Larval intervention in the chronic wound

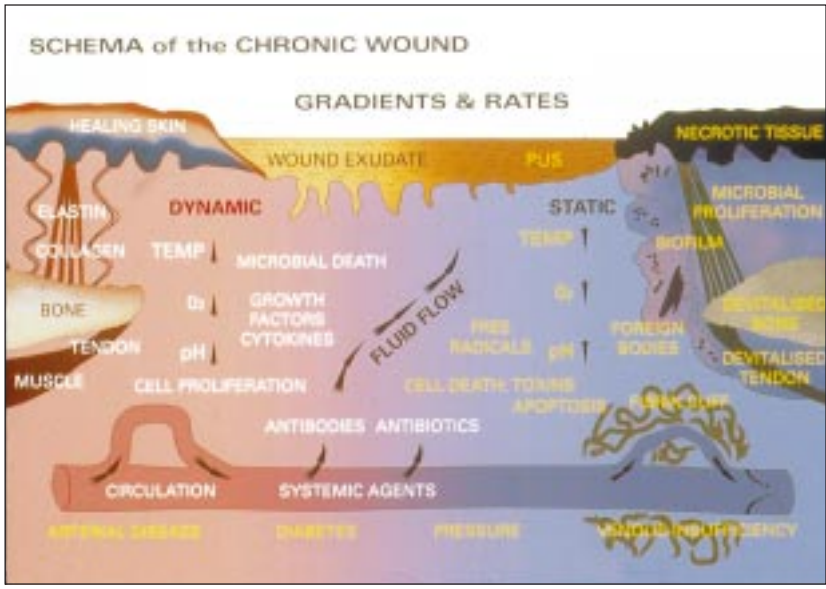

Figure 1.
Schema for Chronic Wound.

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SUMMARY
A schema is presented, studying the nature of the chronic wound, and the effects on it of larval intervention, in clinical practice.

INTRODUCTION
Normal primary wound repair is complex, but under optimal conditions proceeds rapidly to healing, by 'first intention'. The chronic wound is more complex. The potential for healing remains, but is hindered or arrested, by general or local factors, so that a 'stale mate' situation develops.

Lindblad summarises the wound environment thus: It is '... an extremely complex assemblage of cells, soluble mediators, extra-cellular matrix molecules, and lipids, which does not lend itself to refined analysis'. The question is then posed; 'Given this level of intricacy, how should clinicians and researchers reduce the complexity in order to develop a deeper understanding of the processes in repair? One can use the reductionist approach ... or one can use a more holistic approach and attempt to keep the system intact ...' [1]

The introduction of intact living organisms – larvae – into wounds represents a holistic approach. Their efficacy, as agents of debridement and bacterial attack, is now well established [2-5]. These larvae however, bring their own complex biology into the wound environment, further complicating the picture.

THE SCHEMA
Fig. 1 is a schematic overview, depicting some features of the chronic wound. *Fig. 2* amplifies this, adding further features, with the intervention of larval feeding. This provides a basic bio-physio-pathological 'scaffolding', within which must be set the complex, and often rapidly changing, interplay of a wide range of cellular activities, and micro-environmental factors.

The host healing mechanisms are depicted on the left hand side of the diagrams, the non-healing on the right, with a complex 'no man's land' in the centre. All type-face in white denotes positive factors, that in yellow depicts negative factors.

Dynamism – Stasis. There is a vigorous biological dynamism in the healing acute wound, with some cell multiplication rates exceeding those in a tumour mass. The cell types involved exhibit a range of dynamism, from amoeboid motility, as in phagocytic white cells, to contraction as in the myofibroblasts, and migration along extra cellular matrix planes, as exhibited by epithelial cells.

By contrast, necrotic and decomposing organic material is relatively inert and static. The only dynamism there is that of the contained organisms. Bacteria exhibit their own range of dynamism, from the relatively static, within the 'ghettos' of the sessile bio-film, to the mobility, both active and passive, of their planktonic forms.

Within a chronic wound there is an interface between the living tissues with their dynamism, and the inert dead tissue. This may be clear-cut, with an abrupt transition from the living to the necrotic, often dessicated, tissue. But more usually

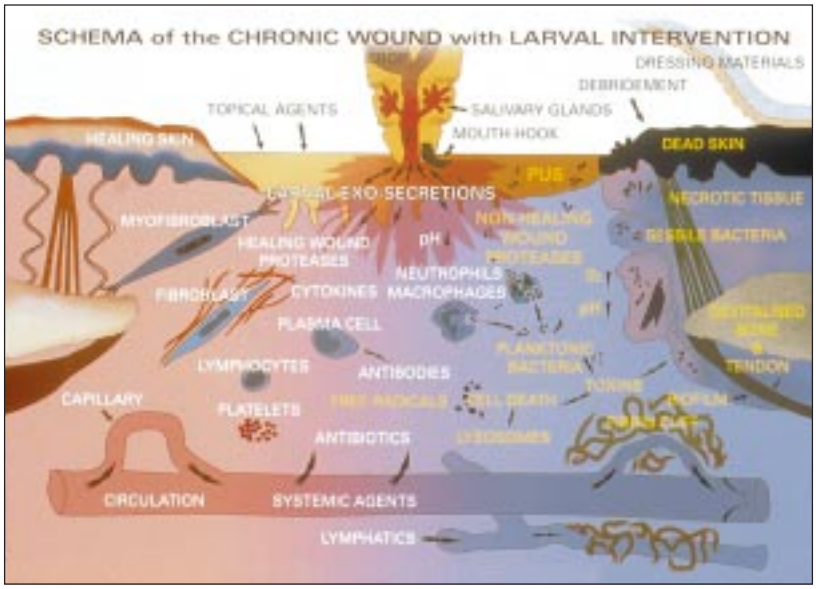

Figure 2.
Schema of the Chronic Wound with Larval Intervention.

the interface is a confused 'no-man's-land', often appearing yellow in a wound, exposed to all the variations of the different factors being considered here. This is extremely difficult to evaluate, whether by biopsy, or by biochemical and microbiological means, let alone on clinical grounds. So there is a '*dynamism gradient*', from active host cells, down to damaged and then dead host cells and bacteria, then back up into that of planktonic and sessile bacteria, in their respective environments.

Cellular turnover. There is a wide range of cell turnover, even under 'normal' physiological conditions, with 'active death' – apoptosis – of most cell types that retain the capability of mitosis. Injury and infection create situations where high cell proliferation is vital, particularly of the inflammatory and connective tissue cell lines. But adverse conditions in the wound environment, such as bacterial toxins and free radicals, will lead to higher death rates, particularly of white cells. These dead cells then become the main constituent of pus.

GRADIENTS

There are a number of gradients in the chronic wound:

- Temperature. Normal skin, particularly at the extremities, is cooler than the body core temperature, in most climates. Wound temperature depends on a number of factors; its blood supply, whether it is exposed or covered, whether the part is artificially heated or not. These factors can cause significant gradients of temperature. Necrotic tissue is 'cold', and will remain more or less at ambient temperature.
- Oxygen. Skin repair is very demanding of Oxygen. The oxygen supply to the chronic wound essentially comes from two sources, the circulation and the atmosphere. An obvious oxygen gradient exists from the active circulation to ischaemic areas of the wound. But the micro-environmental dynamics of oxygen distribution are more difficult to define. Aerophilic, micro-aerophilic, even anaerobic organisms, can co-exist, particularly when protected by bio-film. Intermittent treatment with hyperbaric oxygen will skew these gradients.
- pH. pH gradients are ubiquitous; within the living cell, across cell membranes, within the dying cell, across the base of the chronic wound, within the wound exudate. The pH powerfully affects all enzymic activity, whether host derived or 'parasite' derived. In the chronic wound the pH is characteristically low, lowest where there is necrosis, and 'normal' where there is viable host cellular activity.

RATES

- Fluid dynamics. The most obvious dynamic in a chronic wound is the fluid outflow. This is generated by efflux, whether from healthy or damaged capillaries, in the wound base. It is contributed to by tissue fluid pressure, which may be considerably raised in dependant extremities or various general or local circulatory disorders, especially those resulting in venous and/or lymphatic obstruction. Wound exudate may be serous, copious, and largely acellular, or may be thick glutinous pus, with a heavy content of dead cells and tissue debris. Much depends on the size of the wound, the position of the affected part, and constraints provided by dressing materials, especially those that are relatively impermeable, or allow control by vacuum drainage of the wound.
- Micro-organisms. These are found in two states; sessile, relatively static within their 'ghettos' in the bio-film; and planktonic, mobile and free. Most of

these organisms are invaders, from the patient's skin or mouth, or from the environment. In the sessile 'ghetto', organisms are relatively protected from the host's defence mechanisms. Cells and antibodies can not penetrate the bio-film. Neither can pharmaceutical agents such as antibiotics. Bacterial population dynamics depend on the immediate micro-environment. Planktonic organisms can proliferate exponentially, sessile organisms less dramatically.

Chronic wounds are routinely covered by dressing materials. They are generally absorbant. They can however provide an environment for bacterial proliferation, with absorbed wound exudate as substrate, distanced from host cellular attack.

- Metabolites. Active host cells elaborate metabolites which diffuse into the surrounding tissue. Their diffusion rates are affected by size, degree of ionisation, and the nature of the local extra-cellular matrix. They will also be subservient to the tissue fluid dynamics, outlined above. A vital group, the 'growth factors' and 'cytokines', must encounter their target cells, in adequate concentration, to become effective.
- Extra-cellular matrix (ECM) This is elaborated by host cells which generally remain within it, or on it. The essential ingredients are muco-polysaccharides, with fibrous components such as collagen, elastin, fibrin, and laminins, fibronectins, and other components of basement membranes, and 'ground substance'. The ECM in normal tissue is structured and ordered, optimally controlling the location and migratrion of cell populations within it. The ECM elaborated by wound healing cells, is also structured and ordered, but in a more random fashion than normal tissue. By contrast, the ECM in a chronic wound where there is infection and cell death, is disordered, and 'chaotic'. The complex fibrin cuffs formed in chronic venous ulcers are an example of this.

  Sessile bacteria elaborate an alien ECM, their protective bio-film, normally firmly attached to any ischaemic or foreign material. The composition and nature of this ECM is presumably distinctive, and specific to each species forming it.

Clinical application of *Lucilia sericata* larvae. When larvae are introduced into a wound, their active intervention causes a whole array of further biological changes. These can be considered in the context of the environmental factors already summarised above.

Oxygen. Larvae are air-breathing, mostly through their posterior spiracles, which must thus be clear of fluid surfaces. They are semi-aquatic feeders, with their mouthparts immersed. They can survive for periods under water, or in other adverse conditions, though they will not then feed. Larval activity seems to 'improve oxygen supply within granulation tissue', as shown by monitoring wound granulation tissue with remittance spectroscopy [6].

pH. Larval feeding activity, whether in a wound or otherwise, creates a relatively alkaline medium. Their feeding is optimal once this level of alkalinity is reached. Despite this however, they actively migrate into, and feed in, those parts of a wound which contain necrotic and decomposing organic material. Thus they are, at least initially, exposed to a low pH.

Temperature. Larval metabolism, as with most insect biology, is temperature dependent. In their third instar phase they characteristically exhibit a group feeding behaviour, closely packed, head down and tail up. They thus generate an increase in local temperature, accelerating their enzyme production, and with it their feeding rates.

Attractants and Repellents. Larvae are photophobic. This is an advantage clinically, in that it drives them in under necrotic over overhanging tissue, to deeper levels of the wound. They are attracted to decomposing material within chronic wounds, probably by biochemical signals, which may also arise from bio-film and the micro-organisms which elaborate it. Equally, they are repelled by some biochemical agents. Propylene glycol, a stabilising agent in hydrogels, inhibits maggot growth [7]. Pharmaceutical drugs, whether administered topically or systemically, or their breakdown products, will be present in the wound. Some of these, such as cytotoxic drugs, must be deleterious to larvae in a wound.

Dynamism. When larvae are placed in a wound they are undoubtedly the most dynamic agents in it, not only in their rapid physical migration, but also in the abrading feeding behaviour, using their oral hooks to draw food particles towards them. Their feeding action can be compared to a 'carpet shampoo', with the rapid and forceful ejection of exo-secretions from their salivary glands, to the equally rapid and piston-like action of their mouthparts, swallowing largely liquidised food. This is passed on to the crop, situated at the junction of the foregut and midgut. Being semi-transparent, it is clinically possible to moni-

tor larval feeding status, with the normally brown-stained contents indicating the crop size.

When larvae are fully developed, at the end of the third instar, they stop feeding. They will then actively attempt to leave the wound area, seeking a suitable environment for pupation. This need never happen clinically, as they should be removed from the wound before they reach the pre-pupal stage.

Larval death. In clinical practice larval numbers often decline whilst they are in the wound. This can be due to adverse local physical factors, such as pressure, or toxic agents in the wound secretions. Specific dressing materials are used to confine the larvae, but the wound may then become swamped with exudate, or conversely become dessicated.

Debridement. The most obvious activity that larvae exhibit in a human wound is the relatively rapid atraumatic physical removal of necrotic or semi-decomposed material. Once this is disposed of, a number of gradients that are adversely affected by the continuing presence of necrotic material are altered for the better. Clinical observation would indicate that the chronic wound reverts to a more acute state.

Antimicrobial activity. Larval activity in a human wound regularly leads to the reduction of bacterial numbers. Larval exo-enzymes not only inhibit the production of bio-film, but also actively strip established bio-film, releasing the contained organisms [8]. These would thereby be exposed not only to the further direct action of these enzymes, but also to other anti-microbial factors in the wound, such as host scavenger cells, antibodies and antibiotics.

It has been shown that certain organisms are destroyed in the gut tube of these larvae [9]. Recent studies on the effect of *Lucilia sericata* larvae on bacteria and yeast isolated from wounds, have demonstrated that all wound organisms tested were 'totally cleansed', with the exception of Pseudomonas aeruginosa cultures. These were apparently unaffected by larvae, and the larvae died. Antimicrobials used in these tests had no effect on the larvae. [10].

Larval exo-enzyme penetration into host tissues. Whilst larval feeding is generally directed at decomposing material, the feeding process must lead to some exo-secretion penetration of surrounding wound tissues. Growth factors and cytokines of larval origin presumably enter the wound with the other exo-secretions. Being smaller molecules, these would penetrate further, and thus could have direct or indirect effects on host cells. The universal clinical observation, that after successful larval debridement, the wound granulation tissue is exuberant and healthy, would bespeak some cytokine 'kick-starting' of host cell populations. The efflux of wound secretions would tend to oppose the penetration of active agents being elaborated by the larvae.

Host immunological responses presumably occur following larval 'challenges' in the wound. Little is known however of the extent of these host responses, or whether they might lead to hypersensitivity in some patients. No serious clinical adverse immunological responses have yet been reported.

The enhancement of larval activity in the human wound. With advance in our understanding of the micro-environment of the chronic wound, and in turn how this is affected by the intervention of larvae, it will be possible to develop clinical strategies to enhance the activity of the larvae whilst they are in the wound. This presents an on-going challenge, but can only result in improved clinical results, and a development of the range of indications for, and the art, of larva therapy, in both acute and chronic wounds, as much in Western society as in the Tropics.

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