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WOUND HEALING IN THE SKIN
an interaction between environmental conditions and intrinsic factors

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Injuries to the skin are an inevitable tribulation of life, and over many centuries scholars of human medicine from all parts of the world have studied herbal remedies, physical procedures, and surgery to aid healing and to prevent disfigurement. Cauterisation and wound debridement was practised in the sixteenth century, but not until much later, following the classical studies of Alexander Fleming did antibiotics appear in the clinicians' vademecum.

Recent years have seen a surge of research and investigative techniques in the study of wound healing with the introduction of new techniques in cellular and subcellular analyses, and tissue culture assays. Nowadays, the researcher is allowed an insight into the molecular biology of tissue regeneration and chemotactic pathways.[1,2] Significant achievements have been made in understanding the sequence of events following the induction of shallow abrasions or whole thickness skin wounds, but considerable controversy exists as to the mechanisms regulating cell proliferation and maturation. We know that the mammalian epidermis exhibits a remarkable plasticity and ability to regenerate under normal conditions of health and nutritional adequacy, but mechanisms for triggering the repair process are unclear. Recent studies are only now beginning to shed some light on the contribution of the water content of the tissues (the state of hydration), oxygen concentrations, physical pressures or conditions in the local environment, and how these may be modified by the use of dressings, healing creams and topical administration of nutrients and growth factors.

MAMMALIAN SKIN : HOMEOSTATIC MECHANISMS AND INTER-CELLULAR RELATIONSHIPS

The skin is composed of ectodermal and mesodermal tissues with specialised cells of neural crest origin (melanocytes and Merckel cells). From before birth, the epidermis and dermis are well defined, being separated by a semipermeable glycoprotein-rich basement membrane.[3] The location of epidermal appendages (hair follicles, sweat ducts etc) is determined.

Whereas the epidermis is a well defined stratified epithelium, the dermis is a more loosely structured tissue with bundles of collagen fibres interlaced with blood vessels and nerve fibres. The dermis also contains varaible numbers of mast cells, macrophages lymphocytes and neutrophils. Normally, there is no cellular interchange between the epidermis and dermis but the diffusion of chemical modulators in the form of growth factors and hormones seems increasingly likely.[4] Thus, a central role is conferred upon the basement membrane in regulating whatever interaction might exist between the two main tissues. Where the basement membrane is absent or fragmentary in pathological states (eg viral warts), epidermal cells proliferate irregularly and maturation is incomplete.

The epidermis exists in a state of dynamic equilibrium with the external environment, such that cells desquamated from the surface are balanced by mitosis in the basal layer (Fig 1). Basal cells have the option of retaining their embryonal features to remain in a proliferative state, or they may enter a vertical migration pathway of keratinisation, maturation and ultimately death at the surface of the skin, as the occasion demands.[5-7] Mitotic inhibitors (possibly of the autocrine type), hormones and growth factors have been implicated in this feedback process, but the regulatory mechanisms involved are unknown.

The balance between epidermal cell loss at the skin surface and basal cell mitosis leads to the concept of "critical mass". Thus, for a particular region of the body, the unit skin mass (epidermis and dermis) is constant in individuals of similar age, sex and genotype under optimal conditions of health and nutrition. Although the dermis forms at least 75% of the skin mass, the dermal thickness is proportional to that of the epidermis. Contrasting examples are seen in the thick skin of the palmar and plantar surfaces, and the incredibly thin epidermis of the human eyelid. As cell proliferation declines with advancing age, so the skin becomes progressively thinner and more fragile. Wound healing is usually slower than in younger people.[8]

CHEMICAL AND ENVIRONMENTAL INSULTS AND SKIN INJURIES

Damage to the skin may occur through exposure to a variety of environmental insults (Table 1). Many will penetrate subepidermal tissues and be accompanied by haemorrhage. Where skin damage occurs, alterations in cell-cell and tissue-environment relationships are inevitable. They initiate a complex sequence of events involving autocrine and paracrine mediators leading to regeneration and ultimate recovery - to restore the critical mass.[1,4]

Skin wound healing may be complicated by disease processes such as diabetes or psoriasis.[9,10] Ultraviolet light or ionising radiation can give rise to burn type wounds but may also be associated with mutagenic changes which are potentially tumorigenic. These subjects are well documented but outside the scope of the present review.

Full thickness skin wounds involving damage at all levels are associated with a complex sequence of events which are identified under four main phases according to the cellular and biosynthetic events.[11]

- haemostasis
- inflammation
- proliferation and regeneration
- remodelling and normalisation

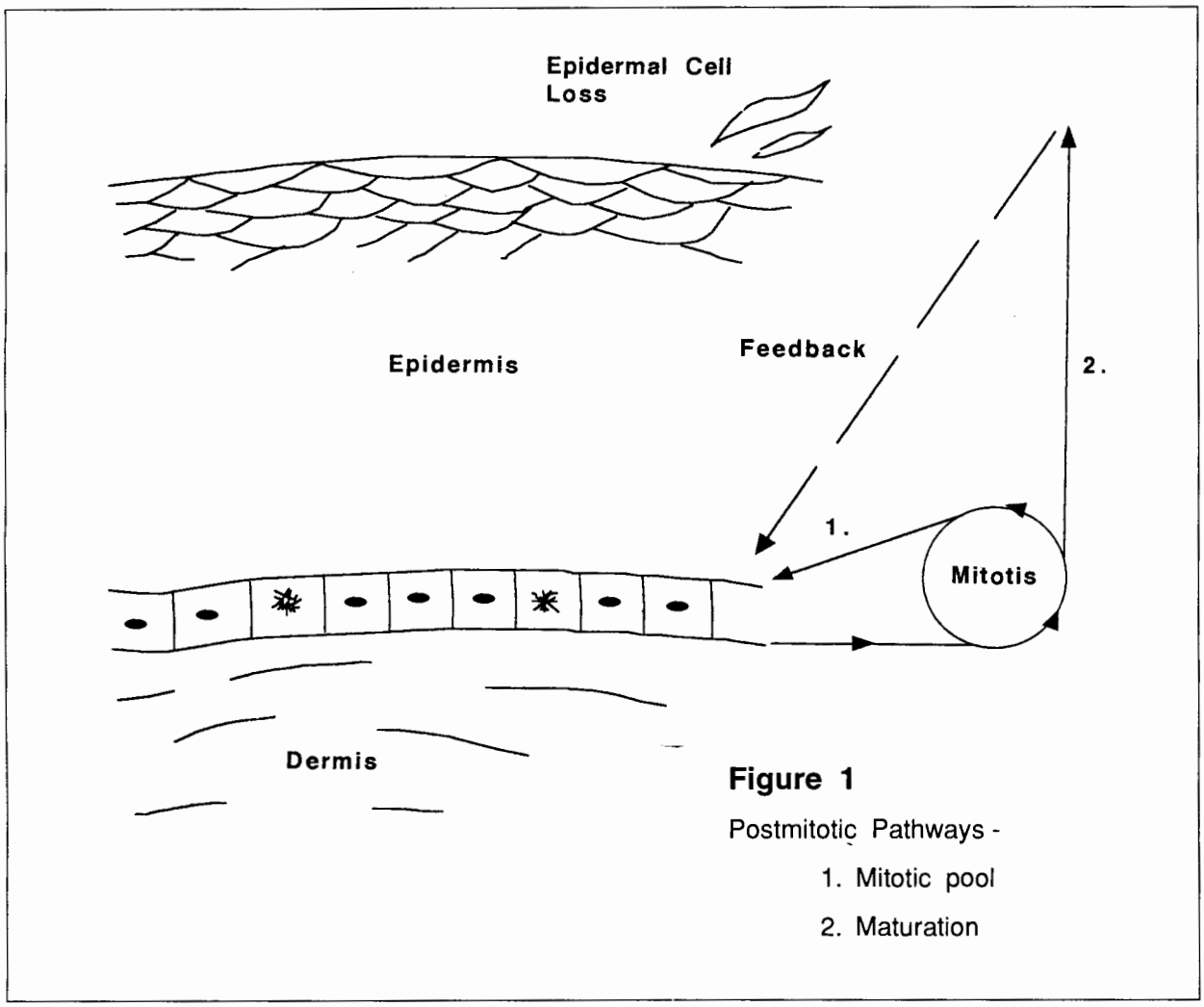

Figure 1

Postmitotic Pathways -

1. Mitotic pool
2. Maturation

Under normal conditions of health and nutrition, the sequence of events is simillar for all injuries, but the onset and duration of the constituent steps varies according to the nature and severity of the wound.

Skin wounds deprived of a protective stratum corneum and exposed to the air, soon become subject to dehydration, hardening and scab formation.[12, 13] These scabs obstruct the formation of new tissue and serve no useful purpose other than to protect underlying tissues. This function is beneficially taken by occlusive or semiocclusive dressings which also preserve the state of hydration of the skin to the benefit of the early regenerative process. The dehydrated skin also facilitates the percutaneous absorption of substances applied to the surface of the wound to advance healing.[14] This is taken into account in the design of dressings containing nutritional supplements (zinc, vitamins etc).

1. HAEMOSTASIS

The period of haemorrhage and haemostasis is the shortest of the post-wounding phases but is prolonged in patients having genetically determined defects in the coagulation process. Platelets initiate the haemostatic process by adhering to one another, to blood vessels and to damaged tissue fragments.[15] They degranulate to release a coagulation factor that is known for its action in the adhesion of platelets to collagen and endothelial tissues with the subsequent release of substances like thromboxane A2,

TABLE 1

Environmental Causes of Skin Damage

1. Contact with substratum, environmental pressures
2. Physical insult - UV-light, burns, scalds, cuts, abrasions, surgical incisions etc
3. Chemical insult - corrosive acids and alkalis, epidermal denaturing agents, carcinogens, mutagens, vesiculants, sensitisors, irritants
4. Biological - infections, parasites, insect bites and stings etc

ADP and thromboplastins.[8] These promote the transformation of prothrombin to thrombin and the polymerisation of fibrinogen to fibrin.[18] Ultimately, the fibrin mesh serves as a matrix for macrophage infiltration and collagenesis. Calcium ions, vitamin K and an appropriate microenvironment are critical in these early events involving the release of growth factors responsible in modulating later steps in healing.[2] Contaminants like excess zinc ions (which compete for calcium binding sites on carrier proteins or on plasman membranes)[19] or dicoumarol which impairs vitamin K metabolism, inhibit enzymic pathways and delay healing.

Soon after wounding, eicosanoids, thromboxane A2 and other mediators induce a vasoconstrictor effect to aid haemostasis.[20,21] In due course, this effect is reversed by the action of histamine and serotonin released from activated mast cells. The increased blood flow so achieved, alleviates increasing oxygen tensions associated with the development of inflammatory changes. Histamine also increases vascular permeability with the serous exudate providing a microenvironment for the synthesis, activation and receptor binding of growth factors and cytokines.

Platelet activation stimulates the release of platelet derived growth factor (PDGF), transforming growth factor (TGF-b) and peptides resembling epidermal growth factor (EGF).[1,2,17,22,23] **These are mitogenic and chemotactic in inflammatory cell infiltration and granulation tissue formation (Table 2).** The chemotaxis may operate in the form of a concentration gradient towards the centre of the wound, but this view remains to be substantiated.

2. INFLAMMATION

Inflammation involves a complex interaction of many soluble substances and other components in the skin leading to classical symptoms of warmth, redness, oedema, pain and pruritis.[24] Despite overt signs, inflammatory cells do not begin to infiltrate the wound site until several hours after wounding probably through the mediation of metabolites of arachidonic acid (eicosanoids), and Hageman's factor (Factor XII). Initial cellular events include an infiltration of neutrophilic polymorphonuclear cells which perform the critical role of detoxifying and disinfecting the wound bed, that is, the elimination of materials that may inhibit subsequent biosynthetic events. Later, macrophages, fibroblasts, lymphocytes and mast cells accumulate. The macrophage is of central importance in the inflammatory stage, not only as a phagocytic cell but one which synthesises a variety of cytokines and growth factors capable of modulating and promoting the activity of epidermal, mesodermal (fibroblasts) and endodermal (vascular endothelial cells) into mitosis and biosynthesis (Fig 2).[25,26]

TABLE 2
Growth Factors with a Role in Wound Healing in Mammalian Skin

Growth Factor	Source	Target cell	Presumed Action
Platelet-derived GF (PDGF)	Blood platelet	Mesenchymal cells (fibroblasts, smooth m. cells, neutrophils) endothelial cells epidermal cells	Induction of inflammation granulation tissue, angiogenesis, epidermal mitosis
Epidermal GF (EGF)	Salivary gland blood platelets	Epidermal cells fibroblasts endothelial cells	Epidermal regeneration, fibroblast proliferation, angiogenesis
Transforming GF-α (TGF-α)	Epidermal cells macrophages	Endothelial cells	Angiogenesis, chemotactic for inflammation and granulation tissue
Transforming GF-β (TGF-β)	Macrophage lymphocytes	Epidermal cells endothelial cells	Down-regulates mitosis in epidermal and endothelial cells, activates fibroblasts
Fibroblast GF (FGF) - basic - acidic	Fibroblasts endothelial cells smooth muscle cells	Epidermal mesenchyal and endothelial tissues	Chemotactic for angiogenesis and epidermal regeneration, induces granulation tissue, collagenesis synthesis of extracellular matrix
Insulin-like GF (IGF)	Fibroblasts macrophages	Fibroblasts endothelial cells	Mitogenic chemotactic for angiogenesis
Tumour necrosis factor (TNF)	macrophages	Fibroblasts endothelial cells	Modulates activity in cytokine production, angiogenic, fibroblast maturation

Much has been learned about events during the inflammatory period through the analysis of wound fluid.[27] Thus, placement of inert sponges or indwelling canulae allows sequential sampling of wound infusates, proteins, peptide hormones, growth factors and extracellular matrix for analysis.[17] Marker proteins such as laminin, actin, myosin and desmin have been useful in identifying cell type and states of maturation.

Monocytes arrive at the wound site under the influence of TGF-b to be transformed to macrophages in the moist medium of the occluded wound.[1,22,28-30] Through chemotactic action and fibronectin labelling, they eliminate cell debris and to create favourable conditions for fibroblast activity. Macrophages produce many growth factors and cytokines of which interleukin-1 (Il-1), TGF-a, TGF-b, b-FGF and tumour necrosis factor (TNF) are instrumental in granulation tissue formation.[31]

Granulation tissue is characterised by an accumulation of myofibroblasts 32 and capillary buds embedded in a fibronectin / collagen rich hyaluronic acid matrix. Some capillaries involute in due course but others mature to form venules or arterioles.

Evidence for the presence of angiogenic factors has been accumulating in the last 40 years. They include factors that influence angiogenesis (by acting on endothelial cells) and those that exert an indirect effect. The former class include an acidic-FGF, TGF-a, TNF, and other macrophage derived growth factors. The indirect factors identified so far are TGf-b and other peptides and eicosanoids. Mechanisms of action are not known.

3. REGENERATION

The regeneration phase is characterised by a surge in dermal collagenesis and angiogenesis, and epidermal regeneration. The wound site becomes re-epithelialised and the basal lamina reformed. Hair follicles and sweat ducts lost in the injury do not reform but viable epithelial cells from both sources are available for epidermal repair.

Regenerative changes may commence several hours or even days after the injury.[11] In the epidermis, cellular differentiation and migration achieve an exteriorisation and elimination of tissue debris and scab tissue. Whereas the tissue debris and scab protect the re-epithelialisation process in an open wound, they obstruct the migration of epidermal cell buds and increase the scarring effect.[33] Debridement of infectious material, jagged wound margins and tissue debris provides a "clean wound" and improves healing.

Re-epithelialisation requires appropriate modulation (probably by PDGF,TGF-b, EGF and b-FGF), availability of zinc and other nutrients, and suitable conditions in the ambient environment.[1,2,22,28,34,35] Chemotactic pathways and motivating influences for basal cells to deviate from their vertical differentiation pathway to a lateral mode to replace damaged cells are not understood. Also, the ability of regenerating epidermal cells to undergrow the scab and cellular debris (which may include dermal tissue) is unclear. Whatever the mechanism, cells from the epidermal cell pool in the wound margin do divide and migrate (in a horizontal plane in the occluded wound) to meet those migrating from the opposing margin. The basement membrane composed of collagens, glycoproteins, fibronectin and glycosaminoglycans is resynthesised.[3]

Re-epithelialisation patterns are largely dependent upon environmental conditons including dressings, drugs and pressures. 12,13 Suboptimal conditions created by excessive dehydration, oxidation and premature cell death impair mitosis, migration and functional differentiation of epidermal cells and alter cell membrane receptor activity to retard healing. Later events leading to vertical maturation parthways and reforming of the epidermal barrier function probably involve mediators and mechanisms of which we are unclear at present.

Regenerative changes in the dermis are more complex but formation of scar tissue is the major event. Collagenesis, angiogenesis, and formation of extracellular matrix are dependent upon the interaction of growth factors, hormones (oestrogens, androgens, insulin and corticosteroids) and the availability of enzyme substrate.[2,23,36] Thus, collagenesis proceeds under the modulation of FGF and TFG, with the elaboration of proteoglycan ground substance and fibrin. Iron, copper and vitamin C are necessary. Procollagen chains twisted in a helical pattern become converted to tropocollagen and thence to collagen with further polymerisaion. In scar tissue, these collagen fibres tend to be arranged irregularly and not in bundles as in normal skin. Scar tissue tends to be denser than normal and is less vascular. Hypertrophic scars can be overtly disfiguring on account of their ability to project healed wounds beyond the normal contour of the skin.

Interesting differences seen between foetal and adult skin wounds suggest that conditions in the microenvironment are important in scar tissue formation.[37] Full thickness foetal skin wounds exhibit minimal scar tissue formation, possbly due to a reduced vascularity and blood pressure, or an immaturity in biosynthetic pathways or growth factorreceptor binding. in vitro studies have demonstrated that TGF-b modulation of fibroblasit is necessary in fibrin/collagen production. This has provided the impetus for recent studies in which TFG-b activity was blocked using specific neutralising antidbodies. Rats with full thickness skin wounds treated with TGF-b antibody produced minimal scar tissue.[38] Wounds healed with a tensile strength similar to those seen in control animals. This, of course, has considerable clinical relevance.

4. REMODELLING AND NORMALISATION

The ability of a skin wound to remodel and recover normality depends upon the extent of the initial injury and the regenerative capacity of the tissues. Management of environmental factors is important. Earlier studies have demonstrated that optimal conditions in the microenvironment of the wound change as healing progresses.[11]

Wound occlusion during haemostasis, inflammation and regeneration is beneficial providing sufficient pO_2 levels are maintained. However, prolonged occlusion following re-epithelialisation can be detrimental. Epidermal mitosis is suppressed, and those cells that do enter the maturation pathway fail to complete their keratinisation profile. Phospholipid and cysteine accumulate but subsequent changes leading to keratohylin and keratin formation fail to materialise. This may be due to oxidative failure, a lack of surface cell dehydration, or inadequate environmental pressures.[39]

In re-epithelialisation, there tends to be an overproductin of tissue and hence a thicker than normal wound site.[40] This might imply a defect in the cut-off point in regulatory processes - a defect in "down regulation". Normalisation will therefore demand some involutionary change, possibly through the mediation of environmental influences, such as

increased contact with substrata etc. In vitro studies suggest that hormones, growth factors and cytokines (involucrin, laminin) may also be involved. Similar observations of down-regulation may operate in the dermis in slowing down and ablation of collagenesis and angiogenesis.

WOUND MANAGEMENT, CONTROL OF FACTORS IN THE WOUND ENVIRONMENT

Wound management is designed to create a milieu for optimal healing. Dressings, creams and other occlusive materials can significantly alter the state of hydration of the surface layers of the skin, free gaseous exchange, and the percutaneous absorption of substances applied topically to aid healing.

Tissue oxygenation is critical in wound healing from an early stage, oxygen being necessary for phagocytic activity and the release of microbiocidal free radicals. Later events include collagenesis, immune function, and presumably, the release and receptor binding of growth factors.[41,42] Excessive pO_2 tends to inhibit angiogenesis however, suggesting that certain growth factors like b-FGF are not oxygen dependent. In fact, high pO_2 leads to a local hypertension due to a thickening of vascular walls and reduced lumena.

Wounds exposed to the environment rapidly dry out with the formation of disfiguring scabs, inflammatory changes and discomfort to the patient. These wounds heal more slowly than occluded wouns on account of the increased re-epithelialisation necessary, and also the greater repair needed to replace cells lost through dessication and abrasion. Increased water loss from the surface of open wounds is thought to be detrimental in the central modulator function played by growth substances and cytokines.

In an attempt to improve healing, experimental and clinical studies have evaluated the effect of increasing local concentrations of vitamins, essential trace metals and growth factors by topical application.[22] Although vitamin A is necessary in normal skin development, the benefits of supplemental vitamin in wound therapy are equivocal. It is possibly beneficial in improving the body's resistance to infection, and may have a role in epithelial growth and collagensis. Also unclear at the present time, is the action of vitamin C in wound healing. Historically, patients with scurvy have shown poor wound healing, but vitamin supplementation may only be indicated for wound healing in cases of nutritional deficiency. Skin wounds in vitamin deficient guinea pigs tend to haemorrhage and heal more slowly than in normal vitaminaemic animals.

Topical zinc administration can be beneficial in advancing wound healing providing the zinc is available in a metabolisable form and can reach target tissues.[43] Zinc aids wound closure and reepithelialisation, and as it is a constituent of more than 40 enzyme systems, additional zinc probably aids other synthetic processes. The value of zinc in medicated bandages is questionable.

In the past 20 years, intensive commercial enterprise has led to the development of a wide range of inert, bioactive and interactive wound dressings.[44] For many of these, there is little information to show how they influence the cellular or molecular events in the wound, other than through a modification of the state of hydration of the tissue and the gaseous exchange with the environment. Cotton wool, lint and tulle gras are inert but they are are absorbent and aid haemostasis. Bioactive and interactive dressings are designed to regulate gaseous exchange, to prevent infections and to protect the wound from environmental insult.

Whereas fully occlusive dressings provoke exudation in the early wound, this hyperhydration is beneficial in promoting granulation tissue and autolytic debridement of tissue debris. Inflammatory changes are less and dermal fibrosis reduced. It is suggested that occluded wounds "capture" the exudate to create a conducive milieu for subsequent events(?) [46] Where occlusive dressings are applied, antiseptic measures are essential - wound fluid makes an excellent cuture medium for bacteria! Antibacterial agents may be effective in controlling infection, but they are antimitotic and healing.[47]

Hydrogels and hydrocolloids are absorbent and permit gaseous exchanges. The relative benefits of most are adequately detailed in the clinical and scientific literature. Clinically, the selection of a dressing will be matched to the type and severity of the wound. Where absorbent dressings are used, they will be suffcient to absorb excess moisture for early wounds, but insufficient to dessicate or otherwise impair tissue regeneration. Hydrocolloids tend to absorb fluid slowly at first, but ultimately form a gelatinous mass at the wound surface. Hydrogels aborb wound fluid in to a sponge-like structure which allows evaporation without compromising the state of hydration. One hydrocolloid product is reported to aid angiogenesis, but how true this is of similar products is not clear.

Bioactive and interactive products may serve to create favourable conditions for wound repair, but in certain cases they also aid healing by providing structural support and nutrition.[48] For example, a dressing containing collagen is likely to promote fibronectin and intercellular matrix formation. Certain alginates avidly absorb wound exudate but in so doing involve an ionexchange process leading to the release of calcium ions as required in haemostasis. Other products form fibrous matrices to aid cellular migration.

Despite the many advances in product design, scientists and clinicians still need to acquire knowledge of the influence of various constituents of dressings upon fine cellular processes and biosynthetic events. Information is lacking or questionable as to the biodegradability of some products available.

LABORATORY MODELS FOR WOUND HEALING STUDIES

Mammalian skin exhibits a remarkable range in form with adaptations enabling different species to occupy a wide variety of environmental niches and conditions. Despite wide variations in hair cover, thickness, vascularity and sensory apparatus, the integument conforms to a simple basic pattern Responses to injury vary in the rate of healing rather than the type.

In most wound healing studies, biologists have preferred to use the rat, mouse, rabbit, guinea pig or pig mainly through their convenience as laboratory animals. Although rat skin is overtly quite different to human skin, it has been employed in many exploratory studies including that to demonstrate the inhibitory role of neutralising antibodies to TGF in scar tissue formation.[38]

Porcine skin is hypotrichous and histologically similar to human skin. It is less sensitive than human skin for irritants owing to its robust stratum corneum, but has been widely used in the evaluation of wound dressings.[12,13] Occlusive conditions can be readily achieved and manitained. Importantly, the pig is a convenient experimental model and one from which results can be extrapolated to man. Because of its size in comparison to other laboratory species, many research groups perform initial studies in rat or mouse before proceeding to the pig for definitive preclinical studies.

Considerable advantage is taken in laboratory studies of the ability to study wound healing, irritancy, immunogenicity and safety evaluation of surgical materials, under strictly controlled conditions. Thus, experimental species of known genotype, age, microbiological and nutritional status are maintained under critical environmental conditions. This enables the elimination of variables which could complicate the interpretation of studies designed to identify the role of growth factors, cytokines and other modulators. A detailed analysis of lag phases, the onset of inflammatory changes, collagenesis etc. is also possible for evaluating the relative efficacy of new products.

Presently, when there are compelling reasons to reduce, refine and replace live animals in research, much use is made of cultured cell and tissue systems. Whilst these have provided much fundamental information on the nature and action of growth factors, the relevance and extrapolation of the information to an in vivo system is questionable. Factors in the external environment so necessary for maintaining normal homeostasis cannot be realistically reproduced in a test tube. Cultured epidermal cells (or keratinocytes as they are commonly but erroneously called) cannot be maintained in dynamic equilibrium with the environment and dermal tissues lack the opportunity to function and differentiate in relation to adjacent tissues (vascular channels, infiltrating cells). Growth factors exhibiting a mitogenic or modifying role in cultured cells may not have the same effect *in vivo!*

CONCLUSION

Wounds arising through accidental trauma, surgical incision, burning or other physical or chemical insult, undergo a well defied sequence of degradative, adaptive and morphogenic events leading to functional differentiation and restoration of the prewounding state. This involves a complex interaction between molecular and cellular factors, the interaction of which is only now becoming clearer through the availability of new investigative techniques and biotechnology. Whereas the progression of cellular events following skin damage has been appreciated for some time, recent research shedding light on the role of cytokines and growth factors opens up a range of opportunities for clinical treatment. This approach will be important, not only in the treatment of acute wounds but in improving our knowledge of the aetiology and treatment of long term and recurrent skin damage, and lesions prevalent in diseased conditions.

SUMMARY

Wound healing in the skin is a complex process involving inflammation, mitosis, angiogenesis and remodelling. New technology allowing the identification of growth factors, cytokine expression and cell surface receptors allows a greater appraisal of fine cellular and molecular events than was possible five years ago. A researcher aware of morphogenic events in the regeneration and remodelling process is allowed new insight into the chemotactic, biochemical and regulatory processes proceeding wound healing. Since the skin normally exists in dynamic equilibrium with its environment, an understanding of how environmental manipulation including the use of wound dressings influences conditions within the wound and hence the healing process is clinically important.

REFERENCES

1. Bennett, N.T. and Schultz, G.S. (1993) Am. J.Surg. 165, 728-737
2. Bennett, N.T. and Schultz G.S. (1993) AM.J. Surg 166, 74-81
3. Rosdy, M., Pisani, A. and Ortonne, J.P. (1993) Br.J. Derm. 129, 227-234
4. Lansdown, A. B. G. (1990) Proc. 7th Fed. Asian Vet. Ass. Cong., Pattaya, Thailand 563-578
5. Bullough, W. S. (1975) Biol. Rev. 50,99-127
6. Argyris, T. S. (1972) Am. Zool. 12, 137-149
7. kariniemi, A-L (1997) Acta. Derm. Venereol. 57, (Suppl.78), 7-26
8. Asmussen, P.D. and Sollner, B., eds (1993) in Wound Care: Principles of Wound Healing Beiersdorf Medical Bibliothek, Hamburg
9. Levenson, S. M. and Demetriou, A. A. (1992) in Wound Healing : Biochemical and clinical aspects (Cohen, I. K., Diegelmann, R. F. and Lindblad, W.J., eds), pp. 248-273, W.B. Saunders
10. Heng, M. C. V., Heng, H.L. and Allen, S. G. (1990) Clin. Exp. Dermatol. 15, 83-90
11. Lansdown, A. B. G. (1996) Bioloist 33, 253-260
12. Winter, G. D. and Scales, J. T. (1963) Nature 197, 91-92
13. Winter, G. D. (1962) Nature 193, 293-294
14. Grasso,P. and Lansdown, A. B. G. (1972) J. Soc. Cos. Chem. 23, 481-521
15. Lynch, S. R. (1991) in Clinical and Experimental Approaches to Dermal and Epidermal Repair; NOrmal and Chronic wounds *Barbul, A., Caldwell, M. D., Eaglestein, W. H., Hunt, T. K., Marshall, D., Pines, E. and Skower, G., eds), pp. 341-357, Wiley-liss
16. Pessa, M. E., Kirby, L. I. and Copeland, E. M. (1987) J. Surg. Res. 42, 207-217
17. Greenhalgh, D. E., Hummel, R.P., Albertson, S. and Breedon, M. P. (1993) Wound Rep. Reg. 1, 69-81
18. Doolittle, R. F. (19981) Sci. Am. 245, 126-135
19. Heng, M K ., Song, M.K. and Heng, M.C.Y. (1993) Br.J. Derm. 129,280-285
20. Martin, D. E., Reece, M. C. Maher, J. E. and Reece, A. C. (1988) Arch. Dermatol. 124, 226-229
21. Moncada, S., Needleman, P. and Bunting, s. (1976) Prostaglandins 12, 323-335
22. Kingsnorth, A. N. and Slavin, J. (1991) Br. J. Surg. 78, 1286-1290
23. Malcherek, P., Lindholm, C., Christensen, O. B. and Zederfeldt, B. (1993) Wound Repair and Regeneration 1, 63-68
24. Llioyd, D. H. (1990) in Veterinary Dermatology (Vol. 1) (von Tscharner, C. and Halliwell, R. E. W., eds), pp. 163-175, Balliere Tindall
25. Persinger, M. A., Lepage, P., Simard, J. P. and Parker, G. H. (1983) Br. J. Derm. 108, 179-187
26. Phillips, G. D., Aukerman, S. L., Whitehead, R. A. and Knighton, D.R. (1993) Wound Rep. Reg. 1,3-9
27. Viljanto, J. A. (1991) in Clinical and Experimental Approaches to Dermal and Epidermal Repair: normal and chronic wounds (Barbul, A., Caldwell, M. D., Eaglestein, W. H., Hunt, T. K., Marshall, D., Pines, E. and Skover, G., eds), pp. 279-290, Wiley-Liss
28. Uhl, E., Barker, J. H. Bondar, I., Galla, T. J., Leiderer, R., Lehr, H. A. and messmer, K. (1993) Br. J. Surg. 80, 977-980
29. Browder, W., Williams, D., Lucore, P., Pretus, H., Jones, E., MacNamee, R. (1988) Surgery 104, 224-230
30. Leibovich, S. J. and Ross, R. (1975) Am. J. Pathol. 78, 71-100

31. Cheqini, N., Rossi, M. J., Schultz, G. S., Dunn, W. A. and Masterson, B. J. (1993) Wound Rep. Reg. 1, 28-40
32. Gabbiani, G., (1992) Kidney International 41, 530-532
33. Krawczyk, W. S. (1971) J. Cell. Bio. 49, 247-263
34. Sarret, Y., Woodley, D. T., Grigsby, K., Wynn, K. and O'Keefe, E. J. (1992) J. Invest. Dermatol. 98, 12-16
35. Nanney L. B. (1990) J. Invest. Dermatol. 94, 624-629
36. Grando, S.A. (1993) J. Am Acad. Dermatol. 28, 981-992
37. Krummel. T. M., Ehrlich, P.H., Nelson, J. M., Michna, B. A., Thomas, B. L., Haynes, J. H., Cohen, I. K. and Diegelmann, R. F. (1993) Wound Rep. Reg. 1, 15-21
38. Shah, M., Foreman, D. M. and Ferguson, M. (1992) Lancet 339, 213-214
39. Fisher, L. B. and Maibach, H. I. (1972) Br. J. Derm. 86, 593
40. Argyris, T. S. (1981) C. R. C. Critical Rev. Toxicol. 9, 151-200
41. Newson, L. (1991) New Scient. (23 November) 132, 50-53
42. Knighton, D. R., Silver, I. A. and Hunt, T. K. (1981) Surgery 90, 262-270
43. Lansdown A. B. G. (1993) Int. J. Cos. Sci. 15, 83-85
44. Morgan, D. A. (1992) in Formulary of Wound Management Products, a guide for health care staff (5th Edn), Materia Medeca Publications
45. Varghese, M. C., Balin, A. K., Carter, M. and Caldwell, D. (1986) Arch. Dermatol. 122, 52-57
46. Katz, S., McGinley, K. and Leyden, J. J. (1986) Arch Dermatol. 122,58-62
47. Niedner, R. and Eschopf, E. (1986) Br. J. Derm. 115, (Suppl. 31), 41-44
48. Jonkman, M. F., Hoeksma, E.A. and Nieuwenhuis, P. (1990) J. Invest. Dermatol. 94, 477-484