

## CHAPTER 1

### MODE OF ACTION

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#### Introduction

Honey is a substance produced by bees to store as a sugar food source collected as nectar from flowers (and occasionally from the sap of plants). Since the earliest recorded times, humans have taken this honey for use, not only as a food product, but also as a medicine, especially for wound care (Zumla and Lulat, 1989). The bees concentrate the dilute sugar solutions they collect from the plants by evaporating off most of the water. (Honey is typically 17% water, 80% sugars.) They also add enzymes, so that as the honey ripens in the comb its composition changes and it becomes impossible for microbes to grow in it and spoil the stored food. One of these enzymes converts sucrose, the major sugar in nectar and sap, into a more soluble mixture of glucose and fructose. This makes the saturated or supersaturated solution of sugars that is stored as honey. (The difference in texture between liquid honey and solid or 'creamed' honey is due to fine crystals of solidified glucose being suspended in the saturated syrup.) The sugar molecules in solution bind up water molecules, thereby denying microbes the water that is essential for their survival. Another enzyme added is glucose oxidase. This converts some of the glucose to gluconic acid, making honey too acidic for microbes to grow (honey has a pH of about 3.5) and, as a by-product of this reaction, forms hydrogen peroxide. This is a sporicidal antiseptic that sterilises the honey that is sealed in the comb. (When subsequently extracted from the comb, honey can be contaminated with microbial spores as the enzyme that produces hydrogen peroxide is inactive at that time because there is insufficient free water. These can survive the acidity and high sugar content.) These factors, which ensure the preservation of honey in the comb, are also useful in suppressing microbial growth when honey is applied to a wound.

Additionally, there are 'herbal' factors present which may be of benefit in wound care. Being the concentrated juice from plants, honey contains the various nutrients and herbal chemicals that come from the plants, such as: amino acids, other organic acids, enzymes, vitamins, acetylcholine, flavonoids, carotenoids, polyphenols, minerals and a wide variety of organic chemicals in trace quantities. These are what give different honeys their characteristic colours, flavours and aromas. The colour of honey is also due to products of the Maillard reaction<sup>1</sup> and caramelisation of its sugars, hence it gets darker in colour as it ages. Some of the plant-derived chemicals have antioxidant properties and some are known to have antibacterial properties.

It is for topical treatment of infections that honey has been most widely used as a medicine throughout history, and now that its use as a wound dressing has been 'rediscovered' by the medical profession, it is on infected wounds that it is mostly being used. The clinical definition of infection is that the area of the wound is showing the classical signs of inflammation (redness, swelling and pain). The clinical focus in treating wounds is to remove the cause of the inflammation by killing the infecting bacteria and removing any pus or dead tissue that provide a medium for their growth. Inflammation in a wound causes many problems, such as making the wound uncomfortable and difficult to manage, but the major problem is that it prevents the tissue repair processes from healing the wound. The various bioactivities of honey work through all of these facets to give rapid healing — honey rapidly debrides wounds (ie. cleans the wound by releasing pus or dead tissue), kills bacteria, directly suppresses inflammation, and stimulates the growth of the various types of cells involved in the production of new tissue to repair the wound.

## **The scourge of excessive inflammation**

Inflammation is a vital part of the normal response to infection or injury, and is what starts the healing process. It normally lasts for one day but, if it is prolonged or if the inflammatory response is excessive in intensity, it can prevent healing or even cause further damage to tissues. Prolonged inflammation, at a level below that which causes damage, is the cause

1. Maillard reaction: one of a group of non-enzymatic reactions in which sugars react with amino acids, peptides or proteins to produce a brown colour in honey.

of fibrosis, seen as hypertrophic scarring in wounds. Inflammation in wounds also causes discomfort for patients and problems in dressing the wounds, because of the large amounts of exudate associated with it.

The prostaglandins are part of the inflammatory response. They give rise to pain and cause small arteries to open, increasing blood pressure locally and giving rise to oedema in the area of the wound, and exudation of plasma from the wound. Suppression of inflammation, as well as reduction of pain, reduces the oedema and exudate. The pressure building up in tissues from oedema can slow the healing process, as it restricts the flow of blood through the capillaries (Chant, 1999), thus starving the tissues of the oxygen and nutrients that are vital for leukocytes to fight infection and for fibroblasts to multiply for wound healing. The swelling also increases the distance for diffusion of oxygen and nutrients from the capillaries to the cells (Sinclair and Ryan, 1994).

The inflammatory response is initiated by the reaction of leukocytes when in contact with substances from bacterial cells, or to tissue factors released when there is physical damage. (In the case of burn injuries, there are large quantities of these tissue factors released, which accounts for the severe inflammation associated with burns.) Phagocytes that are activated as part of the initial inflammatory response produce hydrogen peroxide to destroy the bacteria and debris that they engulf. Some of this hydrogen peroxide leaks out of the cells, which serves to give a feed-back amplification of the inflammatory response, as the hydrogen peroxide attracts and stimulates other leucocytes to proliferate (Flohé *et al*, 1985). If this continues unchecked, the feed-back amplification can result in a vicious cycle that gives excessive levels of inflammation. Hydrogen peroxide was once widely used as an antiseptic, but has gone out of favour because it causes this inflammation. Although the hydrogen peroxide produced in honey may have the potential to cause inflammation, it is produced at very low levels, equivalent to about one thousandth of that in the 3% solution of hydrogen peroxide used as an antiseptic (Bang *et al*, 2003). However, there is also protection via the anti-inflammatory properties of honey (see below).

The hydrogen peroxide released by phagocytes can also be converted to reactive oxygen species (free radicals) in the tissues (Flohé *et al*, 1985). These free radicals are very reactive and can break down proteins, nucleic acids and cell membrane lipids, thus damaging or destroying tissue. Far greater damage to tissues results from the activation of proteases in the wound tissues by reactive oxygen species (Weiss *et al*, 1985; Ossanna *et al*, 1986; Peppin and Weiss, 1986). These protein-digesting enzymes are normally present in an inactive form (in the case of the matrix

metalloproteases), or are kept inactive by the presence of an inhibitor (in the case of the neutrophil serine protease). But, once activated, can destroy wound tissue. Thus, a wound can become ulcerated and a partial-thickness burn can become full-thickness. These activated proteases also have the potential to destroy the tissue growth factors which, being proteins, are essential for activation of the repair process.

In the case of reperfusion injury, hydrogen peroxide produced by a biochemical process is the initiator of the inflammatory response and the ongoing vicious cycle. When tissues are deprived of oxygen through obstruction of circulation, production of xanthine and alteration of the enzyme xanthine dehydrogenase to catalyse an oxidase type of reaction occurs. When the circulation is subsequently restored and the tissue is reperfused, the oxygen now available is used by the enzyme to oxidise xanthine, setting up production of hydrogen peroxide and reactive oxygen species (Bostek, 1989). This mechanism of initiation accounts for the inflammation in pressure ulcers (where circulation is cut off by pressure on the tissues then restored by relief of the pressure), and in varicose ulcers (where circulation is restricted by venous stasis then restored by elevating the legs), with the subsequent activation of proteases by the inflammatory reaction contributing to ulceration of the tissue.

Where the inflammation is less severe and insufficient to give erosion of tissue by activation of wound proteases, it can give excessive activation of fibroblasts, causing fibrosis, hypertrophic scarring and contractures. (Fibroblasts are the precursors of muscle cells, and use the same contractile fibres as muscle cells to pull the edges of a wound together. They also produce collagen fibres which form scar tissue.)

Although excessive or prolonged inflammation is a major problem in wounds, pharmaceutical anti-inflammatory substances are not generally used in wound treatment because they can impair healing through adverse effects on proliferating cells. Honey, however, has a potent anti-inflammatory action that not only has no adverse effects on the growth of cells, but actually gives a positive stimulation of their growth.

## **The anti-inflammatory properties of honey**

Many clinical observations have been reported of reduced symptoms of inflammation when honey is applied to wounds (Burlando, 1978; Dumronglert, 1983; Efem, 1993; Hejase *et al*, 1996; Subrahmanyam,

1996; Subrahmanyam, 1998), and of it having a soothing effect when applied to wounds (Burlando, 1978; Keast-Butler, 1980; Subrahmanyam, 1993) and burns (Burlando, 1978; Subrahmanyam, 1993). The (reported) reduction of exudate in wounds dressed with honey is a great help when managing inflamed wounds (Burlando, 1978; Efem, 1993; Hejase *et al*, 1996; Al-Waili and Saloom, 1999; Betts and Molan, 2001; Alcaraz and Kelly, 2002; Ahmed *et al*, 2003). The anti-inflammatory action of honey is also seen in the reports of reducing scarring (Subrahmanyam, 1991; Efem, 1993; Subrahmanyam, 1994; Al-Waili and Saloom, 1999; Dunford *et al*, 2000a, b) and contractures (Subrahmanyam *et al*, 2001). As well as these clinical observations, it has been demonstrated in animal models that honey gives reduced inflammation compared with various controls; histological studies finding reduced numbers of inflammatory cells present in deep (Postmes *et al*, 1997) and superficial (Burlando, 1978) burns and in full-thickness wounds (Gupta *et al*, 1992; Kumar *et al*, 1993; Oryan and Zaker, 1998). These effects are due to components other than the sugar in honey (Burlando, 1978; Postmes *et al*, 1997). Similar evidence has also come from a study of biopsy samples from burn wound tissue of hospital patients (Subrahmanyam, 1998). The anti-inflammatory action of honey is not just a consequence of removing the stimulus for inflammation by clearing infection and debriding the wound, as has been observed in experimental wounds in which there were few or no bacteria present (Burlando, 1978; Kandil *et al*, 1987; El-Banby *et al*, 1989; Gupta *et al*, 1992; Kumar *et al*, 1993; Postmes *et al*, 1997; Oryan and Zaker, 1998). There has also been a direct demonstration of the anti-inflammatory properties of honey in a standard test for anti-inflammatory agents, where it decreased the stiffness of inflamed wrist joints of guinea pigs (Church, 1954). It has also been reported that, when given orally, honey lowers plasma prostaglandin concentrations in normal individuals (Al-Waili and Boni, 2003).

The component of honey responsible for its anti-inflammatory activity has not been identified, but it may be due to the antioxidant activity of honey. There are significant levels of antioxidants in honey (Frankel *et al*, 1998; Gheldof and Engeseth, 2002; Gheldof *et al*, 2002; Gheldof *et al*, 2003; Schramm *et al*, 2003), including some which complex with iron to stop it catalysing the Fenton reaction<sup>2</sup>. This reaction forms free radicals from hydrogen peroxide (Bunting,

2. Fenton reaction: the formation of free radicals from the non-enzymatic reaction of iron ( $\text{Fe}^{2+}$ ) with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ); a reaction of importance in the oxidative stress in blood cells and various tissues.

2001); these free radicals serve to recruit more leukocytes into areas of inflammation, as a self-amplification of the inflammatory response (Flohé *et al*, 1985). The mechanism of this self-amplification of the inflammatory response is oxidative activation of the nuclear transcription factor NF- $\kappa$ B, which then promotes the production of pro-inflammatory cytokines by leukocytes (Gimble, 1994) and stimulate the activity of the fibroblasts, thus giving hypergranulation and fibrosis (Murrell *et al*, 1990). It is the free radicals formed from hydrogen peroxide, rather than hydrogen peroxide itself, that are responsible for the activation of the transcription factor NF- $\kappa$ B (Schreck *et al*, 1991), and this activation can be prevented by antioxidants (Gimble, 1994). A study carried out on burn wounds has shown that application of antioxidants to mop up free radicals reduces inflammation (Tanaka *et al*, 1995). In a clinical trial it was found that honey dressings prevented partial-thickness burns from converting to full-thickness burns which would have needed plastic surgery (Subrahmanyam, 1998).

## Clearance of infection

Applying honey dressings to wounds has been reported to:

- clear infection rapidly (Cavanagh *et al*, 1970; Armon, 1980; Braniki, 1981; Phuapradit and Saropala, 1992; Efem, 1993; Anoukoum *et al*, 1998; Robson *et al*, 2000; Betts and Molan, 2001; Kingsley, 2001; Subrahmanyam *et al*, 2001; Alcaraz and Kelly, 2002)
- heal deeply infected surgical wounds (Cavanagh *et al*, 1970; Armon, 1980; Bergman *et al*, 1983; McInerney, 1990; Phuapradit and Saropala, 1992; Vardi *et al*, 1998; Al-Waili and Saloom, 1999; Cooper *et al*, 2001)
- halt advancing necrotising fasciitis (Efem, 1993; Hejase *et al*, 1996).

Wounds not responding to conventional therapy with antibiotics and antiseptics have been healed by application of honey dressings (Efem, 1993; Harris, 1994; Wood *et al*, 1997; Vardi *et al*, 1998; Dunford *et al*, 2000a, b; Cooper *et al*, 2001), including wounds infected with methicillin-resistant *Staphylococcus aureus* (MRSA) (Dunford *et al*, 2000a; Natarajan *et al*, 2001), *Pseudomonas aeruginosa* (Dunford *et al*, 2000b) and other bacteria resistant to antibiotics (Al-Waili and Saloom, 1999).

The laboratory evidence for the potent broad-spectrum antimicrobial activity of honey is covered in *Chapter 2*, as is the stimulatory action of honey on leukocytes — another mechanism by which honey may work to clear infection from wounds. There is no clear evidence of the ability of the antibacterial activity of honey to diffuse down into wound tissue when applied as a wound dressing. But, the suppression by honey of growth of any bacteria already present on the surface of the wound means that there is not the problem of malodorous dressings when hydrocolloid dressings are used. Being a source of toxins and pyrogens, honey also removes the problem of bacteria growing on the wound surface. However, the rapid clearance of a deep-seated infection (Cooper *et al*, 2001) and of boils with unbroken skin (Betts and Molan, 2002) by topical application of honey indicates that the antibacterial activity of honey probably does diffuse through skin. If this is so, it is important to use a honey with a high level of antibacterial activity (*Chapter 2*) to achieve an effective level of antibacterial activity below the surface. When diffusion occurs, there is a gradient formed of decreasing concentration from the source (in this case, the dressing on the surface of the wound). As illustrated in *Figure 1.1*, the minimum concentration of antibacterial component that will stop bacterial growth will be deeper down in the wound tissue if the source has a higher concentration. It is also important to keep a substantial quantity of honey on the surface (eg. by using a dressing pad soaked with honey), so that the concentration of antibacterial activity on the surface does not become low through dilution by exudate, or depletion by diffusion into the underlying tissue.

## Deodorising action

Honey rapidly deodorises wounds (McInerney, 1990; Subrahmanyam, 1991; Phuapradit and Saropala, 1992; Efem, 1993; Subrahmanyam, 1993; Hejase *et al*, 1996; Subrahmanyam, 1996; Dunford *et al*, 2000a, b; Kingsley, 2001; Alcaraz and Kelly, 2002; Stewart, 2002; Ahmed *et al*, 2003). On fungating (malignant) wounds where no other treatment could control the malodour, dressing the wound with honey was found to remove the malodour within twenty-four hours (Julie Betts, Waikato Hospital: personal communication). Honey is now being used routinely at Waikato Hospital on fungating wounds, not only for odour control, but also because it reduces the inflammation and level of exudate that is



a common problem with this type of wound.

The rapid deodorising of wounds from honey dressings is probably due to more than just antibacterial action. The malodorous substances that bacteria produce in wounds, such as, ammonia, amines and sulphur compounds, are formed from the metabolism of amino acids derived from decomposed serum and tissue proteins. Bacteria metabolise glucose in preference to amino acids, thus, in the presence of honey (composition of 30%–40%), the malodorous compounds are not formed (Nychas *et al*, 1988).

## Barrier function

The high viscosity of honey provides a physical barrier to infection of wounds from external contamination, the effectiveness of which is increased by the antibacterial activity of the honey (as long as the honey used is selected to have good antibacterial activity). This feature is particularly useful where it is preferable to avoid occluding highly exudative wounds, such as burn wounds, and thus encourage growth of bacteria, particularly *Pseudomonas spp*, in the moist conditions created. Prophylactic use of honey dressings has been found to solve a problem of skin grafts frequently becoming infected with *Pseudomonas spp* (Robson, 2000). This raises the suggestion of using honey dressings routinely on surgical wounds to protect at-risk patients from acquiring nosocomial infection with MRSA — the demonstrated sensitivity of MRSA to honey (*Chapter 2*) and the reports of honey dressings healing wounds already infected with MRSA (Dunford *et al*, 2000a; Natarajan *et al*, 2001) indicate that it is likely to be effective as a prophylactic treatment. It is also likely to be effective as a prophylactic treatment for the other major route of nosocomial infection with MRSA, ie. sites where medical devices penetrate the skin, as indicated by the favourable results reported from a trial conducted on central vein catheter exit sites (Mutjaba Quadri, 1999).



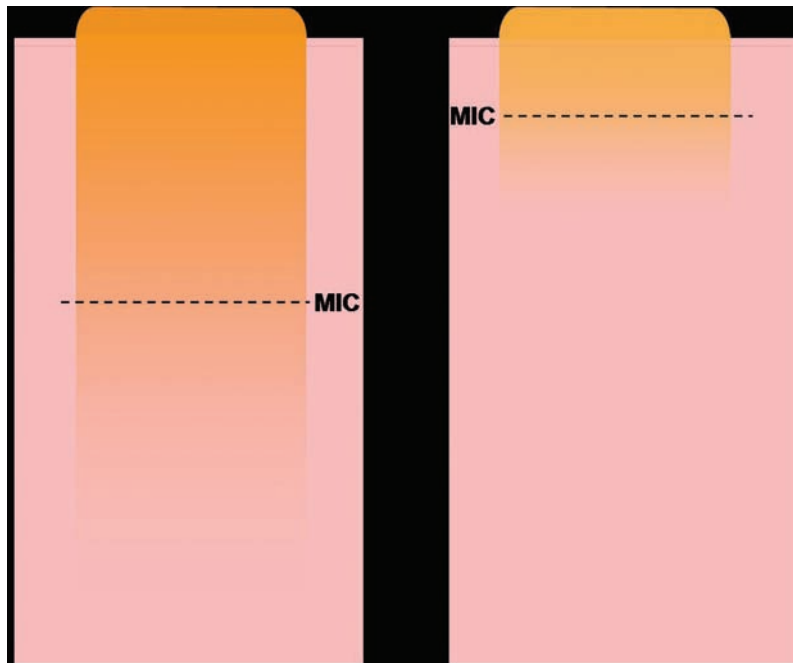


Figure 1.1: Illustration of concentration gradients set up by diffusion into underlying tissue of antibacterial activity of honey from high-activity and low-activity honey on the surface of the skin. 'MIC' shows the position on the gradients of the minimum inhibitory concentration of the antibacterial activity (ie. the minimum concentration that will stop bacterial growth). Thus, the functional components of a high activity honey will diffuse deeper into the tissues and have a greater range of antibacterial activity

## Provision of the optimum moist healing environment

Honey dressings on wounds provide a moist healing environment: a feature sought in most modern wound dressings to obtain conditions necessary for optimum growth of the cells involved in the repair process. But, unlike other dressings, honey does this without the associated problems of encouraging microbial growth and maceration of surrounding skin that can result from the moist conditions. The antibacterial activity of honey prevents bacterial growth without any cytotoxic effects which

could otherwise slow healing; and the osmotic action of its high sugar content will tend to draw fluid out from skin rather than let it soak in, even if honey gets diluted by large amounts of exudate. This means that there is no need to trim honey dressings to the shape of the wound bed or to use protective coverings on peri-wound skin, and there is no restriction on using occlusive secondary dressings. It has been suggested that the osmotic effect would dehydrate wound tissues but, in a paper on the use of sugar as a wound dressing, it has been pointed out that where there is a circulation of blood underneath to replace fluid lost from cells, the osmotic effect of sugar on the surface just creates an outflow of fluid (Chirife *et al*, 1982).

Also, unlike other dressings, honey dressings are easy to remove (Blomfield, 1973; Wadi *et al*, 1987; Farouk *et al*, 1988; Dunford *et al*, 2000a, b; Robson *et al*, 2000; Betts and Molan, 2001; Cooper *et al*, 2001; Alcaraz and Kelly, 2002; Richards, 2002; Stewart, 2002; Misirlioglu *et al*, 2003), causing no pain on changing dressings (Bulman, 1955; McInerney, 1990; Weheida *et al*, 1991; Subrahmanyam, 1993; Subrahmanyam, 1998; Dunford and Hanano, 2004). This is because the outflow of fluid from the wound bed created by the osmotic action of the honey forms a layer of diluted honey in contact with the wound bed. This fluid interface allows the dressings to be lifted off painlessly, preventing the tearing away of newly regenerated tissue which can happen when an adherent wound dressing is pulled off.

## Debriding action

There are many reports that wounds dressed with honey are rapidly debrided to give a clean granulating wound bed (Cavanagh *et al*, 1970; Armon, 1980; Braniki, 1981; Efem, 1993; Subrahmanyam, 1998; Dunford *et al*, 2000b; Alcaraz and Kelly, 2002; Ahmed *et al*, 2003), slough and necrotic tissue painlessly lifting off (Subrahmanyam, 1991; Efem, 1993; Subrahmanyam, 1993; Hejase *et al*, 1996; Subrahmanyam, 1996). Although induction of autolytic debridement is a feature of all wound dressings that give a moist environment, the debriding action of honey is faster than with other dressings and, on sloughy wounds, is about as rapid as that experienced using maggots (Julie Betts, Waikato Hospital: personal communication). Where there is hard eschar, maggot therapy provides a faster method of debridement as the maggots appear

to burrow under the edges of the eschar, but honey dressings will remove hard eschar, especially if this is scored to aid penetration of the honey, and if the honey is diluted with water or saline so that it softens the eschar. Furthermore, using honey instead of maggots, removes the problems of obtaining a supply of freshly prepared material, of ensuring that the wound is not too wet (causing drowning) or too dry (killing by dehydration), or of keeping the maggots in place, and of overcoming patients' unease with the squirming movement of the maggots.

The debriding action of maggots is due to the proteolytic enzyme activity they secrete, but with honey there does not appear to be a direct proteolytic activity involved: the existence of protein-digesting enzyme activity in honey has not been reported. Therefore, there must be a mechanism by which honey activates dormant proteolytic enzyme activity within the wound tissue, but in a controlled way so as not to cause unwanted digestion of the tissue. There is a strong association between high protease activity and impaired wound healing, and dressings are now being produced that inhibit or bind up and inactivate excessive protease activity in wounds that would otherwise be digesting wound tissue (Edwards *et al*, 2001; Cullen *et al*, 2002; Edwards *et al*, 2004). As mentioned earlier, the tissue-digesting collagenase and elastase enzyme activity in wounds is activated by oxidation. The antioxidant activity of honey can be expected to suppress this, just as the anti-inflammatory activity suppresses the infiltration of elastase-secreting neutrophils. This decreases the amount of enzyme released and, subsequently, the activity of that enzyme. The most likely explanation of the debriding activity induced by honey is that it promotes conversion of inactive plasminogen in the wound matrix to the active form, plasmin. This is an enzyme that functions to break down fibrin clots which attach slough and eschar to the wound bed. This action of honey could also be attributed to its anti-inflammatory activity, as inflammation causes inhibition of fibrinolysis by elevating the level of PAI-1 (plasminogen activator inhibitor 1) and thus preventing plasminogen from being converted to the active enzyme plasmin (Esmon, 2004). Fibrin is very common in chronic wounds (Schultz *et al*, 2003). (The enzyme streptokinase that is often used to debride wounds functions by activating plasminogen.)

The osmotic action of honey may also assist, as drawing out lymph from the wound tissues gives a constantly replenished supply of plasminogen at the interface of the wound bed and the overlying slough. Another advantage of this osmotic action of honey is that it washes the surface of the wound bed from beneath. This would account for the long-

known feature of honey dressings to remove dirt with the dressing (Zaiß, 1934), making such dressings an excellent way of cleaning up grazes in which grit has become embedded.

## Stimulation of healing

Wounds dressed with honey often have a rapid rate of healing. Honey is also able to start the healing process in dormant wounds. Honey has been reported to promote the formation of granulation tissue and to stimulate the growth of epithelium over wounds. Clinical evidence of the use of honey on wounds is reviewed in *Chapter 9*. It has also been reported that honey is a reliable alternative to conventional dressing for the management of skin excoriation around stomas (ileostomy and colostomy), giving a more rapid epithelialisation of the raw surface (Aminu *et al*, 2000). These clinical observations of the stimulatory effect of honey on tissue growth in wounds have been confirmed by measurements and histological observations in studies of experimental wounds in animals (Burlando, 1978; Bergman *et al*, 1983; Gupta *et al*, 1992; Kumar *et al*, 1993; Suguna *et al*, 1993; Postmes *et al*, 1997), where honey treatment has been shown to give statistically significant improvements. In these experimental wounds, honey has also been shown to stimulate the synthesis of collagen (Suguna *et al*, 1992) and other connective tissue components (Suguna *et al*, 1993), and to stimulate angiogenesis (development of new blood vessels) (Gupta *et al*, 1992; Kumar *et al*, 1993).

Stimulation of angiogenesis is an important feature for promotion of healing, as the supply of oxygen is the rate-limiting factor in tissue repair (Silver, 1980), granulation tissue being granules of fibroblasts growing where new capillary beds form. The anti-inflammatory activity of honey would also assist by decreasing oedema and, consequently, the pressure on capillaries which restricts blood flow and the supply of oxygen to the regenerating wound tissues. The acidity of honey would also help with oxygenation, as acidification of wounds speeds the rate of healing by increasing the release of oxygen from haemoglobin (Kaufman *et al*, 1985). The newly-formed capillaries supply essential nutrients to growing fibroblasts, another factor limiting the rate of healing — it has been demonstrated that wounds heal faster if a nutrient mixture is applied topically (Viljanto and Raekallio, 1976; Niinikoski *et al*,

1977; Silveti, 1981; Kaufman *et al*, 1984). There is a wide range of minerals, including the trace elements of nutrition, and of amino acids and vitamins contained in honey (Haydak *et al*, 1975; White, 1975). Although these are present in amounts too low to be of nutritional significance when compared with the recommended daily intake, they are, on average, present at levels like those circulating in the blood. This topical supply of nutrients would be augmented by the osmotic action of honey drawing lymph from the underlying capillaries, thus creating a constant flow of nutrients for cells which may be somewhat distant from the functioning capillaries deeper down. Another way in which honey may promote healing is by supplying glucose to the epithelial cells, as these have to build up an internal store of carbohydrate to provide the energy they need to migrate across the surface of a wound to restore skin cover (Silver, 1980). The level of glucose in the wound fluid of chronic wounds is very low (Schultz *et al*, 2003). The sugars in honey would also provide an energy source for the macrophages working in the wound, as glycolysis is their major mechanism for energy production, and is dependent on a supply of glucose or fructose. Glycolysis is the only means of cells obtaining energy in the absence of oxygen, so the supply of sugars from honey would allow them to function in damaged tissues where the oxygen supply is often poor (Ryan and Majno, 1977).

Another possible way that honey may work to stimulate wound repair is through its production of hydrogen peroxide, as hydrogen peroxide activates the insulin receptor complexes on cells (Czech *et al*, 1974; Helm and Gunn, 1986; Koshio *et al*, 1988). Activation triggers a chain of molecular events in the cell that stimulates the uptake of glucose and amino acids, and promotes anabolic metabolism, giving cell growth. Topical or intravenous application of insulin to wounds, stimulates the rate of wound healing (Lopez and Mena, 1968; Belfield *et al*, 1970; Pierre *et al*, 1998). By this mechanism, honey may stimulate the uptake and anabolic metabolism of the nutrients it supplies to wound tissues.

The stimulation of angiogenesis by honey, that has been observed experimentally when honey is applied to wounds, could also be via its production of hydrogen peroxide, as topical application of hydrogen peroxide has been found to enhance cutaneous blood recruitment in ischaemic ulcers (Tur *et al*, 1995). In vascular, smooth muscle cells, hydrogen peroxide is endogenously produced as part of the process of response to stimulation by platelet-derived growth factor, and exogenous hydrogen peroxide in the concentration range of 0.1 to 1.0 mmol/l will also function in the response (Rao and Berk, 1992). The promotion of formation of granulation tissue by honey may also be via the stimulation

of growth of fibroblasts by the hydrogen peroxide produced in honey, as hydrogen peroxide has been found to stimulate the proliferation of fibroblasts (Chung *et al*, 1993). There is a large amount of evidence that hydrogen peroxide is involved in many cell types in the body as a stimulus for cell multiplication, by acting at various points in the mechanisms of the cells that control the cycle of cell growth and division (Burdon, 1995). It has been proposed that low concentrations of hydrogen peroxide might be used to stimulate wound healing in place of the expensive cell growth factors used for this purpose (Burdon, 1995; Postmes and Vandeputte, 1999). However, this is feasible only if the concentration could be carefully controlled to avoid tissue damage (Chung *et al*, 1993). This is possible with the controlled sustained release of hydrogen peroxide that occurs in honey.

Another possibility is that the stimulation of tissue repair is a down-stream effect of the stimulation by honey of cytokine production by leukocytes. The production of cytokines, as part of the initial inflammatory response, normally starts off the healing process. There is good evidence for the ability of honey in quite dilute solution to stimulate such a response in leukocytes in cell culture. This is discussed in *Chapter 2*.

## Safety in use

Honey is extremely safe to use. In the 500-plus cases reported in publications on using honey on wounds, and the 140-plus cases reported of using honey in ophthalmology, there has been no mention of any adverse effects. A large number of other cases that have not been published are known to the author and, again, there have been no adverse effects observed in any of these, with the exception of one case where there appeared to be an allergic reaction on the skin around the wound. With honey, there are no reported cytotoxic effects that would slow the healing process, whereas all antiseptics in common use can be harmful to body tissues (Tatnall *et al*, 1991), including silver as released from nanocrystalline silver dressings (Poon and Burd, 2004).

There have been reports of honey causing a stinging pain when applied to wounds (Dunford *et al*, 2000b; Robson *et al*, 2001; Ahmed *et al*, 2003). This appears to be due to the acidity of honey, as pain is not experienced when neutralised honey is used (Dunford *et al*, 2000b;

Betts and Molan, 2001). The pain experienced does not seem to be indicative of damage being done to the wound, as wounds have healed rapidly in cases where patients have endured the pain to benefit from the stimulation of healing that they see, and in cases where analgesia has been used (personal communications from numerous clinicians). There is evidence that honey stimulates nociceptors (Al-Swayeh and Ali, 1998), nerve endings that create a pain sensation in response to heat, acidity and some organic chemicals such as those in ginger and chilli. It is of interest that patients have been reported to experience a 'peppery' sensation from application of honey to their ulcers (Oluwatosin *et al*, 2000). It may be that it is not a direct effect of the acidity of honey, as neutralising honey could affect the ionisation of some of its components and make them unable to fit in the nociceptors. It is possible that in some patients these nerve endings are sensitised and are more responsive to the acidity and/or the component organic chemicals of honey.

However, there are many reports of honey relieving pain (Al-Waili and Saloom, 1999; Dunford *et al*, 2000a, b; Cooper *et al*, 2001; Subrahmanyam *et al*, 2001; Richards, 2002; Stewart, 2002; Misirlioglu *et al*, 2003). In a trial in which pain was measured on a visual analogue scale, the pain experienced with a honey-soaked gauze dressing was found to be one-third less than with saline-soaked gauze and paraffin gauze, but slightly more than with a hydrocolloid dressing (Misirlioglu *et al*, 2003). In another trial, where the comfort of honey dressings on chronic venous leg ulcers was investigated, six patients experienced a transient stinging pain, and eight experienced a lasting pain, but only some of the times the dressing was applied (Dunford and Hanano, 2004). However, in this trial, the overall result was that pain was significantly reduced by the honey dressings, and the patient satisfaction with the honey dressings was high.

Other cases where honey is reported to cause pain are few. In one of these, there was pain experienced by the patient for the first twenty to thirty minutes (Dunford *et al*, 2000b). In another case, a patient experienced moderate pain for fifteen to thirty minutes after honey was applied (Robson *et al*, 2001). In a clinical trial of honey dressings, one of the sixty patients treated with honey withdrew because the dressings caused pain (Ahmed *et al*, 2003). In a pilot trial in which the author participated (Betts and Molan, 2002), six of the twenty patients recruited withdrew because of the pain caused by honey on the wound. This probably reflects the recruitment criterion of infected or heavily colonised wounds for the trial, as it was observed that pain was experienced only in inflamed wounds. Patients who found honey very painful when their



wounds were inflamed experienced no problem with pain once the inflammation had subsided. Similarly, in the trial where the comfort of honey dressings on chronic venous leg ulcers was investigated, the six patients who withdrew from the trial because of the honey being painful (out of a total of forty participants), had a higher than average pain level before the start of the honey dressings (Dunford and Hanano, 2004).

## Conclusion

The concerted action of the various physical properties and bioactivities in honey explains the remarkable results obtained clinically, especially when appropriately selected honeys are used and sufficient honey is held in place on the wound for these factors to work. Although some of the mechanisms of action are speculative, there is, nevertheless, a great deal of published evidence for honey having such actions on wounds. The excellent clinical evidence of overall effectiveness is presented in *Chapter 9*. The use of honey on human beings over a period of more than 4,000 years, with no adverse effects coming to light, is evidence of its effectiveness as a healing agent.

## References

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