CHAPTER II

LITERATURE REVIEWS

1. *Centella asiatica* (Linn.) Urban

Centella asiatica (Linn.) Urban (CA) is a perennial herb belonging to the family Umbelliferae. It is locally known as Asiatic pennywort, Bua bok, Pa-na-e-khaa-doh (Karen-Mae Hong Son), Phak waen (Southern), Phak nok (Northern). The stems are long, creeping and rooting at the nodes. Leaves are simple, 2-10 fascicled at the node, orbicular reinform, 1-7 cm long, 1.5-9 cm broad, entire, crenate or lobulate. Petioles are 4-10 cm long. Flowers are 3-4 flowered umbels. There are 2-5 umbels arising in axillary. Peduncles are 0.5-5 cm long, erected at first then curved; pedicels are almost none. Each flower has 2-3 bracts, 5 sepals; 5 petals which are 1-1.5 cm long, purple, 5 stamens alternate with the petals. Fruits are flattened, 2-3 mm long, 3-4 mm broad. It is a tropical weed, found especially in wet places of tropical and subtropical regions (Farnsworth and Bunyapraphatsara, 1992).

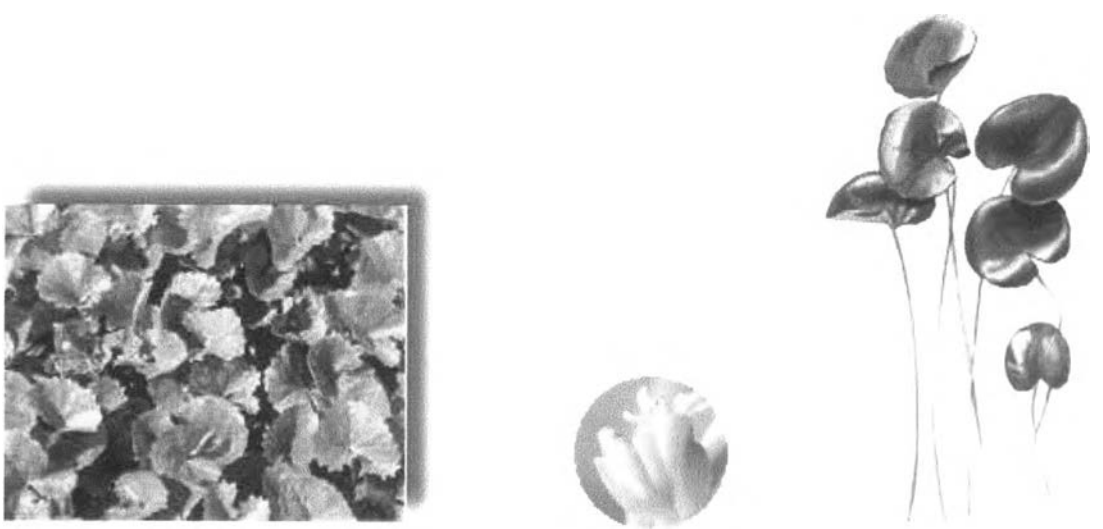

Figure 2.1 *Centella asiatica* Linn. Urban

1.1 Ethnomedical uses (Farnsworth and Bunyapraphatsara, 1992)

The claimed efficacies in Thai traditional text books are as follows:

Whole plant: for health promotion; treatment of skin diseases, wounds and vaginomycosis; as cardiotonic, diuretic, antidysentery, antidiarrhoeal, blood purifier and antisnake venom

Trunk: for health promotion; treatment of skin disease, wound; as diuretic, cardiotonic, antidysentery, antidiarrhoeal, treatment of leprosy

Leaf: for health promotion; treatment of skin disease, burned wound, bunion, wart; as diuretic, cardiotonic, antidiarrhoeal, antidysentery, relief of menorrhagia

Seed: antidysentery, antipyretic, analgesic

1.2 Chemical constituents (Farnsworth and Bunyapraphatsara, 1992).

Leaves: asiaticoside, bicycloelemene, borneol acetate, campesterol, β-caryophyllene, α-copaene, β-elemene, germacrene, kaempferol, kaempferol-3-0-β-D-glucoside, kaempferol-1-7-0-β-D-glucoside, linamarase, myrcene, α-pinene, β-pinene, quercetin-3-0-β-D-glucoside, β-sitosterol, stigmasterol, γ-terpinene, β- trans-farnesene.

Whole plant: asiatic acid, asiaticoside, betulinic acid, brahmic acid, brahminoside, brahmoside, centella asiatica compound BK, centellic acid, centellose, glucose, hydrocotyline, indocentelloside, indocentoic, isobrahmic acid, isothakunic acids,isothankuniside, madecassic acid, madasiatic acid, madecassoside, mesoinositol, methyl-5-hydroxy-3, 6-diketo-23-norurs-12-en-28-oate, phellandrene.

Petioles: asiatic and madacassic acids, asiaticoside.

Stems: asiatic and madacassic acids, asiaticoside.

Not specified part used: alkaloids, D-arabinose, asiatic acid, asiaticoside, brahmic acid, brahminoside, brahmoside, carbohydrates, centellic acid, centellose, centic acid, centoic acid, D-glucoside, madecassic acid, madecassoside, mesoinositol, oxyasiaticoside, pectins, resins, L-rhamnose, starch, thankunic acid, thankuniside, vitamin C.

1.3 Pharmacological study

The uses of CA led to many pharmacological studies as follows.

1.3.1 Wound healing activity

The healing property of CA was shown in patients with wound infection after operation (Muangmun and Rattanaoran, 1982) and chronic ulcers (Kosalwatna et al., 1988). An *in vitro* experiment showed that the triterpenoids, both individual component and the mixture, from CA increased human collagen I synthesis (Bonte et al., 1993). The experiment in rats revealed that the ethanolic extract of CA could impart a marked protective action against the 8 hours restraint stress induced ulcerization method, the protection afforded to the treated animals was comparable to that of the diazepam treated animals (Sarma et al., 1994). Another experiment in rats showed that the ethanolic extract of CA was effective on wound healing when administered orally and topically by increasing cellular proliferation and collagen synthesis at the wound site. Quicker and better maturation as well as crosslinking of collagen, faster epithelialization and enhancement of the rate of wound contraction has also been reported by Suguna et al. (1996). In 1998, Sunikumar et al. found that the topical formulations (ointment, cream, and gel) of aqueous extract of CA increased cellular proliferation and collagen synthesis at the wound site and the effects of the gel formulation was comparatively better than the other two formulations. It has also been demonstrated that the triterpenes from CA were able to stimulate collagen synthesis in the wound (Maquart et al., 1999). In 1999, Shukla et al. found that asiaticoside isolated from CA exhibited significant wound healing activity in normal as well as in delayed healing models. Moreover, CA showed the ability to use as a topical anti-psoriatic agent by inhibiting keratinocyte replication (Sampson et al., 2001). Lu et al. (2004) reported that asiaticoside isolated from CA could promote fibroblast proliferation and extracellular matrix synthesis by a close correlation among the gene profile, mRNA and protein production.

1.3.2 Cardiovascular activity

Dhorranintra and Sangsirinavin (1982) reported that intravenous administration of the CA glycoside solution produced a marked fall of blood pressure and decrease in heart rate in anesthetized dogs. The actions were recovered completely within a few minutes. By perfusion of the blood vessel in isolated rabbit ears, the glycoside caused direct relaxation of the vascular smooth muscle. The glycoside also decreased the force and rate of cardiac contraction in isolated perfused rabbit hearts. These potent cardiovascular actions could not be blocked by atropine, hexamethonium, propranolol or antihistamine. The glycoside itself could not block the ganglion or the alpha-adrenergic receptor. Therefore, it is safe to conclude that, the CA glycoside caused hypotension and cardiodepression by direct actions on the vascular smooth muscle and the heart.

Recently, Gnanapragasam et al. (2004) has reported that CA has cardioprotective effect in adriamycin induced cardiomyopathy. Pre-co-treatment with CA (200 mg/kg body weight, orally) significantly prevented alteration in serum marker enzymes (LDH, CPK, GOT and GPT) and antioxidant enzymes (SOD, CAT, GPx, GST) and restored the enzyme activities to near normal levels.

1.3.3 Antinociceptive activity

The water extract of CA revealed significant antinociceptive activity in the two models, namely, acetic acid-induced writhing and hot- plate method. The activity was similar to aspirin but less potent than morphine. Moreover, the inhibition of antinociception by naloxone, and opioid receptor antagonist, in both models suggested that the mechanism of CA antinociception might involve opioid receptors (Somchit et al., 2004).

1.3.4 Antiinflammatory activity

The antiinflammatory activity of CA was shown in acute radiation dermatitis experiment by reducing severity of the acute skin reaction (Chen et al., 1999). Somchit et al. (2004) found that the water extract of CA revealed significant antiinflammatory activity in prostaglandin E_2-induced paw edema. The extracts elicited antiinflammation in a dose-dependent manner. The activity of the extract (4 mg/kg, i.p.) was found to be similar to the non-steroidal antiinflammatory drug, mefenamic acid (10mg/kg, i.p.). Interestingly, the extract at a dose of 10

mg/kg, i.p. showed a significantly higher effect when compared to mefenamic acid (10 mg/kg, i.p.).

1.3.5 Antioxidant activity

The methanolic extract of CA showed as antioxidant agents when tested in lymphoma-bearing mice by significantly increasing the antioxidant enzymes, like superoxide dismutase (SOD), catalase and glutathione peroxidase (GSHPx), and the antioxidants like glutathiones (GSH) and ascorbic acid (Jayashree et al., 2003).

In the other report, CA exhibited free radical scavenging activity by ability to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical, the stabilized free radical. Furthermore, the total antioxidant power of the extract, as assayed by ferric reducing antioxidant power (FRAP) method revealed that the extract had reducing power that was less than ascorbic acid and $FeCl_2$, the references antioxidant. In addition, the extract significantly decreased the formation of oxygen radicals generated in rat peritoneal macrophages (Pakdeechote et al., 2003; Rababah et al., 2004).

1.3.6 Neurological activity

Bradwejn et al. (2000) evaluated the effects of CA on the acoustic startle response (ASR) in healthy subjects that randomly assigned to receive either a single 12 gram orally administered dose of CA or placebo. The results revealed that in comparison to placebo, CA significantly attenuated the peak ASR amplitude at 30 and 60 minutes after treatment. CA had no significant effect on self-rated mood, heart rate, or blood pressure. These findings suggest that CA has anxiolytic activity in humans as revealed by the ASR.

Shobi et al. (2001) revealed that CA showed the protective effect in radiation-induced behavioral changes during clinical radiotherapy.

The aqueous extract of CA has an effect on cognitive functions by improving learning and memory in both shuttle box and step through paradigms. Furthermore, it has been shown that there was a significant decrease in the brain levels of malondialdehyde (MDA) with simultaneous significant increase in levels of glutathione and catalase. These findings indicate that the aqueous extract of CA has cognitive enhancing effect and an antioxidant mechanism is involved (Veerendra et al., 2002).

Besides the activity on cognitive function, the aqueous extract of CA also decreased the PTZ kindled seizures and showed improvement in the learning deficit induced by PTZ kindling as evidenced by decreased seizure score and increased latencies in passive avoidance behavior. Moreover, the extract showed significant lower levels of MDA and significant higher levels of glutathione comparing to the control group (Gupta et al., 2003).

1.3.7 Gastrointestinal activity

Aqueous extract of CA have been reported to significantly inhibit gastric lesions formation (58% to 82% reduction) and decreased mucosal myeloperoxidase (MPO) activity in a dose dependent manner on ethanol induced gastric mucosal lesions in both gastric *ex -vivo* chamber model and *in vivo* models (Cheng and Koo, 2000).

Additionally, Cheng et al. (2004) found that the water extract of CA and asiaticoside isolated from CA were effective as antigastric ulcer drugs on acetic acid induced gastric ulcers model by reducing the size of the ulcers at day 3 and day7 in a dose-dependent manner, with a concomitant attenuation of myeloperoxidase activity at the ulcer tissues. Epithelial cell proliferation and angiogenesis were promoted. The expression of basic fibroblast growth factor, an important angiogenic factor, was also upregulated in the ulcer tissues.

1.3.8 Antimutagenic activity

Antimutagenic activity of CA was reported by Yen et al. (2001). Water extract of CA showed non-cytotoxicity to *Salmonella typhimurium* and no mutagenicity was found. Furthermore, the extract showed weak to moderate inhibition of mutagenicity.

1.3.9 Anticancer activity

In 1995, Babu et al. described that both methanolic extract and partially purified fractions (AF) of CA revealed the antitumour effect by both *in vitro* short and long term chemosensitivity and *in vivo* tumour model test systems. AF dose dependently inhibited the proliferation of the transformed cell lines more than those did by the methanolic extract and also significantly suppressed the multiplication of mouse lung fibroblast cells in long term culture. However, practically no toxic effects were detected in normal human lymphocytes. Moreover, the extract retarded the development of solid and ascites tumours and increased the life span of these

tumours bearing mice. Results from tritiated thymidine, uridine and leucine incorporation assay suggest that the fraction acts directly on DNA synthesis.

Bunpo et al. (2004) found that the water extract of CA could possibly be used as a chemopreventive agent against colon cancer development in azoxymethane (AOM)-induced aberrant crypt foci (ACF) and intestinal tumorigenesis model. It significantly decreased the number of larger ACF in the large intestine in the early stage, while the number of methylated DNA adducts was not decreased. In the post-initiation stage, the extract significantly decreased the total number of ACF and the number of larger ACF, accompanied by a decrease in the 5-bromo-2'-deoxyuridine-labeling index and an increase in the induction of apoptotic cells in the colonic mucosa. Incidences of neoplasm, the numbers of adenocarcinomas in the small intestines and entire intestines, and sizes of neoplasm in the entire intestines were smaller than those in control group. Furthermore, the extract at dose of 100 mg/kg significantly reduced the multiplicity of neoplasms in the small intestine. These results suggest that the inhibition of the formation of AOM-induced ACF by CA extract is associated with modification of cell proliferation and induction of apoptosis in colonic crypts and that the extract has a chemopreventive effect on colon tumorigenesis.

In the other report, the water and ethanolic extract of CA was tested on the production of nitric oxide (NO) and tumour necrosis factor-α (TNF-α) by J774.2 mouse macrophages. With the water extract, NO production was increased in a dose-dependent manner and an increase also occurred when the extract was administered with lipopolysaccharide (LPS), a known macrophage activator. In contrast, an ethanolic extract had no effect on NO, and when administered with LPS the extract suppressed production of NO. Moreover, the water extract significantly increased TNF-α, consistently in parallel with the increase in NO production while, the methanolic extract actually suppressed the production of TNF-α. These studies showed that the extract of CA can either increase or decrease NO production by macrophages and these effects are predominantly mediated through an effect on TNF-α expression (Punturee et al., 2004).

Recently, asiatic acid isolated from CA has been reported to exert anticancer effect by decreasing viability and inducing apoptosis in human melanoma SK-MEL-2-cells in a time- and dose-dependent manner, with a marked increase in intracellular reactive oxygen species (ROS) level and enhancement of the expression of Bax but not Bcl-2 protein in the cells. In addition, asiatic acid induced activation of caspase-3 activity in a dose-dependent manner and this activity was significantly blocked by trolox, an antioxidant. Furthermore, the apoptosis induced

by asiatic acid could be prevented by Ac-DEVD-CHO, a specific caspase-3 inhibitor and trolox. Asiatic acid did not elevate p53 nuclear protein levels that are present in a mutant form in SK-MEL-2 cells. These results suggested that asiatic acid induced apoptosis may be mediated through generation of ROS, alteration of Bax/Bcl-2 ratio and activation of caspase-3, but p53-independent (Park et al., 2005).

1.3.10 Toxicity assessment

Toxicity studies of CA glycoside cream were carried out on guinea pig skin. The toxic symptoms were observed when 1% extract cream was tested by applying repeatedly on the same area, 2.5 cm diameter of pre-shaved guinea pig normal skin once a day for a period of one month. In general, there was no detectable abnormality of the skin. In skin biopsy, the glycoside cream slightly increased keratinization and mild degree of papillomatosis and cellular swelling of the epidermis were observed. There was small round cell infiltration of the dermis, and some swelling of upper dermis. There were no noticeable changes of the hair follicles, glands or cutaneous vessels (Dhorranintra et al., 1984).

Many reports revealed that CA extracts were clinically effective and results from sensitizing capacity test showed that it was a very weak sensitizer (Eun and Lee, 1985; Izu et al., 1992; Hausen, 1993; Danese et al., 1994; Bilbao et al., 1995).

2. The skin

2.1 Structures of the skin (Marieb, 1981; Mast, 1992; Falkel, 1994)

The skin, also known as the cutis or integument, is the largest organ in the body, occupying almost 1.5 to 2 m^2 of surface area, and yet in most places is less than 2 mm in thickness. It has a complicated structure and serves many functions. It is composed of three layers: the epidermis, dermis and hypodermis as shown in figure 2.2.

2.1.1 Epidermis

The epidermis is the outer layer of epithelial tissue and avascular, having no blood supply. It consists of five typical layers. The stratum corneum or horny layer, which is an upper layer, consists of flattened keratinized cells (keratin). The keratin is a protein with waterproofing properties, preventing water loss from the deep tissue. Keratinized cells are dead, and so they are constantly rubbing and flanking off and being replaced by the division of deeper

cells. Beneath the stratum corneum is stratum lucidum, which consists of the flat, translucent layers of dead cells that contain a protein, called eleidin (a keratin precursor). The stratum lucidum appears only in the palms of the hands and sole of the feet, acting as a protective shield against the ultraviolet ray of the sun. The stratum granulosum; below the stratum lucidum, is the area in which the cells begin to die due to their accumulation of eleidin and their increasing distance from the dermal blood supply. The stratum spinosum contains 8 to 10 layers of closely packed cells, joined together by spiny projections, hence prickle cell. These cells have limited capacity for mitosis, and the stratum basale consists of a single layer of columnar or cuboidal cells. It undergoes continuous cell division to produce new cells to replace those being shed in the exposed superficial layer. It also contains melanocytes, cells that produce melanin (a pigment that helps protect from UV radiation).

2.1.2 Dermis

The dermis consists of two principle regions: the papillary and reticular layers. The papillary layer of the dermis consists of loose connective tissue with thin bundles of collagenous fibers. The reticular layer is made up of dense connective tissue with coarse collagenous fibers and fiber bundles that crisscross to form a strong and elastic network. The cells of the dermis are mostly fibroblasts, fat cells and macrophages. Blood vessels, lymphatic vessels, nerve endings, hair follicles and glands are also present.

2.1.3 Hypodermis

The hypodermis (subcutaneous) is beneath the dermis; composed of loose, fibrous, connective tissue. The hypodermis is generally much thicker than the dermis and is richly supplied with lympathic, blood vessels and nerves. Also within the hypodermis are the coiled ducts or sudoriferous (sweat) glands and the base of hair follicles. The boundary between the epidermis and dermis is distinct but that between the dermis and the hypodermis is not (Marieb, 1981).

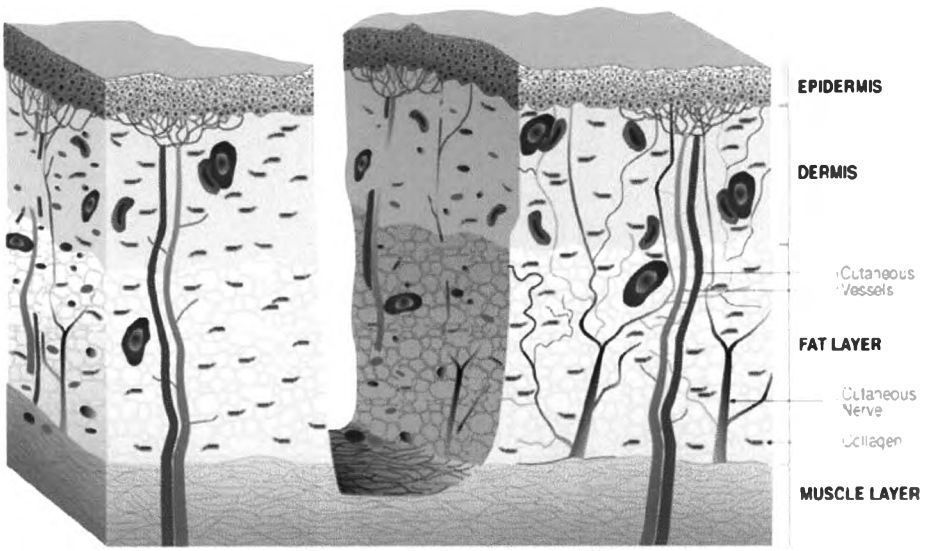

Figure 2.2 The skin

2.2 Function of skin

Falkel (1994) and Wynsberghe et al. (1995) described that the function of the skin are as follows:

1) protection : the skin acts as a stretchable protective shield that prevents harmful microorganisms and foreign materials from entering the body.
2) temperature regulation : by excreting through the pores of sweat glands
3) excretion : through perspiration, small amounts of waste materials such as urea are excreted through the skin.
4) sensory reception : the skin contains sensory receptors that respond to heat, cold, touch, pressing and pain
5) synthesis of vitamin D
6) provision of a cosmetic covering for personal identity
7) allowance for absorption of selected substances

3. The wound

3.1 **Definition of wound** (Leaper and Gottrup, 1998)

A wound may be defined as the loss of continuity of epithelium, with or without loss of underlying connective tissue (including muscle, bone and nerves for example), following injury. The injury may follow direct violence or be inflicted by a non-mechanical injury which may also be responsible for delay in healing. There may be extensive tissue damage (e.g., contusion or hematoma) with minimal tissue loss.

3.2 **Types of wounds** (Leaper and Gottrup, 1998)

Wounds can be broadly categorized as having either an acute or chronic etiology. Acute wounds are caused by external damage to intact skin and include surgical wounds, bites, burns, minor cuts and abrasion and more severe traumatic wounds such as lacerations and those caused by crush or gunshot injuries, acute wounds are expected to heal within a predictable time frame. Chronic wounds are most frequently caused by endogenous mechanism associated with a predisposing condition that ultimately compromises the integrity of dermal and epidermal tissue. Pathophysiological abnormalities may predispose to the formation of chronic wounds such as leg ulcers and pressure sore. Chronic wound types heal slowly and in an unpredictable manner.

3.2.1 Incised wounds

Incised wounds involve no loss and minimal damage to tissue. There are two types, surgical incision and non-surgical wound.

1) Surgical incisions are usually clean, except when made to treat an infective condition such as an abscess or fecal peritonitis. They are usually placed anatomically to avoid major vessels and nerves, but poor surgical technique can cause undue tissue damage and tissue hypoxia.

2) Penetrating, non-surgical wounds are caused by injuries inflicted by a knife or other sharp instrument. The random nature of such wounds may involve any tissue or organ and be accompanied by extensive hemorrhage. Hemorrhage may be more life threatening after a penetrating wound because contraction of muscular vessels is less likely.

3.2.2 Lacerated wounds

With this type of wound there may be tissue loss with some tissue damage. There is always a traumatic element, usually a blunt compressive force on tissues over an unyielding bony surface beneath. The scalp or face wound following a blow to the head is typical. The degree of tissue damage may be extensive with a degree of devitalization and bruising or extensive hematoma. The extravasation of blood activates platelets and the coagulation cascade, which in turn promotes repair through release of mediators.

3.2.3 Abrasions

Injury resulting in this type of wound is associated with loss of the superficial layers of epithelium (usually skin). Nerve endings are exposed and the wounds are painful. When extensive, the blood or plasma loss may be substantial and mimic a burn injury.

3.2.4 Contusion

These wounds are a more severe form of laceration and follow a much greater energy exchange. The tissue layers are separated and there is often tissue loss leaving an open wound.

3.2.5 Ulcer

An ulcer may be defined as a loss of an epithelial surface together with a variable degree of underlying connective tissue. The majority of acute ulcers follow trauma or pyogenic infections and there is usually some loss of connective tissue. The defect is made good by wound contraction and epithelialization (regeneration) and formation of scar tissue in the subepithelial layers (repair). The chronic ulcer is a lesion which fails to heal and its aetiology may be very diverse. The ulcer needs management of the underlying cause.

3.3 **Wound healing** (Regan and Barbul, 2000)

The process of wound healing can be divided into 3 overlapping phases that merge into a continuous process as follows.

3.3.1 Inflammatory phase (day 0-5)

Coagulation : injury causes hemorrhage from damaged vessels and lymphatics. Vasoconstriction occurs almost immediately as a result of release of catecholamines. Other various vasoactive compounds, such as bradykinin, serotonin, and histamine are released from tissue mast cell. They initiate the process of diapedesis, a passage of intravascular cells through vessels walls and into the extravascular space of the wound. Platelets are derived from the hemorrhage or a hemostatic clot. Platelets release clotting factors to produce fibrin, which is hemostatic and which forms a mesh for the further migration of inflammatory cells and fibroblast. Platelets are also extremely important because they are the first cells to produce several essential cytokines, which modulate most of the subsequent wound healing events.

Inflammation : within 6 hours, circulating immune cells appear in the wound. Polymorphonuclear leukocytes (PMNs) as neutrophils are the first blood leukocytes to enter the wound site. They initially appear in the wound shortly after injury and subsequently their numbers increase steadily, peaking at 24-48 hours. The role of the neutrophils is to kill organisms and to facilitate breakdown of debris by extracellular release of their enzyme. The next cell that appears in a wound is the macrophage. The role of macrophage, just like neutrophils, is to phagocyte and digest pathological organisms and tissue debris. Macrophages regulate tissue repair through the release of several growth factors and cytokines that stimulate fibroblast proliferation, collagen production and other healing process. Among these are TNF-α, PDGF, TGF-β, IL-1 and others.

3.3.2 Proliferative phase or regenerative phase (day 3-14)

The proliferative phase is characterized by the formation of granulation tissue in the wound. Granulation tissue consists of a combination of cellular elements, including fibroblasts and inflammatory cells, along with new capillaries embedded in loose extra cellular matrix of collagen, fibronectin and hyaluronic acid. Fibroblasts first appear in significant numbers in the wound on the third day post-injury and achieve peak numbers around the seventh day. Fibroblast produces large quantities of collagen, which subsequently reorganized, by cross-

linking, into regularly aligned bundles oriented along the lines of stress in the healing wound. The process of fibroblast proliferation and synthetic activity is known as fibroplasia.

Revascularization of the wound proceeds in parallel with fibroplasia. Capillary buds sprout from blood vessels adjacent to the wound and extend into the wound space. On the second day post-injury, endothelial cells from the side of the venule closest to the wound begin to migrate in response to angiogenic stimuli, angiogenesis occurred.

Re-epithelialization of the wound begins within a couple of hours of the injury. Epithelial cells, arising from either the wound margins or residual dermal epithelial appendages within the wound bed, begin to migrate under the scab and over the underlying viable connective tissue.

Wound contraction begins within 1 to 2 weeks of injury, resulting from fibroblast movement and myofibroblast interaction. Fibronectin and other factors regulate the process.

3.3.3 Maturation phase or remodeling phase (day 7 to 1 year)

The final phase of tissue repair is the maturation or remodeling phase, which continues for up to 1 year or even longer. Collagen is still synthesized but collagenase productions also increase to create a balance between collagen production and degradation. Wound has been closed by connective tissue and epithelialization. Vascularity decreases, fibroblasts shirk and collagen fibers alter red, granulation tissue to white and thickened wound.

The final product of the healing process is a scar formation. Scar tissue is a matrix of cells and fibers embedded in a ground substance. This relatively a vascular and a cellular mass of collagen serves to restore tissue continuity, strength and function, while abnormalities of the healing process may lead to abnormal scar formation as keloids and hypertrophic scars. Hypertrophic scars enlarge in size or bulk, most common within loose skin and over convex surface, whereas keloids enlarge by cellular proliferation, can occur anywhere, but almost often in areas such as the upper back, shoulder, anterior chest and upper arm.

3.4 Types of wound healing (Cotran et al., 1994; Greenhalgh and Staley, 1994; Kumar et al., 2003)

3.4.1 Healing by first intention

One of the simplest examples of wound repair is the healing of a clean, uninfected surgical incision approximated by surgical sutures. This is referred to as primary union or healing by first intention (Figure 2.3). The incision causes only focal disruption of epithelial basement membrane continuity and death of a relatively few epithelial and connective tissue. As a result, epithelial regeneration predominates over fibrosis. The narrow incisional space rapidly fills with fibrin-clotted blood; dehydration at the surface produces a scab to cover and protect the healing repair site.

Within 24 hours: neutrophils are seen at the incision margin, migrating toward the fibrin clot. Basal cells at the cut edge of the epidermis begin to exhibit increased mitotic activity. Within 24 to 48 hours, epithelial cells from both edges have begun to migrate and proliferate along the dermis, depositing basement membrane components as they progress. The cells meet in the midline beneath the surface scab, yielding a thin but continuous epithelial layer.

By day 3: neutrophils have been largely replaced by macrophages, and granulation tissue progressively invades the incision space. Collagen fibers are now evident at the incision margins, but these are vertically oriented and do not bridge the incision. Epithelial cell proliferation continues, yielding a thickened epidermal covering layer.

By day 5: neovascularization reaches its peak as granulation tissue fills the incisional space. Collagen fibrils become more abundant and begin to bridge the incision. The epidermis recovers its normal thickness as differentiation of surface cells yields a mature epidermal architecture with surface keratinization.

During the second week: there is continued collagen accumulation and fibroblast proliferation. The leukocyte infiltrate, edema, and increased vascularity are substantially diminished. The long process of "blanching" begins, accomplished by increasing collagen deposition within the incisional scar and the regression of vascular channels.

By the end of the first month: the scar comprises a cellular connective tissue largely devoid of inflammatory cells and covered by an essentially normal epidermis. However, the dermal appendages destroyed in the line of the incision are permanently lost. The tensile strength of the wound increases with time.

3.4.2 Healing by second intention

When cell or tissue loss is more extensive, as in burn wound, infarction, inflammatory ulceration, abscess formation, or even just large wounds, the reparative process is more complex. In these situations, regeneration of parenchymal cells alone cannot restore the original architecture. As a result, there is extensive in growth of granulation tissue from the wound margin, followed in time by accumulation of extracellular matrix and scarring. This form of healing is referred to as secondary union or healing by second intention (Figure 2.3).

Secondary healing differs from primary healing in several respects:

1) *Large tissue defects intrinsically have a greater volume of necrotic debris, exudates, and fibrin that must be removed.* Consequently, the inflammatory reaction is more intense, with greater potential for secondary inflammation-mediated injury.

2) *Much larger amounts of granulation tissue are formed.* Larger defects accrue a greater volume of granulation tissue to fill in the gaps in the stromal architecture and provide the underlying framework for the regrowth of tissue epithelium. A greater volume of granulation tissue generally results in a greater mass of scar tissue.

3) Secondary healing exhibits the phenomenon of *wound contraction.* Within 6 weeks, for example, large skin defects may be reduced to 5% to 10% of their original size, largely by contraction. This process has been ascribed to the presence of *myofibroblasts,* modified fibroblasts exhibiting many of the ultra structural and functional features of contractile smooth muscle cells.

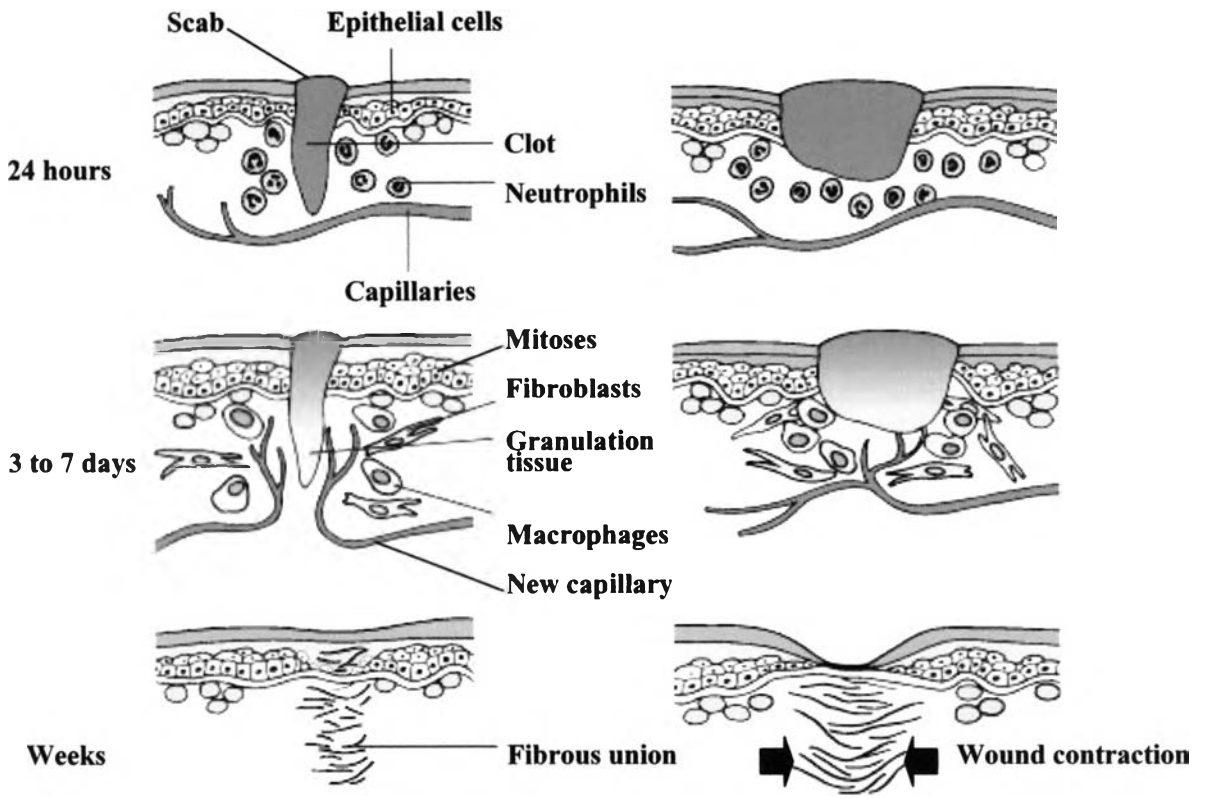

Figure 2.3 Steps in wound healing by first intention (*left)* and second intention (*right*). In the latter, the resultant scar is much smaller than the original wound, owing to wound contraction.

3.5 Factors that affect healing (Mast, 1992; Greenhalgh and Staley, 1994; Mulder, 1998)

A consequence of the complexity of wound healing as a multiple integrated process is the variety of conditions that can impair it. Several systemic conditions and therapeutic interventions that adversely affect the healing response, leading to ways in which tissue repair might be improved, are as follows:

3.5.1 Bacterial infection

Gross bacterial infection of a wound certainly delays or even reverses the healing response. Local destruction of tissue by bacterial growth and enzymatic action as well as prolongation of the inflammatory phase of healing is responsible. However, the abundance of normal bacteria flora of the skin suggests that virtually all wounds are contaminated to some degree. Yet contamination does not always lead to infection or deficient healing.

3.5.2 Aging

Age affects many aspects of wound repair. Overall, the rate of healing appears to slow with increasing age. Several investigators have documented that fibroblasts proliferate at a slower rate in older animals and individuals. Older cells also have decreased synthetic capability. The characteristics of collagen also change in elderly skin, soluble collagen decreases while insoluble collagen increases. The amount of elastin increases but the number of elastic fibers decreases to make skin less elastic. Wound contraction also proceeds at a slower rate in the elderly.

3.5.3 Malnutrition

Severe protein malnutrition affects body protein metabolism and thus may exert effects on collagen synthesis and connective tissue deposition. Moreover, the severely malnourished patient is immunosuppressed, thus affecting the inflammatory phase of healing. Local control of bacterial contamination may not be as efficient, leading to an increased likelihood of wound infection. This suppression can also blunt the proliferative phase of healing as macrophage infiltration and the cytokines are reduced.

3.5.4 Vitamins and trace elements

Deficiencies in vitamins and trace elements may also cause clinical healing problems. The roles of vitamins and trace elements in wound healing are shown in table 2.1 and table 2.2, respectively.

Table 2.1 The effects of vitamins on wound healing

Vitamin	Action related to wound healing
C	Required for protocollagen hydroxylase function. This intracellular enzyme creates intramolecular bonds between collagen α- helices. Deficiency (scurvy) results in abnormal collagen synthesis, markedly weakened wounds, and scar breakdown.
A	Induces fibroblast differentiation and increased collagen synthesis. It counteracts the ill effects of steroids and local radiation on wound healing.
E	The role of this superoxide scavenger in wound healing is uncertain. Some studies suggest that vitamin E may inhibit healing.
B_1 (thiamine)	Required for normal cell energy metabolism. Thiamine deficiency may inhibit wound healing.
B_5 (pantothenic acid)	Required for metabolism of carbohydrates, proteins, and fats. Pantothenic acid deficiency may inhibit wound healing.

Table 2.2 The effects of trace elements on wound healing.

Trace element	Action related to wound healing
Iron	Required for protocollagen hydroxylase function. This intracellular enzyme creates intramolecular bonds between collagen α-helices. Anemia has to become severe before wound healing is affected.
Zinc	Required for numerous enzyme reactions, including DNA and RNA synthesis. Zinc is required for proliferation of the reparative cells. It is also necessary for lysyl oxidase function, the enzyme responsible for intermolecular collagen bond formation (between tropocollagen molecules) and is important in scar maturation.
Copper	Required for lysyl oxidase activity to form intermolecular collagen cross-linkages. As with zinc, copper is important for collagen maturation.
Manganese	Required for galactosyl and glucosyl transferase, which add carbohydrate side chains to collagen α-helices. These carbohydrates stabilize collagen molecules.
Calcium*	Required for the endopeptidases that cleave the nonhelical ends (propeptides) from procollagen to form troprocollagen. Calcium has numerous other physiologically important functions.
Magnesium*	Required for enzymes that participate in protein synthesis. Magnesium has many other activities that are related to those of calcium.

* Calcium and magnesium are electrolytes that exist in relatively high concentrations in the body and are not really "trace elements".

3.5.5 Tissue oxygenation

Oxygen is required for the efficient supply of energy necessary for the various steps of healing. Furthermore, oxygen is directly involved as a substrate in oxidative destruction of bacteria by neutrophils and macrophages and in the hydroxylation of proline and lysine. Therefore, conditions that result in reduced tissue oxygenation may impair healing.

3.5.6 Disease

Diabetes mellitus is well known as being associated with altered healing. The effects of diabetes on the body are multiple, as are the effects on healing such as alteration on the function of leukocytes in such a way that chemotaxis, phagocytosis, and intracellular bacterial killing are all diminished. These effects would be expected to lead to impaired healing due to a less efficient inflammatory response, and the altered functions of neutrophils, macrophages, and lymphocytes can cause diminished fibroblast proliferation and collagen deposition.

Occlusive peripheral vascular and microvascular disease are more common in diabetics. These conditions result in reduced cutaneous blood flow and can potentially impair healing. Diabetics requires a higher distal perfusion pressure compared to nondiabetics to heal lower extremity amputation sites and also causes peripheral neuropathy, and the resultant impaired sensation leading to repeated trauma to tissue and wounds combined with poor perfusion may cause a further delay in healing.

Other chronic diseases are associated with wound healing problems. Chronic renal failure and liver failure predispose patients to impaired healing. Malignancy also contributes to healing abnormalities, probably by indirectly competing for nutrients. Any diseases that are associated with malnutrition will predispose the patients to abnormal healing.

3.5.7 Medication : corticosteroids, cytotoxic agents and immunosuppressives

Systemic corticosteroid therapy has multiple effects on wound healing. The inflammatory response necessary for healing may be blunted. This anti-inflammatory effect is thought to be mediated via stabilization of lysosomal enzymes, thereby preventing the secretion of acid hydrolases. The blunted inflammatory response may, in turn, result in the observed impairment in capillary budding, inhibition of fibroblast proliferation, decreased protein synthesis, and diminished epithelialization.

A variety of anti-neoplastic drugs have been shown to reduce the breaking strength of cutaneous wounds in experimental animals when administered at therapeutic levels. These cytotoxic agents render their therapeutic effect by interfering with DNA or RNA synthesis, cell division, or protein synthesis. Consequently, their effect on healing occurs primarily during the proliferative phase. Additionally, many patients receiving chemotherapy are systemically neutropenic and more prone to wound infection, which further impairs healing.

Immunosuppressives such as steroids, azathioprine, and cyclosporin A are used in the treatment of a variety of conditions most often for the prevention of rejection following organ transplantation. The therapeutic effect of these drugs is due to a blunting of the normal immune response, and cells involved in the inflammatory response of healing are likewise affected, causing a potential deficiency in tissue repair.

3.5.8 Radiation

The healing impairment found after acute radiation exposure is also due to inhibition of proliferating cells. As for other healing impairments, vitamin A or growth factors may ameliorate the altered healing. Delayed healing or even chronic wounds may result from previous radiation therapy. Radiation may cause a chronic vasculitis that impairs local blood supply and predisposes the patient to impaired healing. Chronic wounds in areas of previous radiation are extremely difficult to manage.

4. The burn wound

4.1 Pathology of burn (Boykin and Molnar, 1992; Johnson, 1994)

There are many types of burns: electrical, chemical, thermal, radiation and nuclear explosion burns. Among these types, thermal burn is a common case. Thermal injury results in non-uniform burn wound. At the time of injury, some tissues are totally coagulated. Other tissues are seriously damaged but not immediately destroyed. Still others are only transiently affected. The burn wound consists of three zones as shown in figure 2.4.

4.1.1 The outermost zone, **the zone of hyperemia**, is the area of the burn least affected and is usually healed by the seventh day. This area is furthest from injury, vascular integrity is maintained and blanches on pressure. No cell death occurs and the area appears pink. In other words, it is equivalent to a superficial burn. This indicates that it has a circulation and that

metabolism is taking place. This characteristic does not change: the zone becomes deeper red by the fourth day and is dry and healed by seventh day. A biopsy of this zone shows almost complete loss of the epidermis without apparent structural damage to the dermis, the subpapillary plexus and capillary loops are patent.

4.1.2 **The intermediate zone of stasis** is initially moist, red, blistered, and blanches on pressure. The subpapillary plexus is patent and contains oxygenated blood. At first after burning circulation and metabolism are diminished or ceased. By the end of twenty four hours the circulation through these superficial vessels has ceased. Petechial haemorrhages may be present visible to the naked eye, and on microscopical examination the superficial capillaries are dilated and packed with red cells. Complete stasis has occurred. Between the third and seventh days the red and white mottling of the zone of stasis turns white, so that only two zones are then apparent, these are the red hyperaemic outer zone and an inner white area consisting of the two zones of stasis and coagulation together. The zone of stasis at this stage is white because the superficial surface of the dermis is avascular and necrotic and the red cells which previously coloured the zone have haemolysed.

4.1.3 **The central zone of coagulation** represents the bull's eye of the target and is the area of greatest destruction. It is characterized by complete obliteration of the lumina of the vessels in the subpapillary plexus and the capillary loops; only an occasional solitary red cell can be seen under microscopic examination. Starting at about the seventh day, epithelialization begins to take place from the inner edge of hyperaemic zone. It became visible when the superficial dead dermis has been shed and in most burn this necrotic layer is thicker at the centre. Also fewer foci for epithelial regeneration occur in the deeper parts of the burn.

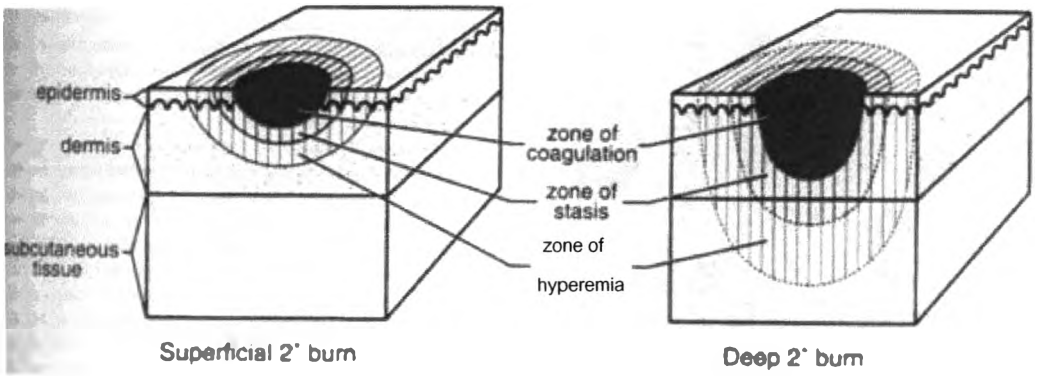

Figure 2.4 The intensity of cellular impairment of three zones

The burn wound is made up of varying degrees of cellular impairment following exposure to thermal energy. The mechanisms involve denaturation of cellular protein, inhibition of cellular metabolism, and secondary interference of local vascular supply. The factors that determine the extent of injury are: 1) the heat intensity to which the cells are exposed, 2) the duration of the exposure, and 3) the conductance of the tissue involved (Bonaldi and Frank, 1987).

4.2 Classification of burns (Bonaldi and Frank, 1987; Johnson, 1994)

4.2.1 Traditional classification

Traditionally, burns have been differentiated into first, second, third, and fourth degree injuries. Diagnosis at the time of injury or for several days after is not always possible with any degree of assurance. This classification is in many respects retrospective. If the skin healed by peeling, without blistering, it is said to have been a first degree injury. If blisters formed and the wound healed by re-epithelialization, it is labeled a second degree injury. A wound which fails to heal by regeneration of epithelium from within the wound margins is assumed to be a full thickness skin loss and is called third degree. Fourth degree wounds are those which are discovered to involve muscle or other tissue deep to the skin after the skin has been removed.

In 1992, Boykin et al. have classified the intensity of burning according to pathophysiological observation into 3 levels: first, second or partial and third or full thickness.

4.2.1.1 First-degree burns

First degree burns are those in which the deep part of the epidermis, particularly the basal cell layer remains viable and only the upper portion of the epidermis is affected by heat coagulation. In the affected areas, the nuclei appear either pyknotic and possess a perinuclear halo or in a more advanced stage, stain faintly eosinophilic or not at all, appearing as "architectural ghosts".

4.2.1.2 Second-degree burns

Second degree burns often show subepidermal blisters and are characterized by partial-thickness dermal necrosis, which leaves intact the lower portion of the cutaneous appendages from which re-epithelialization can occur. One may distinguish between superficial and deep secone-degree burns. Superficial second-degree burns are associated with necrosis of the surface epidermis and of only a small amount of superficial dermal collagen. In deep second-degree burns, much of the dermal collagen and the cutaneous appendages is injured. At a later stage, an inflammatory reaction develops at the junction of viable and nonviable tissue.

4.2.1.3 Third-degree burns

Third degree burns show full-thickness dermal necrosis with destruction of all cutaneous appendages. The coagulation necrosis may even extend on the subcutaneous tissue and to the underlying muscle.

4.2.2 Functional and descriptive classification

While the above traditional classification is still present, it is being rapidly abandoned for a functional and descriptive classification that allows a more precise description of the burn wound. Burn wounds are now classified into superficial, partial thickness, or full thickness wounds. The partial thickness wounds are frequently separated into superficial and deep subgroups. The spectrum of both eventual outcome and treatment varies greatly from the superficial burns to those of full thickness. It is thus important to choose a grading system that will allow the most complete understanding of the pathophysiological process taking place in the burn wound.

4.2.2.1 The superficial burn wound

These wounds are frequently the result of either prolonged exposure to low intensity heat, e.g., sunburn, or to a short duration flash exposure to a high intensity heat source. Erythema of the skin with edema confined to the basal layers is the result. Irritation of naked nerve endings occurs but swelling is not consistently present. In some cases, cell death at the level of the stratum granulosum does occur. This results in desquamation for two or three days following the burn and is recognized as the typical peeling following sunburn. There is no significant, early clinical consequence to burns at this level. The wounds heal rapidly without leaving a trace of scar. Late changes, such as an increased rate of neoplastic degeneration, are well recognized following solar and ionic radiation, but have not been documented for flash explosion burns.

4.2.2.2 The partial thickness burn wound

A superficial partial thickness burn is equivalent to the classic second degree blister burn. These injuries are the result of either increased exposure time or higher intensity flash exposure, and imply further cellular destruction than present in a simple superficial burn. The basal layer of the skin provides the line of demarcation between the deep and superficial partial thickness injury. In the superficial (second degree) burn, the basal layer is not totally destroyed. As in the first degree burn, erythema is a prominent feature; however, blistering is the hallmark of this level of burn. Cellular destruction of the stratum granulosum and stratum corneum occurs forming the covering of these blisters. The vascular response of the subpapillary plexus within the dermis results in edema formation at the dermal-epidermal junction. As fluid accumulates, the junction separates forming the blister. The epidermis itself may become swollen and edematous. Again, nerve endings are irritated and those can be extremely painful injuries.

The deep partial thickness burn consists of a wound with complete disruption of the epidermis and destruction of most of the basal layer. Sparing of dermal appendages such as hair follicles and sweat glands allows the wound to potentially regenerate and is thus partial thickness. The events that occur in the subpapillary plexus characterize the major histologic changes in this injury. Edema fluid infiltrates the dermal-epidermal junction. Ischemic (coagulation) necrosis of the epidermis occurs, followed by an inflammatory cellular response incited at the basilar level resulting in further tissue destruction. Blistering may occur; however, this is not an essential component. The wound is more often characterized by eschar formation.

Microhygrometer readings of deep partial thickness burn eschar demonstrate massive fluid loss. Of major clinical importance and a significant difference from the superficial burn is the loss of the cellular barrier which protects against bacterial invasion and wound sepsis.

4.2.2.3 The full thickness burn wound

The differences between deep partial thickness and full thickness burns can be broken down into five characteristics (Table 2.3).

Table 2.3 Characteristics of deep partial and full thickness burn wounds

Characteristic	Deep partial	Full thickness
Vascularity	Never totally ischemic	Arterial occlusion and devitalization
Dermal cellular inflammation	Present	Absent
Revascularization	Arterial patency re-established one week postburn	Neovascular granulation tissue three weeks postburn
Granulation tissue	In the dermis	In the fascia
Healing by	Epithelial growth	Eschar separation No epithelial growth Requires autograft

It is evident from the above that in the full thickness injury the epidermis is destroyed along with dermal appendages and supporting structures. The wound is characterized by coagulation necrosis of cells, and only at the edges of the wound do edema and cellular infiltrates occur. As with the deep partial thickness wounds, a thick, leathery eschar forms that allows copious fluid losses and fails to prevent bacterial invasion and wound sepsis. Granulation

tissue consisting of new fibroblasts and endothelial tissue develops as the result of an inflammatory response at the margin of the wound (both the edges and the undersurface of the coagulated eschar). The eschar becomes loosened and will eventually slough. Because of the lack of skin appendages, the wound will heal by contraction and epithelial growth from the edges. For most wounds of any size, the result of such natural wound healing is a contracture deformity and unstable scar. In clinical practice, skin grafts are applied to seal the wound and speed the healing process.

4.3 **The estimation of burn size** (Miller et al., 1994)

A body diagram and chart are used to estimate burn size. Commonly, the Rule of 9s is used because it is easy to remember. The body surface of an adult is divided into 11 segments of 9 percent, or multiples of 9 percent, which 1 percent reserved for the perineum (Figure 2.5). An alternative method is to use the palm of the patient's hand, which represents approximately 1 percent of the patient's body surface. Small areas can be estimated by using the relative size of the patient's hand as a reference.

Frequently, a modified chart, devised by Lund and Browder, is used to estimate burn size in children because this chart may be more accurate (Table 2.4). Many clinicians also use it for estimating burn size in adults. During the first year of life, a child's head is approximately twice as large as an adult's in proportion to the total body surface area. When using the Rule of 9s in children, 9 percent is taken from the legs and added to the head for a child up to 1 year of age. Each subsequent year, 1 percent is returned to the legs until, at approximately age 9, the child's head is an in proportion to that of an adult's.

Calculated burn sizes are only estimates. Superficial areas of burn that are pink or red, such as sunburn, are not used in the estimate. Only partial- and full- thickness areas of burn are used in estimating the extent of burn.

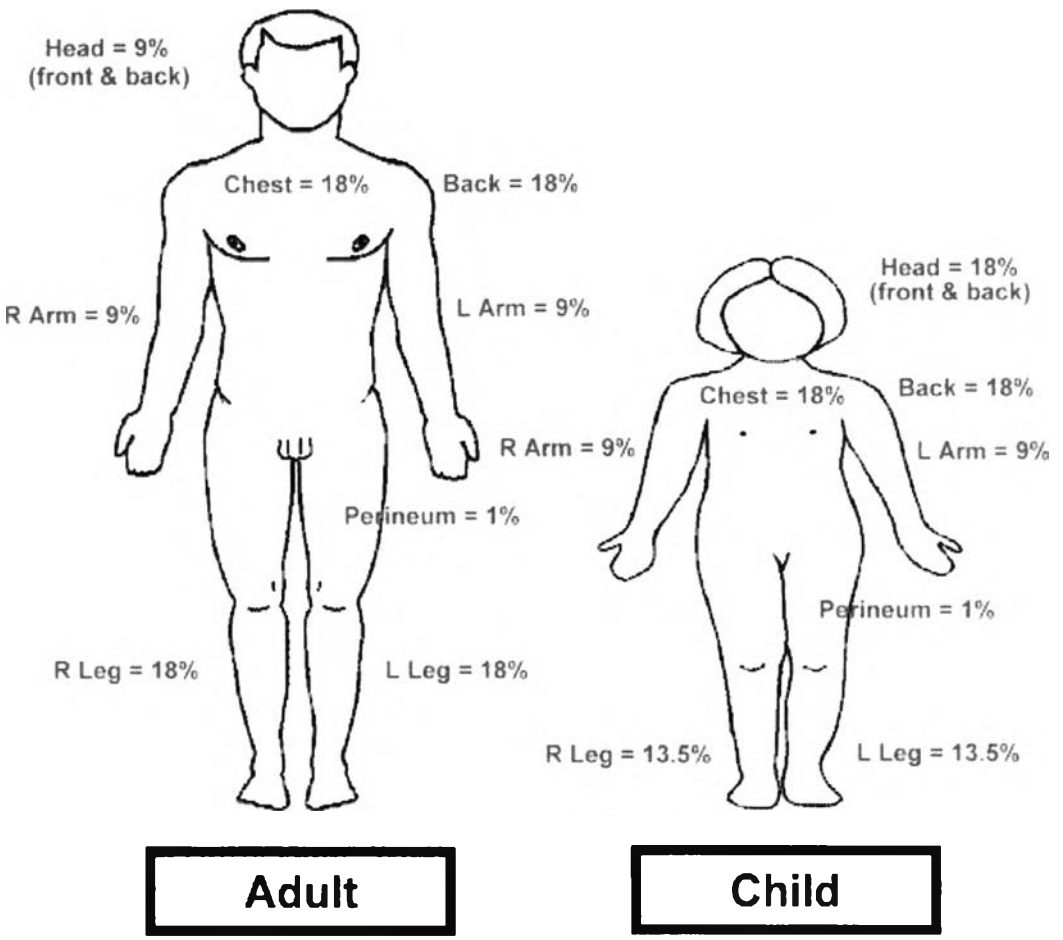

Figure 2.5 Rule of 9s to estimate extent of burn injury by percentage

Table 2.4 Child burn size estimation (percent total body surface area)

Burn area	Age (years)				
	1	1-4	5-9	10-14	15
Head	19	17	13	11	9
Neck	2	2	2	2	2
Anterior trunk	13	13	13	13	13
Posterior trunk	18	18	18	18	18
Genitalia	1	1	1	1	1
Upper extremity (each)	9	9	9	9	9
Lower extremity (each)	14.5	15.5	17.5	18.5	19.5