposure. Vesication is not usually seen until the second day and progresses thereafter for several more days.

The degree to which the skin is affected depends on the moistness of the surrounding area, with wide variations in the severity of burns seen amongst individuals. Environmental humidity during deployment plays a large part in this. Conflicting evidence surrounds the protective effect of skin pigmentation, however. The density of sebaceous and sweat glands does have some bearing on how densely the skin is affected. The groin is considered to be ten times more sensitive than other skin surfaces. In a study of 6980 casualties studied in the First World War (Blewett 1986) the common regions affected were the eyes (86%), respiratory tract(75%), scrotum(42%), face(27%) and perineum(24%). The thick glabrous skin on the soles and palms together with the scalp, are particularly resistant to damage.

Figures 1.6.3-7 These illustrations show the severity of vesicant burns seen on casualties from the Gulf War. Various stages of presentation are seen since there were often lengthy delays between injury and treatment Fig. 1.6.3 Axilla . Fig.1.6.4 & 1.6.5 Right thigh with detailed close-up. Fig.1.6.6 & 1.6.7 Torso and groin burns © Crown copyright 2003 Defence Science and Technology Laboratory UK

Fig.1.6.3 © **Crown copyright 2003**

Fig.1.6.4 © **Crown copyright 2003**

Fig.1.6.5 © **Crown copyright 2003**

Fig.1.6.6 © **Crown copyright 2003**
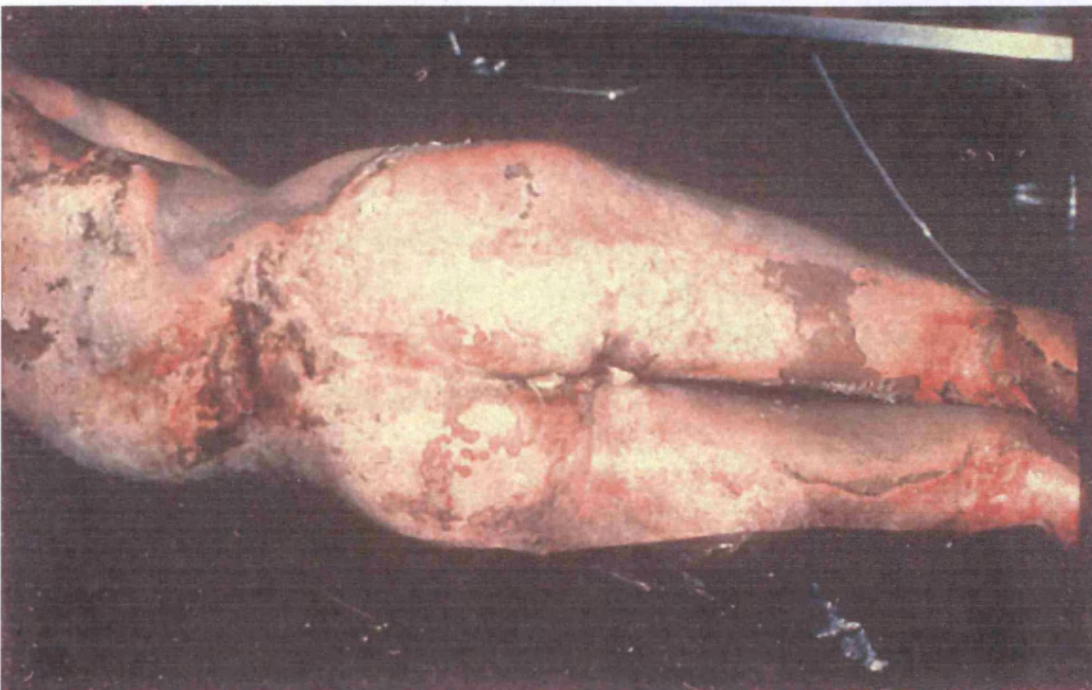
Fig.1.6.7 © Crown copyright 2003

The skin may be made to vesicate in areas of erythema by slight trauma, e.g. on rubbing, and this phenomenon is known as Nikolsky's sign. However, it does not imply the persistence of active vesicant (Sullberger and Katz,1943).

Between 4 to 6 days after exposure, necrosis is complete. Eschar formation begins within 7 days once the early blisters begin to degenerate and separation of necrotic slough begins. The accompanying oedema and erythema may persist. By 16-20 days, separation of slough is complete and re-epithelialisation has begun.

Healing may take 3-8 weeks post-exposure to be complete and the casualty is often left with depigmented areas surrounded by zones of hyperpigmentation. This can provide a startling contrast and is due to the leaching of melanin when the melanocytes are destroyed. Subsequently, this is taken up by reactive histiocytes with sufficient accumulation to provide the blackened discoloration. This phenomenon is referred to as 'melanocyte incontinence'. Permanent hypopigmentation and tenderness to mechanical pressure are common late

sequelae along with the progression to scarring.

The quality of this scarring is thought to be softer than that associated with thermal burns and is not usually hypertrophic unless the burn was deep. Late onset Bowen's Disease has also been implicated.

For those casualties unfortunate enough to suffer repeat exposure to vesicants, sensitisation of the skin will mean that the reaction will be more rapid and occur at lower concentrations of agent. However, the resolution is thought to be swifter as well.

1.6.6.4   *Gastrointestinal*

Any exposed mucosal surface within the tract can undergo necrosis even leading to perforation of a viscus. Punctate bleeding in the bowel occurs, and nausea with vomiting usually occurs within a few hours (Willems 1989). Diarrhoea and abdominal pain will accompany the sloughing of mucosa. Paralytic ileus and stress ulceration may develop.

1.6.6.5   *Other Systemic Effects of Vesicants*

At high doses, mustard can cause bone marrow depletion suppressing the immune response. The nadir is between five to twenty days. This renders the exposed individual more susceptible to infection: patients may not be able to mount a reactive leucocytosis (Newman-Taylor 1991). Depression of the haemopoietic system is manifested as neutropenia, thrombocytopenia and anaemia. Lewisite toxicity will also lead to changes in capillary permeability leading to haemoconcentration and hypovolaemic shock. Massive intravascular haemolysis is induced, leading to abdominal pain, haematuria and jaundice with renal failure. Accumulation of toxic metabolites within the bile causes focal necrosis of the biliary tract and liver. Hepatic failure and acute renal tubular necrosis will ensue. The seizure threshold is lowered with convulsions seen at high doses.

1.6.7 Wound Healing of Vesicant Burns

As described in Section 1.3.3., burn wounds will heal by secondary intention. Surface re-epithelialisation of ulcerated lesions following the rupture of blisters relies partly on regeneration from undamaged epidermis at the wound edge, and on regeneration from intact adnexal structures, such as the hair follicles in a manner akin to thermal deep dermal burns (Willems 1989).

The healing rate of these burns is slower, however, compared to an equivalent sized thermal burn. It has been proposed that the differing kinetics of healing in Lewisite and sulphur mustard burns are explained by the fact that with the latter, alkylation of epidermal cells extends *beyond* the immediate region of exposure. Although epidermal cells in this area may not ultimately die, the level of damage to cellular DNA may be sufficient to delay or prevent effective replication (Rice & Brown 1999).

Achieving effective epidermal regeneration further requires the presence of an appropriate matrix on which the epidermal cells are able to migrate (Shakespeare & Shakespeare 1987). The papillary dermis and basement membrane are vital in this respect, not only providing a structural scaffold for the epidermis but also acting as molecular signals for the subsequent maturation of the overlying new epidermis (Fleming 1991).

Collagen within the papillary dermis is *altered* by exposure to sulphur mustard and in this state may no longer function normally. Laminin is the principal target for proteolytic degradation at the basement membrane leading to dermoepidermal separation. A histological picture of coagulative necrosis is seen (Lindsay & Rice 1995).

Unlike sulphur mustard, there is no evidence that Lewisite alkylates DNA and the increased spontaneous healing rate of these lesions may be explained purely in terms of the absence of alkylation of the DNA of keratinocytes at the wound edge.

The difference in healing rate cannot be explained in terms of variations in either the size or biological severity of the two types of vesicant lesions. The histological appearances of these burns will be discussed later.

1.6.8   Carcinogenesis/Genotoxicity and Vesicants (Papirmeister 1991)

Sulphur compounds are cytotoxic even at low doses. The carcinogenicity of these compounds has been well documented in animal models such as rats where administration of sulphur mustard has been shown to cause localised skin and soft tissue sarcomas, carcinomas and distant pulmonary tumours, damaging RNA by alkylation and producing DNA lesions.

In small doses, these may be reparable. By its alkylating action on guanine, the resultant lesions are thought to cause the mutagenic consequences in animal models.
The genetic toxicology of Lewisite has been poorly studied. A by-product of Lewisite metabolism however, is arsenite. This has been shown to block DNA binding sites and inhibits DNA repair proteins leading to chromosome aberrations. Cutaneous Lewisite exposure causing Bowen's disease has been reported (Watson & Griffin 1992). It may be possible that a synergistic effect is seen with concomitant dosing of both sulphur mustard and Lewisite.

Nitrogen mustard has been used as a therapeutic cytotoxic for cancer chemotherapy. Associated with this unfortunately, has been a rise in the number of associated secondary skin malignancies and leukaemias. Occupational exposure to the manufacture of vesicating agents has been linked to an excess of respiratory tract neoplasias, most notably of the larynx and pharynx.

Teratogenicity studies in human workers and animal models exposed to sulphur mustard or Lewisite have remained inconclusive.

1.6.9   Treatment Strategies

1.6.9.1   Prophylaxis

Prophylactic measures should be undertaken ideally with protective clothing in the form of a suit and respirator. The boots should, ideally, be lined with Fuller's earth. This is very fine china clay that has a relatively large surface area to volume ratio. As such, it is able to adsorb the lipophilic vesicant on the skin. However, protection is only afforded for up to six hours after which the clothing will need decontamination. Protective barrier creams are still in their experimental phase of development.

1.6.9.2   Treatment (HMSO 1987, Kadivar 1991, NATO 1996, Evison et al 2002) is on the following principles:

a) First aid

Standard recommendations are that upon contamination, first aid would need removal of clothing and copious wet decontamination with water having been moved to a well-ventilated area (Heyndrickx 1990). If available, a dilute chlorine solution should be used instead, followed by normal saline. A coating of Fuller's earth to the skin would reduce any further vesicant action. Eyes should be lavaged with water or isotonic (1.26%) sodium bicarbonate. Blisters should be punctured and de-roofed before dressing the wounds with Flamazine™ (silver sulphadiazine) cream and dry dressings. Intravenous sodium thiosulphate (500mg/kg) may be beneficial if given within 30 minutes of exposure. Inhalational injuries may be lessened with the use of inhaled corticosteroids prior to the manifestation of an airway injury.

b) Tetanus Prophylaxis

Adsorbed tetanus toxoid should be given for those not immunised within the previous twelve months. Immunoglobulin should be added if the patient's tetanus status is unknown.

c) Airway management

Head and neck burns are at risk of developing airway compromise, with hoarseness and stridor heralding life-threatening upper airway obstruction. This requires a high index of suspicion with early endotracheal intubation if an inhalation injury is considered. There should be a low threshold for the commencement of antibiotics since secondary infection is common. Respiratory support may be indicated with moderate to severe infections. Acute respiratory distress syndrome (ARDS) is a recognised sequela of severe pulmonary injury and a marker of poor prognosis.

d) Specific Antidotes

Systemic absorption of Lewisite should be treated with intramuscular BAL(British Anti-Lewisite) which can, itself, cause localised muscle necrosis. BAL is dimercaprol (2,3-dimercapto-propranol), a chelating agent specifically for Lewisite. Other side effects include a tachycardic, hypertensive response, chest pain, lacrimation, rhinorrhoea and nausea with vomiting. These are seen in up to 50% of patients. It has no action on the mustard vesicants. Topical BAL ointment was recommended until recently for percutaneous Lewisite exposure but the product has a very short shelf life, hence its withdrawal. Furthermore, there have been concerns that the ointment may trap and further propagate the Lewisite. As a prophylactic countermeasure, it has been mooted whether the ointment would, again, speed up the process of Lewisite exposure by providing a moistened environment. However, BAL does not fully protect against the haemolytic effect of systemic Lewisite; after intravenous fluid resuscitation, exchange transfusion may be indicated. Pressor agents should be utilised to maintain perfusion if hypotension is persistent.

e) Cardiovascular Effects

Intravenous fluids should be instituted since large fluid losses are incurred with the blistering. Colloidal or albumin solutions may be indicated, depending on the extent of loss. This fluid shift is not as rapid as with thermal burns although it can become significant to the point of cardiovascular collapse without supportive measures.

f) Limb-threatening Injuries and Escharotomy

Circumferential burns can lead to the formation of a constricting eschar with the development of ischaemia and gangrene of distal extremities if unrecognised. This may require escharotomy, an incision through the full-thickness burn which is painless. Chest burns can cause respiratory embarrassment unless treated in a similar manner.

g) Gastric Decompression

With extensive burns, insertion of a nasogastric tube decompresses the stomach contents. Haemorrhagic gastritis with ulceration (Curling's Ulcers) and paralytic ileus may develop with severe burns and monitoring of these conditions is possible with the tube. Antacids, H2-receptor antagonists (cimetidine or ranitidine), proton pump inhibitors (omeprazole or lansoprazole) and prokinetic agents such as metoclopramide may be administered via the tube to overcome these effects. In the acute phase, nutritional support in the form of total parenteral nutrition (TPN) may be indicated until the gut has recovered sufficient function.

h) Adjuvant treatments

As a result of the various hospitals that the casualties of the Iran-Iraq conflict from 1984 were treated at, alternative strategies at the secondary treatment stage have been developed. The burning pain of the recovering skin wounds responds to carbamazepine 200mg t.d.s. with eye lesions responding favourably to topical dexamethasone (Newman-Taylor 1991).

1.6.10 Experimental Treatments

Antidote research has concentrated on anti-oxidants, cyclo-oxygenase inhibitors and nucleic acid enzyme inhibitors (Zhang 1995). Immunomodulatory compounds such as pyrimethamine have also been proposed (Ebtekar 1993). It has been found that melanin may have a role in reducing the severity of sulphur mustard blistering (Brown 1998) by acting as a nucleophile target. The use of intra-lesional superoxide dismutase prior to exposure reduces the

severity of the lesions thus implying that the injury leads to the formation of oxygen free radicals (Eldad 1998).

Topical protective agents have been investigated and comprise of synthetic polymers such as PTFE (Teflon®) mixed with chloramide as a deactivating agent (Smith & Graham 1997). Povidone-iodine ointment has been shown to have a therapeutic role post-exposure: applied up to twenty minutes after cutaneous exposure, treated burns did not develop to the extent of their untreated controls. The mechanism of action remains unclear, however (Wormser et al 1997). Acetylcysteine and sodium thiosulphate have been proposed as an antidote administered intravenously with the intention of binding and de-activating any systemic vesicant agent. Ideally, this should be done soon after exposure for maximal benefit. However, no objective evidence has justified the usefulness of these agents (Willems 1989).

Similarly, oral administration of activated charcoal and laxative has been tried to see whether it would absorb any ingested agent. However, since only 10% of agent is excreted in the faeces (the majority will undergo renal excretion), the benefit of this intervention was found to be minimal. Haemoperfusion and haemodialysis have been tried but morbidity often outweighed any benefit obtained (Willems 1989).

1.6.11   The Ideal Experimental Study Model

The only published human experiments have been from Cameron's series in 1946 from Porton Down where Lewisite was applied and then skin biopsies taken from volunteers (Cameron et al 1946). With the known toxic effects of lewisite, human volunteer studies are now precluded. Vesicant burn pathology can be investigated with various mammalian experimental models. Flesch *et al.* (1952) believed that the relative thinness of the epidermis common to most laboratory animals, and the anchoring of the epidermis afforded by a high

density of hair follicles, were important factors in explaining the differences in response to challenge between human and animal skin.

Guinea pig, rat, hamster and rabbit skins show a rapid and deeper reaction, resulting in more extensive damage to the dermis than is seen with human skin. However, no vesicles form. Typically, the burns appear as nonvesicating, necrotic, encrusted lesions. Microblister formation was thought to be a uniquely human response to vesicant agents due to the dearth of hairs and follicles to anchor the epidermis to the dermis.

The best available model for this had been human keratinocyte skin grafts on athymic nude mice. Although the grafts would take, no follicular units grew, limiting its use as a realistic model. In addition, the abnormally situated epidermis together with its underlying scar tissue was predisposed to premature blister formation (Papirmeister et al 1984). Explanted human skin has been tried with moderate success since only small areas ($\sim 1 cm^2$) can survive in this manner for a limited period of time (Lindsay and Rice 1996).

Porcine skin has been used for wound healing research because it has a number of similarities to that of man (Montagna & Yun 1964) and has been recognised as a valid model for a range of cutaneous toxicity tests (Khan 1984). With its thicker epidermal and dermal components, porcine skin was developed as an effective model since the formation of microblisters is seen both in vitro (Zhang 1995) as well as in vivo (Mitcheltree 1989, Lindsay and Rice 1995). The structure comprises of a multiple layered epidermis, reticular ridge pattern, dermal organisation and even similar epidermal lipids to human skin. Studies have shown that the pig can be used to model both the development and healing of Lewisite and sulphur mustard vapour-induced lesions in man (Brown & Rice 1997; Rice 1997: Rice et al 1999). In addition, pig skin vesicates at doses matching those that would be seen in human skin.

1.6.12   Histological Appearances of Vesicant Burns

Although similar in nature to thermal burns in terms of clinical features such as erythema, oedema and blistering, the kinetics of vesicant burns are much slower. Vesicant burns may heal in up to twice the time that it would take an equivalent thermal burn to heal, with mustard burns being slower to heal than Lewisite. Moreover, large areas of eschar can harbour localised abscess formation acting as infective foci causing systemic toxicity in much the same manner as thermal burns.

The pathological and toxicological effects of Lewisite were initially investigated *in vivo* on human volunteers in the early half of the last century (Cameron et al 1946). Blisters were noted to be subepidermal with coagulative necrosis of the dermis - a full thickness burn. This has been re-confirmed on a pig model, showing coagulative necrosis of the epidermis and papillary dermis complete within twenty-four hours post-exposure (Rice & Brown 1999). At forty-eight hours, the lesions appeared as full thickness burns with necrosis extending into the deep subcutaneous connective and adipose tissues. It was found that by three weeks approximately two-thirds (66.8%) of the original burn area had been covered by regenerative epidermis.

The pathological changes observed with sulphur mustard are very similar on the large white pig model which has been investigated at Porton Down (Brown & Rice 1998). Vesicant burns cause characteristic coagulative necrosis of the epidermis with shrinkage of dead epidermal cells. Some areas of focal extension as far down to the subdermal fascia and fat may be seen. Dermoepidermal cleft formation leading to microblisters is noted, particularly with sulphur mustard, together with diffuse epidermal degeneration (Rice 1999).**(Fig.1.6.8)**

This comprises of hydropic degeneration of basal keratinocytes, epidermolysis and pyknosis. Vacuolisation of the basal cells is also seen with loss of the rete peg structure. In particular, the laminin network appears to be vulnerable. Cleavage of the adhesion molecule which anchors the basal cell layer to the

basement membrane is seen clearly with sulphur mustard burns (Lindsay & Rice 1995).

The open structure of laminin may expose the structure to attack compared to the tightly bound bundles of Type IV collagen. The proportions of collagen in porcine skin may explain its hardiness to vesicle formation, compared to the gross epidermal separation seen in humans. Vesicant-induced proteases responsible for this action may provide a key to a treatment. The necrosis affects the papillary and reticular dermis (i.e. deep dermal to full thickness). Vascular damage and hair follicle degeneration is also seen with sulphur mustard, manifesting as dermal haemorrhage and thrombosis with follicular necrosis (Mitcheltree 1989). This capillary thrombosis would most certainly contribute to the extent of the lesion, with the maximal depth of lesions being reached by 5-10 days (Vogt 1984).

The boundary between the necrotic eschar above and the viable tissues beneath is well demarcated by a prominent band of acute inflammatory cells. The surface of these burns is covered by a well-developed eschar composed of necrotic cellular debris and organising fibrinous exudate. Oedema is seen within all layers (more so with Lewisite) and an inflammatory neutrophil infiltrate is seen in the papillary dermis (Brown 1997)(**Fig.1.6.9**). The melanocyte is also sensitive to vesicant challenge with selective necrosis of these cells within a few hours (Brown & Rice 1998).

The natural healing course of Lewisite burns is more rapid than with sulphur mustard. The epidermis shows focal basal cell vacuolation with associated acute inflammation as early as one hour post-exposure. Coagulative necrosis of the epidermis and papillary dermis is complete by 24 hours following the appearance of multiple coalescent blisters between six and 12 hours post-exposure.

By 48 hours, the lesions have become full thickness burns with necrosis extending into the deep subcutaneous connective and adipose tissues. Early epithelial regeneration seen at the wound edge probably reflects a lack of alkylation of DNA and RNA as discussed previously. This translates into a faster rate of spontaneous re-epithelialisation (Rice and Brown 1999) **(Fig.1.6.10)**.
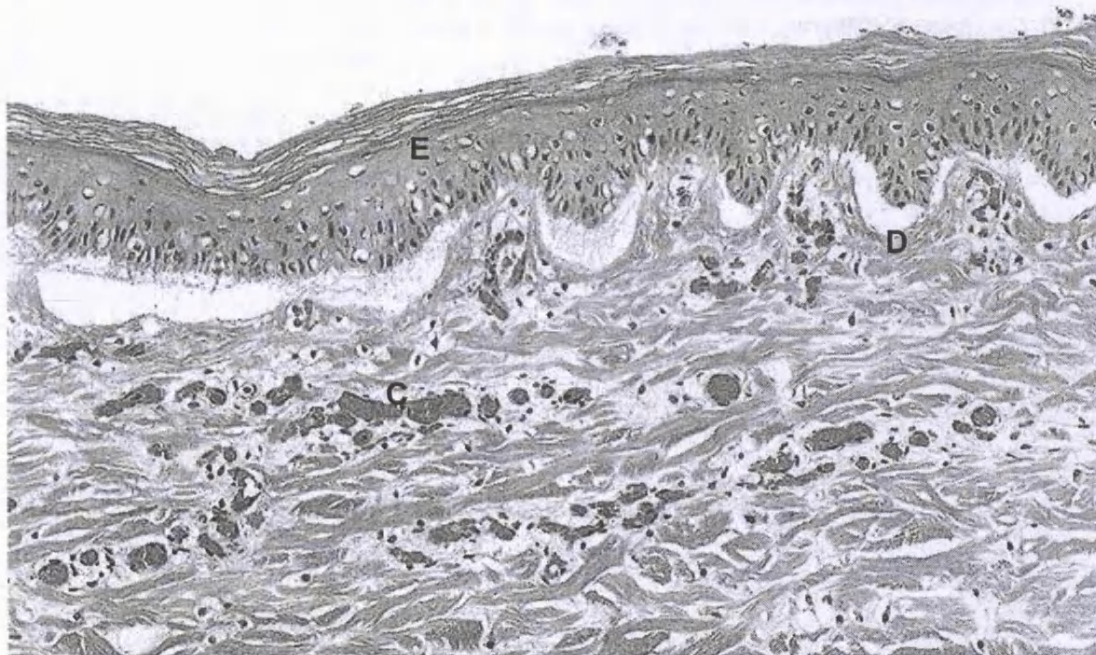
Fig.1.6.8. Optical micrograph of pigskin following sulphur mustard challenge. The roof of the blister has intact epidermis(E) and the floor has coagulated dermal collagen(D) supporting thrombosed capillaries(C) . Haematoxylin & Eosin x500. *Reproduced with permission of Dr. Paul Rice, Dstl, Porton Down.*
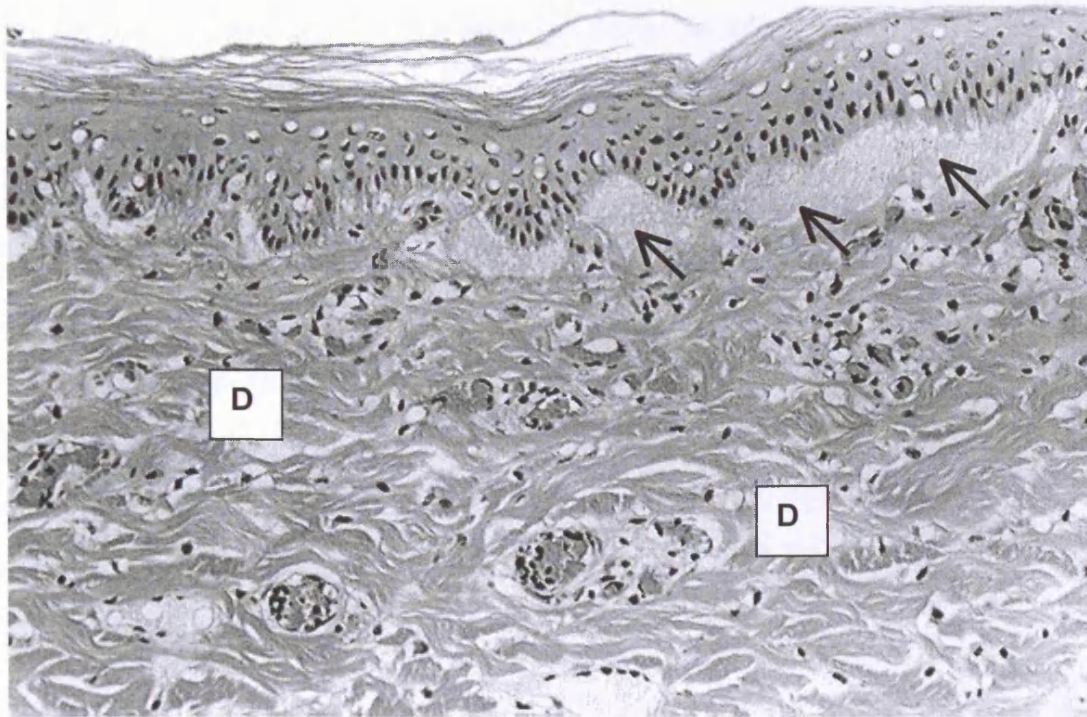
Fig.1.6.9. Section of dorsal pigskin showing advanced epidermal degeneration and obvious areas of dermo-epidermal separation (arrows). The underlying dermis (D) is diffusely infiltrated by acute inflammatory cells and individual collagen bundles are separated by oedema. Haematoxylin & Eosin x250. *Reproduced with permission of Dr. Paul Rice, Dstl, Porton Down.*
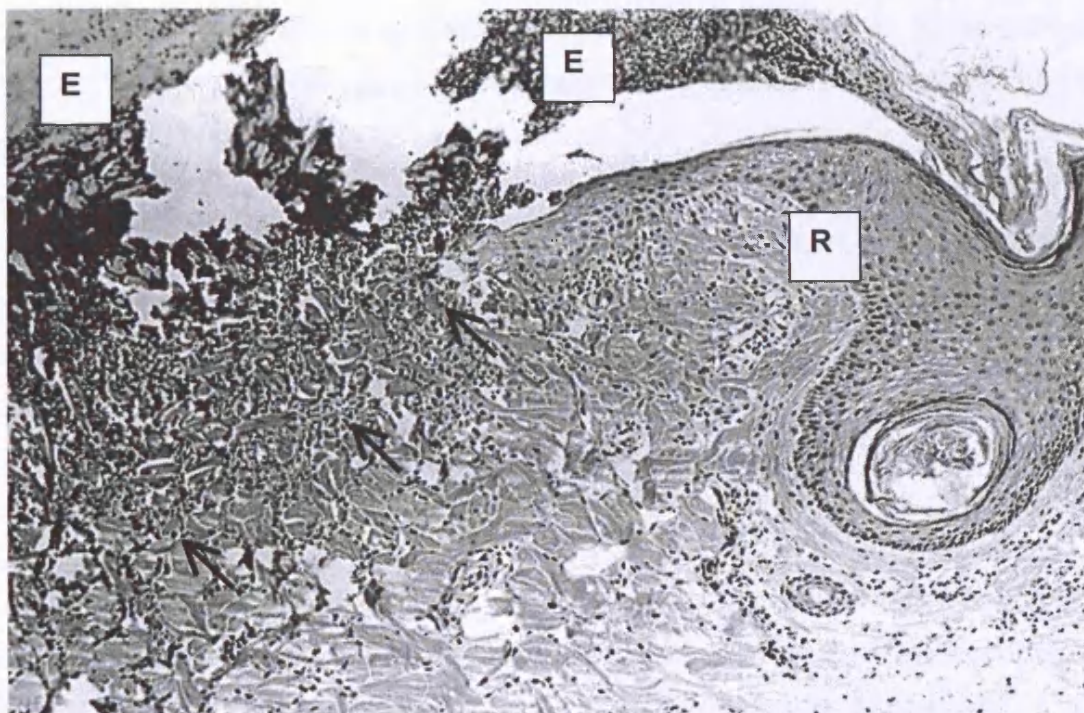
Fig.1.6.10. A section of dorsal pigskin showing early migration of a tongue of regenerative epidermis (R) at the wound edge beneath the eschar (E). There is a clear delineation between the nonviable surface eschar and viable dermis (arrows). *Reproduced with permission of Dr. Paul Rice,Dstl, Porton Down.*

1.6.13   The Search for a Suitable Surgical Intervention

13.1   Excision with or without grafting

Surgical intervention would mandate early tangential excision of the whole burn in a manner analogous to thermal burns. Although obvious for full thickness burns, controversy exists regarding debridement of deep dermal and mixed depth vesicant burns (Willems 1989). In 1998 Eldad et reported a comparison of early treatment modalities performed on nitrogen mustard burns using a guinea pig model. These included tangential excision, laser ablation and chemical debridement. Tangential excision to bleeding dermis without the use of a skin graft proved to be disappointing compared to non-treated controls, although enzymatic debridement using trypsin based dressings was useful in accelerating healing or at least providing a suitable recipient site for skin grafting. This paper is discussed further in Section 1.3.4. 'Laser Debridement'.

Excision of burn lesions with direct closure has been suggested (Kjellström 1997), although this would only be feasible for small burns (of less than 2-3cm in diameter) provided that sufficient laxity of the skin allows this. In the guinea pig model used for this study full thickness skin grafts were unreliable, with poor uptake resulting in the grafts acting only as biological dressings. With these two techniques, the amount of donor sites that will yield adequately sized full thickness skin grafts, with the relative inelasticity of the skin, severely limits the applicability to large burns.

13.2   Dermabrasion

Dermabrasion has been shown to be effective in healing sulphur mustard burns on a porcine model *without* the need for skin grafting (Rice 1997, Rice et al 1999). Re-epithelialisation was seen within 3 weeks in this study as opposed to the untreated controls (86% healed versus 16%). The important concept of this study was that merely a *reduction* in the amount of necrotic eschar was necessary to accelerate healing, obviating the need for skin grafting.

Dermabrasion simply removes the majority of the eschar without denuding the healing undersurface. This mechanical damage stimulates epithelial cells in the epidermis to migrate and rapidly mitose to cover the defect (Gillman 1955). At the wound edges, short tongues of regenerative epidermis are seen to undermine the surface scab within a few days. These
are supported by loose, highly vascular granulation tissue containing active fibroblasts which begin to migrate towards the centre of the lesion. The only discernible difference is the magnitude of this epithelial regeneration at the wound edge for the non-treated and dermabraded burns (Rice, Brown & Lam 1999).

13.3   Enzymatic Debridement

Eldad et al (1998) used enzymatic debridement with Debridase™ gel on a guinea pig model 24 hours post-exposure to sulphur mustard and found this dramatically accelerated healing compared to both tangential sharp excision with no skin grafting (80% epithelialisation by 6 days versus 30%). In turn, at a lower dose was more effective than tangential excision (70% versus 30% by day 6).

13.4   Laser Debridement

The application of lasers for thermal burn debridement has already been discussed in Section 1.5.6 Current research has concentrated on the ability to debride vesicant eschar without the need for skin grafting (Graham 1997, Smith 1997, Eldad 1998). This would provide shorter recovery phases for the burn victim since no donor sites are required, which has logistical implications in a war situation.

Pulsed CO_2 lasers have been used to debride sulphur mustard burns on a porcine model with reportedly accelerated healing (Smith et al 1997, Graham 1997). This was performed at various time points up to 48 hours post-exposure to the vesicant agent. The subjects were culled at 14 days and then the wounds were assessed. Only two laser passes were used which would not have removed the complete eschar, yet this appeared to be effective in a manner similar to dermabrasion. No skin grafting was employed. It was concluded that

laser debridement resulted in a more organised epidermis via the removal of cytologically atypical cells, which encouraged healing compared to untreated controls. However, all wounds in this study, whether treated or not, had re-epithelialised by two weeks. This is in contradiction to the generally accepted fact that sulphur mustard burns are slow to heal, taking twice as long as an equivalent thermal burn (Willems 1989, Mellor 1991). This may have been due to the burns being only superficial, the dosing method not accurately modelling a human casualty.

Similarly, Eldad et al (1998) performed laser debridement on a guinea pig model 24 hours post-exposure to sulphur mustard and found this dramatically accelerated healing compared to both tangential sharp excision with no skin grafting (80% epithelialisation by 6 days versus 30%). In turn, at a lower dose enzymatic debridement with Debridase™ gel was more effective than tangential excision (70% versus 30% by day 6). This potentiation of epidermal healing may be due to the removal of alkylated, damaged debris together with any remaining viable keratinocytes that possess mutated DNA. By reducing the degree of inflammatory infiltrate, the healing process is not hindered by the presence of excessive proteolytic enzymes resulting in healed lesions with little cytological atypia and minimal inflammatory infiltrate (Smith 1997, Smith 1999).

However, in a theatre of war, it would be unlikely that injured victims could be treated at an adequately equipped facility within this short time-frame of up to 48 hours.

This thesis will investigate the role of modern lasers in the debridement of Lewisite vesicant burns.

Aims and Basis of this Research

1.7.1 The Surgical Treatment of Vesicant Burns

Burns induced by vesicant agents (Lewisite and sulphur mustard) have been the subject of little research in terms of their surgical treatment as outlined in Section 1.6.13. However, the pathological and toxicological effects are well documented as a result of their use as chemical warfare agents.

Using a porcine model, it has already been noted that mechanical dermabrasion is a useful modality in accelerating the healing of these types of burn injuries without the need for additional split skin grafting (Rice 1997 and Rice, Brown, Lam et al 1999). It would appear that a decrease in the necrotic eschar alone is able to expedite the healing process. In this study, the depth of eschar debridement was just to the point of the appearance of punctate bleeding to be effective. Formal debridement to fresh bleeding (usual surgical practice for thermal burns) mandates the use of skin grafting to avoid an ulcer. Left untreated, the burns heal slowly by secondary intention. By removal of a portion of the necrotic eschar, the edges of the wound re-epithelialise at an accelerated rate. No skin grafting is employed since this is only a partial debridement.

It has been established in Section 1.5.6 that laser technology has now improved to the point where high fluences can be delivered accurately to burn eschar with the minimum of residual thermal damage. Hence, it is now possible to laser debride burn wounds such that the remaining bed can accept a skin graft with minimum loss.

The corollary of this is that it should be feasible to *partially* laser debride a vesicant burn and thus accelerate healing. This 'lasablation' should be more precise and therefore encourage rapid healing compared to dermabrasion. Therefore, it is proposed to use the 'lasablation' method on the same animal

model that has already used at our laboratories to model the dermabrasion work.

The purpose of this thesis is to investigate the validity of applying laser debridement to Lewisite burns in order to establish if there is a similar, if not enhanced, acceleration of wound healing *without* the use of skin grafts.

1.7.2 Proposed Animal Model

The animal model proposed is the Large White pig, which has been used for previous exposures to Lewisite. A reproducible method of inducing a Lewisite lesion will be used which was also the same method for the dermabrasion study by Rice's group.

Using this methodology, Lewisite burns have already been studied at various points over a three-week period to observe the histological patterns of healing in such burns (Rice and Brown 1999). They will provide the basis for historical controls in the experiments.

1.7.3 Laser ablation – 'Lasablation'

It is proposed to use modern medical class lasers, namely the Ultrapulse™ CO_2 (Coherent, Palo Alto, California, USA) and Continuum Biomedical Erbium-YAG (Continuum Biomedical, Dublin, California, USA), to investigate the use of lasers for Lewisite burn debridement in the Large White pig. These instruments have the selective ability to vaporize *thin* layers of tissue with the minimum of residual thermal damage.

Removal of non-viable tissue in this way should accelerate wound healing in a manner akin to dermabrasion. However, the precision of the lasers allows for a more tightly controlled level of debridement. This should ensure that the rate of spontaneous epithelialisation is superior compared to mechanical dermabrasion since the risks of inadvertent damage to healthy tissue are reduced.

After initial chemical insult with Lewisite as described previously, the burns will be laser debrided at the fourth day post-exposure, and then examined over three successive weeks to monitor the rate of wound healing in terms of macroscopic size and histological characteristics. The objective of the histological examination will be to investigate the contribution of different cellular components to the healing of the burn wounds, at 0, 1,2 and 3 weeks post-'lasablation'. These results will be compared with the control data already acquired for no treatment (Rice & Brown 1999).

There will also be a concurrent study of the effects of laser debridement of unburnt skin on the same subjects which should provide some insight into the normal healing patterns of skin post-laser resurfacing in comparison to burn tissue.

The analysis of the experimental results should provide detailed information as to whether 'lasablation' has any role in the treatment of Lewisite and vesicant burns in general.

All animal work will be carried out under the conditions set out by the Animals (Scientific Procedures) Act 1986 and relevant Home Office Licences will be obtained.

Experiment 1

The Effect of CO2 and Erbium-YAG Lasers on Ex Vivo Porcine skin: A Comparison of Thermal Injury

2.1.1 Introduction

Laser resurfacing has become an invaluable treatment modality for facial rhytids and other superficial skin lesions such as acneiform scarring, photodamage and pigmented lesions (Apfelberg 1996). The uptake of these instruments has been particularly rapid within the last five years, replacing the previous modalities of sharp excision, dermabrasion and chemical peeling since they are able to offer a degree of controlled precision with regard to depth of desired resurfacing.

Much has been documented about the properties of the CO_2 laser (wavelength 10.6µm), regarding its behaviour in terms of depth of penetration and the amount of residual thermal damage pertaining to each type of laser (Green 1990,Payne 1998,Zweig 1990,Walsh 1988) as mentioned in Section 1.5.

These were the predecessors of the ultrapulsed type of CO_2 laser, which is now capable of generating microsecond long pulses. The ability to produce multiple short duration pulses allows these lasers to selectively ablate thin layers of tissue with a minimal layer of residual thermal damage. It has been advocated that the precision of this method is superior to both dermabrasion and chemical peeling for resurfacing surgery (Stuzin1998).

Similarly, the Erbium: YAG laser has recently been developed with a specific operating characteristic of a shorter wavelength (2.94µm) with a superior water absorption coefficient compared to CO_2. This is claimed to lead to more selective ablation of target cells with less unwanted transmitted energy.

The choice of laser type was governed by use of the latest technology, namely the Ultrapulse CO_2 laser (Coherent Medical, Cambridge, UK) and the ConBio Erbium:YAG laser (Continuum Biomedical Systems, California, USA). These recent variants are the most technically advanced lasers of their type.

With this background, the purpose of this experiment is to directly compare the effects of these two types of laser medium on porcine skin. Differences in technical specifications and usage, together with histological analysis are to be investigated. The purpose of this is to provide the optimal criteria for laser parameters in Experiments 2 and 3, where they will be used for skin resurfacing and the debridement of burn eschar, respectively.

2.1.2 Methods

Standard safety precautions were adopted throughout all laser usage in accordance with local health and safety requirements, detailed in **Appendices 2 & 3**. This included the use of safety goggles and smoke evacuators to remove plume. All work was carried out within a designated operating theatre armed with circuit breakers to the laser power supply in case of inadvertent entry by unprotected staff.

Porcine skin was obtained from a fresh post-mortem animal, shaved and prepared with a saline moistened swab before being laid on an even surface. Circular targets were marked using a template with a $10cm^2$ surface area. At least ten targets were used for each laser, all with individual fluence, pulse settings and number of passes as shown in **Table 2.1.1**. During laser usage a smoke evacuator was employed and masks worn.

After each pass, the burnt tissue was wiped off with a saline swab and any changes noted.
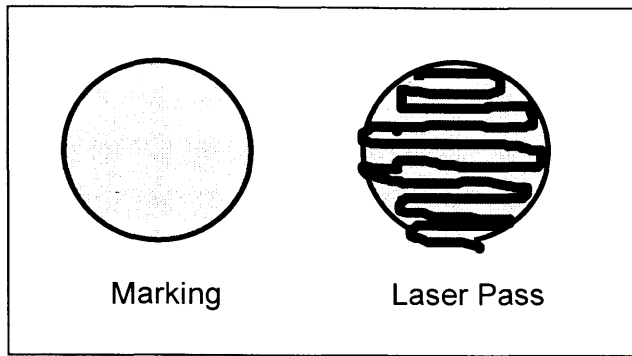
Figure 2.1.1.
The scanning raster pattern of the laser over the skin.

2.1.2.1   Ultrapulse resurfacing

Energy was first set at 500mJ as for human skin resurfacing which developed a fluence of 7 J/cm^2. Initial passes were made with the Ultrapulse 3mm Truespot guided freehand in an airbrush type fashion. Immediate colour changes were noted with the production of grey-yellow residual surface debris. After wiping with the swab, a pale area of denuded dermis was revealed underneath. With the increased repetition rate, it soon became apparent that the quality of passes was extremely operator dependent such that some passes could be made inadvertently deep by mild inco-ordination (see **Fig. 2.1.1**).

The decision was made to try the Computer Pattern Generator (CPG) device. This is a robotised device that allows multiple shots to be fired precisely within a set pattern. A variety of patterns were selectable for a single pass (**Fig. 2.1.2**). Being generated electronically, this removed human error and achieved extremely even coverage.

The spot diameter for the CPG of 2.2mm with energy of 300mJ per pulse equated to a fluence of 7.5J/ cm^2 using Pattern 19, the hexagonal pattern (at the bottom left of the figure).

The working characteristics of the CPG are such that a 300mJ pulse equates to 500mJ using a 3mm Truespot. Using a size 19 hexagon with a spot density overlap of 60% developed an equivalent fluence of around 7.5 J/cm^2. This was

chosen for its size in relation to the test area and for the fact that the degree of overlap would ensure even coverage.
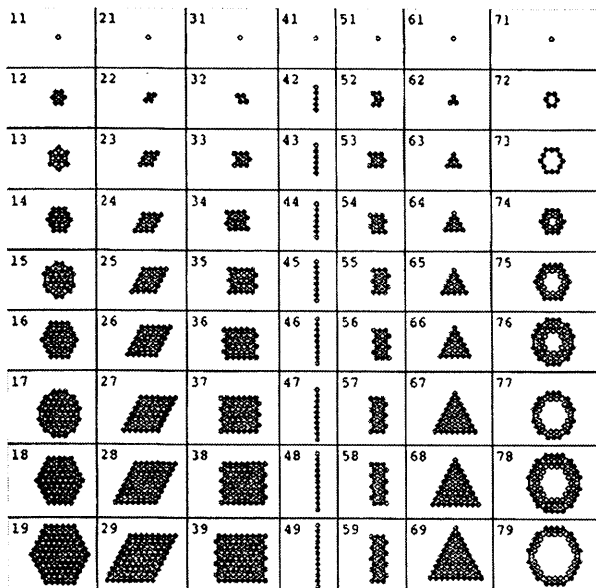
Fig.2.1.2 Computer Pattern Generator Beam Profiles

Hence with a similar fluence but with more accurate delivery, increasing numbers of successive passes were tried over the same area followed by wiping of tissue debris post-debridement. Up to five passes were tried, which is more than the normal clinical practice in skin resurfacing. This was in order to investigate the effect of depth of penetration.

2.1.2.2 Erbium:YAG Resurfacing

With the different characteristics of this laser, it was decided to fix a manageable pulse repetition rate of 8Hz and modify the energy per pulse from 1000mJ to 1900mJ developing fluences between 10 to 20Jcm^{-2}. It was decided to try these high fluence levels since it was clear from initial test passes that there was only a very superficial depth of penetration achieved. The explosive effects required good plume evacuation to be present.

2.1.2.3   Histological Analysis

The skin was then fixed in neutral buffered formalin before embedment in paraffin to analyse histological architecture. The specimens were cut into 4μm sections and stained with haemotoxylin and eosin during preparation. Sections were taken through the burn crater to view the edge on both sides and at the centre **(Fig. 2.1.3. a & b)**. These were analysed on a Zeiss (Carl Zeiss GmbH, West Germany) light microscope at 20 and 40X magnification. Using a calibrated graticule (Graticules Ltd, UK), it was possible to gauge the thickness of the zone of residual thermal damage (ZRTD) and compare its position with that of the adjacent intact epidermis.

Watts et al (2001) have previously described burn depth assessment using histological methods. This relies on looking for both the depth of microvascular occlusion within the dermal circulation and erythrocyte extravasation as markers of non-viability. In our following studies a similar methodology was used.

To ensure reproducibility, both the author (DL) and Project Supervisor (Dr. Paul Rice) analysed the samples on separate occasions.

The ZRTD was characterised by a homogeneous, eosinophilic appearance to the eschar, which became less clearly defined in the transition zone (TZ). A distinct plane of cleavage marked the TZ where the eschar was beginning to separate from the underlying dermis. Normal dermis was judged by the appearance of patent dermal vessels contained within regular connective tissue architecture.

These were measured to the nearest 5μm at six different sites to obtain mean values and standard deviations, as tabulated in **Table 2.1.2**
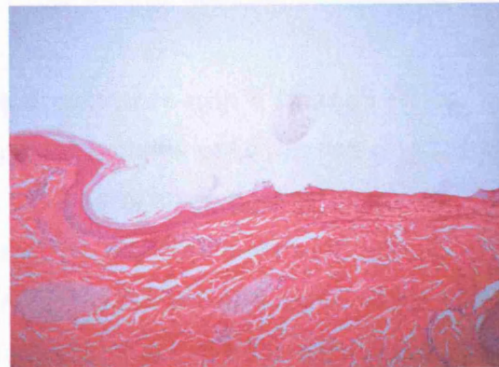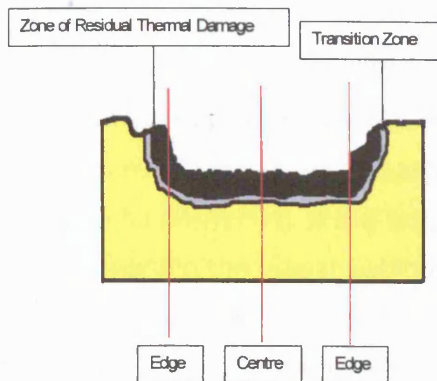
Fig. 2.1.3 a) Diagrammatic representation of sections

b) LM of Crater edge showing ZRTD (H&Ex50) © Crown Copyright 2003 Defence Science and Technology Laboratory UK

2.1.3 Results

Each successive UPL pass revealed a fresh, pale layer of tissue. After deeper passes, pale white dermis was visualised and there was obvious tissue contraction noticed during the pass. Visual differences were clearly seen compared to the EYL which did not penetrate as deeply, and caused small explosive effects with minimal residual char. After a single pass, a yellow-brown colour was revealed with minimal charring.

Under microscopy an obvious transition zone separated the homogeneous staining of the ZRTD, implying consistent heat damage to the layers, before merging into normal tissue. Cell nuclei were not seen within the ZRTD, only beginning to appear in a scanty and shrunken form within the transition zone. Here, cells were shrunken with decreased cytoplasmic volume and nuclear contraction, before changing to the appearance of normal dermis. At higher fluence levels actual cleavage of the ZRTD began in the plane of the transition zone, implying a disruption of the bond between dermis and epidermis. The

histological appearance with the related depths for each type of laser is shown in **Tables 2.1.3 & 2.1.4**.

A series of typical photomicrographs of the appearance with EYL and UPL is shown overleaf. Gradually increasing the power settings of Ultrapulse (**Fig.2.1.4 a-c**) resulted in stepwise progression with the loss of all epidermal elements and smoothening of the rete ridges to involve the papillary dermis. Gradually increasing the power settings of Erbium:YAG (**Fig.2.1.5 a-c**) showed stepwise progression in loss of just the epidermal elements and smoothening of rete ridges; the highest power, EYL 10 only just began to affect the papillary dermis.

Fig.2.1.4 Cross sections of ex vivo specimen a) UPL 1 b) UPL6 c) UPL 9 (H & E x 200) © Crown copyright 2003 Defence Science and Technology Laboratory UK
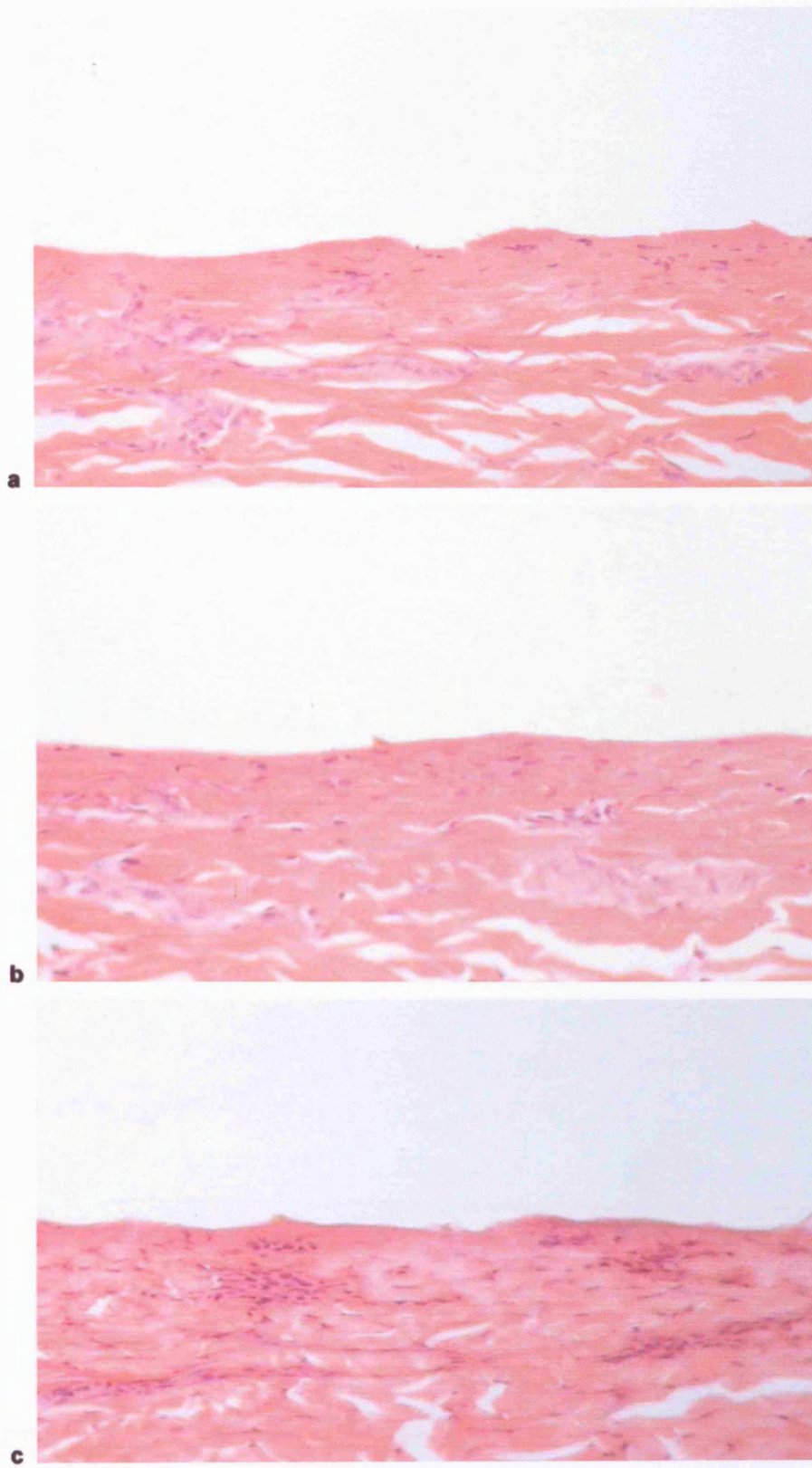
Fig.2.1.5. Cross sections of ex vivo specimen a) EYL 1 b) EYL6 c) EYL 10 (H & E x 200) ©
Crown copyright 2003 Defence Science and Technology Laboratory UK
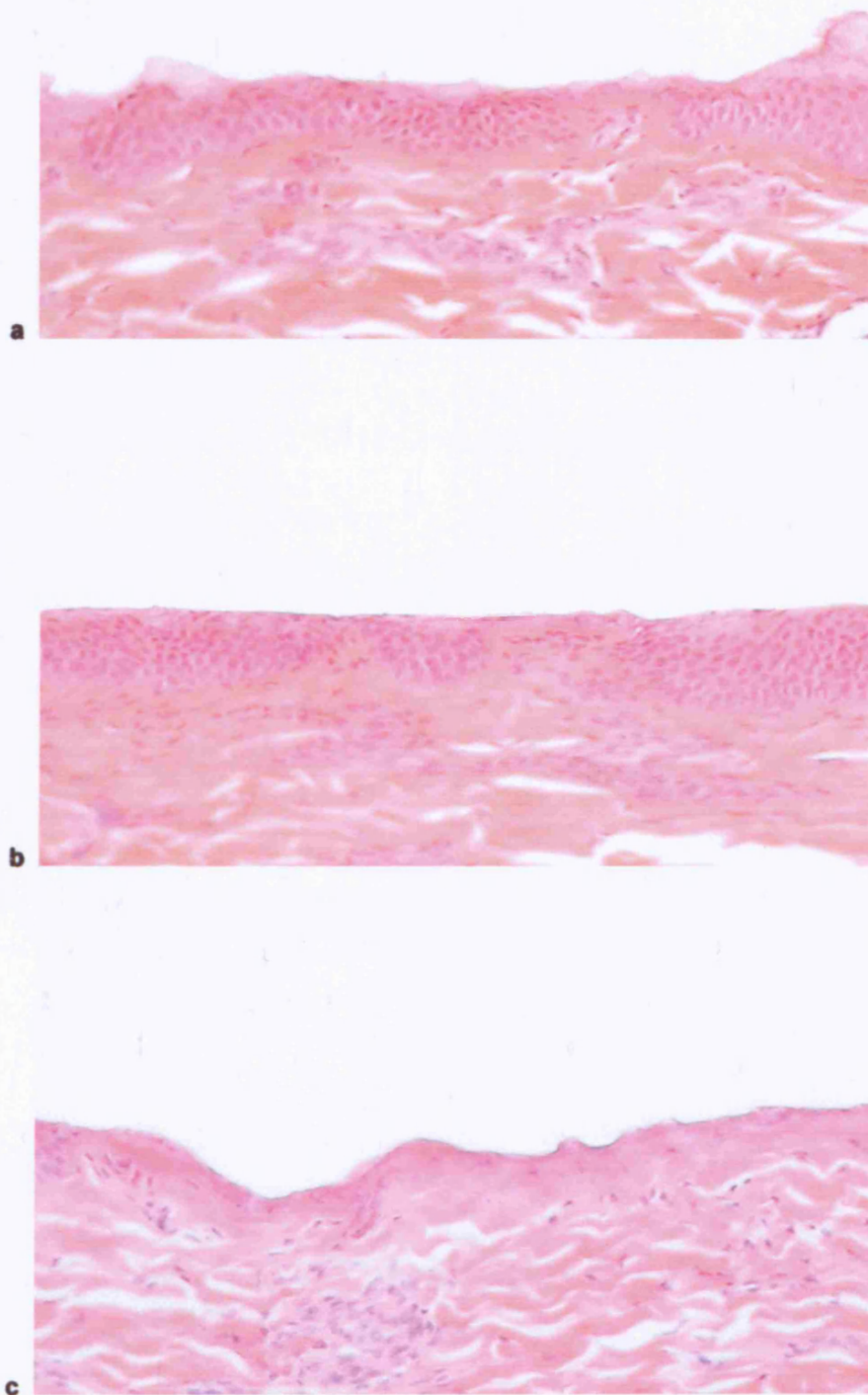
2.1.4 Discussion

2.1.4.1 Ultrapulse

The Ultrapulse CO_2 laser leaves a residual zone of damage between 70 to 200μm, sufficient to lead to haemostasis (Apfelberg 1996). This was confirmed by our experiment. The fluences used were between 5-7J/cm^2 although the repetition rates were much higher (20-175Hz) than normally recommended for human work (6-10Hz).

Higher repetition rates tended to coagulate the ZRTD such that it became more homogeneous without a marked increase in depth despite the number of passes. This was in keeping with the diminishing return effect that has been reported with the CO_2 laser. Settings using the CPG lead to a much more homogeneous and even residual zone, implying even coverage compared to the freehand small beam spot.

Although the CPG was superior in terms of a reproducible control of depth, multiple passes did not yield any further beneficial effects. Histological analysis revealed the development of a homogeneous ZRTD layer implying transmission of a more severe thermal injury. This is because the initial ZRTD and any remaining unwiped tissue char can act as heatsinks, absorbing energy and with random transmission of heat. Subsequently, burning of the tissue occurs with little ablative effect.

The colour changes reported with CO_2 by Reid in 1988 start with the first pass removing the epidermis, leaving a pink superficial papillary dermis. Successive passes will penetrate more deeply revealing a whitened papillary dermis before deep penetration to reticular dermis with its resultant chamois leather appearance. This represents the end-point of safe treatment beyond which scarring is inevitable. The absence of an intact dermal circulation in this experiment meant that these changes were absent. Tissue contraction is thought to be a result of collagen denaturation coupled with desiccation of the water content in the skin as a result of thermal challenge. It is believed that the

heating of collagen to 55°C results in a shrinkage of 20% to 30% (Apfelberg 1996). In addition, the skin comprises of 70% water, the principle chromophore for CO_2, explaining why shrinkage was clearly visible even on this ex vivo specimen.

2.1.4.2 Erbium:YAG

EYL has previously been shown to penetrate only a thin layer of tissue, 1-2μm, as opposed to 18μm for CO_2 in guinea pig skin (Walsh 1988). On ex vivo pig skin the depths sustained with each pass have previously been shown to be between 10 to 40μm depending on settings, with a resulting residual zone of thermal damage up to 50μm (Kaufman 1996). This narrow ZRTD was also noted in our findings, as seen in **Table 2.1.4**. It is this property which allows punctate bleeding to occur since the residual thermally damaged layer is so minimal that there is no haemostatic effect.

The fluences used (10-20J/cm^2) were in the higher range of that recommended for EYL work. This was tried in an attempt to elucidate the effect of high fluence alone on the depth of penetration. High repetition rates have been linked to desiccation with subsequent coagulation of ex vivo human dermis whereas increasing fluence has a lesser effect (Hohenleutner 1997). Usual values recommended are 5 J/cm^2 for the delicate periorbital area and up to 12-20 J/cm^2 for deeper rhytids and heavily photodamaged skin (Alster 1999, Weinstein 1999). Even at this high fluence only the papillary dermis was breached and the remaining nuclei within the dermis appeared to be relatively intact. Whether the uppermost cell layer is viable and capable of regeneration or destined to undergo necrosis will be established in Experiment 2.

EYL behaves in a different manner with clinical usage (Perez 1998, Bass 1999). The end point for adequate penetration is either until the lesion is obliterated or punctate bleeding is seen indicating penetration into the papillary dermal plexus. A transudate has been described where the trauma of the laser-tissue interaction causes vasodilatation in the dermal vessels with subsequent fluid release (Weinstein 1999). Due to the absence of a circulation in this ex vivo specimen, these changes were not seen. In the clinical situation, it has been

found that a single EYL pass will ablate down to the stratum granulosum, to the basal cell layer after two passes with a yellow-brown colour, and papillary dermis within three to four passes heralded by a pink appearance. The reticular dermis is reached after five to six passes where follicle openings become very apparent (Perez 1998, Weinstein 1999). By increasing the fluence only and using a single pass, penetration was possible just to the dermo-epidermal junction in our experiment.

The safety margin depends on the theory of selective photothermolysis being observed. This would be valid if the pulse duration was less than the thermal relaxation time of skin, Tr. However, UPL pulses are around 1ms (Tr = 695µs) and the EYL generates pulses of 250 to 350µs (Tr =1.9µs). Since these pulse lengths are in excess of their respective Tr values this clearly means that some thermal build-up and excess thermal energy is being transmitted into the remaining non-vaporised tissue.

2.1.5 Conclusion

In this ex vivo skin sample, the Erbium:YAG laser ablated at a superficial level leaving a thin zone of residual thermal damage of the order 40µm at most. Only the papillary dermis was breached with intact adnexal structures. Despite increasing fluences by a factor of two there was very little change in the depth of penetration. There is still a ZRTD since the pulse length of this device (250-350µs) still exceeds the thermal relaxation time (Tr) of 1.9µs, although it is very thin. Smoke evacuation was problematic, with a theoretical risk of viral transmission within the plume.

In contrast, the CO_2 laser left zones of residual damage to a depth of up to 130µm with the papillary dermis being breached with ease even at low fluence settings. Using the freehand method with the laser did show that inconsistent penetration depths were easily generated with high repetition rates. This could account for up to 80µm of inadvertent damage and certainly demonstrated the

consistent nature achievable with the computer pattern generator when used. Unintentional damage of this magnitude would account for the ease with which hypertrophic scarring is induced with injudicious passes. These differences in working characteristics would certainly account for the clinical findings. However, it has been suggested that depth for depth, the lasers are identical in terms of their tissue effects (Burns 1999). In practice, this would require multiple passes of the Erbium:YAG to achieve the same depth.

The lack of a circulation in this ex vivo sample masked the effects of any punctate bleeding that would have been seen with penetration into the papillary plexus. Similarly, any protective effect of an intact dermal circulation would not be realised.

In the light of these findings it was decided that the CPG gave the most reproducible results. The setting of UPL 9 (**Fig.2.1.4c**) was chosen for the main experiments i.e. 300mJ at 2.2mm spot size on hexagonal pattern 19 with a rate of 200Hz developing a fluence of 7.5 J/cm^2. This was due to the fact that with only a single pass, there was an even ZRTD left reaching down to the papillary dermis.

With the EYL, it was found that the plume was very extensive at the fluence settings used. Despite this, penetration was poor compared to UPL. It was decided that instead of a single pass at 8Hz, two passes of 4Hz would be used at the same 1900mJ setting, EYL 10 (**Fig.2.1.5c**) on a 3.5mm spot in order to reduce the degree of plume. Cumulatively, this would involve the same energy transfer.

The importance of these settings will be investigated in Experiment 2.

Experiment 2

The Resurfacing of Normal Skin with CO_2 and Erbium:YAG Lasers : the effect of circulation and sequential wound healing

2.2.1 Introduction

The depth of injury by laser ablation has been quantified with ex vivo specimens of skin in Experiment 1. These showed a distinct zone of residual thermal damage (ZRTD) that extended up to 140μm for the CO_2 and 40μm for the Erbium:YAG lasers separated by a distinct transition zone of 30μm and 20μm respectively, from the underlying viable dermis.

Although this gave meaningful data on the depths of injury for each type of laser, it did not show the effects of the dermal circulation, namely, the papillary and dermal plexuses. It may be that the circulation has a protective effect by dissipating heat via conduction, thus minimising the ZRTD produced.

In order to establish the effects of the skin circulation, a repeat set of laser passes was done on an in vivo model. Obtaining skin at 'time zero' will reveal whether the depths of injury are similar compared to injury without an intact circulation.

Additionally, the progress of wound healing will be monitored over a period of three weeks post-laser pass. This should provide an insight into the differences between the effects of the Ultrapulse and Erbium:YAG lasers on porcine skin during the healing process.

2.2.2 Methods

Eight female Large White domestic pigs were used in total. The animals each weighed between 25-30kg at the commencement of each experiment. Sites

were marked in numerical order, with sites C-1, L-1 and L-3 being planned for Erbium:YAG laser and sites C-2, L-2 and L-4 for Ultrapulse CO_2 laser.(Fig.2.2.1)

C-1 and C-2 sites were used to investigate laser passes on intact, normal skin and the rest of the sites were used for the Lewisite exposure detailed in Experiment 3. This generated sixteen sites of normal skin exposed to laser passes, equally divided between the two types of laser. It was planned that skin would be obtained at zero, one, two and three weeks post-laser pass by euthanasia of the subject at the appropriate time.

2.2.2.1   Anaesthetic

Each pair of animals received a general anaesthetic consisting of inhaled halothane (1-3% at 0.5L/min.) administered in a stream of nitrous oxide and oxygen (4-6L/min) for induction and maintenance. Whilst anaesthetised, an area measuring approximately 35 by 25cm ($875cm^2$) of dorsal skin was prepared by gentle wet shaving and drying in readiness for dosing chamber template application as seen in Fig.2.2.2.
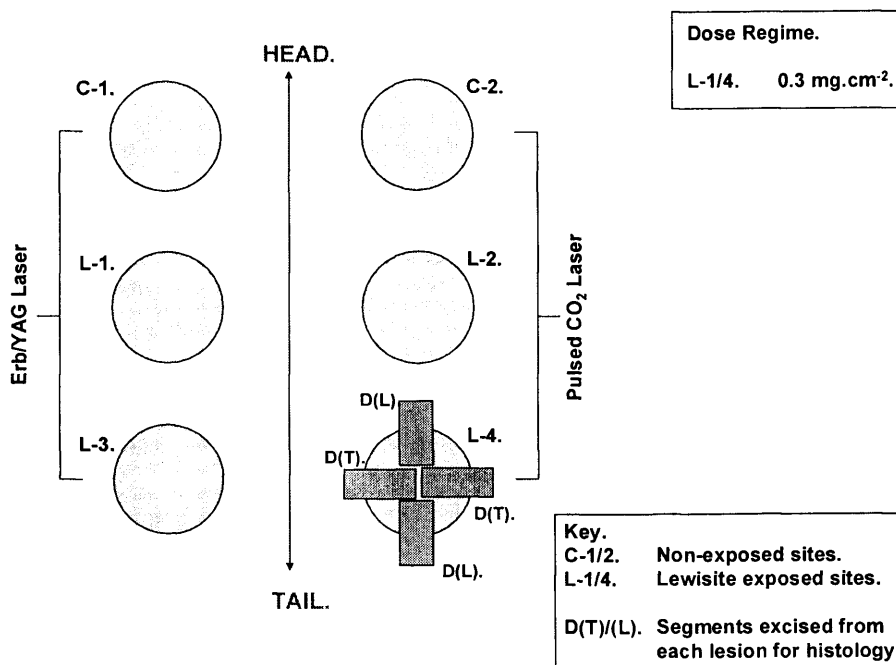
Fig. 2.2.1 Dosing regime for Lewisite
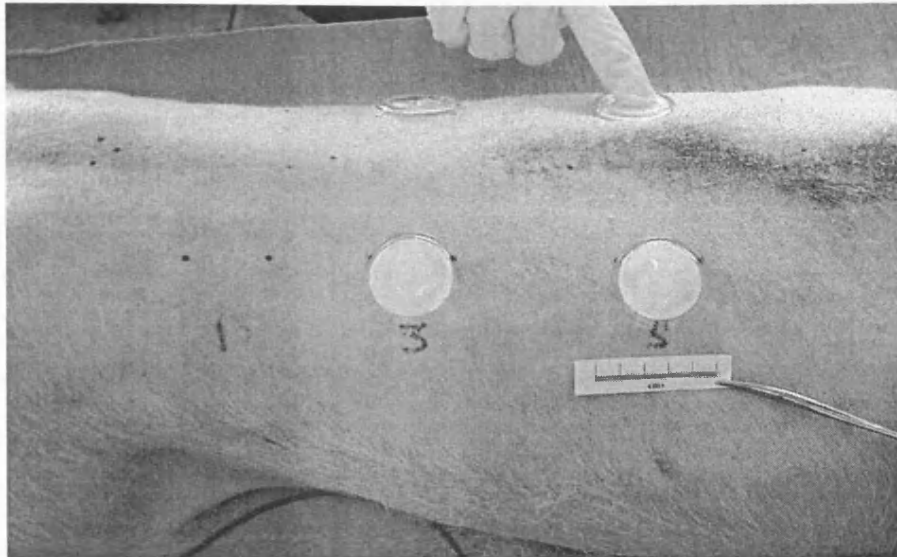
Fig.2.2.2 Surface markings on dorsal surface showing dose chambers and normal exposure pattern
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2.2.2.2 Monitoring

Vital functions were monitored throughout the procedure by means of electrocardiogram (ECG), pulse rate, pulse oximetry, respiratory rate and in-line capnometry with a Propaq106EL Monitor. Adjustments were made to the levels of anaesthesia administered based on these signs. Trends of those vital functions such as electrocardiogram (ECG), oxygen and carbon dioxide fluctuation were taken **(Fig.2.2.3)**
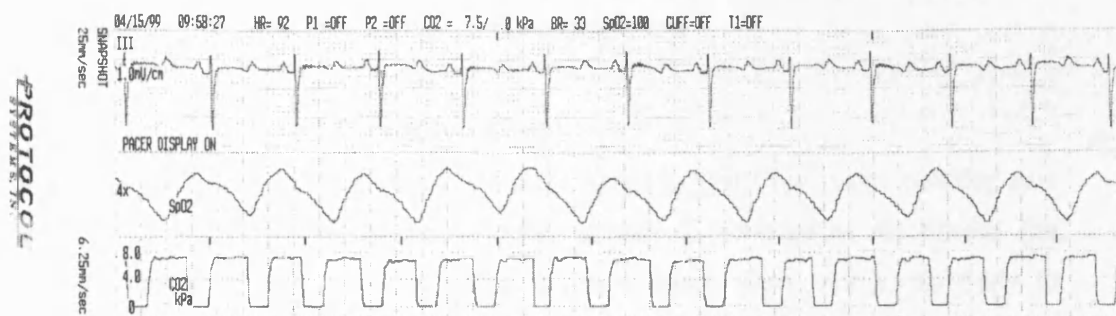
Fig.2.2.3 Intra-operative Vital Function Trace

2.2.2.3   Laser Passes

The lasers were set up in the designated safe area and smoke evacuation was used throughout.

Site C-2 was lasered with the Ultrapulse Laser (UPL) at a setting of 2.25mm spot size, 300mJ per pulse with a repetition rate of 200Hz. The computerised pattern generator was set to produce a hexagonal shape (Pattern no. 19) as per Experiment 1, configured to produce a 60% overlap of individual beams. This was equivalent to a fluence of 7.5 Jcm^{-2}.

The C1 site was treated with the Erbium:YAG Laser (EYL) on a setting of 3.5mm spot size, 1900mJ per pulse with a repetition rate of 4Hz. A fluence of 20J cm^{-2} was developed.

Epidermis was removed with a single pass of the UPL, exposing the papillary dermal vessels that had a minimal amount of bleeding. The freshly ablated char debris was wiped with a gauze swab moistened with saline. With EYL, two passes were used for the whole area. Again, the char was wiped between each pass as before.

Following 'lasablation', each lesion was dressed using Flamazine™ (silver sulphadiazine) ointment and a Melolin™ non-adherent dressing was secured over this with Hypafix™ tape. An intramuscular injection of 50μg buprenorphine was given at the end of the procedure before recovery from general anaesthetic. The animals were then returned to their home pen and observed for two hours to ensure all was well before being fed.

2.2.2.4   Post-Operative Care

The animals were observed twice daily to ensure that they were healthy and that the dressings remained intact. After dressing removal at 48 hours, the wounds were then left exposed. The animals were then monitored daily to observe any changes in wound progression.

2.2.2.5   Control Excision

At various time-points from Day 0 (zero timepoint) to Day 21 post-exposure, the animals were humanely killed with an intravenous overdose of Euthatal (pentobarbitone sodium, 200 mg/ml) and all burn sites excised for histological appraisal and calculation of areas of regrowth of new skin.

Thus, specimens were obtained at 0, 1, 2 and 3 weeks post 'lasablation'. The sections were all placed in neutral buffered formalin prior to embedment in paraffin for histological section. Prepared specimens were sectioned at 4μm thickness and stained with haematoxylin and eosin. Sections were taken through the eschar at representative quadrants to view the edge on both sides and at the centre as shown in **Fig.2.2.1 (bottom right).**

The specimens were then sectioned into longitudinal strips taken from each quadrant of the circular burn for measuring the widths of the ingrowing tongues
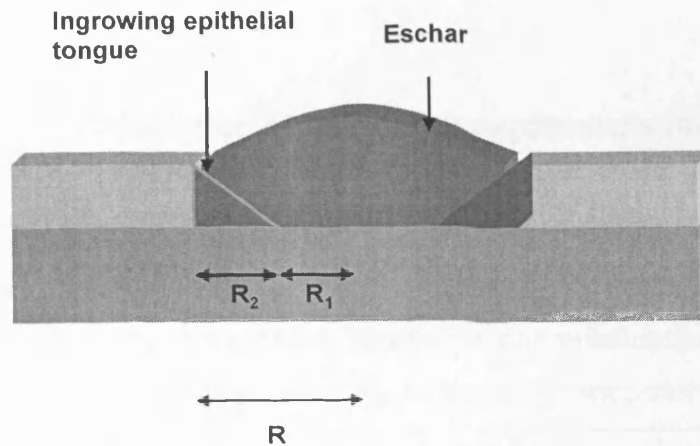
Fig.2.2.4 Diagrammatic representation of a healing control burn

of epithelium as R_2., represented diagrammatically as the red triangle in **Fig.2.2.4**

2.2.2.6   Histological Analysis

The analysis was carried out on a Zeiss Axioplan Light Photomicroscope (Carl Zeiss GmbH, West Germany) at 20 and 40X magnification. Using a calibrated graticule (Graticules Ltd, UK), it was possible to gauge the width of the regenerating tongue of new epithelium and compare its position with that of the adjacent intact epidermis. The architecture of the eschar, the junction between non-viable and necrotic tissue and the healthy bed were all examined.

In particular, examination centred around the junction of viable skin and non-viable eschar, the ZRTD. Epidermal loss and changes in the structure of the papillary and reticular dermis were documented.

Four readings were taken which were then tabulated **(Table 2.2.1)** and averaged to obtain a mean value for percentage re-epithelialisation using the formula given next. To ensure reproducibility, the analyses were carried out separately by both the author (DL) and the Project Supervisor (PR).

2.2.2.7   Calculation of Rates of Re-epithelialisation

Since all of the sections of the exposed sites showed evidence of epithelial regeneration, calculations of the area and percentage re-epithelialisation were possible for each site. The area of the zone of re-epithelialisation **(Fig.2.2.5)** was calculated by measuring values of R_2 at four different points around the circumference of the lesion and then calculating the average area of re-epithelialisation (A) using the following expression,

$$A = \pi(R)^2 - \pi(R_1)^2$$

where, $R_1 = (R-R_2)$

Zone of epithelial regeneration.

Unhealed lesion centre.

R_2. R_1. R.

R.   Radius of initial exposure.
R_1.   Radius of unhealed lesion centre.
R_2.   Radius of zone of re-epithelialisation.
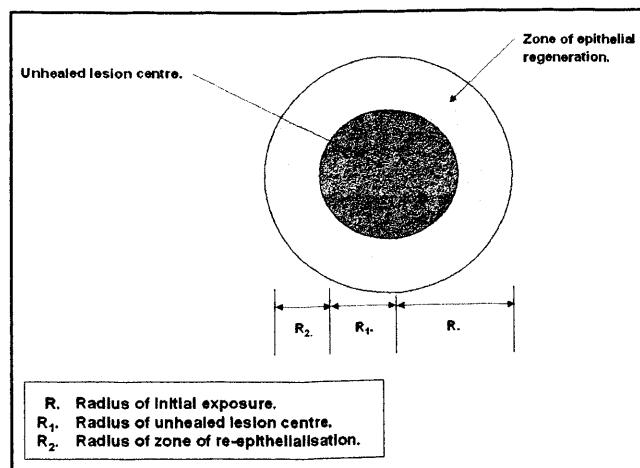
Fig.2.2.5 Diagrammatic representation of the zone of healing

R   = radius of initial exposure

= 1.784 cm

and $(R)^2$ = area of initially exposed site= 10.00 cm^2

Therefore,

$A = 10 - \pi(R-R_2)^2$ and since the original exposed area = 10.00 cm^2,

then the % re-epithelialisation (P) is given by,

$P = 10A.$

All procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986.

2.2.3 Results

2.2.3.1   Appearance during laser procedure
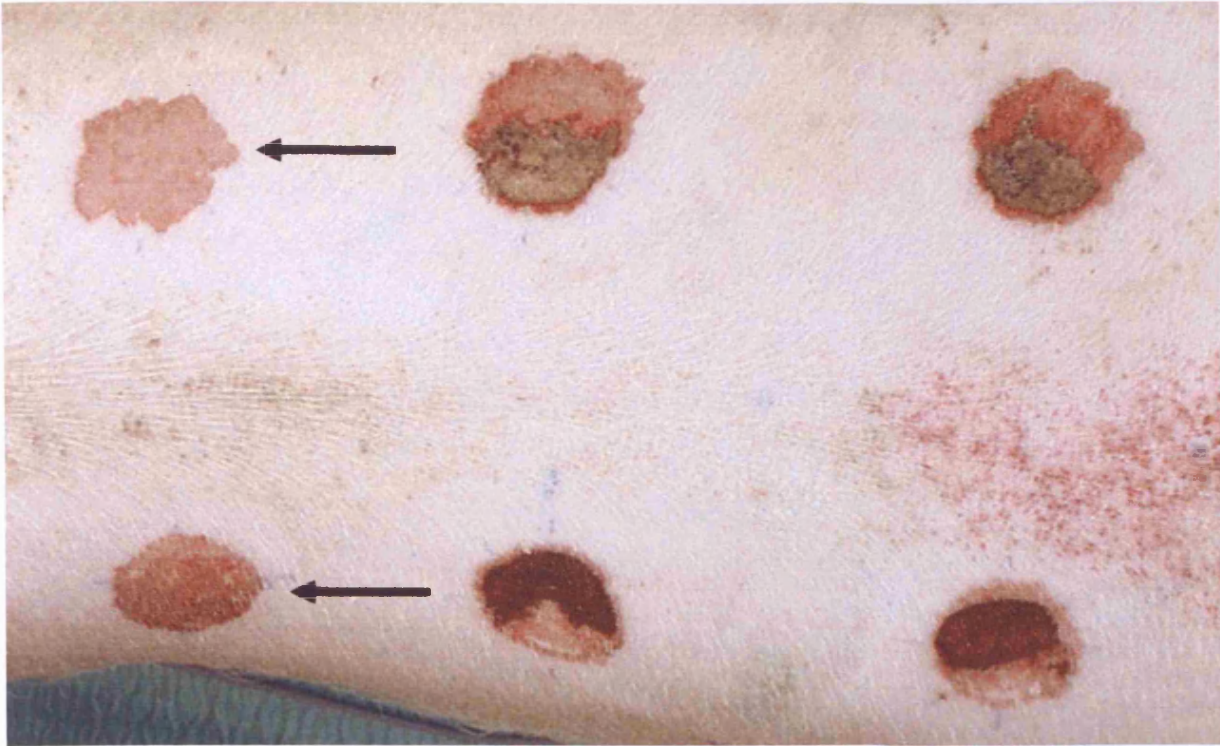
Fig. 2.2.6   Post-lasablation appearance of whole subject (N.B. Normal skin resurfacing is marked with arrows.) © Crown Copyright 2003 Defence Science and Technology Laboratory UK

Fig. 2.2.7 Close-up appearance after Ultrapulse laser pass © Crown Copyright 2003 Defence Science and Technology Laboratory UK
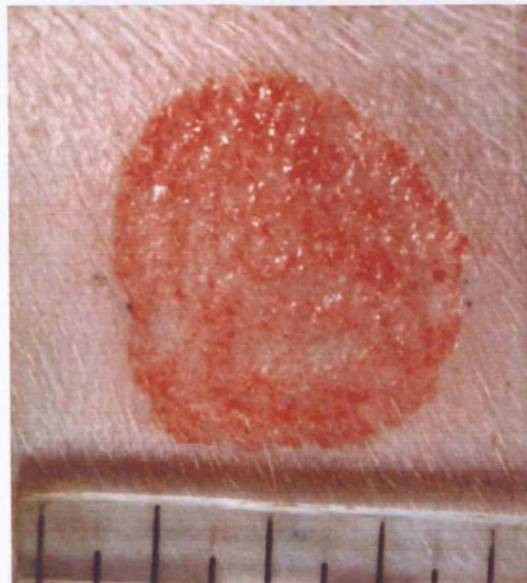
Fig. 2.2.8 Close-up appearance after Erbium:YAG laser pass © Crown Copyright 2003 Defence Science and Technology Laboratory UK

After a single UPL pass, a thick yellow-grey char developed which needed to be rubbed off quite firmly before successful removal. Underneath, the papillary dermis was initially blanched although erythema and sparse punctate bleeding were noted within a few minutes (**Fig. 2.2.7**). The characteristic hexagonal shape of the computer pattern generator can be seen, together with blanching of the papillary dermis, and an obvious loss of the epidermis and a distinct edge.

EYL caused an extensive plume to be formed and left only a thin char. The major difference was that the erythema was almost immediately noted in the surrounding tissue and was revealed upon wiping of tissue char. Areas of punctate bleeding were more numerous followed by a small amount of straw coloured exudates, giving a shiny appearance to the surface (**Fig. 2.2.8**).

2.2.3.2   First wound inspection

At the first change of dressings after 48 hours, all wounds were noted to be clean and dry with superficial, crusting eschar present (**Fig. 2.2.9**).

There were no signs of gross infection and therefore it was decided to leave these sites exposed. The subjects were then observed daily for any evidence of developing wound problems.
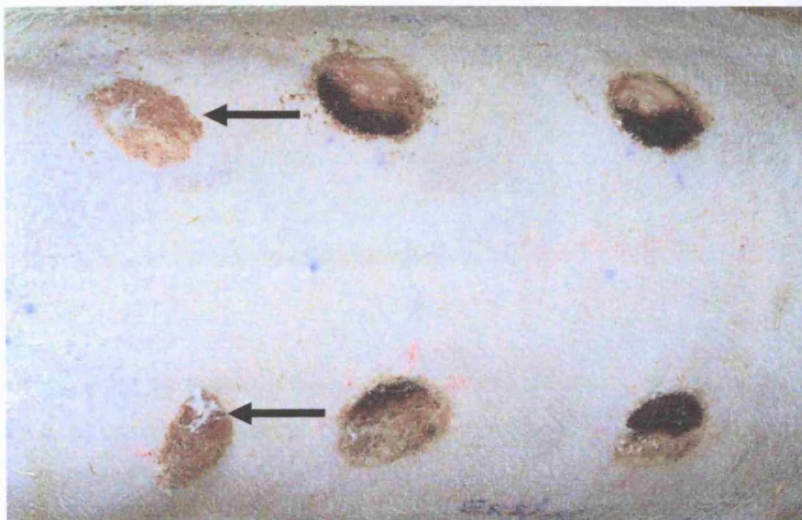
**Fig. 2.2.9
Typical appearance of burns at Day 4.**

N.B. The control burns are on the left (arrowed)

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UK**

As healing took place over the next three weeks, the eschars gradually fell away, revealing freshly healed skin underneath. No specific aftercare was needed since there were no gross wound infections observed. The sequential appearance of these healing wounds is shown in **Fig.2.2.10**:
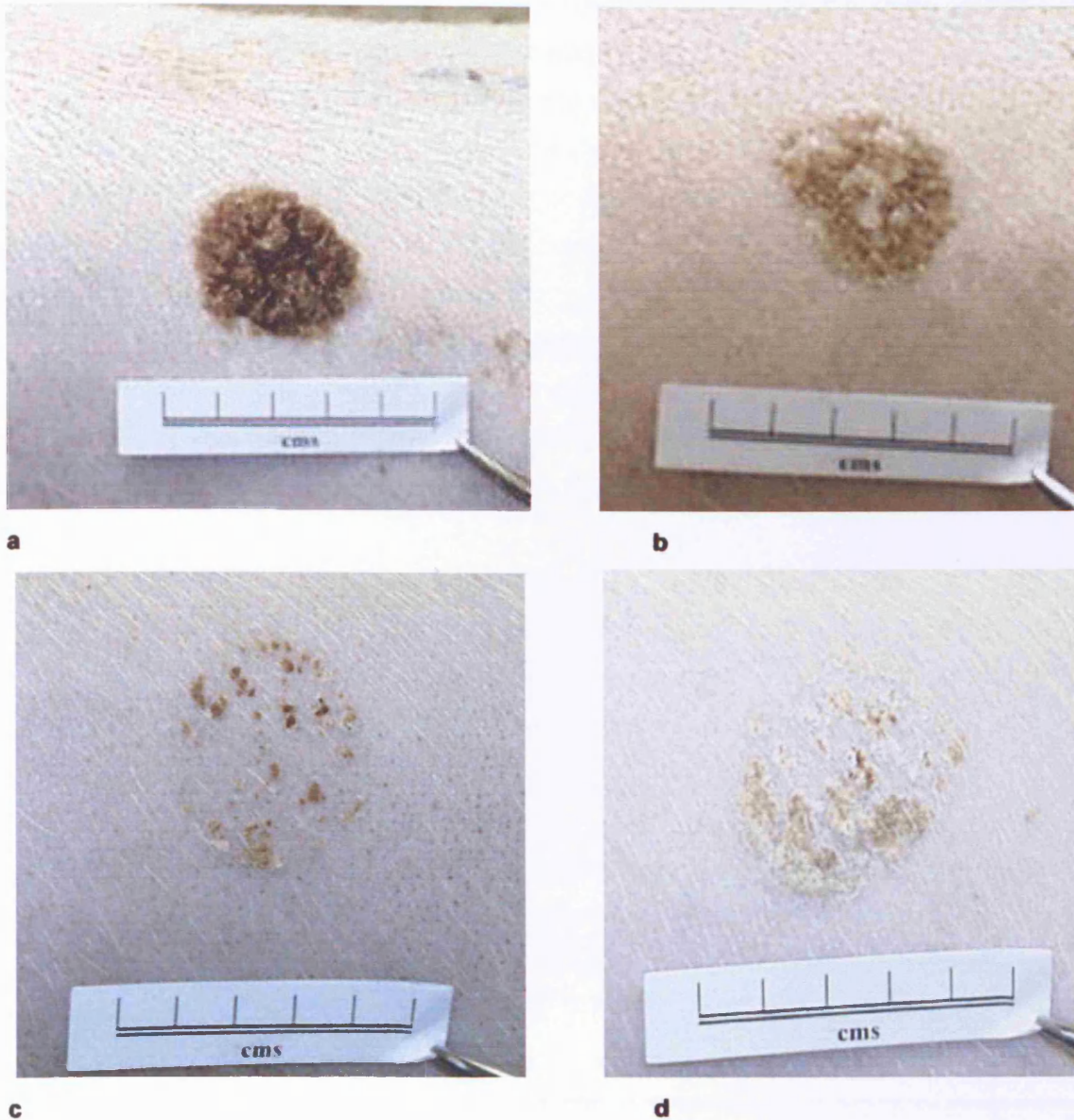
Figure 2.2.10 Healing laser ablated lesions: a & b) illustrate typical healing at one week; c & d) wound appearance by two weeks. © Crown Copyright 2003 Defence Science and Technology Laboratory UK

2.2.3.3   Histological appearances of EYL

Immediately following lasablation, the histopathological changes of epidermal denuding and loss of the superficial layers of the papillary dermis were observed. A zone of residual damage was seen which averaged between 300-1200μm thick, penetrating into the papillary dermis.

By 7 days, the lesions were covered with variable amounts of surface eschar comprising of necrotic epithelial and papillary dermal debris. The rete ridges were disorganised and elongated. At the level of the epidermis, acanthosis (large numbers of cells), hyperkeratosis (large amounts of keratin) and parakeratosis (nucleated effete keratinocytes in the stratum corneum) were noted with the concomitant rise in the number of mitotic figures.

A moderate amount of lymphocytic infiltrate was noted within the papillary dermis together with oedematous changes and the presence of granulation tissue. Associated neoangiogenesis and multiple fibroblasts were visible. However, epithelial regeneration, when measured, was nearly complete.

By 14 days, the epidermis was well differentiated with a homogeneous thickness associated with a decrease in inflammatory oedema and fewer fibroblasts. The maturation of the papillary dermal granulation tissue continued and the collagen was noted to be mature, with disappearance of the eosinophilic character noted previously.

By 21 days, further maturation of the papillary dermal granulation tissue was seen, with resolution of oedema and inflammation. More mature capillary networks were noted together with deposition of mature types I & III collagen.

Fig.2.2.11 a)Example of EYL resurfaced lesion at a) time zero b) Week 1 healed, with residual eschar evident c) Week 2 d) Week 3 (H & E x100) © Crown Copyright 2003 Defence Science and Technology Laboratory UK
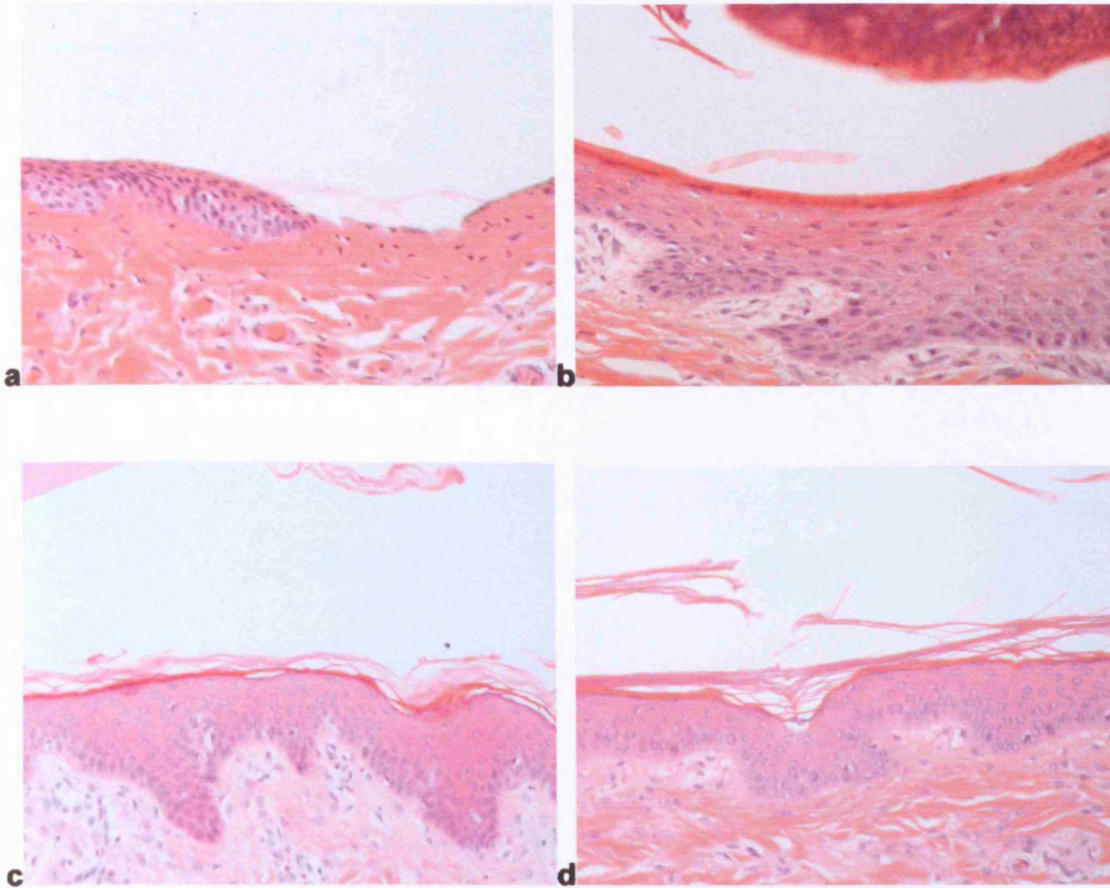
2.2.3.4 Histological appearances of CO_2

At time zero, there were similar findings to the above with a more narrow eschar of 400-1000µm and the presence of epidermal denuding with loss of the superficial layers of the papillary dermis. Dyskeratosis (early abnormal keratin formation in the stratum spinosum) was evident. Usually, this would be limited to the stratum granulosum. Penetration into most of the papillary plexus was noted.

By 14 days, the epidermis was of normal appearance with healing. Some immature collagen was noted.

Again, by 21 days, further maturation of dermal granulation tissue with normal appearance was seen.

Fig.2.2.12 a)Example of UPL resurfaced lesion at a) time zero b) Week 1 with residual eschar evident, c) Week 3 (H & E x100) © Crown Copyright 2003 Defence Science and Technology Laboratory UK
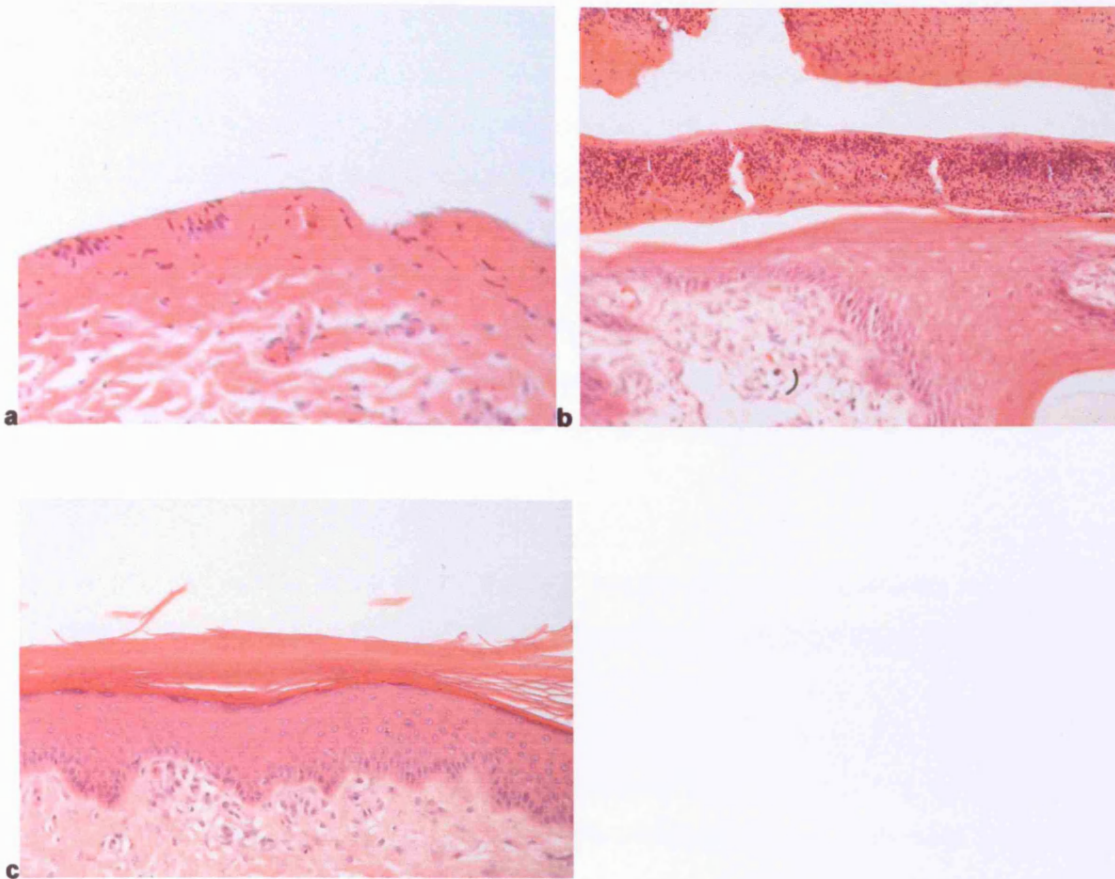
2.2.4 Discussion

2.2.4.1   The Effect of Circulation at Time Zero

Comparison with the ex vivo skin from Experiment 1 did show some startling contrasts to the in vivo specimen with an intact circulation. The depths of injury were different with deeper zones of residual thermal damage after Erbium:YAG laser in the intact skin. The presence of a circulation led to deeper burns (100µm) being created within the papillary dermis as opposed to just breaching

the epidermis (40μm) where post-mortem skin was used. This was thought to be due to several reasons:

- Ex vivo tissue without its underlying fascial support and tension becomes more densely packed, the phenomenon of 'primary contraction'. Subsequently, the tissue is more resistant to damage compared to the live skin which is stretched out and subsequently thinner.

- The ex vivo tissue would have only been warmed to room temperature, thus requiring more energy to cause heat damage compared to that of tissue at body temperature. With water being the principle chromophore, the paucity of this in the relatively desiccated ex vivo specimen would have meant less efficient laser-tissue interaction.

- The circulation acts as a heatsink, attempting to dissipate the heat but in doing so, extends the thermal damage to a deeper zone.

The general appearance of the freshly ablated tissue in this animal model was very similar to human skin in the clinical setting, since they have similar morphology as discussed previously.

2.2.4.2   The Progress of Wound Healing

In general, the alterations observed were similar following both laser treatments. By 7 days the laser-induced lesions were similar in histological appearance irrespective of the laser treatment employed. The lesions were covered with variable amounts of surface eschar composed of necrotic epithelial and papillary dermal debris. The eschars were undermined by well developed regenerative epidermis showing elongation of the rete pegs, acanthosis, increased mitotic activity in the basal epidermal cell layer, hyperkeratosis and focal parakeratosis and dyskeratosis. The presence of oedematous granulation tissue and active proliferation of small blood vessels indicated earlier damage to the superficial papillary dermis. At 14 days, the degree of maturation of

papillary dermal granulation tissue was greater than that observed at 7 days. The amount of re-epithelialisation on the laser-induced lesions was calculated to be nearly complete at just 7 days (99.95 - 100%) in the presence of surface eschar.

The overall rates of healing were such that re-epithelialisation was virtually complete within one week. This attests to the fact that both Erbium:YAG and Ultrapulse lasers ablate only the epidermis and superficial, papillary dermis i.e. a superficial partial thickness burn. Enough dermal elements remain to regenerate a new epithelial surface as well as from the edges of the burn wound.

These healing times are similar to those achieved from human aesthetic skin resurfacing. In such procedures, the skin is resurfaced, causing a controlled thermal injury to remove rhytids (wrinkles). Wound care after this usually involves covering the wounds with a moist emollient daily until healed. This prevents desiccation and thus improves the speed of healing. No such care was used for our animal models, yet despite this, epithelial regeneration was evidenced at one week.

2.2.5 Conclusions

It has been demonstrated that the use of either type of laser results in a superficial burn, the majority of which is healed within a week. The burn depth is in the order of 150 µm, thus removing epidermis and breaching the papillary dermis. The superficial nature of the burn allows for rapid healing and this is certainly consistent with other animal models and with human skin in clinical practice.

The next step is to use the same method on a burn eschar to investigate the effects of laser debridement on wound healing.

Experiment 3

The Effect of CO_2 and Erbium:YAG Laser Debridement ('Lasablation') on the Healing of Lewisite Burns

2.3.1 Introduction

The vesicant agents, Lewisite and sulphur mustard, were developed at the beginning of the last century as chemical weapons; sulphur mustard was widely deployed by both sides during the First World War. The agents cause immediate blistering followed by deep dermal to full thickness burns on the skin.

As discussed in Section 1.6.13, methods that have been investigated for enhancing the healing of mustard burns include the use of enzymatic debridement with dressings (Eldad et al, 1988), surgical excision (Kjellström et al, 1997) and CO_2 laser debridement (Smith et al, 1997,Graham et al, 1997).

Using our porcine model, mechanical dermabrasion has been shown to be a useful modality in accelerating the healing of mustard burn injuries *without* the need for additional split skin grafting (Rice et al 2000).This study demonstrated that a decrease in the volume of necrotic eschar alone was able to expedite the healing process. By removing the non-viable tissue that would normally be cleaved away and separated by epithelial regeneration, the healing rates were accelerated by up to threefold.

Full thickness burns would necessitate treatment similar to that of a thermal burn with accepted practice involving surgical debridement with tangential excision, covering the defect with a split skin graft.

The use of the CO_2 laser in the debridement of thermal burns has been studied to prepare a surface suitable for skin grafting, as described in Section 1.5. Following a similar rationale, in 1997 Smith et al have shown accelerated

healing in a porcine model of sulphur mustard burns with a pulsed CO_2 laser without the use of skin grafting. `

As with sulphur mustard, no specific treatment modalities exist for the management of Lewisite-induced burns. In view of the fact that these vesicant agents cause similar skin burns, it was proposed that laser debridement ought to have a useful role for treating Lewisite burns.

The Ultrapulse™ CO_2 (Coherent, Palo Alto, California, USA) and Continuum Biomedical Erbium-YAG (Continuum Biomedical, Dublin, California, USA), were used to investigate the role of laser debridement for Lewisite burns in the large white pig. As a result of their operating characteristics, these instruments have the selective ability to vaporize *thin* layers of tissue with the minimum of residual thermal damage. Removal of non-viable tissue in this way should accelerate wound healing in a manner akin to dermabrasion, again *without* the need for skin grafting.

The precision of these lasers allows for a more precisely controlled level of debridement which should ensure that the rate of spontaneous epithelial healing is greater than that achieved with mechanical dermabrasion since the risk of damage to healthy tissue at the wound edges is reduced. By selective laser ablation of only eschar without damaging the remaining healthy bed, the need for skin grafts should be avoided in this situation. We have termed this partial debridement as **'lasablation'** to distinguish it from formal surgical debridement.

The ideal operating characteristics for both types of laser have already been investigated in Experiment 1; these will be used for this current experiment. All the procedures were subject to ethical review and carried out in accordance with the Animals (Scientific Procedures) Act 1986.

2.3.2 Methods

A series of six Large White domestic pigs were each exposed, under identical conditions, to four Lewisite burns carefully positioned either side of the mid-back. Twenty-four burn areas were studied in total with half of these undergoing

lasablation with the Ultrapulse™ CO_2 and the remainder with the Erbium-YAG laser.

2.3.2.1   Anaesthetic & Lewisite Dosing

Each animal received a general anaesthetic consisting of inhaled halothane (1-3% at 0.5 L.min^{-1}) administered in a stream of nitrous oxide and oxygen (4-6 l.min^{-1}) for both induction and maintenance. Whilst anaesthetised, an area measuring approximately 35 by 25 cm (875 cm^2) of dorsal skin was prepared by gentle wet shaving and drying in readiness for dosing chamber template application. Four 10 cm^2 areas were marked out **(Figures 2.3.1 & 2.2.2)**.
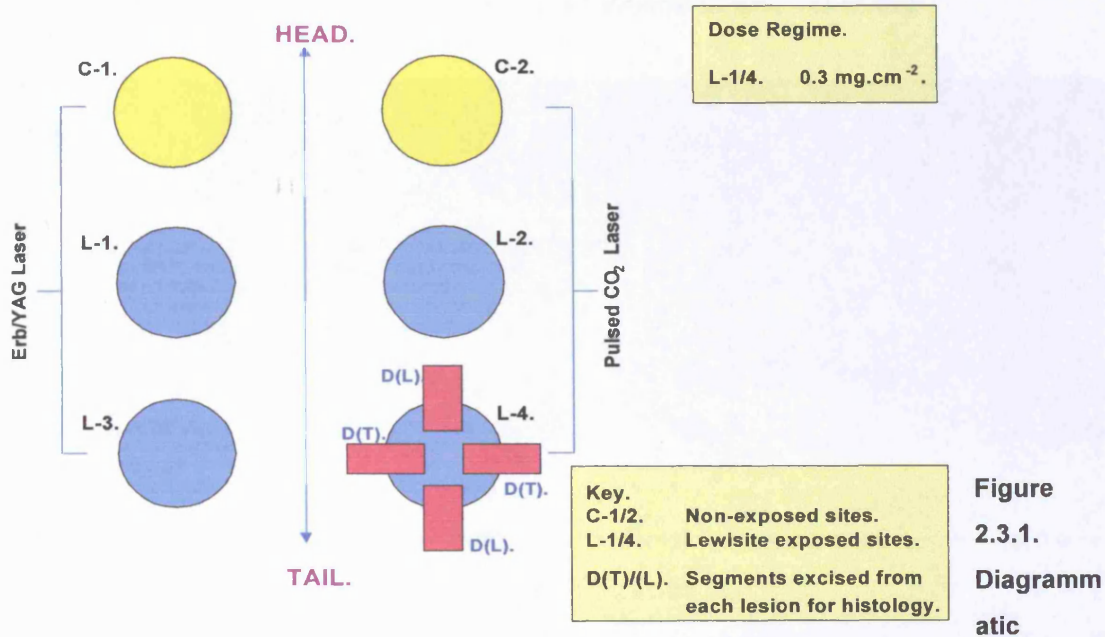
Figure 2.3.1. Diagrammatic representation of the dosing and lesion excision protocol for the lasablation study.

3.57 cm diameter = 10 cm ^{2}.

(A).

(B).

(E). **(C).** **(D).**

KEY.

(A). **Glass dosing chamber.**
(B). **Glass fibre filter disc.**
(C). **Area of depilated skin (exposure site).**
(D). **Normal, non-exposed area.**
(E). **Rim of acrylic adhesive.**
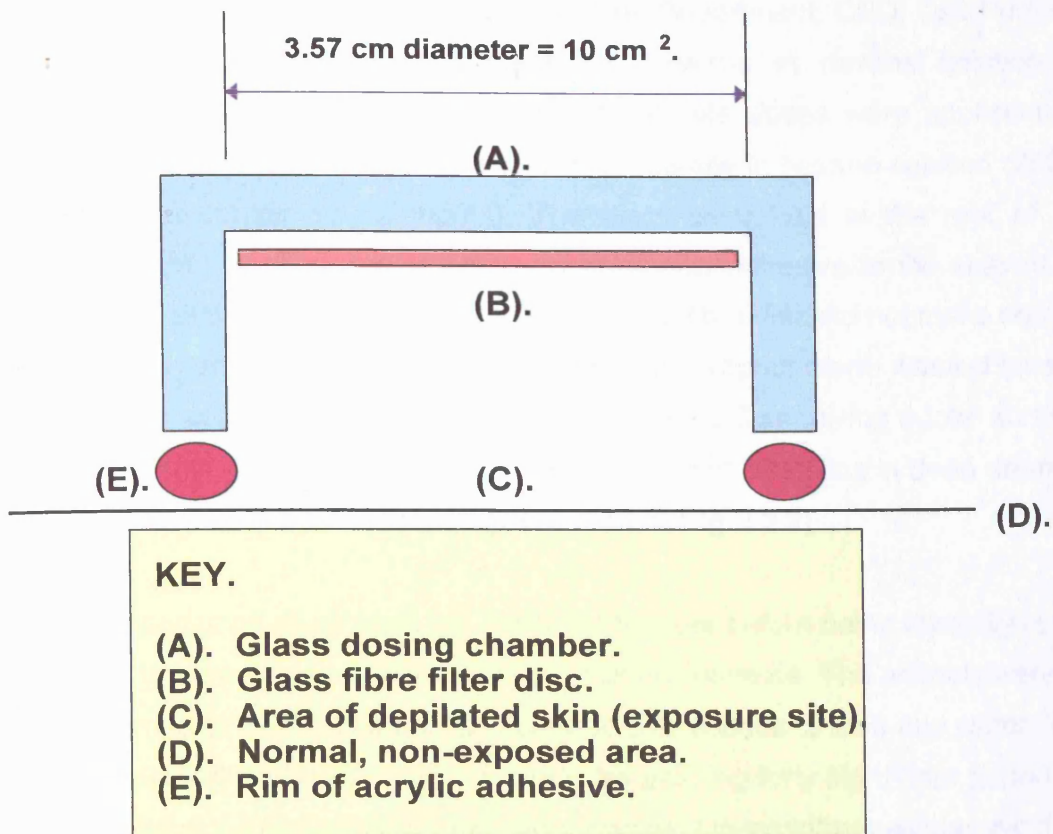
Figure 2.3.2. Diagrammatic representation of the Lewisite dosing apparatus
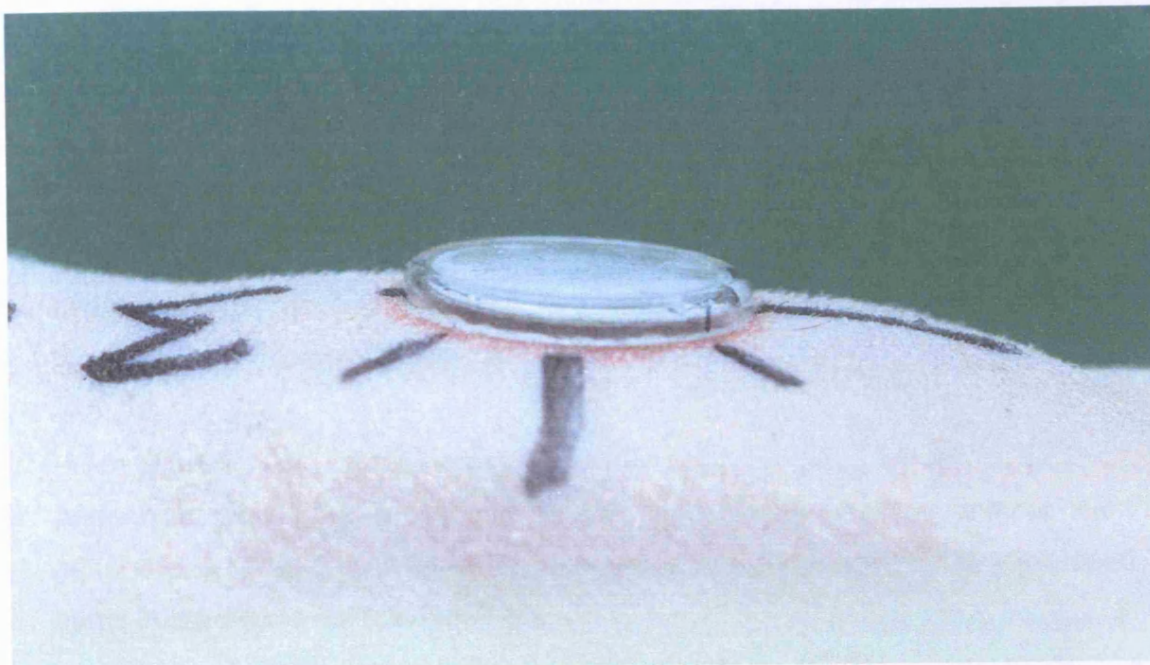
Fig. 2.3.3 Lewisite dosing acrylic disc in situ

Lewisite was synthesised at CB Systems Department, CBD, Dstl Porton Down in readiness for the experiments as Lewisite in hexane solution with a concentration of 12mg/ml. Individual Lewisite doses were applied using an occluded glass fibre disc saturated with Lewisite in hexane solution (250 µl of a solution containing 12 mg/ml). The discs were held in the roof of a glass chamber, inverted and glued using an acrylic adhesive to the shaved area of dorsal skin. This ensured that that the glass fibre disc did not make contact with the skin and that even exposure to saturated vapour alone was achieved. Each circular disc had a diameter of approximately 3.6cm, giving a total surface area of 10 cm^2. This generated a dose of 0.3mg/cm^2, resulting in deep dermal to full thickness chemical burns. **(Figures 2.3.2 & Fig.2.3.3)**.

The occluded discs were left in situ for 6 hours before being carefully removed and the animals recovered from general anaesthesia. The animals were then returned to their home pen and allowed free access to food and water. The animals were closely monitored over the ensuing forty eight hour period for clinical signs of discomfort and were routinely given intramuscular injections of buprenorphine 50µg (Temgesic®) over the first twenty four hours whilst the burn developed.

2.3.2.2   Monitoring

Vital functions were monitored throughout the procedure by means of electrocardiogram (ECG), pulse rate, pulse oximetry, respiratory rate and in-line capnometry with a Propaq106EL Monitor. Adjustments were made to the levels of anaesthesia administered based on these signs. Trends of those vital functions such as electrocardiogram (ECG), oxygen and carbon dioxide fluctuation were taken **(Fig.2.2.3)**

2.3.2.3   Lasablation

After initial exposure to Lewisite vapour, all animals received another short general anaesthetic on the fourth post-exposure day and their fully developed burns subjected to lasablation.

Two sites were lasablated with the Ultrapulse laser (UPL) at a setting of 2.25 mm spot size, 300mJ per pulse with a repetition rate of 200 Hz. The computerised pattern generator was set on a hexagonal shape that was configured to produce a 60% overlap of individual beams. This was equivalent to a fluence of 7.5 Jcm^{-2} and only a single pass was employed. Burn eschar was removed with one pass of the UPL, exposing the papillary dermal. The freshly ablated char debris was wiped with a gauze swab moistened with saline before a second pass was performed. The resulting char was again wiped off.

The other two sites were treated with the Erbium:YAG laser (EYL) on a setting of 3.5 mm spot size, 1900 mJ per pulse with a repetition rate of 4 Hz. A fluence of 20Jcm^{-2} was developed for two passes of the EYL beam. A single pass was used for the whole burn area and then a second pass was done over the actual eschar. Again, the freshly ablated char debris was wiped away with a gauze swab moistened with saline. All laser treated burn sites were then dressed with Flamazine™(silver sulphadiazine) cream and Melolin™ non-adherent dressings.

2.3.2.4   Post-Operative Care

An intramuscular injection of 50µg buprenorphine was given at the end of the procedure before recovery from general anaesthetic. The animals were then returned to their home pen and observed for two hours to ensure all was well before being fed. The animals were observed twice daily to ensure that they were healthy and that the dressings remained intact.

After dressing removal at 48 hours, the wounds were left exposed (Figure 2.2.9). Daily monitoring was performed to observe any changes in wound progression.

2.3.2.5   Burn Excison

The animals were culled humanely by an intravenous overdose of Euthatal® (pentobarbitone sodium 200 mg.ml^{-1}) at one, two and three weeks post-surgery. All treated skin lesions were excised and the samples fixed in neutral buffered formalin before embedding in paraffin and staining with haemotoxylin and eosin. The eschar was divided into quadrants to view the wound edge on four sides of the circular lesion (**see Figure 2.3.1**).

2.3.2.6   Histological Analysis and Interpretation of Healing

The analysis was carried out on a Zeiss Axioplan Light Photomicroscope (Carl Zeiss GmbH, West Germany) at magnifications of up to x450 . Using a calibrated graticule (Graticules Ltd, UK), it was possible to gauge the radii of regenerative epithelial tongues and compare their position with that of the adjacent intact epidermis. The architecture of the eschar, the junction between non-viable and necrotic tissue and the healthy bed were all examined.

Regenerative epithelial tongues were located at the junction of the burn eschar and normal skin. Where these had begun migrating and undermining the eschar was deemed to be the width R_2. Eschar was visible by the basophilic staining characteristics of the non-viable cells. A typical example is shown at low power (**Fig.2.3.4**)

In cases where eschar was absent, regenerative epithelium was complete with no tongue to be seen at the wound crater. This then meant that interpretation of healing was deemed to have been complete, with the value of R_2 being taken as 1.784cm since it was assumed that the burn wound was a circular pattern of 10cm^2 (i.e. $\pi(R_2)^2$. If there were small islands of eschar with no epithelium underneath, the cumulative total width of these within the examined field was subtracted from 1.784cm, the value of R. Typical examples of these are shown in Figures 2.3.10. & 2.3.11.

A sample of four readings was taken which was then tabulated (**Table 2.2.1**) and averaged to obtain a mean value for percentage re-epithelialisation using the formula given next. This allowed calculation of the overall percentage

healing rate of each burn which were then compared with the healing rates for untreated Lewisite burns derived from historical controls (Rice & Brown 1999).To ensure reproducibility, the analyses were carried out separately by both the author (DL) and the Project Supervisor (PR) for comparison.

Fig.2.3.4 Micrograph of healing burn lesion. The arrow denotes the width of the tongue of regenerating epithelium, R_2.

© **Crown Copyright 2003 Defence Science and Technology Laboratory UK**
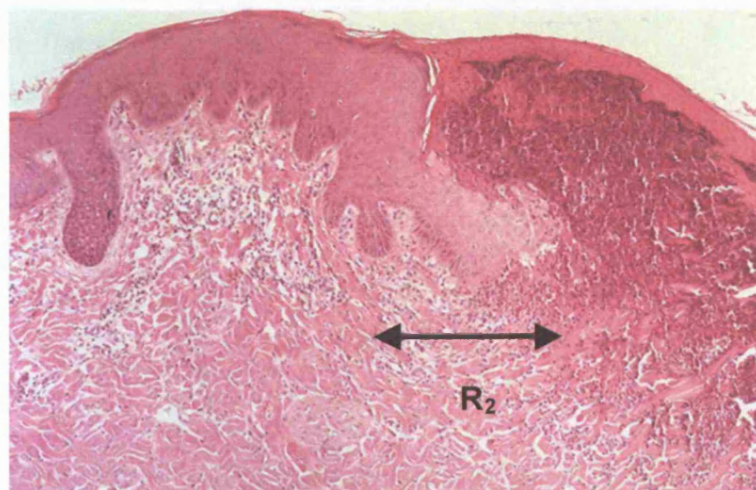
2.3.2.6 Calculation of Rates of Re-epithelialisation

Since all of the sections of the exposed sites showed evidence of epithelial regeneration, calculations of the area and percentage re-epithelialisation for each site was performed.

The area of the zone of re-epithelialisation (**see Figure 2.3.5**) was calculated by measuring values of R_2 at four to six different points around the circumference of the lesion and then calculating the average area of re-epithelialisation (A) using the following expression,

$$A = \pi(R)^2 - \pi(R_1)^2 \qquad \text{where } R_1 = R - R_2$$

As the area originally exposed = 10.00 cm^2,

$$A = 10 - \pi(R-R_2)^2$$

and the % re-epithelialisation (P) is given by

P = 10A

2.3.2.7 Statistical Analysis

The data from Table 2.2.1 was analysed using a two factor analysis of variance (ANOVA) for each time point. Statistical significance was defined as being $p < 0.05$ (Microsoft Excel for Windows 98)
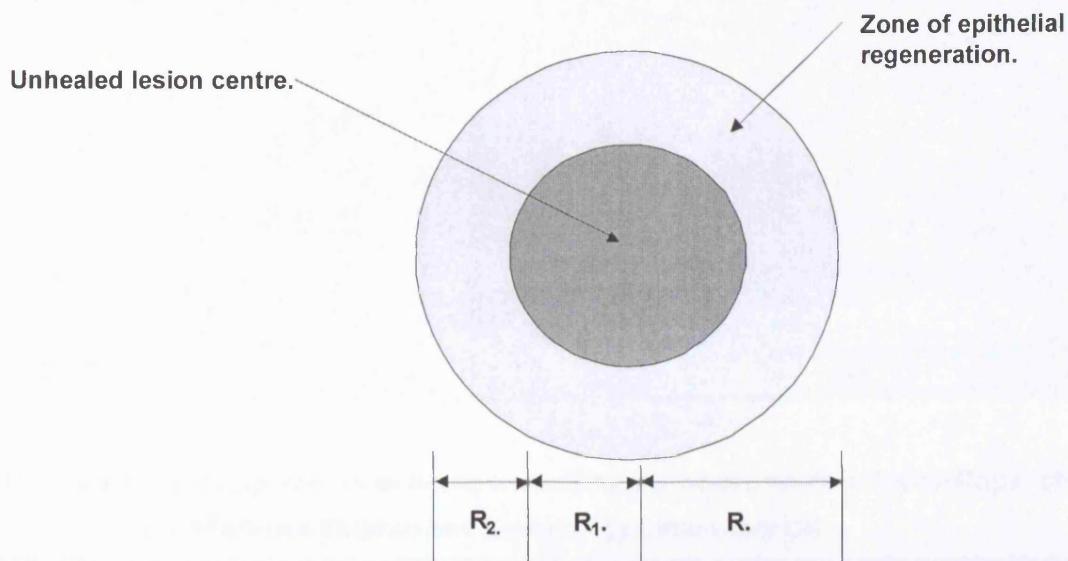
R.   Radius of initial exposure.
R_1. Radius of unhealed lesion centre.
R_2. Radius of zone of re-epithelialisation.

Figure 2.3.5 Diagrammatic representation of the Lewisite healing zones and the calculation of the percentage area of healing.

2.3.3 Results

2.3.3.1 Post-exposure appearance

Immediately after the discs were removed, an erythematous reaction was noted to develop. By the 4th day these had progressed into tough eschars, the appearance of which is shown in **Figures 2.3.6 and 2.3.7**.
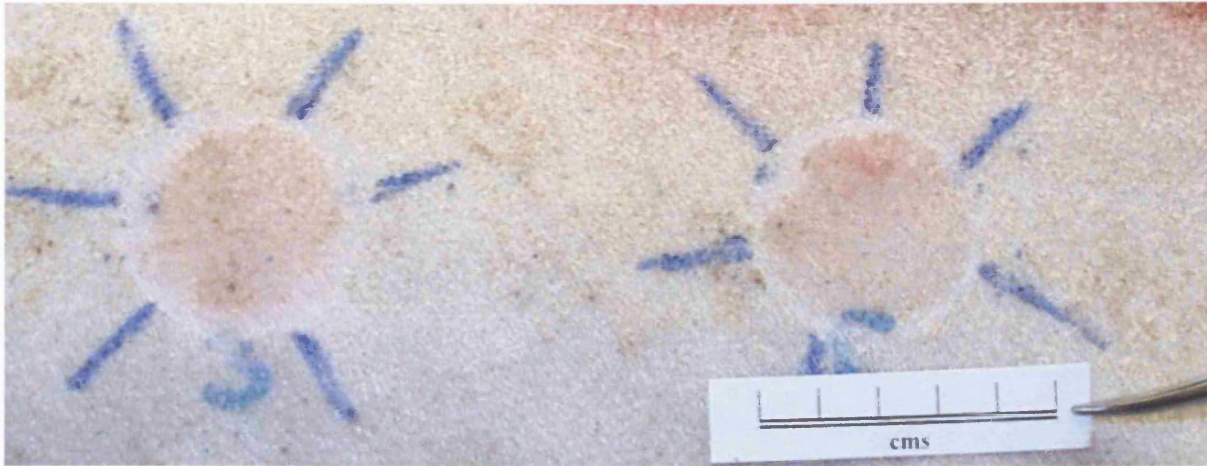
Figures 2.3.6 Typical appearance of burns immediately post-exposure © Crown Copyright 2003 Defence Science and Technology Laboratory UK

Fig.2.3.7 Lewisite lesions at day 4 showing leather-like appearance © Crown Copyright 2003 Defence Science and Technology Laboratory UK

2.3.3.2   Intra-operative appearance

After a single UPL pass, a thick yellow-grey char was noted which needed to be rubbed off quite firmly before successful removal. Underneath, the papillary dermis was initially blanched although erythema and sparse punctate bleeding were noted within a few minutes. Remaining eschar was softened and beginning to lift off at the edges. Of significance was the fact that the laser, along with the usual associated tissue contraction seen during a pass, noticeably smoothed the eschars. However, after ten to fifteen minutes some oedema did return to deform the eschar.

EYL caused an extensive, acrid plume to be formed and left only a thin char. There was an obvious difference in that the erythema was noted almost immediately after in the surrounding tissue, being seen upon wiping of tissue char. Punctate bleeding was seen, proceeded by a small amount of straw coloured exudate.

The appearance is shown below in **Figures 2.3.8 and 2.3.9**
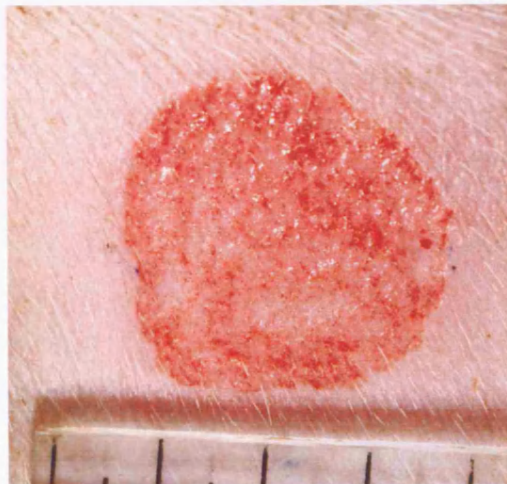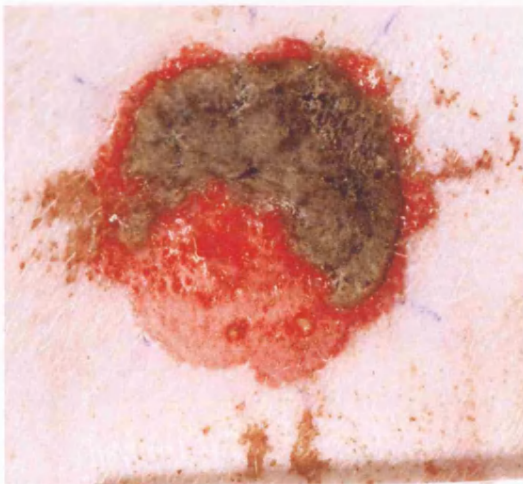
Fig. 2.3.8 Post-Ultrapulse Lasablation   Fig. 2.3.9 Post-Erbium:YAG Lasablation

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2.3.3.3   Skin Sites Excised at 7 Days Post-Lasablation

The histological features observed in all sites can be summarised as follows;

- The lesions were covered with variable amounts of surface eschar composed of necrotic epithelial and papillary dermal debris.

- The eschar was undermined by well developed regenerative epidermis showing elongation of the rete pegs, acanthosis, increased mitotic activity in the basal epidermal cell layer, hyperkeratosis, focal parakeratosis and dyskeratosis.

- There was evidence of earlier damage to the superficial papillary dermis as evidenced by the presence of oedematous, slightly inflamed granulation tissue showing active proliferation of small blood vessels.

The only significant difference between those sites treated with the Erbium/YAG laser and those by pulsed CO_2 was that the latter appeared to be slightly deeper with lesions involving most of the papillary dermis. In terms of degree of surface healing, there appeared to be no significant differences between the two treatment modalities.

The histological changes observed in the burn were well demarcated from the wound edge and measured values of the radii of re-epithelialisation already indicated *near complete* epithelial healing at one week post-surgery (**see Table 2.3.1**).

In comparison, control sites revealed a burn with an established eschar and coagulative necrosis extending focally into the deep reticular dermis and subcutaneous tissues. In these sites, the measured radii of re-epithelialisation indicated an overall healing rate of approximately 22%.

2.3.3.4   Skin Sites Excised at 14 Days Post-Lasablation

These sites also showed similar features to their counterparts at one week post-surgery and only differed from them in terms of the maturity of the granulation tissue supporting the regenerative epidermis.

An exception to this generalisation was one site treated with the Erbium/YAG laser that developed a wound infection. This lesion showed evidence of significant suppuration of the surface eschar and underlying regenerative epithelium with the focal formation of small intra-epidermal pustules. The pathology of this site was reflected in the measured values of the radii of re-epithelialisation, which when used to calculate the overall percentage healing for this site, only indicated approximately 47% healing. This was borne out in the average rate of healing for all similarly treated lesions (**see Table 2.3.1**).

2.3.3.5   Skin Sites Excised at 21 Days Post-Lasablation

Again, these sites showed similar features to those described at one and two weeks but showed evidence of further maturation of dermal granulation tissue.

In contrast, the controls remained covered by a well-developed eschar composed of necrotic debris and coagulated fibrin. The eschar was undermined in all cases by tongues of regenerative epidermis that showed features of recent proliferation including acanthosis, loss of the rete ridge pattern, hyperkeratosis and parakeratosis. The measurement of these regenerative tongues implies an overall epithelial healing rate of approximately 55% by three weeks.

2.3.3.6   Statistical Significance of Results

Statistical analysis of the differences by means of a two factor analysis of variance (Anova) generated the graphs shown in **Figures 2.3.11 and 2.3.12.** It was demonstrated that lasablation significantly enhanced the natural rate of healing of Lewisite-induced burns by up to a factor of four within the first week ($p = 0.006$ for CO_2 and $p = 0.012$ for Erbium:YAG).

Fig.2.3.10 EYL (Left) & UPL (Right) sequential healing of Lewisite burns, a,b) Week 1, c.d) Week 2, e,f) Week 3. © Crown Copyright 2003 Defence Science and Technology Laboratory UK

EYL   UPL
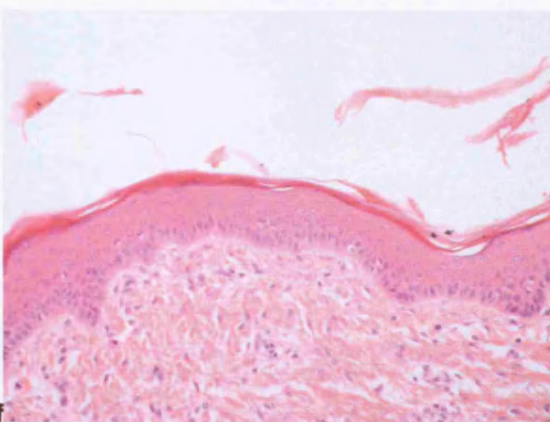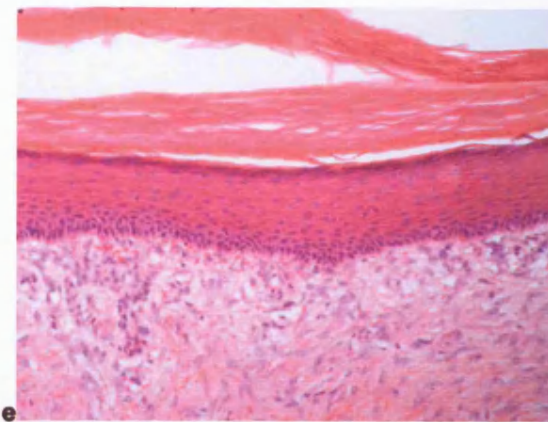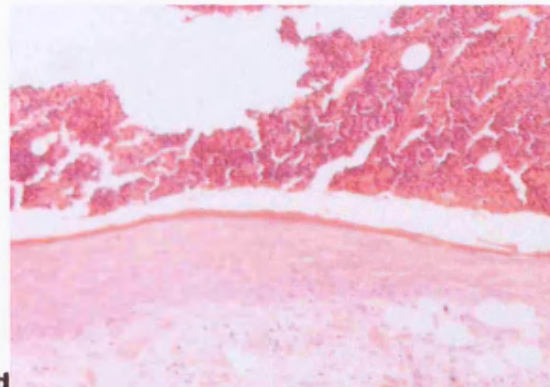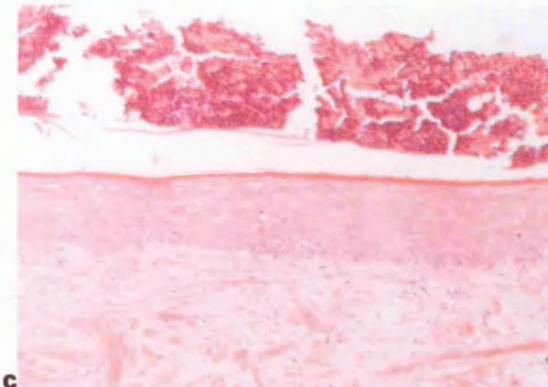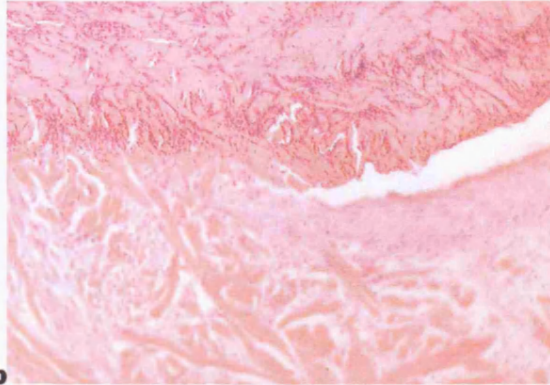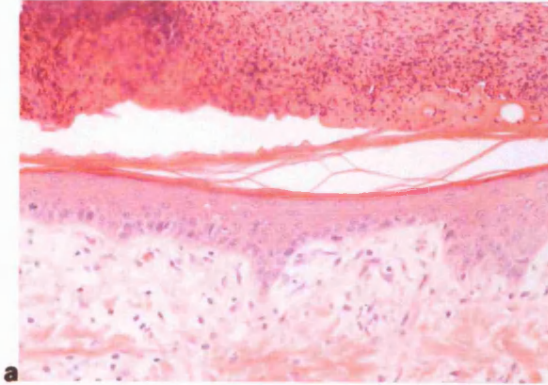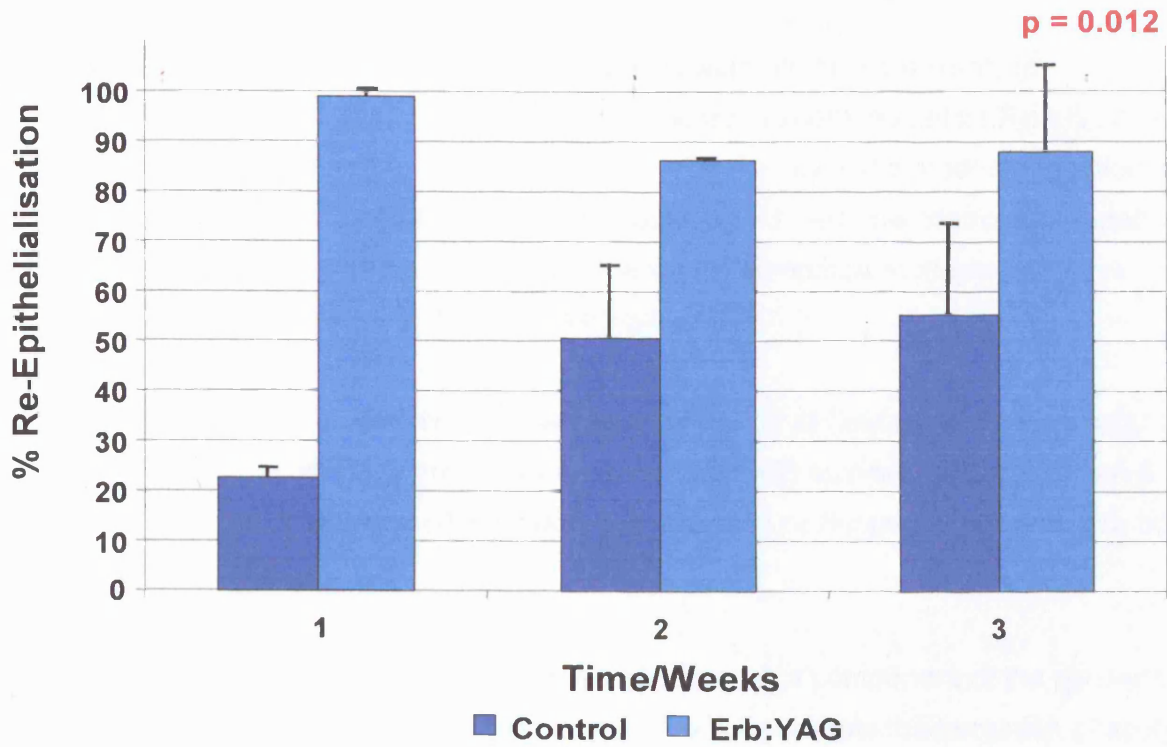
Figure 2.3.11. Comparative rates of healing for untreated and Erbium:YAG laser treated Lewisite lesions.
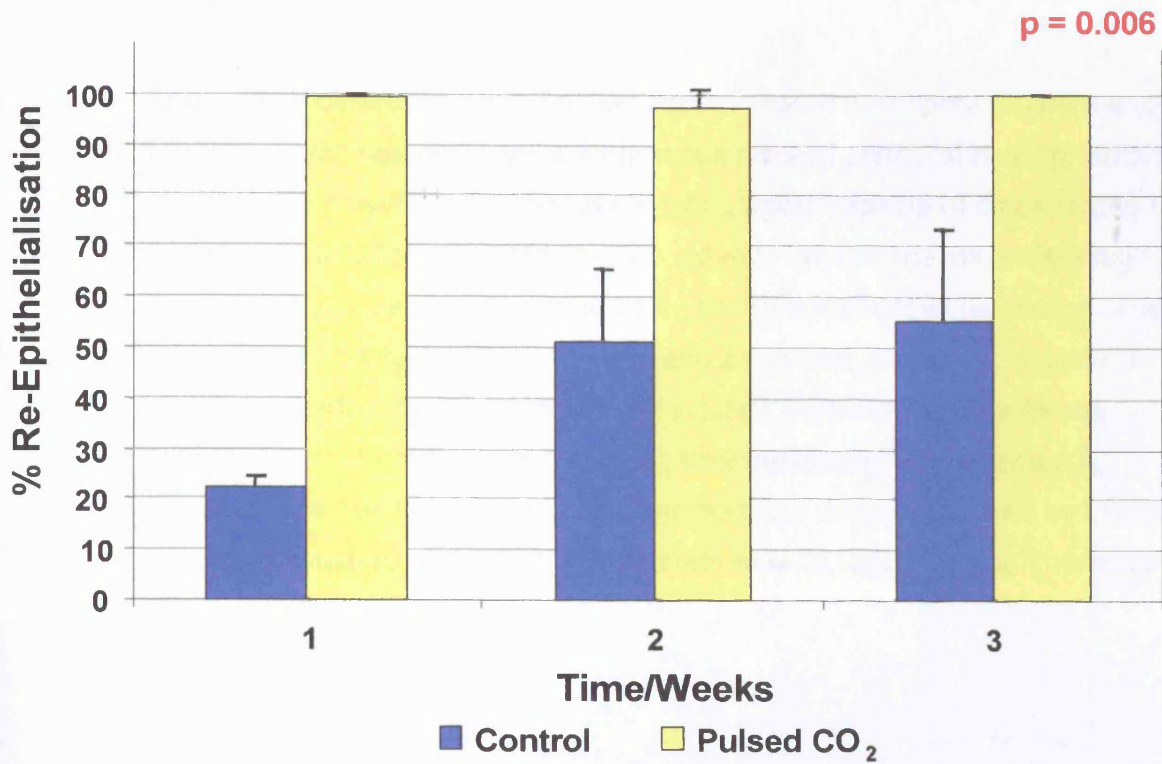
Figure 2.3.12. Comparative rates of healing for untreated and pulsed CO$_2$ laser treated Lewisite lesions

It was apparent that the lasablated sites were almost completely re-epithelialised within a week (99%), compared to controls (24%)(Rice & Brown 1999). This *fourfold* improvement was achieved by just a modest reduction in the volume of necrotic eschar by lasablation. At best, the control, untreated sites were two thirds healed by three weeks compared to almost 100% re-epithelialisation with the lasablated group.

The pattern of histological changes in this study of Lewisite action is similar to those that we have previously demonstrated with sulphur mustard (Brown & Rice 1997), although the timeframe involved in development of a Lewisite burn is shorter.

Lewisite is thought to bind to dihydrolipoic acid, a component of the pyruvate dehydrogenase complex (Peters 1953) which prevents the formation of acetyl coenzyme A and build up of pyruvate. As a result, rapid onset irreversible cell membrane damage, with disruption of the cellular energy cycle is seen within the first twenty-four hours (Rice & Brown 1999). Sulphur mustard has a slower onset of action in this respect.

The second difference between skin burns induced by sulphur mustard and Lewisite is with respect to the spontaneous rates of epithelial healing. Sulphur mustard is a powerful bifunctional alkylating agent capable of cross-linking DNA and RNA. Alkylation of epidermal cells extends beyond the immediate region of the burn wound edge, sufficient to delay or prevent effective replication. The presence of an intact papillary dermis and basement membrane provide a structural scaffold for the epidermis and act as molecular signals for the subsequent maturation of the overlying new epidermis (Shakespeare & Shakespeare 1987). Collagen within the papillary dermis is altered by exposure to sulphur mustard and in this altered state may no longer function normally (Lindsay & Rice 1999).

Unlike sulphur mustard, there is no evidence that Lewisite alkylates DNA, and the increased healing rate of these lesions may be explained purely in terms of the absence of alkylation of the DNA of keratinocytes at the wound edge.

The exact mechanism through which such significant enhancement in the rate of healing is achieved remains uncertain but this study demonstrates the importance of the following factors :

- It may be that the eschar-wound bed interface is sufficiently disrupted by the laser treatment allowing regenerating epithelium to migrate earlier. The normal process of events would involve proteolytic degradation of the fibrinous bonds between eschar and wound base *before* the onset of any migration (Clark 1985).

- In addition to the proteolytic digestion of the eschar, direct phagocytosis is also clearly involved in the repair process. Lasablation, and its reduction in overall eschar volume, would tend to favour increased phagocytosis without the need for prior proteolytic cleavage.

- Lasablation is a clean surgical technique, the thermal effect generated sealing the surface of the eschar thus minimising secondary infection that would otherwise impede healing. This was borne out by the very low rate of observed infection in these burns.

Of interest was the transient smoothening of the eschar post-UPL debridement. The tissue contraction seen during a pass is thought to be partly due to collagen contraction and desiccation of the skin. However, with these subjects some oedema returned to deform the eschar within minutes. This implied that the water content of the dermis and *not* collagen contraction, is the main contributing factor to the visible shrinkage seen during the procedure. On rehydration of the skin, this effect would be lost. The Lewisite lesions rehydrated within a short time due to the fact that they were hyperaemic as a

result of the inflammation caused by the burn. Indeed, this rehydration effect was not seen to the same extent with the control normal skin.

The current study clearly demonstrates that both CO_2 and Erbium:YAG lasers have a similar effect on burn eschar. The laser settings had been originally chosen from Experiment 1 to minimise the effect of any residual thermal damage, i.e. the Lewisite burn depth would not be further increased by lasablation. Operating time was extremely quick for both lasers, the Ultrapulse™ laser having a slight advantage with the wide beam pattern of the Computer Pattern Generator and this would have implications for the overall operating time.

Moreover, the study confirms that the zone of residual thermal damage incurred with these lasers is extremely operator-dependent. Fluence, pulse density and the number of passes can all be adjusted to ensure a comparable clinical effect that is not dependent upon the lasing media. The laser settings used in this study would not be advocated for cosmetic skin resurfacing.

The speed and minimal thermal damage characteristics of these lasers lend themselves to further assessment as a tool for both vesicant burn debridement and lasablation of thermal burns. Their use for larger area burns requires further appraisal.

2.3.5 Conclusions

A fourfold improvement in re-epithelialisation of Lewisite burns was achieved at one week post-surgery with lasablation. There appears to be little clinical or histological difference between the lesions treated by these lasers with regard to the enhanced healing rate.

The mechanism of this clear therapeutic benefit of lasablation has been postulated as being due to the physical disruption of the eschar-wound base interface with the concomitant reduction in necrotic eschar volume.

Lasablation would appear to be a useful adjunct in the surgical management of Lewisite vesicant burns. In addition to its use in a military context, it seems likely that this technique will offer benefits to the management of chemical and thermal burns seen in a civilian setting.

Discussion

3.1.1 Laser debridement of thermal burns

This thesis has reviewed the current literature on the use laser technology with burn treatment; translating the application to the treatment of vesicant lesions has involved studies with laser debridement of Lewisite lesions with good effect.

Lasers have the effect of being able to coagulate and vaporise tissue in the path of the beam and Hall (1971) first demonstrated the potential for use as a scalpel. However, a residual zone of necrosis was noted at the wound edges and hence the use of the laser for skin incisions was not recommended.

The gold standard of sharp tangential debridement of burn eschar is effective at creating an adequate bed for skin graft uptake at the cost of considerable blood loss. In this respect, the perceived advantages of lasers include the prevention of significant haemorrhage with accurate debridement of burn eschar.

In 1971, Stellar et al reported their preliminary success with a CO_2 laser in debriding full thickness burns on an animal model (pig) of up to 20% TBSA. Achieving 90 to 100% skin graft take, this was affirmed by the groups' subsequent work in 1973. By using the laser as a scalpel rather than as an ablative tool, decreased blood loss was seen and with similar graft take compared to sharply excised controls.

Levine (1974) at the Brooke Army Medical centre in Texas reported on the use of the CO_2 laser for debridement of a full thickness burnt human neck with immediate autograft. One half of the burn was sharp excised as a control and there was no difference in graft take between the sides. It was noted that blood loss was dramatically reduced but that operation time was slower.

The same group performed a comparison of electrocautery, laser and scalpel burn debridements where blood loss with laser was reduced by up to one third with operating time doubled. These results were still superior to that of electrocautery (Levine 1975). Fidler of the Shriner's Institute in Ohio reported a series of fifteen paediatric burn excisions with the CO_2 laser with immediate autografting. Blood loss was only a third of conventional sharp debridement (Fidler 1976).

Jackson (1977) also reported that the technique took longer and was more physically traumatic to the wound with the need for excised burn slough to be pulled off with considerable traction. The graft take in this series was slightly less compared to scalpel tangential excision, attributed to the thin layer of surface necrosis left behind.

With the introduction of pulsed CO_2 lasers the perceived favourable advantages of minimal blood loss and a rapid mode of action were coupled with less thermal damage (Green 1990).

Rapid scanning CW(continuous wave)-lasers were another modification to minimise thermal damage. Blood loss was virtually nil with graft take comparable between laser debrided and sharp debrided control sites (93% vs. 96%) (Glatter 1998).

Following on from this the same group at Harvard Medical School in Boston performed a trial on 21 children with full thickness burns, again comparing the rapid scanning CW-laser with sharp debridement as control, of full thickness burns with immediate skin grafting. Equal graft uptake was noted (94% vs. 94%) with no difference in a scar assessment at up to eight months of follow up (Sheridan et al 1999). Operating times were similar for the small area of wound involved although the authors did admit that the laser would take longer when dealing with circumferential and extremity burns, due to size limitations

The benefits of the latest lasers include minimal blood loss and a rapid mode of action, coupled with less thermal damage. The ability to deliver large amounts of energy within a short pulse has only been possible recently with the development of the Ultrapulse Laser and similar devices. This ensures efficient

photothermolysis with minimal transmitted heat damage to the underlying healing dermis.

3.1.2 Debridement of Vesicant Burns

The treatment of vesicant injuries has not been well established since there has only been limited experience of such injuries. The only credible clinical information to date has been a comprehensive report of the outcome of 65 of the 170 or so casualties evacuated to European hospitals from the Iran-Iraq conflict during 1984-6 (Willems 1989). These were evacuated between 4 to 17 days post-exposure, providing a unique opportunity to treat these casualties with up-to-date therapy. A US report of the experiences gained from treating these casualties revealed burns of up to 70% TBSA inflicted by a mixture of mycotoxins and conventional chemical agents (sulphur mustard, Lewisite and tabun). Their recommendations were to sharp debride full thickness vesicant burns, covering the defects with skin grafts in the same manner as with thermal burns (Kadivar & Adams 1991).

3.1.3 Techniques to reduce eschar volume

Dermabrasion has been shown to be effective in healing sulphur mustard burns on a porcine model *without* the need for skin grafting (Rice 1997, Rice et al 1999). Re-epithelialisation was seen within 3 weeks in this study as opposed to the untreated controls (86% healed versus 16%). The important concept of this study was that merely a *reduction* in the amount of necrotic eschar was necessary to accelerate healing, obviating the need for skin grafting.

By removing the majority of the eschar without denuding the healing undersurface, this mechanical damage stimulates epithelial cells in the epidermis to migrate and rapidly mitose to cover the defect (Gillman 1955).

Using this concept of eschar volume reduction, experiment current research has concentrated on the ability to debride vesicant eschar without the need for skin grafting (Graham 1997, Smith 1997, Eldad 1998). This would provide shorter recovery phases for the burn victim since no donor sites are required, which has logistical implications in a war situation.

Pulsed CO_2 lasers have been used to debride sulphur mustard burns on a porcine model with reportedly accelerated healing (Smith et al 1997, Graham 1997). This was performed at various time points up to 48 hours post-exposure to the vesicant agent. The subjects were culled at 14 days and then the wounds were assessed. Only two laser passes were used which would not have removed the complete eschar, yet this appeared to be effective in a manner similar to dermabrasion. No skin grafting was employed. It was concluded that laser debridement resulted in a more organised epidermis via the removal of cytologically atypical cells, which encouraged healing compared to untreated controls. However, all wounds in this study, whether treated or not, had re-epithelialised by two weeks. This is in contradiction to the generally accepted fact that sulphur mustard burns are slow to heal, taking twice as long as an equivalent thermal burn (Willems 1989, Mellor 1991). This may have been due to the burns being only superficial, the dosing method not accurately modelling a human casualty.

Similarly, Eldad et al (1998) performed laser debridement on a guinea pig model 24 hours post-exposure to nitrogen mustard and found this dramatically accelerated healing compared to both tangential sharp excision with no skin grafting (80% epithelialisation by 6 days versus 30%). In turn, at a lower dose enzymatic debridement with Debridase™ gel was more effective than tangential excision (70% versus 30% by day 6). This potentiation of epidermal healing may be due to the removal of alkylated, damaged debris together with any remaining viable keratinocytes that possess mutated DNA. By reducing the degree of inflammatory infiltrate, the healing process is not hindered by the presence of excessive proteolytic enzymes resulting in healed lesions with little cytological atypia and minimal inflammatory infiltrate (Smith 1997, Smith 1999).

All these earlier studies used laser debridement at periods of up to 48 hours post-exposure. It is unlikely that casualties from a theatre of war would reach an adequately equipped facility within that time.

3.1.4 Proposed Experimental Model

The natural time-course of Lewisite lesions was already investigated by Rice's group in 1999 at Porton Down. The spontaneous healing rate of Lewisite burns is more rapid than with sulphur mustard, with early epithelial regeneration seen at the wound edge reflecting a lack of the alkylation of DNA and RNA that occurs with sulphur mustard. This translates into a faster rate of re-epithelialisation compared to sulphur mustard (Rice and Brown 1999). The study revealed an overall percentage re-epithelialisation of 66% at 3 weeks. Sulphur mustard injuries were at best achieving 3.6% by the same time interval.

The data from this provided an ideal control for the investigation of laser-accelerated healing of Lewisite burns.

The reasons for using historical of controls were as follows:

- The experimental protocol using the glass chambers was a reproducible technique and a well established model which had been used for several other vesicant studies at Porton Down. . Seeking ethical approval to repeat the Lewisite Time Course lesions would have been difficult to justify.

- Each subject could only safely tolerate four Lewisite lesions; a total of 12 milligrams of Lewisite per subject. This still only allowed two lesions for studying each type of laser per subject. A third lesion purely as a control measure (i.e. an intra-subject control) would have incurred a toxic dose for the subject.

3.1.5 Methodology of Lewisite Iasablation

Minor flaws within the technique used were as follows:

- The use of historical controls incurred a flaw in results from the comparison of treated versus untreated burns. In performing lasablation four days post-exposure and allowing seven days for healing before analysis a stricter control comparison would have required an eleven day old untreated vesicant burn. In other words, both burns had the same Time Zero.

- This is a purely pedantic measure had intra-subject controls been used, and would have involved comparison of a three-day old treated burn compared to one week untreated burn, a ten-day old treated burn compared to two weeks untreated burn and so on.

Burn wounds do progress and starting experiments at Day 4 ensured a fully-developed burn to assess the usefulness of Iasablation. A pragmatic definition of a control should compare the healing of a lesion without treatment from time zero and a treated lesion from *time zero of Iasablation* and this was the case for our experiments.

3.1.6 Histological analysis

The sectioning method involved has measurement limitations because each section is not a pure quarter of the $10cm^2$ area of burn. Also dividing the lesions into pure quarters was based on the assumption that each lesion was a true circle. In reality, there was some variance with the lesion shape.

The burns were often not perfectly circular and quadrants for analysis were taken as evenly as was possible. Artefacts from lost, separated eschar, twisted epithelium & squashed specimens also contributed to measurement errors.

For reproducibility, histological analysis was performed by the by the supervisor, Dr Paul Rice, a qualified Consultant Histopathologist and repeated separately by the author. Hence, both intra-observer and inter-observer errors were minimised. The raw data analysis should average out as a result of these processes.

3.1.7 Lasablation is a viable option for accelerating the healing of Lewisite burns

This thesis investigated the application of modern CO_2 and Erbium: YAG lasers for debriding Lewisite burns on a representative model. A large white pig model (n=6) was used to investigate the effectiveness of CO_2 and Erbium: YAG lasers in ablation of established Lewisite burns. Burns underwent treatment at four days post-exposure and were assessed at one, two and three weeks thereafter for the rate of epithelial healing.

The re-epithelialisation rates in the laser debrided groups were accelerated by a factor of four compared to untreated, historical controls by the first week. (Anova $p = 0.006$ for pulsed CO_2 and $p = 0.012$ for Erbium: YAG).

Ablation of the burn eschar was thought to accelerate the rate of healing by causing partial debridement. This method has been termed *'lasablation'*.

Benefits of this technique include the fact that no donor sites are required, and that logistics during military confrontation are much more simplified. The treatment can be realistically started at a four days post-exposure and still give good results. This allows adequate time for evacuation of casualties from the frontline situation. With the reduced blood loss associated with this technique, there is a much reduced need for blood replacement products with the inherent risks associated from blood transfusions; a logistic benefit.

3.2 Conclusions & Recommendations for further work

3.2.1 Experiment 1 – Ex vivo power settings

In this ex vivo skin sample, the Erbium: YAG laser ablated at a superficial level leaving a thin zone of residual thermal damage of the order 40μm at most. Only the papillary dermis was breached with intact adnexal structures. Despite increasing fluences by a factor of two there was very little change in the depth of penetration.

In contrast, the CO_2 laser left zones of residual damage to a depth of up to 130μm with the papillary dermis being breached with ease even at low fluence settings.

The lack of a circulation in this ex vivo sample masked the effects of any punctate bleeding that would have been seen with penetration into the papillary plexus. Similarly, any protective effect of an intact dermal circulation would not be realised.

In the light of these findings it was decided that the CPG gave the most reproducible results. The setting of UPL 9 was chosen for the main experiments i.e. 300mJ at 2.2mm spot size on hexagonal pattern 19 with a rate of 200Hz developing a fluence of 7.5 Jcm^{-2}. This was due to the fact that with only a single pass, there was an even ZRTD left reaching down to the papillary dermis.

With the EYL, it was found that the plume was very extensive at the fluence settings used. Despite this, penetration was poor compared to UPL. It was decided that instead of a single pass at 8Hz, two passes of 4Hz would be used at the same 1900mJ setting on a 3.5mm spot in order to reduce the degree of plume. Cumulatively, this would involve the same energy transfer.

It would have being useful to have performed the same study on an in vivo model since it would have allowed a *direct* comparison to be made.

3.2.2 Experiment 2 - Resurfacing of Normal Skin

It was demonstrated that the use of either type of laser produced superficial lesions, the majority of which were healed within a week. The burn depth was in the order of 150 μm, thus removing epidermis and breaching the papillary dermis. The superficial nature of the burn allows for rapid healing and the rate of this was certainly consistent with other animal models and with human skin in clinical practice.

3.2.3 Experiment 3 - Lasablation of Lewisite Lesions

A fourfold improvement in re-epithelialisation of Lewisite burns was achieved at one week post-surgery with lasablation compared to historical control data. There appears to be little clinical or histological difference between the lesions treated by these lasers with regard to the enhanced healing rate.

Lasablation would appear to be a valuable adjunct in the surgical management of Lewisite vesicant burns. In addition to its use in a military context, it seems likely that this technique will offer benefits to the management of chemical and thermal burns seen in a civilian setting.

3.3 Further feasible studies

- The impact of scarring has not been investigated with this technique. Obviously on a porcine model with such a short time-course it was not possible to assess the outcome of eventual scarring. Hence, it would be

useful to assess the long-term appearance of healed scars from this technique.

- In view of these promising results, human vesicant trials could be justified especially if the casualties had sustained small surface area burns. With the changing pace of technology, the laser beam size could be much larger and thus cover a wider surface area with each debridement procedure.

- It has already been shown that laser debridement of thermal burns in children is a feasible proposal to provide an adequate bed for skin graft uptake with minimal blood loss (Sheridan et al 1999). Similarly, the lasablation technique could be applied to suitable thermal burns where haemostasis is vital. This is particularly important for children since their blood reserve is not great. Without the use of skin graft cover, it should still be possible to accelerate the healing of small wounds, and thus incur no donor site costs.

3.4   Summary

This thesis investigated the application of modern CO_2 and Erbium: YAG lasers for debriding Lewisite burns on a representative model. A large white pig model (n=6) was used to investigate the effectiveness of CO_2 and Erbium: YAG lasers in ablation of established Lewisite burns. Burns underwent treatment at four days post-exposure and were assessed at one, two and three weeks thereafter for the rate of epithelial healing.

The re-epithelialisation rates in the laser debrided groups were accelerated by a factor of four compared to untreated, historical controls by the first week. (Anova p = 0.006 for pulsed CO_2 and p = 0.012 for Erbium: YAG). Ablation of the burn eschar was thought to accelerate the rate of healing by causing partial debridement. This method has been termed *'lasablation'*.

A concurrent study of CO_2 and Erbium: YAG laser debridement of normal skin resulted in a superficial resurfacing burn, which healed within a week, regardless of the type of laser. The burn depth was in the order of 150 μm. The superficial nature of these burns allows for rapid healing, consistent with previous findings.

Lasablation is a useful adjunct in accelerating the healing of vesicant burns. This works even without the use of skin grafts. Current practice is to treat vesicant burns in the same manner as thermal burns by sharp excision and covering the wounds with split skin grafts. However, in the light of these findings, a trial of lasablation on human casualties using sharp excision as control would be justified. At present, the technique is limited by laser beam size such that only small burns could be feasibly treated.

Similarly, lasablation of conventional thermal injuries themselves may prove to be of benefit. This would be especially useful where there is little reserve with haemorrhage such as in paediatric burns.

Table 1.1.1 Burn Mortality Rates (LD$_{50}$)

Study	Age 0-14	15-44	45-64	>65
1942-52 **Bull & Fisher** **Pts. = 2807**	49%TBSA 1366	46%TBSA 967	27%TBSA 330	10%TB! 144
1967-70 **Bull** **Pts. = 1917**	64%TBSA 962	56%TBSA 565	40%TBSA 246	17%TB! 144
1975-79 **Curreri &** **Abston** **Pts. = 1508**	98%TBSA 1056	70%TBSA 301	46%TBSA 120	19%TB! 30
1980-95 **Shriners Burns** **Institute** **Pts. = 2169**	98%TBSA 1526	70%TBSA 453	46%TBSA 127	19%TB! 63

Percentages expressed as part of Total Body Surface Area(TBSA)

Table 1.4.1 Specific Antidotes for Chemical Injuries

Chemical	Therapy
Acids	
Hydrochloric acid	Sodium bicarbonate irriga
Nitric acid	
Hydrofluoric acid	Calcium gluconate gel or I oxide paste
Chromic, tannic and formic acids	Sodium hyposulphite
Sulphuric acid	Magnesium oxide, lime wa
Alkalis	
Sodium Hydroxide (Caustic soda)	Ammonium chloride or Bu
Potassium Hydroxide	Solution of Potassium and
Calcium oxide (Lime, Cement)	phosphates
Metallic sodium or potassium	Cover with petroleum oil
Sodium hypochlorite (Bleach)	Aluminium hydroxide
Inorganic	
Phosphorus	Wet dressings then a mixt copper sulphate 2% with s bicarbonate 5% in hydroxy cellulose 1%
Cyanide	i.v. sodium thiosulphate if
Organic	
Phenol, Lysol, Cresol	Polyethylene glycol

Table 2.1.1 Settings for Laser Resurfacing of Ex Vivo Porcine Skin

Ultrapulse

	Spot Size/mm	Energy/mJ	Repetition Rate/s	Power/W	Passes
UPL1	3	500	20	10	1
UPL2	3	500	40	20	1
UPL3	3	500	50	25	1
UPL4	3	500	75	37.5	1
UPL5	3	500	100	50	1
UPL6	3	500	125	62.5	1
UPL7	3	500	150	75	1
UPL8	3	500	175	87.5	1
UPL9	CPG	300	200	60	1
UPL10	CPG	300	200	60	2
UPL11	CPG	300	200	60	3
UPL12	CPG	300	200	60	4
UPL13	CPG	300	200	60	5

Erbium-YAG

	Spot Size/mm	Energy/mJ	Repetition Rate	Fluence/ Jcm^{-2}
EYL1	3.5	1000	8	10.39
EYL2	3.5	1100	8	11.43
EYL3	3.5	1200	8	12.47
EYL4	3.5	1300	8	13.51
EYL5	3.5	1400	8	14.55
EYL6	3.5	1500	8	15.59
EYL7	3.5	1600	8	16.63
EYL8	3.5	1700	8	17.67
EYL9	3.5	1800	8	18.71
EYL10	3.5	1900	8	19.75

Table 2.1.2 Experiment 1 The Effect of CO2 & Erbium:YAG Lasers on Ex Vivo Porcine Skin

Depth of Residual Thermal Damage/µm

							Mean	S.D.
UPL1	90	50	80	70	30	50	61.67	22.29
UPL2	80	50	40	70	70	70	63.33	15.06
UPL3	140	80	95	90	40	50	82.50	35.74
UPL4	60	70	60	120	70	90	78.33	23.17
UPL5	100	130	120	105	110	90	109.17	14.29
UPL6	80	85	110	80	120	110	97.50	17.82
UPL7	120	150	180	110	150	80	131.67	35.45
UPL8	50	80	130	80	100	110	91.67	27.87
UPL9	110	90	60	90	100	60	85.00	20.74
UPL10	80	90	100	100	80	80	88.33	9.83
UPL11	100	110	110	140	130	120	118.33	14.72
UPL12	110	120	125	110	120	110	115.83	6.65
UPL13	120	140	120	110	140	110	123.33	13.66
EYL1	10	10	15	10	20	10	12.50	4.18
EYL2	10	15	10	10	10	10	10.83	2.04
EYL3	10	20	10	15	20	10	14.17	4.92
EYL4	20	15	20	15	15	15	16.67	2.58
EYL5	40	30	30	20	30	30	30.00	6.32
EYL6	20	30	50	30	50	40	36.67	12.11
EYL7	20	20	30	30	30	30	26.67	5.16
EYL8	40	30	30	30	30	30	31.67	4.08
EYL9	30	20	30	30	30	20	26.67	5.16
EYL10	40	40	50	30	30	20	35.00	10.49

Depth of Transition Zone/µm

						Mean	S.D.
20	20	30	15	20	20	20.83	4.92
20	20	15	20	15	15	17.50	2.74
30	20	30	20	20	20	23.33	5.16
15	20	20	20	20	20	19.17	2.04
20	30	20	20	20	30	23.33	5.16
20	15	20	20	20	20	19.17	2.04
20	20	30	20	30	20	23.33	5.16
30	20	20	20	20	20	21.67	4.08
20	20	20	30	20	20	21.67	4.08
20	15	20	30	20	20	20.83	4.92
20	15	20	20	20	20	19.17	2.04
20	30	20	25	25	30	25.00	4.47
20	30	30	20	30	20	25.00	5.48
5	5	5	10	5	5	5.83	2.04
5	5	5	5	5	5	5.00	0.00
5	5	5	5	5	5	5.00	0.00
5	5	5	5	5	5	5.00	0.00
5	10	5	10	10	10	8.33	2.58
5	10	5	5	10	5	6.67	2.58
5	5	10	10	5	10	7.50	2.74
5	10	5	10	5	10	7.50	2.74
5	10	5	10	5	5	6.67	2.58
10	10	15	10	10	10	10.83	2.04

Table 2.1.3 Histological Findings for Ultrapulse Laser

	Mean Depth of residual thermal damage / μm	Mean Transition Zone/μm	Comment
UPL 1	61.67	20.83	Papillary dermis expose only, consiste
UPL 2	63.33	17.50	Cleavage of ZRTD noticeable
UPL 3	82.50	23.33	Cleavage noted and papillary dermis bi
UPL 4	78.33	19.17	Even thickness with cleavage of ZRTD
UPL 5	109.17	23.33	TZ splitting seen & inconsistent, with dermal papillae exposure
UPL 6	97.50	19.17	Inconsistent layer of ZRTD
UPL 7	131.67	23.33	Very uneven ZRTD, dermal papillae co:
UPL8	91.67	21.67	Cleavage at TZ & ZRTD uneven
UPL 9	85.00	21.67	Even layer of ZRTD down to papillary d
UPL 10	88.33	20.83	TZ splitting, extremely even
UPL 11	118.33	19.17	TZ splitting with papillary ridges seen,
UPL 12	115.83	25.00	TZ splitting and becoming more homoς
UPL 13	123.33	25.00	TZ split and homogeneous

Table 2.1.4 Histological Findings for Erbium:YAG Laser

	Mean Depth of Residual Thermal Damage / μm	Mean Transition Zone/μm	Comment
EYL 1	12.50	5.83	Coagulated stratum corneum, stratum granulosum exposed
EYL 2	10.83	5.00	Even exposure of stratum granulosum
EYL 3	14.17	5.00	Tips of dermal papillae breached
EYL 4	16.67	5.00	Flattened dermal papillae, rete ridges intact
EYL 5	30.00	8.33	Stratum spinosum exposed
EYL 6	36.67	6.67	Rete pegs still seen with exposed Stratum Granulosum
EYL 7	26.67	7.50	Basement membrane exposed
EYL 8	31.67	7.50	Cleaved at dermal-epidermal junction
EYL 9	26.67	6.67	Cleavage at dermal-epidermal junction
EYL 10	35.00	10.83	Papillary coagulation

Table 2.2.1   Epidermal Healing Rates for Lasablated Normal Skin

Controls (7 Days Post-Laser).

Site.	Description.	R_2 (cms).				Mean R_2 (cms).	A (cm^2).	P (%).
C-1. Non-Exposed, Erb/YAG treated.	0 mg.cm⁻².	1.784	1.696	1.706	1.784	1.743	9.995	99.95
C-1. Non-Exposed, Erb/YAG treated.	0 mg.cm⁻².	1.784	1.784	1.784	1.784	1.784	10.000	100.00
C-2. Non-Exposed, Pulsed CO_2 treated.	0 mg.cm⁻².	1.784	1.784	1.561	1.784	1.728	9.990	99.90
C-2. Non-Exposed, Pulsed CO_2 treated.	0 mg.cm⁻².	1.692	1.499	1.561	1.784	1.634	9.929	99.29

Controls (14 Days Post-Laser).

Site.	Description.	R_2 (cms).				Mean R_2 (cms).	A (cm^2).	P (%).
C-1. Non-Exposed, Erb/YAG treated.	0 mg.cm⁻².	1.784	1.784	1.784	1.784	1.784	10.000	100.00
C-1. Non-Exposed, Erb/YAG treated.	0 mg.cm⁻².	1.784	1.784	1.784	1.784	1.784	10.000	100.00
C-2. Non-Exposed, Pulsed CO_2 treated.	0 mg.cm⁻².	1.784	1.784	1.784	1.784	1.784	10.000	100.00
C-2. Non-Exposed, Pulsed CO_2 treated.	0 mg.cm⁻².	1.784	1.784	1.784	1.784	1.784	10.000	100.00

Controls (21 Days Post-Laser).

Site.	Description.	R_2 (cms).				Mean R_2 (cms).	A (cm^2).	P (%).
C-1. Non-Exposed, Erb/YAG treated.	0 mg.cm⁻².	1.784	1.784	1.784	1.784	1.784	10.000	100.00
C-1. Non-Exposed, Erb/YAG treated.	0 mg.cm⁻².	1.784	1.784	1.784	1.784	1.784	10.000	100.00
C-2. Non-Exposed, Pulsed CO_2 treated.	0 mg.cm⁻².	1.784	1.784	1.784	1.784	1.784	10.000	100.00
C-2. Non-Exposed, Pulsed CO_2 treated.	0 mg.cm⁻².	1.784	1.784	1.784	1.784	1.784	10.000	100.00

Table 2.3.1. Comparative Rates of Healing for Untreated and Laser Treated Lewisite Lesions

Time/Weeks.		Range of R_2 Values/cm.	Mean Value of R_2/cm.	Area of Healing (A)/cm^2.	Percentage Healing (P)/%.
Untreated Lewisite Sites.					
	0.	0	0	0	0
	1.	0.158-0.319	0.212	2.239	22.39
	2.	0.169-1.784	0.532	5.077	50.77
	3.	0.317-1.784	0.591	5.527	55.27
Erb/YAG Laser Sites.					
	0.	0	0	0	0
	1.	1.211-1.784	1.614	9.909	99.09
	2.	0.236-1.784	1.130	8.656	86.56
	3.	0.337-1.784	1.168	8.807	88.07
Pulsed CO_2 Laser Sites.					
	0.	0	0	0	0
	1.	1.410-1.784	1.642	9.937	99.37
	2.	0.820-1.784	1.500	9.747	97.47
	3.	1.784-1.784	1.784	10.000	100.00

Appendix 1   Abbreviations used

ARDS Acute Respiratory Distress Syndrome - Respiratory failure which is
refractory to oxygen therapy

CPG Computer Pattern Generator- a robot device allowing multiple laser
beams to be fired within a controlled, set pattern

DNA Deoxyribonucleic acid

Energy Density (Fluence)
The total energy divided by the cross sectional area of the beam in Joules
per sq.cm

EGF Epidermal Growth Factor - see TGF

EYL Erbium-YAG(Yttrium Aluminium Garnet) Laser

IGF-I Insulin-like growth factor

LCA Laser Controlled Area

LPA Laser Protection Adviser

LSO Laser Safety Officer

MODS
Multiple Organ Dysfunction Syndrome

MPE Maximum Permissible Exposure – the safe limit for exposure to the eye

PDGF Platelet derived growth factor

Power
The rate of performance of work, the energy divided by the time in which
the energy is delivered, expressed as Watts (1Joule per second)

Power Density (Irradiance)
The rate of energy delivery per unit of target tissue derived by dividing the
power by the surface area of the beam in Watts per sq.cm

Pulse Energy
The energy of a single pulse , usually expressed in milliJoules

RNA Ribonucleic acid

RNA Ribonucleic acid

Spot size
> The diameter of the laser cutting beam

SSG Split Skin Graft

SIRS Systemic Inflammatory Response Syndrome

TBSA Total body surface area

TGF-α & TGF-β
> Transforming Growth Factors alpha and beta. TGF-α is also known as Epidermal Growth Factor (EGF)

Tr Thermal Relaxation Time-the time taken for half the heat generated in tissue to be dissipated, about 695 μs for skin

UPL Ultrapulse Laser

Appendix 2   Classes of Laser

Lasers are classed according to their power output and degree of associated hazard set by the British Standards Institute, (1997).

- Class 1. Low risk with a maximum permissible exposure (MPE) which cannot be exceeded.

- Class 2. Visible radiation of low power is emitted. The corneal blink reflex is sufficient to protect inadvertent damage.

- Class 3a. Low risk visible radiation up to 5mW power. Dangerous only if concentrated through an optical system.

- Class 3b. Medium risk with maximal output to 0.5W. May damage the eye if viewed directly.

- Class 4. High power devices where even a diffusely reflected beam may cause damage with an inherent fire risk. The majority of medical lasers are within this category.

Appendix 3 Laser Safety

The use of lasers is governed by specific guidelines (Crumplin 1994):

1. A Laser Protection Adviser (LPA) is appointed to consult on the usage of instruments within a facility and to draft rules regarding this.

2. A Laser Safety Officer (LSO) is a member of the staff utilising the laser with custody of the key.

3. All staff using the device should be adequately trained.

4. A list of nominated users should be drawn up

5. A Laser Controlled Area (LCA) should be established which restricts entry to the area only to relevant staff with appropriate warning signs.

6. Adequate eye protection must be worn at all times when the laser is active. The device should not be fired until it is pointing at the target and an audible warning signal is also present.

7. The laser should be labelled with the correct classification mark and be key-controlled if Class 3 or above. Interlock devices should activate if the laser is damaged or tampered with. Other safety features should include shutter devices, shrouded foot pedals to prevent inadvertent firing and emergency shut-off switches. Aiming beams will be only Class1/2 Helium/neon devices or an attenuated beam of the main laser. Regular maintenance and calibration is mandatory.

8. Environmental considerations within the LCA should include the avoidance of reflective surfaces with adequate ventilation for the laser plume. Fire precautions must be taken since the laser will ignite dry material.

Appendix 4   Intra - operative Safety (Smalley 1994)

- **Beam Hazards.** These encompass fire, burns and ocular damage. Flammable materials should be avoided or kept under cover by damp swabs. Carbon sparking can cause thermal damage and should be irrigated to prevent build-up. Pre-operative preparation of the patient should include the removal of all alcohol-based cosmetics and hair gels or sprays and the operative field should only be cleaned with water-based antiseptics. Both patient and operator should have adequate eye protection conforming to BSI standards to prevent damage from specular and diffuse reflections (**Figure 4.4.1**). Instruments and surfaces should be either roughened to prevent specular reflection or draped with damp cloths

- **Nonbeam Hazards.** The fumes generated by the laser are referred to as plume. This has been shown to contain intact virions and viral DNA during laser tissue vaporisation although no definitive studies of their harm has yet been published. In addition, the explosive spray can lead to an aerosol effect with the debris containing tissue and blood for which universal precautions should be undertaken. Effective smoke evacuation should be employed to minimise these risks.

Diffuse Reflection   **Specular Reflection**
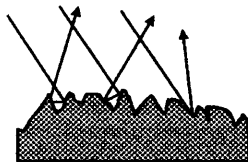
Angle of Incidence = Angle of Reflection
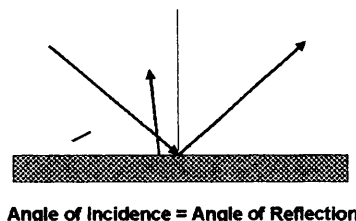
Fig.4.4.1 Comparison between Specular and Diffuse Reflection

The treatment of Lewisite burns with laser debridement—'lasablation'☆

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Dermabrasion — a novel concept in the surgical management of sulphur mustard injuries

P. Rice[a,*], R.F.R. Brown[a], D.G.K. Lam[b], R.P. Chilcott[a], N.J. Bennett[b]

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