Chapter 2

Why honey works

Peter Molan

There is increasing interest in using honey in wound care, but because there is relatively little promotion of honey products for wound care there is a common misconception that there is little evidence to support its use. However, there is in fact far more clinical evidence for the effectiveness of honey than there is for the myriad of modern wound dressings which are heavily advertised (Vermeulen *et al*, 2005). A review of the higher-level evidence for honey (Molan, 2006) revealed a great deal of evidence giving positive results for the use of honey in wound care. Since then, 16 more randomised controlled trials of honey have been published (Nagane *et al*, 2004; Okany *et al*, 2004; Bangroo *et al*, 2005; Johnson *et al*, 2005; Marshall *et al*, 2005; Okeniyi *et al*, 2005; Ingle *et al*, 2006; Mashhood *et al*, 2006; McIntosh and Thomson, 2006; Moolenaar *et al*, 2006; Tahmaz *et al*, 2006; Güneş and Eşer, 2007; Mphande *et al*, 2007; Nilforoushzadeh *et al*, 2007; Gethin and Cowman, 2008; Jull *et al*, 2008) giving the sum of 33 randomised controlled trials involving a total of 3289 participants. The review (Molan, 2006) also summarised the details of five other forms of clinical trials with 97 patients treated with honey, and ten reports of studies of case series (totalling 276 cases), most of which were chronic wounds which healed after being treated with honey.

Although the catch-cry these days is evidence-based medicine, this vast amount of evidence is not sufficient to persuade many clinicians to try using honey. The reason for this has long been present in the medical profession. In 1830 a lament was published by John Renton in the *Edinburgh Medical and Surgical Journal* (34: 101):

> *When no satisfactory explanation can be afforded of the modus operandi of the agent, professional persons, unhappily for the*

> *interests of medical science, are too apt to reason upon the authenticity of the facts averred, instead of adopting the more simple and direct method of determining their value by subjecting them to the fact of further experience.*

This chapter has been written to 'afford a satisfactory explanation of the '*modus operandi*' of honey; to explain biochemically why honey appears to be effective in bringing about rapid healing of wounds.

Formation and composition of honey

Nectar (or occasionally sap) is collected by foraging worker bees and delivered to the hive, where 'house bees' concentrate the harvested watery sugar solution by drying it in the warm draught created by multitudinous bees flapping their wings and generating heat by their 'aerobics'. During the intake of the nectar into the bees' crops, and subsequent regurgitation, enzymes are added by secretion from the hypopharyngeal gland which will help preserve the nectar. One of these enzymes, invertase, breaks sucrose (the principle sugar in nectar) into glucose and fructose, giving a mixture which is much more soluble than sucrose. The saturated or supersaturated solution of these two sugars has a sufficiently high osmolarity when honey is fully dried (i.e. to a point where it is about 17% water) to prevent the growth of any microorganisms.

Another enzyme added, glucose oxidase, generates hydrogen peroxide from the oxidation of glucose, and this serves to sterilise the nectar while it is being turned into honey. The other product of this enzymic reaction is gluconic acid, which gives honey a pH in the region of 3.5 to 4.5. This enzyme becomes dormant once the water content of the honey becomes low, but it will become active again if honey gets diluted. It is at maximal activity when honey is diluted to a concentration of about 50% (Bang, 2003).

Some of the other bee proteins added from the hypopharyngeal gland secretion also have bioactivity of relevance to wound healing (*Chapter 14*). Different types of honey will vary little in respect to their content of these various bee proteins. However, there is great variety in their content of different phytochemicals (plant chemicals), which, often occurring in trace quantities, give the different types of honey their characteristic colours, flavours, aromas, and bioactivities, such as antioxidant and anti-inflammatory properties [illegible]. Being

a concentrated plant fluid (nectar or sap), honey is basically a 'herbal medicine' with some bee proteins added. Anyone familiar with herbal medicines will know that different types of plants produce medicines with different therapeutic properties [illegible].

Nutritional value of honey

As flowering plants have co-evolved with pollinating insects, the nectar they produce has to provide the nutrients necessary to ensure the survival of the pollinators. Honey contains a wide range of nutritional minerals, amino acids and vitamins (Haydak, 1975; White, 1975), although its precise composition varies depending on the floral source. It has been reported that wounds heal faster if a nutrient mixture is applied topically (Kaufman, 1984; Niinikoski, 1977; Silvetti, 1981; Viljanto, 1976), and honey, used as a wound dressing, will be bathing the re-growing cells in the wound bed with such a nutrient mixture.

Honey can also promote healing by supplying glucose to epithelial cells, which require an energy source to migrate across the surface of a wound during reepithelialisation (Silver, 1980) [illegible]. The level of glucose in wound fluid of chronic wounds is low (Schultz, 2003). Honey is 30–40% glucose in its composition.

The sugars in honey provide an energy source for cells in the wound environment that remove bacteria and non-viable tissue, and initiate the cascade of cytokines and growth factors that regulate healing [illegible]. Glycolysis is the major mechanism for energy production in macrophages, and depends on adequate levels which may be limited in areas surrounding the wound where capillaries have been damaged. Glycolysis is the only means by which cells obtain energy in the absence of oxygen; thus, the supply of sugar from honey allows macrophages to function well in damaged tissues where oxygen supply is often poor (Ryan and Majno, 1977 [illegible]).

In individuals with diabetes, the shortage of insulin leads to impaired glucose uptake into cells and this may be a factor that contributes to their characteristically poor wound healing [illegible]. High glucose levels in a wound will establish the diffusion gradient across cell membranes, and thus enhance the entry of glucose by diffusion which is independent of the insulin-controlled active transport mechanism.

Bacteria always metabolise glucose in preference to amino acids. In

the absence of glucose, malodorous sulphur compounds, amines and ammonia arise from the anaerobic bacterial metabolism of amino acids formed from the digestion of proteins in wound fluid, however, in the presence of glucose these are not formed (Nychas *et al*, 1988). This accounts for the rapid deodorising effect of honey in wounds, prior to the inhibitory effects of honey.

Osmotic action of honey

Osmosis is the movement of water across a membrane towards a concentrated solution. Solutions of high osmolarity cause removal of water molecules from cells. The outflow of lymph created by the osmotic action of honey is beneficial to the healing process.

The rate-limiting factor for growth of granulation tissue is the availability of oxygen (Silver, 1980). The surface area of a wound bed is too small to allow atmospheric oxygen to dissolve at a sufficient rate to supply cellular needs. In the lungs the vast surface area of the alveoli allows oxygen to be absorbed and bound to haemoglobin at a high rate. It is then distributed to cells in tissues by blood. Within a wound, irregularities in the capillaries supplying blood, limits the availability of oxygen. The presence of inflammation and concomitant oedema exacerbates this situation. The osmotically induced outflow of lymph from underlying tissue by the addition of honey promotes extra oxygenation. This outflow of lymph also provides an improved supply of nutrients to the growing cells on the wound surface. Furthermore, it flushes away proteases which may be inhibiting the repair process (see below). These benefits have also been demonstrated with topical negative pressure (TNP).

The osmotic action of honey also gives a moist healing environment without promoting bacterial growth if honeys of proven antibacterial activity are used. Provided there is sufficient honey present, its osmotic effects allow the presence of sufficient fluids to prevent the dressing adhering to the wound bed. It is also likely that honey activates proteolytic action which digests any fibrin clot . Thus, the presence of honey next to the wound bed helps to prevent pain at dressing changes and trauma to the newly-formed wound tissue.

Acidity of honey aids wound healing

The level of dissolved oxygen is a rate-limiting factor for fibroblast growth. Haemoglobin passing through the capillaries releases only about 25% of the oxygen it carries if the pH is at the normal blood level (7.4). However, if the pH drops to just 6.8, more than 50% of the oxygen carried is released. It has been observed that acidification of wounds speeds the rate of healing (Kaufman, 1985; Leveen, 1973). An alkaline pH is associated with wounds which are not healing, whereas an acidic pH is associated with healing (*Chapter 9*).

Acidification of wounds may also aid healing by decreasing protease activity which may be preventing wound healing by digesting the extracellular matrix and growth factors which are essential for tissue repair (see below). As discussed in *Chapter 9*, the proteases in wounds work best at neutral to alkaline pH values.

Thus, the acidity of honey is of benefit in wound treatment even though it sometimes causes pain where nocioceptor nerve endings, which detect heat and acidity, have become sensitised by factors produced in inflamed wound tissue.

Antioxidants

Honey contains a high level of antioxidants (Frankel, 1998; Gheldof, 2002; Gheldof, 2002; Gheldof, 2003; Henriques, 2006; Meda, 2005; Vela, 2007; Aljadi, 2004; Al-Mamary, 2002; D'Arcy, 2005; Blasa, 2006). After honey is ingested its antioxidants give protection in the bloodstream and within cells (Schramm, 2003).

Antioxidants neutralise free radicals, which are highly reactive molecules that are damaging to vitally important components of wound tissue such as proteins, cellular DNA and cell membranes. A large amount of free radical production occurs in wounds as a result of phagocytosis. When bacteria or necrotic cells are engulfed by phagocytes, an enzyme in the cell membrane becomes activated and produces the superoxide free radical. This in turn gives rise to hydrogen peroxide and other free radicals which are collectively known as 'reactive oxygen species' (ROS). Hydrogen peroxide is not included in this term, but it can diffuse out of the phagocytes and generate free radicals outside the cell. The superoxide radical is highly reactive and, although it can cross cell membranes, it usually reacts immediately before diffusion.

The chemical attractant is not hydrogen peroxide itself but a peptide produced from complement factor C5 in serum by oxidation by ROS free radicals (Shingu, 1984). This oxidation reaction would

be inhibited by antioxidants. The attraction of neutrophils which results from superoxide produced is similarly not a direct attraction to superoxide itself. The superoxide produced reacts with lipids in serum to create the attractant molecules (Flohé, 1985).

Manuka honey is unusual in containing a high level of methyl syringate, an antioxidant which neutralises the superoxide radical (Inoue, 2005). It also has another unusual type of antioxidant activity. This functions by binding iron and making it incapable of catalysing the formation of the extremely damaging hydroxide radical from hydrogen peroxide (Brangoulo and Molan, unpublished work). This iron-catalysed breakdown of hydrogen peroxide is considered to be a major source of free radical damage in the body (Puntarulo, 2005).

Far greater damage to tissues is caused by the activation of proteases in the wound tissues, than by the ROS themselves (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). The matrix metalloproteases are activated by oxidation (Van Wart, 1990) and the inhibitor of neutrophil protease in wound tissue is inactivated by oxidation (Flohé, 1985). Once activated, these proteases can destroy wound tissue. Thus, a wound can become ulcerated and a partial thickness burn can become full-thickness if there is excessive production of ROS.

Activated proteases destroy both cytokines and tissue growth factors, that have essential regulatory functions. They also destroy the extracellular matrix, which are important for cellular attachment during migration and multiplication. The antioxidant activity of honey could thus be responsible for major protection of wound tissue where inflammation has caused an influx of phagocytes. 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). From the results of another clinical trial of honey dressings on burns, in which oxidative stress was measured, it was concluded that control of free radicals by the antioxidant action of honey was the way in which honey initiates healing in burns (Subrahmanyam, 2003).

Anti-inflammatory action

Excessive inflammation is the scourge of wound healing. A mild, short-lived state of inflammation is required to initiate the healing process, but when it becomes excessive the number of neutrophils and macrophages present increases and the ROS from their phagocytic activity cause damage which slows or prevents healing. If bacteria or any other trigger for inflammation persist, a chronic wound can result because of the activated proteases digesting cytokines, growth factors and extracellular matrix.

The large number of clinical reports where honey was shown to relieve the symptoms of inflammation has been reviewed (Molan, 2002). Some of these reports make it clear that this is not due simply to the clearance of the bacteria present, as decreased inflammation resulting from application of honey was seen in trials conducted with animals with experimentally inflicted wounds in which there were few or no bacteria present (Burlando, 1978; El-Banby, 1989; Gupta, 1992; Kandil, 1987; Kumar, 1993; Oryan, 1998; Postmes, 1997). The anti-inflammatory effect of honey was observed in studies on induced colitis in laboratory animals (Bilsel, 2002; Mahgoub, 2002) and in the wrist-joint stiffness test in guinea-pigs which is standard test for anti-inflammatory activity (Church, 1954).

The antibacterial activity of honey (*Chapter* 3) plays an important part in decreasing inflammation in wounds, as components of the cell wall of bacteria are potent stimulators of the inflammatory response (*Chapter 14*). The presence of slough in wounds also acts as an inflammatory stimulus and its removal following topical application of honey to wounds [illegible] is another anti-inflammatory effect (*Chapter* 9).

The inflammatory response itself can set up a vicious cycle if the inflammatory stimulus is prolonged and superoxide and hydrogen peroxide are continuously produced because they act to recruit more neutrophils by chemical attraction (Flohé, 1985; Klyubin, 1996) (*Figures 2.1* and 2.2). Hydrogen peroxide activates neutrophils, via the activation of the nuclear transcription factor NF-kB, to switch on genes to produce cytokines which amplify the inflammatory response by recruiting and activating leukocytes (Baeuerle, 1996). The recruited neutrophils in turn produce more hydrogen peroxide. Thus there is a feed-back amplification of the inflammatory response, which exists while the stimulus continues.

With this being a cycle, hydrogen peroxide from other sources can trigger it. This explains why reperfusion injury gives rise to inflammation

because the hydrogen peroxide is generated by cellular metabolism when the oxygen supply is restored after circulation-restricting pressure is relieved. It results in the formation of pressure and varicose ulcers. It has been shown that the oxidative species formed from hydrogen peroxide, rather than hydrogen peroxide itself, is responsible for activating NF-kB. Antioxidants prevent this activation (Grimble, 1994), therefore, honeys that contain antioxidants ameliorate this effect (Schreck, 1991).

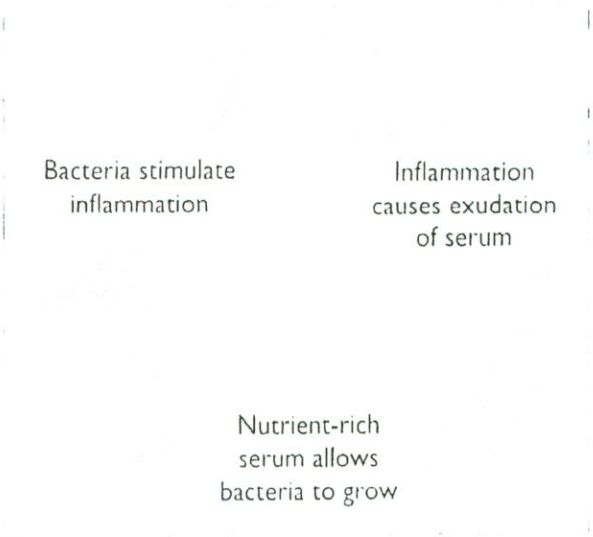

Figure 2.1: The vicious cycle that can result when bacteria are in a wound. It can be started by infection or by inflammation resulting from other causes

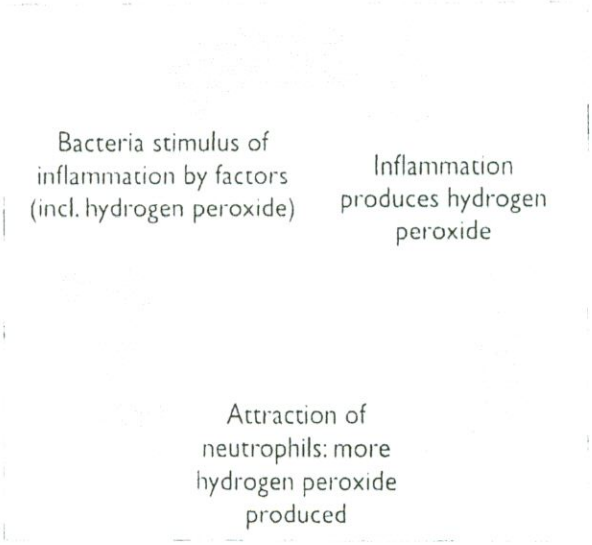

Figure 2.2: The vicious cycle that can result from feed-back amplification of the inflammatory response by the hydrogen peroxide that is produced

The ability of honey to neutralise free radicals has been demonstrated (van den Berg, 2008; Henriques, 2006). Clinically it has been shown that application of antioxidants to burns (Tanaka, 1995) and to corneal ulcers (Alio, 1995) decreases inflammation. However, recent research has revealed that honey also has another mechanism of anti-inflammatory activity, as it directly inhibits the process of phagocytosis itself, thus preventing oxygen free radicals being formed in the first place (Bean, A; University of Waikato: personal communication). In the author's opinion, it is most likely that both mechanisms are involved. Although it may be thought that the antioxidant action neutralising free radicals would be more important once inflammation

has become established, it must be remembered that in the vicious cycle there is ongoing phagocytosis. Inhibition of this would help break the cycle.

Both the prevention of free radical formation and the quenching of free radicals are important factors in the ability of honey to minimise hypertrophic scarring. The ROS formed in inflammation stimulate the activity of the fibroblasts which produce the collagen fibres of scar tissue, and in situations where there is prolonged inflammation, their over-stimulation can lead to hypergranulation and fibrosis (Murrell, 1990). Like with the stimulation of leukocytes, this is via oxidation of NF-kB, and can be inhibited by antioxidants (Murrell, 1990). There have been numerous clinical reports of this therapeutic feature of honey (Dunford *et al*, 2000; Efem, 1993; Subrahmanyam, 1991; Subrahmanyam, 1994). Free radicals have been implicated in hypertrophic scar formation following burn injuries (Wan, 1999).

Immunostimulatory activity

It may be thought that the removal of the impediments to healing by way of the antibacterial, antioxidant and anti-inflammatory actions of honey would account for the rapid rate of healing brought about by honey, and the 'jump-start' of healing in wounds where healing has stalled (Molan, 2002). But there is evidence from studies on experimental wounds in animals that honey stimulates the growth of repair tissues. A study on mice found that honey gave a 114% increase in the extent of epithelialisation and a 69% increase in the thickness of granulation tissue compared with a saline control (Bergman, 1983). Stimulation of angiogenesis by honey has been observed histologically (Gupta, 1992; Kumar, 1993). The most likely mechanism for such promotion of growth is the stimulation of cytokine production by honey, which is covered in *Chapter 14*.

References

Al-Mamary M, Al-Meeri A, Al-Habori M (2002) Antioxidant activities and total phenolics of different types of honey. *Nutrition Res* 22(9): 1041–7

Aho JL, Ayala MJ, Mulet ME, Artola A, Ruiz JM, Bellot J (1995) Antioxidant therapy in the treatment of experimental acute corneal inflammation. *Ophthalmic Res* 27(3): 136–43